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Cover: *Saproamanita praeclara*: Sporocarp in habitat © Kantharaja. R.

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COMMUNICATION

Study on the diversity of birds in the new abode of wetlands created by the 2004 tsunami in South Andaman

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Abstract: Subsidence and upliftment of landmass were encountered in Andaman & Nicobar Islands due to the 2004 tsunami. The subsided landmass at the coastal front was permanently waterlogged ensuring a conducive new habitat for wetland birds. Pre- and post-tsunami Landsat satellite data products were used to demarcate the permanently waterlogged areas. A total of 63 bird species belonging to nine families comprising of five orders were identified and documented through direct observation technique in six stations of the 2004 post-tsunami-created wetlands in South Andaman. Order Charadriiformes and Anseriformes recorded the highest (47.62%) and least (4.76%) taxonomic composition of wetland birds, respectively. Scolopacidae family recorded the highest (56.67%) species composition. Among the six stations, the highest diversity of birds was observed in Sippighat and Ograbraj stations.

Keywords: Andaman birds, geographic information system (GIS), landmass subsidence, remote sensing, Tsunami-created wetlands (TCW), wetland biodiversity.

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Author contributions: NP and VSS—concept, and documentation of manuscript. NP, VSS, SH and CR—Bird watching and documentation. GN—GIS mapping. RPS—reference collection and graphing.

INTRODUCTION

The 26 December 2004 (Sumatra-Andaman) was one of the two recent tsunamigenic mega earthquakes that unleashed a tremendous catastrophic impact on the human race and the environment (Malik et al. 2019) which struck near Indonesia with a magnitude of 9.3 on the Richter scale (Stein & Okal 2005; Garay & Diner 2007). The epicenter was situated 80 km west of the coast of northern Sumatra (at approximately 95.85W and 03.41N). The seismic wave thereafter advanced approximately northward rupturing the 1,300-km Andaman-Sunda plate (with an average rupture speed of 2.5 to 3 km/s) in about 8 to 10 minutes (Ammon et al. 2005; Bilham et al. 2005; Lay et al. 2005; Subarya et al. 2006; Malik et al. 2011) resulting in up to ~6 m of bottom subsidence and ~10 m of upliftment parallel to the rupture and about 100–150 km wide across the subduction area (Malik & Murty 2005; Ioualalen et al. 2007; Malik et al. 2011, 2015). Upliftment and subsidence of landmass were caused as a consequence of earthquake elastic rebound, offshore of Banda Aceh, the northern tip of Sumatra (Bilham 2005). The tsunami waves which surged across the ocean with a velocity of 900 km/h and took a devastating wave height of 10 m with a speed of 40 km/h along the coastal frontiers (Bahuguna et al. 2008) resulting in permanent waterlogging in landmass subsided zones (Shankar et al. 2019).

Andaman & Nicobar Islands (ANI) is the maritime union territory of India in the Bay of Bengal between Myanmar and peninsular India, distributed in the north-south direction. It is a group of 572 islands, stretching over 700 km and bounded by the geographic coordinates (06.00–14.00 N and 92.00–94.00 E). ANI is located close to Myanmar by 190 km in the north, Sumatra by 150 km in the south, and 1,200 km from mainland India (Shankar et al. 2016). ANI enjoys a tropical climate, and classified as warm and humid. The average annual temperature is in the range of 25°C to 30.5°C and relative humidity is 81%. The average annual rainfall is approximately 3,000–3,500 mm with south-west monsoon (May–September) contributing 76.35%, north-east monsoon (October–December) contributing to 22%, and pre-monsoon (January–April) of 1.64% (Meteorological Statistics 2019).

ANI are distinct eco-regions sandwiched between two major landmasses, namely, the Indian subcontinent and the Malaysian-Indonesian. It is one of the 12 biogeographic zones of India showing great diversity, variety, and high level of endemism in flora and fauna. ANI comprises of nine national parks, 96 sanctuaries, and one biosphere reserve (Rao et al. 2013; FSI 2019).

ANI were the first responders to the 2004 tsunami among other territories of India because of its closest proximity to the epicenter. Due to this tsunamigenic mega-earthquake, the ANI experienced both upliftment and landmass subsidence (Nehru & Balasubramanian 2011, 2018) creating permanently waterlogged areas (Shankar et al. 2019). These waterlogged areas were called tsunami wreated Wetlands (TCW) and they became a favorable habitat for the water birds. The avian diversity of ANI is well documented by various studies (Pande et al. 2007; Sivaperuman et al. 2010, 2018). However, there is a lack of studies about the diversity of birds post 2004 in the TCW thus created and this study is a first of its kind. The study was initiated to understand the diversity and distribution of birds in the six TCWs of South Andaman (Figure 1).

MATERIALS AND METHODS

Pre (2003) and post (2018) tsunami Landsat satellite images, ArcGIS 10.5, Garmin 60 CSx handheld global positioning system (GPS), binocular, and camera were used to comprehend the objective of the present investigation.

Landsat (7 & 8) satellite data products before (2003) and after (2018) tsunami respectively for the study were downloaded from the website (www.earthexplorer.usgs.gov/). The study area is covered by the scene with path (134) and row (52). Mangrove patches and water bodies decipherably picked up very well by band-5 and band-6 by the short-wave infrared (SWIR) sensor of Landsat 7 and 8 satellites respectively from other features like forests and human settlements. six TCWs were chosen for the assessment of wetland avian diversity (Figure 1) using the leads from pre- and post-2004 tsunami satellite images. These six birding locations are Stewartgunj, Ograbraj, Sippighat, Wandoor, Chidiyatapu, and Carbyn's Cove (Table 1, Image 1,2). Field visits were carried out from November 2018 to March 2020. Each of these six sites was periodically revisited every month on weekends (Saturday and Sunday) at 0600–0900 h during the aforementioned period. Upon reaching the field using binocular the birds were observed directly (Altman 1974) and identified using the identification keys by Ali (2002) and Grimett et al. (2012). Also, the identified birds were cross verified with the checklist of Pande et al. (2007) and Sivaperuman et al. (2018). Thus, the distribution of wetland birds in TCWs of six villages

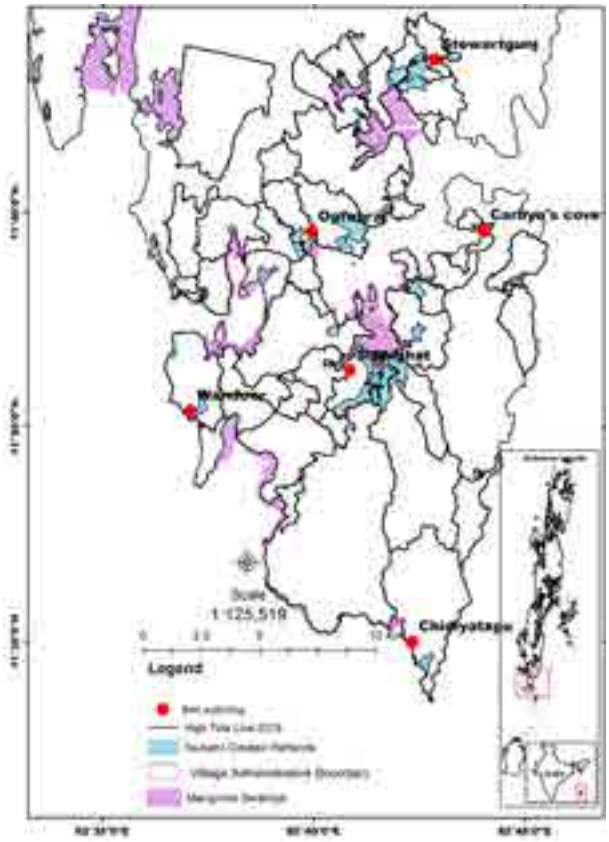

Figure 1. Study area map.

was observed, identified, and documented.

RESULTS AND DISCUSSION

Mangrove swamps were the ancestral abode to the wetland birds of the study area (Figure 1). Physical fury, subsidence of landmass, and permanent water logging due to the 2004 tsunami resulted in massive destruction of mangroves (Roy & Krishnan 2005; Nehru & Balasubramanian 2011; 2018; Shankar et al. 2019). Thus, the wetland birds were flushed out of their original habitat and were resilient to adapt to the post-tsunami newly created habitat. All the six study sites' visual interpretation of pre (2003) and post (2018) tsunami satellite data articulates the loss of mangrove habitat and the areal extent of the TCWs are presented in Table 1. The mangrove swamp at Ograbraj (10.31 ha) was completely wiped out by the 2004 tsunami. On the other hand, the mangrove swamp of Carbyn's Cove (0.66 ha) was spared, while the subsidence of landmass resulted in the creation of wetland after the tsunami at Stewartgunj. Sippighat mangrove swamps were one of the worst affected habitats in the study area (Roy & Krishnan 2005; Yuvaraj & Dharanirajan 2013; Das et al. 2014; Shankar et al. 2019) as only 37.37 ha (2018) of mangroves survived out of the 130.05 ha (2003). The chosen six birding locations have unique importance, Chidiyatapu is an internationally known birding location in Andaman. While Wandoor is covered under the Mahatma Gandhi Marine National Park (MGMNP). Stewartgunj is situated at the foothills of Mt Harriet National Park. Ograbraj located close to Sippighat (~3 km across the sea), massive mangrove habitat destruction was observed in these two locations.

A total of 63 birds were observed through the direct observation technique and identified using keys by Ali (2002) and Grimett et al. (2012). These 63 wetland birds (Figure 2a) encompassed in five orders, viz., Anseriformes (3 species; 4.76%), Coraciiformes (8 species; 12.70%), Gruiformes (9 species; 14.28%), Charadriiformes (30 species; 47.61%), and Pelecaniformes (13 species; 20.63%). Order Anseriformes, Coraciiformes, Gruiformes, and Pelecaniformes comprise of one family each, viz., Anatidae, Alcedinidae, Rallidae, and Ardeidae. Charadriiformes (Figure 2b) was the most diverse order comprising of five families, viz., Burhinidae (1 species; 3.33%), Charadridae (7 species; 23.33%), Jacanidae (1 species; 3.33%), Laridae (4 species; 13.33%), and Scolopacidae (17 species; 56.66%). Among the 63 birds only two species, viz., Andaman Crake *Rallina canningi* and Andaman Teal *Anas albogularis* are endemic.

According to IUCN version 3, of the identified 63 wetland birds majority (57 species; 90.47%) are Least Concern (LC), five species (7.93%) are Near Threatened (NT), and one species (1.58%) is Vulnerable (VU) categories. Chinese Egret *Egretta eulophotes* is the only Vulnerable species belonging to the order

Table 1. Before and after tsunami areal extent of wetlands (Mangrove stand in ha).

Village name	Before Tsunami	After Tsunami	Area of TCW (ha)	Before tsunami land use apart from Mangrove
Chidiyatapu	18.42	2.43	27.83	Agricultural Land & Settlement
Wandoor	31 .7	21.36	28.89	Agricultural Land
Ograbraj	10.31	0	32.42	Agricultural Land
Sippighat	130.05	37.37	136.96	Agricultural Land & Settlement
Carbyn's Cove	0.66	0.66	4.20	Agricultural Land
Stewartgunj	0	0	10.2	Agricultural Land

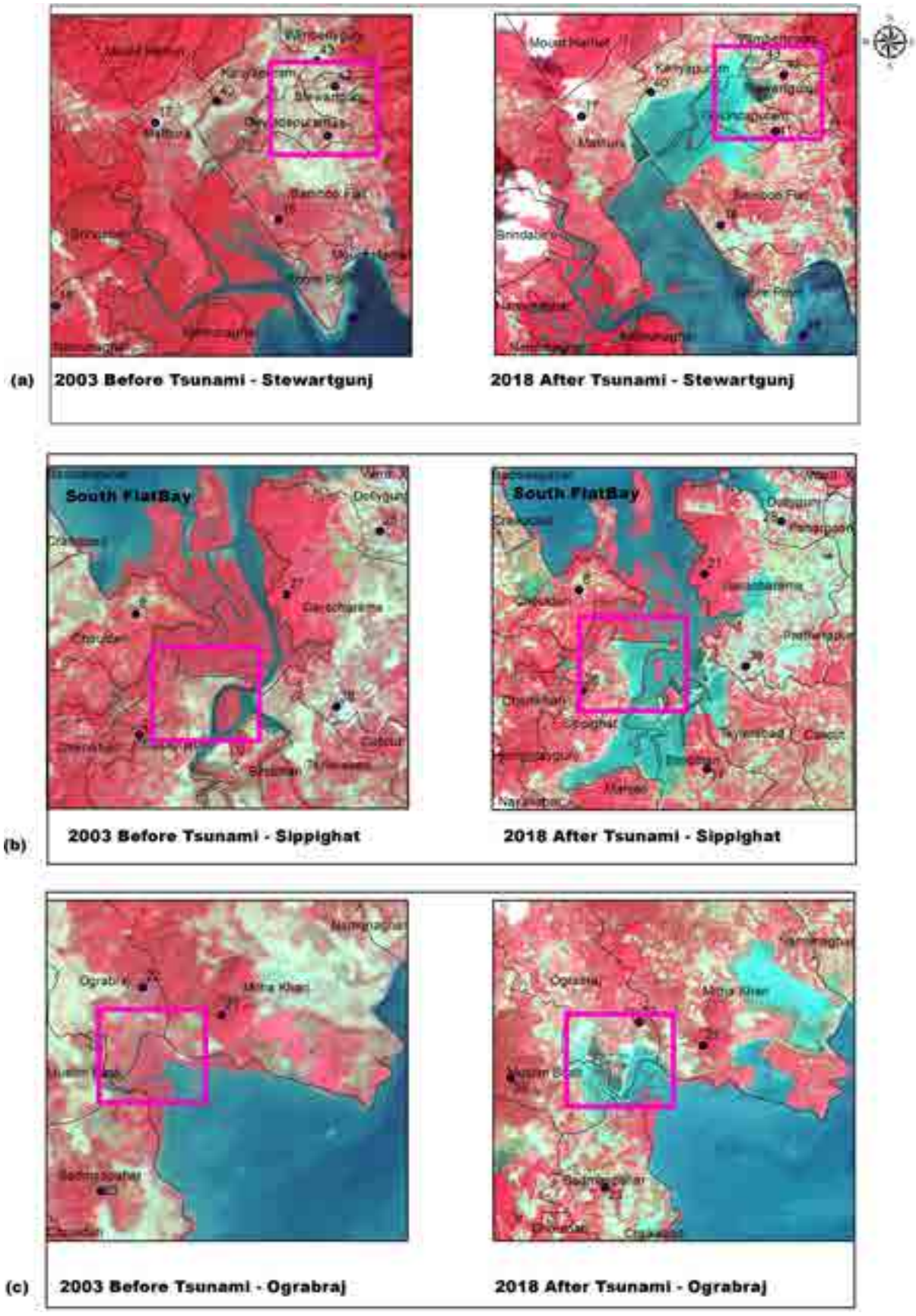

Image 1. Before and after tsunami satellite image: a—Stewartgunj | b—Sippighat | c—Ograbraj.

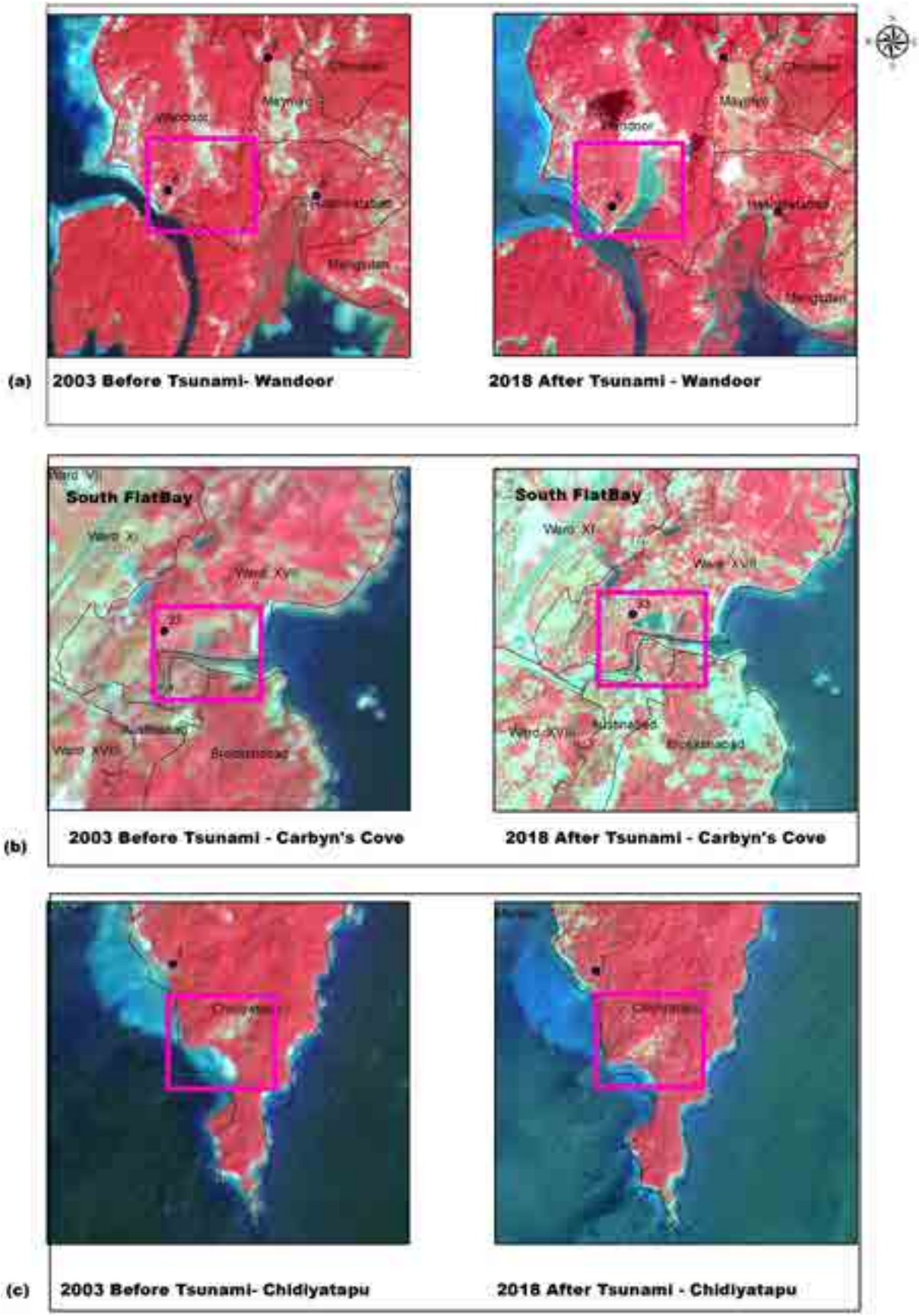

Image 2. Before and after tsunami satellite image: a—Wandoor | b—Carbyn's Cove | c—Chidiyatapu.

Pelecaniformes. Whereas, Andaman Teal *Anas albogularis*, Curlew Sandpiper *Calidris ferruginea*, Eurasian Curlew *Numenius arquata*, Bar-tailed Godwit *Limosa lapponica*, and Beach Thick-Knee *Esacus magnirostris* are Near Threatened. The complete list of birds is in Table 2, Image 3. According to the Indian Wildlife (Protection) Act, 1972, one species (Andaman Teal) was listed under Schedule I; and all the 63 species were included in Schedule IV.

Wards cluster analysis (Figure 2c) articulates two distinct clusters. Cluster 1 comprises Wandoor and Carbyn's Cove. Eleven and 18 wetland birds were identified from two sites of Wandoor and Carbyn's Cove. The species diversity was found to be low in these two sites when compared to the other four locations. Low levels of species diversity in Carbyn's Cove and Wandoor are probably due to the frequent movement of tourist vehicles and fishing boats in these two sites. In addition to tourism, the movement of vehicles and the closest proximity of human settlements around the wetlands has threatened the birds in Carbyn's Cove. Cluster 2 is further classified into two sub-groups. Ograbraj and Sippighat exhibit a high degree of species diversity. These two stations comprise 53 and 54 wetland birds, respectively. Before the 2004 tsunami, Sippighat was the abode for wetland birds. The massive mangrove habitat loss (80%) in Sippighat (Roy & Krishnan 2005) and a complete wipe of the mangrove swamp in Ograbraj (Shankar et al. 2019) flushed the wetland birds to the newly created habitat of TCW. The vast expanse of permanent waterlogging with suitable prey base availability in these sites would have the wetland birds adapt to the new environment. We assume this could be the reason for the high diversity observed in both Sippighat and Ograbraj sites. Stewartgunj and Chidiyatapu form another subgroup of cluster 2. A retrospection of pre-and post-tsunami satellite data products (Image 1) articulates that the shoreline has migrated inwards to Stewartgunj due to the subsidence of landmass thus responsible for the migration of wetland birds to the new habitat.

Before the 2004 tsunami impact, agriculture was extensively practiced on the coastal plains of Sippighat and Ograbraj (Rajan & Pramod 2017). The loss of natural habitat due to tsunami had led to the migration of the wetland birds to the new habitat (permanently waterlogged subsided landmass).

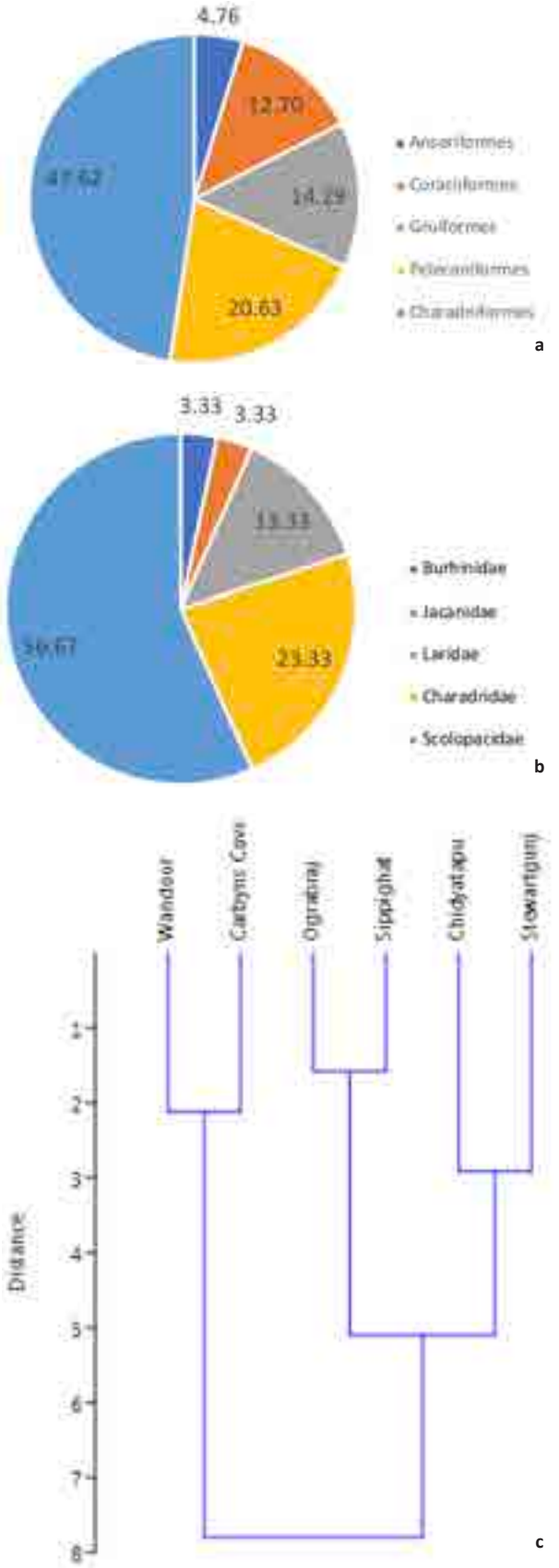

Figure 2. a—Taxonomic composition of wetland birds | b—Species composition of order Charadriiformes | c—Cluster analysis.

Table 2. wetland-wise avian diversity.

Order	Family	Common name	Scientific name	Status	IUCN Red List status	CT	WD	OB	SG	CC	ST
Anseriformes	Anatidae	Lesser Whistling Duck	*Dendrocygna javanica*	R/LM	LC	P	P	P	P	A	P
Anseriformes	Anatidae	Andaman Teal*	*Anas albogularis*	R	NT	P	A	P	P	A	P
Anseriformes	Anatidae	Cotton Teal	*Nettapus coromandelianus*	R	LC	P	A	P	P	A	A
Coraciiformes	Alcedinidae	White throated Kingfisher	*Halcyon smyrnensis*	R	LC	P	P	P	P	P	P
Coraciiformes	Alcedinidae	Stork-billed Kingfisher	*Pelargopsis capensis*	R	LC	P	P	P	P	P	P
Coraciiformes	Alcedinidae	Ruddy Kingfisher	*Halcyon coromanda*	R	LC	A	P	P	P	A	P
Coraciiformes	Alcedinidae	Black capped Kingfisher	*Halcyon pileata*	R/LM	LC	P	P	P	A	A	A
Coraciiformes	Alcedinidae	Blue Eared Kingfisher	*Alcedo meninting*	R	LC	P	A	P	P	A	P
Coraciiformes	Alcedinidae	Common Kingfisher	*Alcedo atthis*	WM	LC	P	P	P	P	P	P
Coraciiformes	Alcedinidae	Collared Kingfisher	*Todiramphus chloris*	R	LC	P	P	P	P	P	P
Coraciiformes	Alcedinidae	Oriental Dwarf Kingfisher	*Ceyx erithaca*	R	LC	P	A	P	P	A	P
Gruiformes	Rallidae	Andaman Crake*	*Rallina canningi*	R	LC	P	P	A	A	A	P
Gruiformes	Rallidae	Baillon's Crake	*Zapornia pusilla*	WM	LC	A	A	A	P	A	A
Gruiformes	Rallidae	Ruddy Breasted Crake	*Zapornia fusca*	WM	LC	P	A	P	P	A	A
Gruiformes	Rallidae	Common Coot	*Fulica atra*	R/LM	LC	P	A	P	P	A	A
Gruiformes	Rallidae	Purple swamphen	*Porphyrio poliocephalus*	R	LC	P	A	P	P	P	P
Gruiformes	Rallidae	Eurasian Moorhen	*Gallinula chloropus*	R	LC	A	A	P	P	A	P
Gruiformes	Rallidae	Slaty-breasted Rail	*Lewinia striata*	R	LC	P	P	P	P	P	P
Gruiformes	Rallidae	Water Cock	*Gallicrex cinerea*	R/LM	LC	P	A	P	P	A	P
Gruiformes	Rallidae	White-breasted Waterhen	*Amaurornis phoenicurus*	R	LC	P	P	P	P	P	P
Charadriiformes	Scolopacidae	Common Snipe	*Gallinaga gallinago*	WM	LC	P	P	P	P	A	P
Charadriiformes	Scolopacidae	Pin tailed Snipe	*Gallinago stenura*	WM	LC	P	P	P	P	P	P
Charadriiformes	Scolopacidae	Curlew Sandpiper	*Calidris ferruginea*	WM	NT	P	A	P	P	A	A
Charadriiformes	Scolopacidae	Common Sandpiper	*Actitis hypoleucos*	WM	LC	P	P	P	P	P	P
Charadriiformes	Scolopacidae	Green Sandpiper	*Tringa ochropus*	WM	LC	P	A	P	P	A	A
Charadriiformes	Scolopacidae	Terek Sandpiper	*Xenus cinereus*	WM	LC	A	A	P	P	A	A
Charadriiformes	Scolopacidae	Wood Sandpiper	*Tringa glareola*	WM	LC	P	A	P	P	A	P
Charadriiformes	Scolopacidae	Eurasian Curlew	*Numenius arquata*	WM	NT	P	P	P	P	P	P
Charadriiformes	Scolopacidae	Little Stint	*Calidris minuta*	WM	LC	A	A	A	P	A	A
Charadriiformes	Scolopacidae	Long-toed Stint	*Calidris subminuta*	WM	LC	P	A	P	P	A	P
Charadriiformes	Scolopacidae	Marsh Sandpiper	*Tringa stagnatilis*	WM	LC	P	A	P	P	A	A
Charadriiformes	Scolopacidae	Bar tailed Godwit	*Limosa lapponica*	WM	NT	A	A	P	A	A	A
Charadriiformes	Scolopacidae	Common Greenshank	*Tringa nebularia*	WM	LC	P	A	P	P	A	P
Charadriiformes	Scolopacidae	Common Redshank	*Tringa totanus*	WM	LC	P	A	P	P	A	P
Charadriiformes	Scolopacidae	Spotted Redshank	*Tringa erythropus*	WM	LC	A	A	P	P	A	A
Charadriiformes	Scolopacidae	Eurasian Whimbrel	*Numenius phaeopus*	WM	LC	P	P	P	P	P	P
Charadriiformes	Scolopacidae	Ruddy Turnstone	*Arenaria interpres*	WM	LC	P	A	A	A	P	A
Charadriiformes	Burhinidae	Beach Thick-knee	*Esacus magnirostris*	R	NT	A	P	A	A	A	A
Charadriiformes	Jacanidae	Pheasant-tailed Jacana	*Hydrophasianus chirurgus*	WM	LC	P	A	P	P	A	P
Charadriiformes	Charadridae	Greater Sand Plover	*Charadrius leschenaultii*	WM	LC	P	P	P	P	P	P
Charadriiformes	Charadridae	Grey-headed Lapwing	*Vanellus cinereus*	WM	LC	A	A	A	P	A	A
Charadriiformes	Charadridae	Grey Plover	*Pluvialiss quatarola*	WM	LC	A	A	A	A	P	P

Order	Family	Common name	Scientific name	Status	IUCN Red List status	CT	WD	OB	SG	CC	ST
Charadriiformes	Charadridae	Kentish Plover	*Charadrius alexandrinus*	WM	LC	A	A	P	P	A	A
Charadriiformes	Charadridae	Lesser Sand Plover	*Charadius mongolus*	WM	LC	P	A	P	P	A	P
Charadriiformes	Charadridae	Little Ringed Plover	*Charadrius dubius*	WM	LC	P	A	P	P	A	A
Charadriiformes	Charadridae	Pacific Golden Plover	*Pluvialis fulva*	WM	LC	P	P	P	P	P	P
Charadriiformes	Laridae	Black Naped Tern	*Sterna sumatrana*	R/LM	LC	P	A	A	A	P	P
Charadriiformes	Laridae	Lesser Crested Tern	*Thalasseus bengalensis*	WM	LC	P	A	A	A	A	A
Charadriiformes	Laridae	Little Tern	*Sternula albifrons*	WM	LC	P	A	P	P	A	A
Charadriiformes	Laridae	Whiskered Tern	*Chlidonias hybrida*	WM	LC	A	A	P	P	A	A
Pelecaniformes	Ardeidae	Black Bittern	*Ixobrychus flavicollis*	WM	LC	A	A	P	P	A	A
Pelecaniformes	Ardeidae	Cattle Egret	*Bubulcus ibis*	R/LM	LC	P	A	P	P	P	P
Pelecaniformes	Ardeidae	Chinese Egret	*Egretta eulophotes*	WM	VU	A	A	P	P	A	A
Pelecaniformes	Ardeidae	Chinese Pond Heron	*Ardeola bacchus*	WM	LC	A	A	P	P	A	P
Pelecaniformes	Ardeidae	Cinnamon Bittern	*Ixobrychus cinnamomeus*	R/LM	LC	P	P	P	P	A	P
Pelecaniformes	Ardeidae	Great Egret	*Ardea alba*	R/LM	LC	P	A	P	P	P	P
Pelecaniformes	Ardeidae	Yellow Bittern	*Ixobrychus sinensis*	WM	LC	P	A	P	P	P	P
Pelecaniformes	Ardeidae	Purple Heron	*Ardea purpurea*	R/LM	LC	A	A	P	P	A	A
Pelecaniformes	Ardeidae	Striated Heron	*Butorides striata*	R	LC	P	P	P	P	P	P
Pelecaniformes	Ardeidae	Pacific Reef Egret	*Egretta sacra*	R	LC	P	P	P	P	P	P
Pelecaniformes	Ardeidae	Little Egret	*Egretta garzetta*	R/LM	LC	P	P	P	P	P	P
Pelecaniformes	Ardeidae	Indian Pond Heron	*Ardeola grayii*	R/LM	LC	P	A	P	P	A	P
Pelecaniformes	Ardeidae	Intermediate Egret	*Ardea intermedia intermedia*	R/LM	LC	P	P	P	P	P	P

*—Endemic to Andaman Nicobar Islands | LC—Least Concern | NT—Near Threatened | VU—Vulnerable | R—Resident | R/LM—Resident with local movement | WM—Winter Migration | —Present | A—Absent | CT—Chidiyatapu | WD—Wandoor | OB—Ograbraj | SG—Sippighat | CC—Carbyn's Cove | ST—Stewartgunj.

CONCLUSION

The present study is the first of its kind to document the diversity of wetland birds in the last two decades. This study contributes to the rich bird diversity recorded in earlier studies of the Andaman & Nicobar Islands. Various tools like GIS, remote sensing, and on-field direct observation were comprehended to achieve the objective of the present investigation in pre and post-tsunami bird diversity and TCW. Land reclamation from these newly created wetlands is on the rise since it is in private ownership thus threatening the diversity of these wetland birds. A conservation drive is recommended for the conservation of these wetland birds.

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Image 3. Field photos of wetland birds. a—Common Moorhen | b—Purple Swamphen | c—Andaman Teal | d—Striated Heron | e—Lesser Whistling Duck | f—Pheasant-tailed Jacana.

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COMMUNICATION

Population abundance of Greater Flamingo *Phoenicopterus roseus* (Aves: Phoenicopteridae) in district Gurugram of Haryana, India

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Abstract: We quantified the population abundance of Greater Flamingo *Phoenicopterus roseus* in Najafgarh Drain (Jheel), Basai Wetland, and Sultanpur flats of district Gurugram, Haryana from October 2018 to December 2020. A total of 72 visits were made to the study sites. In this study, we explored the uses of an unmanned aerial vehicle (UAV) equipped with a 5-megapixel camera to census the population and distribution of Greater Flamingos. The Nikon 10 x 50 field binoculars were used for observations. A photographic record was taken using a Canon Powershot sx70hs camera. To estimate the population size, point count method was used and videos and image analysis were carried out for a more accurate count in densely packed flocks. The mean population of Greater Flamingos was 267 ± 47 observed throughout the study period from the three sites. For three years, the highest mean population of Greater Flamingos recorded was 745 ± 76 at Najafgarh Drain and the lowest was 19 ± 8 at Sultanpur Flats. The Greater Flamingos were found to be residents at Najafgarh Drain. At the Basai Wetland, two major human activities were the construction of highways along wetlands and wetland drainage have been observed that resulted in habitat fragmentation and shrinkage, which is responsible for the huge decline in their population. While at Najafgarh Jheel fishing activities and overgrowth of water hyacinth were a major threat that affect the Greater Flamingo population. The findings in this study will be beneficial for the conservation efforts of the flamingos in this area.

Keywords: Distribution, drone, population abundance, Najafgarh Drain, UAV.

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Author contributions: SR and AK conceived and designed the study. Both the authors equally contribute in field data collection, preparation of draft of the manuscript and data analysis.

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INTRODUCTION

Greater Flamingo *Phoenicopterus roseus* is a significant species of the family Phoenicopteridae, it is one of the long-legged water-wading birds belonging to the order *Phoenicopteriformes* (Ali et al. 1987). Flamingos are gregarious birds that are found in groups, extending from a few to thousands or lakhs and their group is termed 'Pat' (Tere 2005; Johnson & Cezilly 2007). Presently, six species of flamingos are known globally, among which four species namely the Caribbean Flamingo *Phoenicopterus ruber*, the Chilean Flamingo *Phoenicopterus chilensis*, the James Flamingo *Phoenicopterus jamesi*, and the Andean Flamingo *Phoenicopterus andinus* are found in Asia, Europe, South America, and North America (Ogilvie & Ogilvie 1986). Two species of flamingos, namely, the Lesser Flamingo *Phoenicopterus minor* and Greater Flamingo *Phoenicopterus roseus* are found In India (Grimmett et al. 1998).

Flamingo groups extend from a few individuals to thousands and are seen in freshwater, saltwater, brackish water habitats, shallow lagoons, alkaline lakes, saltpans, and mudflats (Del Hoyo et al. 1992; Grimmett et al. 1998). Greater Flamingos continuously cluster in large feeding flocks or groups during the non-breeding season (Allen 1956). Both of these flamingo species are known to breed at Little Rann of Kachchh in Gujarat in large colonies (Rameshchandra 2014). Greater Flamingos are reported continuously from western Africa to southwestern and southern Asia, and throughout the sub-Saharan region of Africa.

The population of western Africa, Iran, and Kazakhstan seem to be expanding, while the Asian and sub-Saharan African populations seem to be constant (Delany & Scott 2006). The Rann of Kutch in Gujarat, India is a well-known breeding location at the India-Pakistan border (Ali & Ripley 2001), where the world's largest aggregation of Greater Flamingos can be seen (Shivrajkumar et al. 1983). Greater Flamingos have been recorded from Gujarat, Andhra Pradesh, Odisha, Maharashtra, Karnataka, Kerala, Rajasthan, Uttar Pradesh, and Tamil Nadu (Grimmett et al. 1998; Ramesh & Ramachandran 2005; Tere 2005; Kidwai & Bhattacharjee 2016; Arjun & Roshnath 2018; Parasharya & Gadhvi 2020).

Limited studies have been reported on the Greater Flamingo in India (Ramesh & Ramachandran 2005; Tere 2005; Arjun & Roshnath 2018) and most of the studies are limited to the coastal wetlands.

From the literature reviewed it was found that no previous work has been made so far in Haryana, to study the distribution pattern and population size of Greater Flamingos apart from Kumar & Rana (2021). The present study was undertaken to assess the pattern of distribution of Greater Flamingos in Gurugram, Haryana. As a part of a detailed ecological study of flamingos, we surveyed the selected sites in Gurugram Haryana; their numbers were counted from 2018 to 2020 to understand their abundance and population size.

Study Area

Primary visits were made to a few wetlands of Gurugram (Haryana) to select a suitable investigation site. Finally, three sites in Gurugram—Basai wetland (28.471N, 76.985E), Najafgarh Drain (28.498N, 76.946E), and Sultanpur Flats (28.455N, 76.890E)—were selected (Figure 1). Basai wetland is a perennial shallow-water wetland, located in the village of Basai on the outskirts of the Gurugram district of Haryana. Najafgarh drain, located at Delhi-Haryana border, is estimated around 7 km long and is the part of dying Sahibi river. Much of the region covered by the Najafgarh Drain is located in two villages, Kherki Majra and Dhankot. As untreated sewage flows into it, the Najafgarh drain is one of the most contaminated, but productive habitats for many species of birds and plants. Sultanpur flats are the area around Sultanpur National Park and include various habitats such as grasslands, fields, and uncultivated lands. Paddy fields, wheat, and mustard were the dominant vegetation observed in each study area.

MATERIAL AND METHODS

Monthly visits were made to selected sites. The unmanned aerial vehicle (UAV) system (drone) was used to study the population and distribution of Greater Flamingos in Gurugram (Haryana). In the present study, the DJI Tello UAV drone (Image 1) in combination with Apple iPhone 11 mobile devices were used. This drone, a quadcopter (having four rotors) equipped with a 5MP HD camera with 720p recording. The drone is controlled by the smartphone app 'Tello'. The Nikon 10 x 50 field binocular was used for observations. Photographic records were taken using a Canon Powershot sx70hs camera. The total count method has been used to count flamingos for small congregations (Bibby et al. 2000; Sutherland 2006). To make an accurate count of densely packed flock drone was used to capture aerial photographs and videos, then an analysis of aerial photographs and videos was done. A manual tally counter

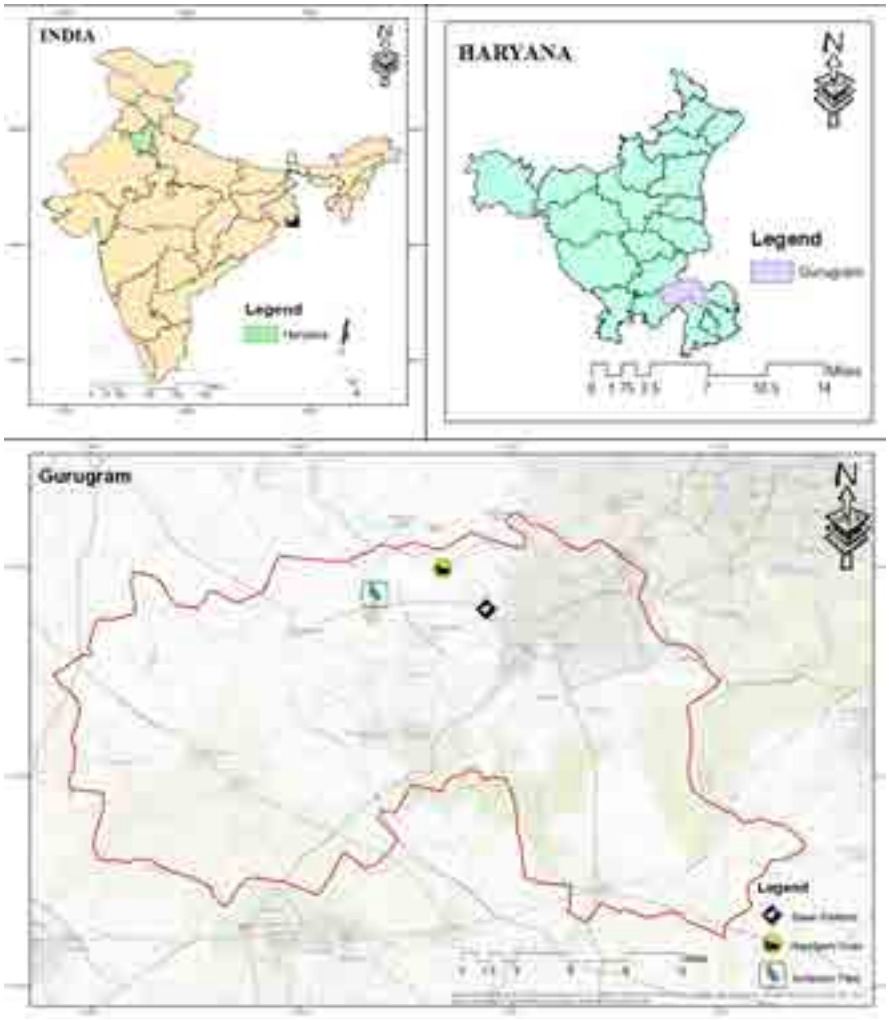

Figure 1. Study area map showing various study sites in district Gurugram.

Image 1. DJI Tello drone used to operate the drone for the aerial population census of Greater Flamingo in large densely packed.

was used for the census of large colonies of Greater Flamingos for an accurate count (Rameshchandra 2014). To reach study sites various modes of transport were used. For each sighting of Greater Flamingos, GPS coordinates were taken using the handheld GPS device Garmin Etrex 30x.

RESULTS

A total of 24 visits were made to each site for three years (Oct 2018–Dec 2020). The summary of Greater Flamingo numbers (N) observed at each site during sampling years (2018–2020) is enlisted in Table 1; 19,230 flamingos were observed during the visit, out of which the highest mean population of flamingos (745 ± 76) was recorded in Najafgarh Drain followed by Basai Wetland (34 ± 6) and Sultanpur Flats (19 ± 8). For the three years,

Table 1. Greater Flamingos in the various reservoirs of Gurugram censused from October 2018 to December 2020.

	Location	Coordinates	Year	Number of visits	Mode of flamingos seen	Mean ± SE
1	Basai Wetland	28.478 N 76.982 E	2018	3	118	56 ± 34
			2019	12	88	46 ± 8
			2020	9	69	19 ± 9
2	Najafgarh Drain	28.774 N 76.622 E	2018	3	957	794 ± 100
			2019	12	1350	969 ± 64
			2020	9	937	430 ± 123
3	Sultanpur Flats	28.468 N 76.892 E	2018	3	170	61 ± 54
			2019	12	51	13 ± 5
			2020	9	70	13 ± 8

*SE = Standard Error

the maximum number of Greater Flamingos counted per visit was 1,350 individuals at Najafgarh Jheel, 118 individuals at Basai Wetland, and 170 individuals at Sultanpur Flats.

Among three years of study, the highest mean population of individuals was observed at Najafgarh Drain in 2019 (969 ± 123) while the highest mean population in Basai Wetland (56 ± 34) and Sultanpur Flats (61 ± 95) was recorded during the year 2018. The mean number of individuals at Najafgarh Drain was similar during 2019 and 2020. In the case of Basai Wetland, the lowest mean population was recorded during the year 2020. The present study shows that Najafgarh Drain holds the maximum number of individuals (> 400) of Greater Flamingos in all three years as compared to other sites. There is a decline in the number of individual sightings observed during 2020 which may be associated with an increase in road construction and fishing activities at Basai Wetland and Najafgarh Jheel.

DISCUSSION

To implement the conservation measures for any species current population size and frequented habitats must be determined before management steps can be implemented. Earlier studies indicated that the Greater Flamingos favored coastal wetlands, but they can live in inland wetlands as well (Tere 2005). They are known to migrate in large flocks to the south-eastern coasts of India during the winter (Nagarajan & Thiyagesan 1996; Balachandran 2006; 2012); and all year surveys are required to differentiate migratory populations from the resident ones. Greater Flamingos are the winter visitors in Lalitpur (Uttar Pradesh). The current research was conducted on various Greater Flamingo inhabiting sites of Gurugram (Haryana) covering the freshwater bodies. The Greater Flamingos were found to be residents at Najafgarh Drain. As previously reported, flamingo abundance fluctuated in response to water level and rainfall patterns (Vargas et al. 2008). Najafgarh Drain is the major stronghold of Greater Flamingos in the area. Flamingos use various parts of the Drain for foraging predominantly in the shallow areas (Image 2A).

The Greater Flamingos were also found to be distributed at Basai Wetland and Sultanpur Flats, but only till water levels were adequate for their sustenance. The unavailability of water in these areas is one of the major reasons for their low abundance. We observed that flamingos leave these sites as water levels start to decline with the end of the rainy season.

It was reported that when the wetland dried up or when food became scarce, they were forced to relocate to a new environment, such as a nearby perennial water body or a location that was a long distance away (Johnson 1989). Najafgarh Drain supports the maximum population of Greater Flamingo as its large area and the availability of water is one of the main reasons for their large aggregation throughout the year. The Najafgarh Drain is a freshwater reservoir that provides a vast expanse of shallow water and food to sustain Flamingos all year round. On the other hand, a minimum number of flamingos counted in Basai Wetland and Sultanpur Flats. The availability of water in these areas is one of the major reasons for their minimum aggregation. At the end of rainy seasons when water levels start to decline and these sites start dry out, flamingos start to leave these sites. Any ecosystem that provides food in sufficient quantity and quality contributes significantly to the survival of its fauna. As a result, differences in the

Image 2. A—Aerial photographs of Greater Flamingos captured at Najafgarh Drain using DJI, TELLO UAV Drone (circle and other shapes are represents to show how a photo interpretation was done to count the flamingos) | B—Flamingos at the dried field | C—Greater Flamingo flocks foraging in shallow water. © Amit Kumar

number of Greater Flamingos inside and among lakes may be due to seasonal access of diet.

Utility of UAVs in surveying flamingoes

UAVs have a wide range of uses in ecological and behavioral studies but have been used infrequently in India. Outside India, UAVs technology has been used

for avian research for example, to count the number of nesting Wood Storks *Mycteria americana* (Rodgers et al. 1995), assessing the nesting success of species of canopy-nesting birds (Weissensteiner et al. 2015), common terns (Chabot et al. 2015), Lesser Black-backed Gulls (Rush et al. 2018), breeding population of Glossy Ibis *Plegadis falcinellus* (Afán et al. 2018), to survey five species of colonial marshbirds (McKellar et al. 2021) and waterfowl (Dundas et al. 2021). UAV technology has been also used for the study of other animal species such as to survey Nile Crocodile populations (Ezat et al. 2018) and basking freshwater turtle species (Bogolin et al. 2021). We used UAV, DJI Tello, for surveying densely packed Flamingos Group at Najafgarh Drain (Image 1). Aerial counting, for example, was more precise and reliable than ground counting using aerial photographs of penguin colonies (Fraser et al. 1999); geese (Boyd 2000), and similar advantages have been shown for imagery obtained by drones (Hodgson et al. 2016).

In this study, recent technological advancements such as the UAV were used. This will be fruitful to develop new field approaches for monitoring the population status and abundance of Greater Flamingos in Haryana, India. The advanced UAV system unmanned aerial vehicles, or drones, collect exceptionally high spatial resolution data with temporal versatility (Anderson & Gaston 2013). Drones equipped with a camera provide new opportunities for the study of population census and distribution of species (Koh & Wich 2012). Analysis of drone video data enabled us to review and pause on larger flocks, making them easier to count and recognize, resulting in more accurate counts (Dundas et al. 2021).

In the aerial photograph, each individual is easily recognizable as shown in Image 2A. Furthermore, the aerial picture made Greater Flamingo enumeration even simpler. Aerial imagery makes counting the densely packed flocks easier as compared to visual ground count. Flamingos were found to forage in various parts of the Najafgarh Drain, particularly in the shallow water areas (Image 2A) whereas in the area of Sultanpur Flats flamingos were found occasionally whenever the availability of water is sufficient for these birds (Image 2C). When compared to traditional ground counts, drone-derived counts are more precise (Dundas et al. 2021).

With the use of UAVs, the accuracy of manual waterbird colony counts has increased drastically. This eliminates visual interpretation bias and ensures that data is similar throughout time. Drones, in particular, can help to census the population of waterbirds and overcome the difficulties of assessing areas that are difficult to reach on foot.

CONCLUSION

The present study was carried out at three selected sites in Gurugram (Haryana) to find out the population of Greater Flamingos. The Greater Flamingos were found to be residents at Najafgarh Drain. There is a continuous decline observed in the population of flamingos. At the Basai Wetland, there was a drastic decline observed in the flamingo population and road construction along the wetland was one of the major threats responsible for the decline in their population. We used a UAV drone to census the population of Greater Flamingos. At Najafgarh Jheel, drones proved to be an excellent tool for surveying the Greater Flamingos population and were found to be more accurate than ground counts. The findings in this study will be extremely beneficial to undertake conservation efforts of the flamingos in this area.

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COMMUNICATION

Freshwater fish diversity in hill streams of Saberi River in Eastern Ghats of Odisha, India

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Abstract: Freshwater fish diversity of the hill streams of Saberi River (a major tributary of the Godavari River system) in Koraput district in Eastern Ghats of southern Odisha was studied from September 2017 to August 2019. Sites for the present study were located between Gupteswar Proposed Reserve Forest (PRF) of Odisha on the eastern side, and Kanger Valley National Park of Chhattisgarh on the western side. A total of 36 species of freshwater fish belonging to 24 genera, 13 families and six orders were recorded from the study sites, of which two species are exotic. Family Cyprinidae dominated with 14 species. Species richness and diversity is greater during the pre-monsoon months followed by post-monsoon and monsoon months respectively. The physico-chemical parameters of water in the study sites during all seasons are within prescribed limits for fish culture. Among the four major types of fish habitats identified in the study sites (riffles, runs, pools and logs), pools were the most preferred, and logs the least preferred habitat for the fishes. Habitat analysis indicated that deep pools and runs are the primary habitats contributing to the maximum species diversity, and therefore, protection of these particular habitats is recommended for conservation and management of ichthyodiversity.

Keywords: Godavari, habitat analysis, ichthyofauna, Koraput district, ichthyodiversity, mountain streams, physico-chemical parameters, species diversity.

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Author contributions: SKP developed the hypotheses. SS and SKP designed the methodology. SS collected the data. SKP performed the statistical analysis. SKP and SS prepared the manuscript. Both the authors contributed critically to the draft and gave final approval for publication.

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INTRODUCTION

Freshwater ecosystems and their resources are indispensable parts of human life, and the health of freshwater ecosystems is often reflected in the structure and characteristics of the fish communities they support (Facey & Grossman 1990). Freshwater habitats in rivers, streams, springs, and headwaters are heterogeneous because of alterations in their altitude, flow rate, dissolved oxygen, physical substrate, and the riparian zones for provision of food, shade and cover (Armantrout 1990). As a result, these habitats harbour diverse fauna, with fish serving as prime indicator of ecosystem status (Karr et al. 1986). A high degree of endemism is exhibited by riverine fauna, with most endemic species living in headwater streams or short stretches of river (Groombridge 1992; Kottelat & Whitten 1997). However, these aquatic bodies are among the least studied, and possibly many species from such ecosystems still await discovery (Kottelat & Whitten 1997).

Hill streams or head waters play a very important role in shaping major rivers, both in terms of physico-chemical conditions and water resources. Streams being fluvial ecosystems play an important role in aquatic faunal diversity, especially for ichthyofauna. Streams are designated as the narrow to broad fixed route through which water flows forming a channel cutting through the ground and rocks. These are classified into the freshwater lotic (running) ecosystem with a sophisticated water flow and diverse habitats with a complex ecological asset (Anonymous 2020). They are located in all latitudes and in all climatic conditions (Singh et al. 2013).

Generally, fish habitat requirements in freshwater streams are related to a number of factors, including the population dynamics of the fish themselves, geomorphology and climate, and the flow regime. In addition, the quality and quantity of riparian and in-stream habitat is vital to fish, particularly with regard to temperature, dissolved oxygen, sediment, and pollutants. Habitat diversity influences the structure and composition of stream fish communities (Golterman 1975; Magnuson et al. 1995). More diverse habitat conditions support a greater range of species and age classes compared to simple habitats. Therefore, the habitat use by stream fish can provide an understanding of this important component of species niches, and the changes in the availability of certain structures of instream habitats. So, it is essential to link fish ecology with the variability of the physical habitat at multiple scales and to detect how the size, persistence, and arrangement of the habitats may influence the distribution of the fish (Gosselin et al. 2010). Additionally, to restore habitats effectively, it is necessary to identify the relevant habitat features and which of them are potentially limiting (Bond & Lake 2003).

India contributes to about 7.7% of global fish diversity, of which, 1,668 species are marine, and 1,027 are freshwater (Gopi et al. 2017; Froese & Pauly 2019). Studies on fish assemblage structure and their requirements in Indian streams are limited, some studies being conducted in Himalayan region (Negi & Negi 2010; Singh & Agarwal 2013; Acharjee & Barat 2014), Western Ghats (Arunachalam 2000; Johnson & Arunachalam 2010; Abraham et al. 2011) and few in Eastern Ghats (Venkateswarlu & Bakde 1986; Ramanujam 2015).

The state of Odisha contributes about 13.92% to the freshwater fish fauna of India (Dutta et al. 1993) and around 186 species of fish (Mogalekar & Canciyal 2018) have been recorded from the state. Koraput district in northern Eastern Ghats of southern Odisha is endowed with hill streams, rivers and reservoirs. Gupteswar Proposed Reserve Forest (PRF) in Koraput bordering the interstate Saberi River is a biodiversity rich region with some significant faunal discoveries in the recent past (Debata et al. 2015, 2018; Mohapatra et al. 2016; Debata & Palita 2017; Purohit et al. 2017). In the present work, an attempt has been made to study the diversity of hill stream fishes in Saberi River at Gupteswar of Koraput district of Odisha.

MATERIALS AND METHODS

Study area

Study on fish diversity was carried out in hill streams of Saberi River at Gupteswar Proposed Reserve Forest (PRF) (18.8194–18.9038 N, 82.1608–82.1791 E) of Koraput district in Northern Eastern Ghats of southern Odisha (Figure 1) from September 2017 to August 2019. Saberi is one of the main tributaries of the Godavari River system and originates from the western slope of the Eastern Ghats of Odisha from Sinkaram Hill ranges at 1,370 m (Anonymous 2016). The river Saberi (also known as Kolab River) forms a 200 km long boundary with Chhattisgarh State, with Gupteswar PRF of Odisha on the eastern side and Kanger Valley National Park of Chhattisgarh on the western side.

Gupteswar forest range falls in the eastern plateau biotic province under the Deccan peninsular biogeographic zone. The topography consists of high land plateau with hills and undulating landscape. The study area experiences a tropical climate with three distinct seasons, pre-monsoon (February–May), monsoon (June–

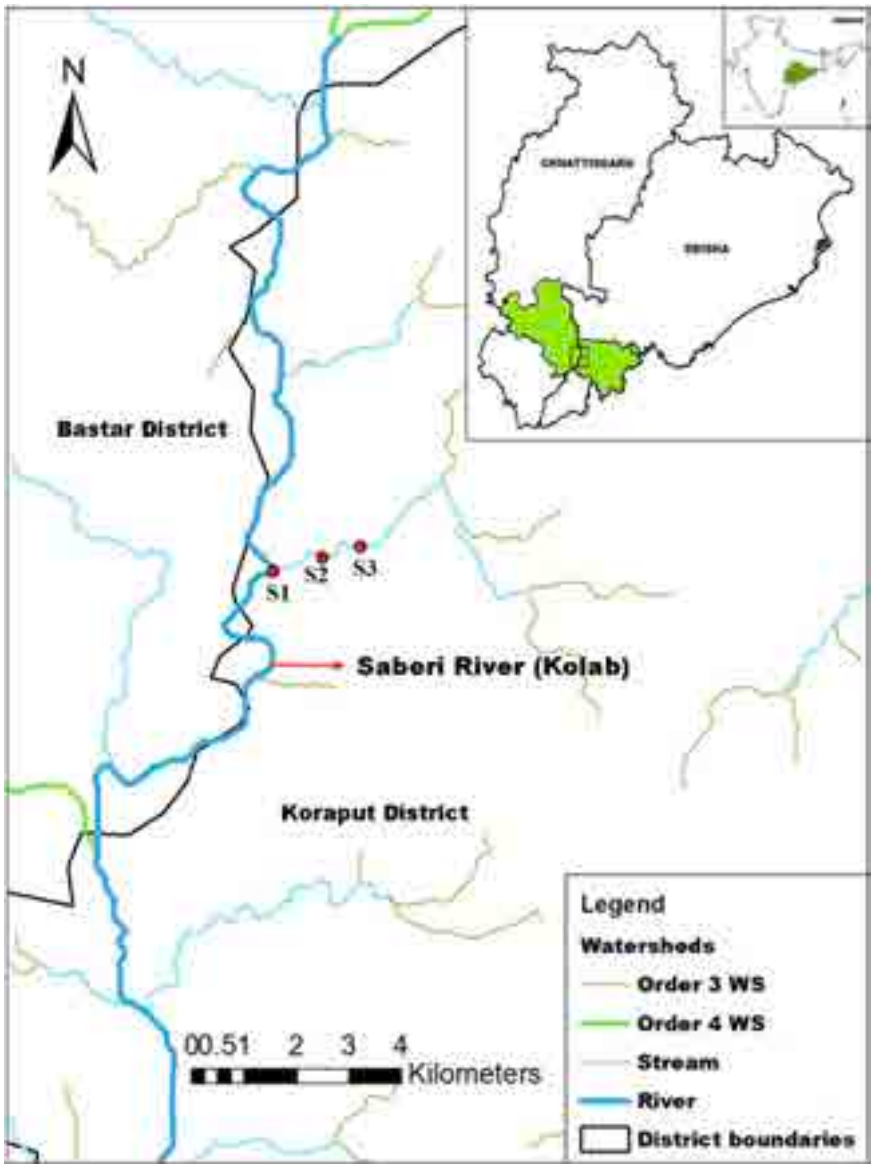

Figure 1. Map showing study sites in the Saberi River at Gupteswar, Koraput, Odisha.

September). and post-monsoon (October–January). The average annual precipitation in the region is around 1,524 mm. The vegetation of the study area at mid elevation is primarily deciduous forest. A good riparian vegetation cover including herbs, shrubs and trees is usually found in this area throughout the year.

Four major types of fish habitat were identified along the entire stretch of stream: riffles, runs, pools and logs (Image 1). These habitats were studied at three sites i.e. S_1 (18.814N – 82.166E), S_2 (18.816N – 82.174E) and S_3 (18.819N – 82.180E) located in the upper and lower stretches of hill stream of the Saberi river over a stretch of two km and with a distance of one km (approx.) from each other. The total stretch between two sites was divided into segments of 100 m length in all the streams which differed substantially in their location, elevation and geomorphology. Sampling for habitat study was done in each of these stretches. The stream bed consists of bed rock, boulders, gravels, sand and mud deposition due to organic detritus. The average stream depth varies from 0.35–0.75 m, and average stream width varies from 8–12 m with sloping stream bank. The water discharge in the river varies depending on the discharge of water from Kolab reservoir, maximum in monsoon, slowly declining through post-monsoon to pre-monsoon.

Sampling Methods

Most of the sampling was undertaken during the

morning hours (0700–1100 h) and occasional night sampling was also carried out. Fishes were collected by various fishing gears such as drag net, scoop net, gill net of varying mesh sizes, and hook and line were used for collection of fishes. Each gear was used at least five times during the entire sampling period. While catching fishes, habitats with greater width were surveyed using the drag net to scrap the benthic substratum for hidden fishes which were lured to the net when underparts of the rocks are disturbed. The scoop nets were used in habitats with narrow width for scooping out the fish. During night sampling, torches were used to attract the fishes into the scoop nets. Geographical coordinates were measured with the help of Garmin GPS (Garmin GPSMAP 64S, Kansas City, KS, USA). Fish were also collected from fishers and local villagers for creating a checklist. Photographs of fresh specimens were taken with a digital camera (Nikon P900).

Fish specimens were brought to the laboratory wrapped in cotton, soaked in 10% formalin solution and packed in polythene bag. Before identification, samples were washed in running tap water for half an hour, after which they were preserved in 70% ethanol for further study and identification. The valid nomenclature of species was adopted as per the Eschmeyer's Catalog of Fishes of the California Academy of Sciences (Fricke et al. 2021). Fishes were identified after referring to Jayaram (2010) and Nelson (2016). The current conservation status of fishes followed the IUCN Red List of Threatened Species. Lagler (1956) classified the fish species on the basis of their economic importance. On the basis of economic importance, fishes have been classified as 'commercial' (species which are prolific breeders, can be cultured and have market value), 'fine food' (having good taste and protein value), 'coarse food' (have less food value and preferred as a food by poor people), and 'aquarium fish' (can be maintained in aquarium for aesthetic and recreational value).

For physico-chemical analysis, water samples were collected between 0800–1100 h and were transported to the laboratory immediately for further analysis. Water temperature, pH, electric conductivity (EC) and total dissoved solids (TDS) was measured with multiple parameter PCS tester (TM35), while dissolved oxygen (DO) was analysed in the laboratory according to the methods suggested by APHA (2005).

Data Analysis

The following diversity indices were calculated to understand fish diversity:

Species Richness Index (Margalef's richness index as modified by Brower & Zar 1977).

$R = S\text{-}1/\ln N$

Where, S = total number of species; N = total number of individuals.

Shannon-Wiener Diversity index (Krebs 1999).

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

Where, H' = Index of species diversity; S = Number of species; *pi* = Proportion of total sample belonging to i^{th} species (*ni*/N); *ni* = number of individuals of i^{th} species in the sample; N = Total number of individuals in the sample = Σni.

Evenness Index (Pielou 1966).

$J' = H' / \ln S$

Where, J' = Evenness index (range 0–1); H' = Shannon-Wiener diversity index.

Relative abundance: The commonness or rarity of a species in the hill stream in the present study was determined using relative abundance (RA) which was calculated as follows:

RA= Number of samples of particular species × 100/ Total number of samples

Statistical analyses were carried out using PAST software version 3.15 (Hammer et al. 2001) for calculating the diversity indices, and ANOVA was calculated using CropStat Vers. 7.2. (IRRI 2007).

RESULTS

Physico-chemical Parameters

Mean seasonal variations of physico-chemical parameters for a period of two years for the study sites are summarized in Table 1. Water temperature was observed in the range of 21–26.8 °C throughout the study period. Highest water temperature was recorded during pre-monsoon season (26.41±0.22 °C), followed by post-monsoon (22.19±0.15 °C), and the least was observed in monsoon season (21.63±0.28 °C). The recorded pH of water in three seasons were acidic and in the range 6.54–6.71, with maximum in monsoon (6.71±0.08) followed by post-monsoon (6.63±0.03) and pre-monsoon (6.54±0.03). Stream water was less turbid throughout the year and in the range of 171.11–221.08 ppm and below WHO standard, i.e., 1,000 mg/l. The highest TDS recorded during pre-monsoon (221.08±1.98 ppm) followed by post-monsoon (192.63±1.56 ppm), and least during monsoon (171.11±1.51 ppm). Electrical conductivity of an aqueous solution is a measure of the ability to carry out an electric current (Ram & Singh 2007). In the present study, EC varied within a range of 452.99 µS/cm to 510.85 µS/cm

Table 1. Physico-chemical parameters in hill streams of Saberi River at Gupteswar, Koraput in three different seasons (September 2017 to August 2019).

Season	Temperature (in °C)	pH	TDS (in ppm)	Conductivity (in µs/cm)	Dissolved oxygen (in mg/L)
Pre-monsoon	26.41 ± 0.22	6.54 ± 0.03	221.08 ± 1.98	510.85 ± 1.12	4.63 ± 0.25
Monsoon	21.63 ± 0.28	6.71 ± 0.08	171.11 ± 1.51	452.99 ± 1.59	5.21 ± 0.18
Post-monsoon	22.19 ± 0.15	6.63 ± 0.03	192.63 ± 1.56	495.98 ± 1.34	6.65 ± 0.24
SE (N = 04)	0.07	0.009	0.36	0.83	0.06
5% LSD	0.23	0.03	1.25	2.89	0.20

SE—Standard Error | LSD—Least Significant Difference.

Image 1. Microhabitats recorded at the study sites of Saberi River at Gupteswar, Koraput, Odisha: a—Riffle | b—Run | c—Pool | d—Log. © Supriya Surachita.

and this may be due to greater ionic concentration of the inlet flow (Jha & Barat 2003). Electrical conductivity varied among seasons, with maximum in pre-monsoon (510.85±1.12 µS/cm), followed by post-monsoon (495.98±1.34 µS/cm) and least by monsoon (452.99±1.59 µS/cm). Dissolved oxygen (DO) is the most essential parameter which can be used as an index of water quality, primary production and pollution. DO values also varied among seasons with highest during post-monsoon (6.65±0.24 mg/l), followed by monsoon (5.21±0.18 mg/l) and minimum values were recorded during pre-monsoon (4.63±0.25 mg/l). All five parameters (water temperature, water pH, turbidity, electrical conductivity and dissolved oxygen) showed variations among seasons that were statistically significant ($p<0.05$) (Table 1). The seasonal study showed that species richness and diversity is high in pre monsoon followed by post monsoon and monsoon respectively. The physico-chemical parameters during all seasons are within the tolerance limits of class 'D' water prescribed (I.S.I. 1982) for fish culture and wildlife propagation. However, growing anthropogenic activities can significantly affect the freshwater fish fauna unless

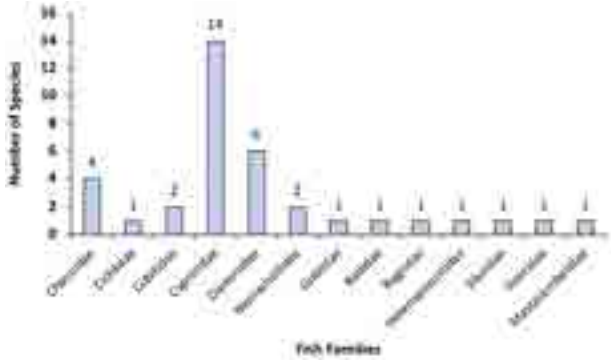

Figure 2. Family-wise fish species richness at study sites in Saberi River at Gupteswar, Koraput, Odisha.

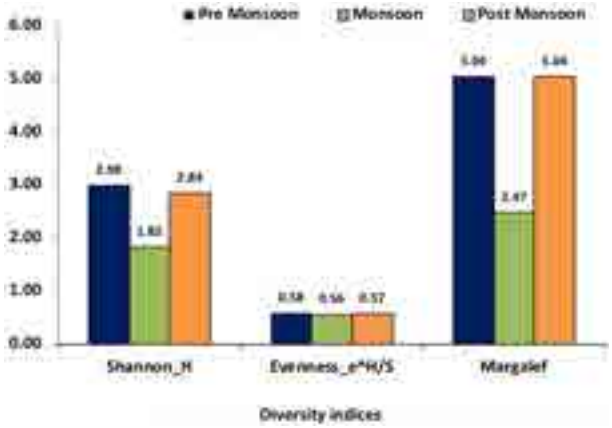

Figure 3. Seasonal fish species diversity at different study sites in Saberi River at Gupteswar, Koraput, Odisha.

conservation measures are adopted urgently.

Species Richness

During the study period a total of 36 species of fish belonging to 24 genera and 13 families and six orders were recorded. Family Cyprinidae dominated with 14 species, followed by Danionidae (six species), Channidae (four species), Cobitidae and Nemacheilidae (two species each) and Cichlidae, Gobidae, Badidae, Bagridae, Heteropneustidae, Siluridae, Sisoridae and Mastacembalidae (one species each) (Figure 2). Maximum species richness was observed in the order Cypriniformes (with 24 species under 16 genera and four families).

Among the species recorded, two species (*Cyprinus carpio* and *Oreochromis mossambicus*) are exotic, one species (*Wallago attu*) was assessed as Vulnerable (VU) and one species (*Bagarius bagarius*) as Near Threatened (NT) in the IUCN Red List. The presence of two exotic species (*C. carpio* and *O. mossambicus*), may pose potential threats to the native species and may cause loss of ecosystem function, habitat disruption, reduction of genetic diversity of native species in future.

Twenty-eight species were assessed as Least Concern (LC) (Table 2).

The site-wise relative abundance (RA) of all the species in different seasons is shown in Table 3. Maximum relative abundance (RA) value (68.18%) was recorded in case of *Devario aequipinnatus* in monsoon from Site S_3. All the major carps were recorded from Site S_1. Among the five major carps, *Labeo rohita* had the highest RA values in all the three seasons (50.0% in monsoon, 38.6% in post-monsoon and 31.25% in pre-monsoon), followed by *Labeo catla*. Next to carps, *Pethia conchonius* showed higher RA in monsoon and post monsoon seasons, and were recorded from sites S_2 and S_3. The RA of *W. attu* at site S_1 during monsoon was 12.50%. The two exotic species *Cyprinus carpio* and *Oreochromis mossambicus* had greater RA i.e. 13.13% and 5.00% respectively at site S_1 during pre-monsoon. The RA of *Bagarius bagarius was* 3.13% which was restricted to site S_1 during pre-monsoon. *Systomus sarana* was found only at site S_2 during all seasons. The RA of *S. sarana* was highest during monsoon (3.70%) followed by post-monsoon (1.16%) and pre-monsoon (0.87%) respectively. *Danio dangila* was only accessible at site S_3 during pre-monsoon and post monsoon with an RA of 2.59% and 2.33%, respectively.

Diversity indices

Higher species diversity in terms of fish species was observed in pre-monsoon with the Shannon–Wiener index value of H′ = 2.98, followed by post-monsoon with H′ = 2.84, and monsoon with H′ = 1.82 respectively. Maximum species richness was observed during pre-monsoon, followed by post-monsoon and monsoon respectively, and ANOVA analysis also indicates that species richness showed significant difference during pre-monsoon in comparison to post-monsoon and monsoon ($p < 0.05$) (Table 4). Similarly, evenness was also highest during pre-monsoon, followed by post-monsoon and monsoon, respectively (Figure 3). Mean abundance of fishes also showed a similar trend, maximum value in pre-monsoon followed by post-monsoon and monsoon it was statistically significant ($p < 0.05$).

Among the three sites studied, species richness was significantly higher ($p < 0.05$) at site S_2 in comparison to sites S_1 and S_3. Though species richness was higher at site S_1 compared to S_3, it was not statistically significant. Mean abundance also showed a similar pattern to species richness among sites (Table 4).

Habitat preference of fish

Four major types of fish habitat were identified at the

Table 2. Checklist of fishes recorded from hill streams of Saberi River at Gupteswar, Koraput, Odisha with their IUCN status and economic importance.

Name of the species	Common name	IUCN Red List status	Economic importance				
			Commercial	Fine food	Coarse food	Aquarium fish	Others
Order- Anabantiformes							
Family- Channidae							
Channa marulius (Hamilton, 1822)	Great Snakehead	LC	+			+	PF, LV
Channa gachua (Hamilton, 1822)	Dwarf Snakehead	LC	+			+	
Channa punctata (Bloch, 1793)	Spotted Snakehead	LC	+	+		+	Bt
Channa striata (Bloch, 1793)	Striped Snakehead	LC	+	+		+	PF, LV
Family- Badidae							
Badis sp.	-	-				+	
Order- Cichiliformes							
Family- Cichlidae							
Oreochromis mossambicus (Peters, 1852)	Mozambique Tilapia	Non-native / Invasive	+	+		+	GF
Order- Cypriniformes							
Family- Cobitidae							
Lepidocephalichthys guntea (Hamilton, 1822)	Guntea Loach	LC				+	
Lepidocephalichthys thermalis (Valenciennes, 1846)	Common Spiny Loach	LC				+	
Family- Cyprinidae							
Cirrhinus mrigala (Hamilton, 1822)	Mrigal Carp	LC	+	+			
Cirrhinus reba (Hamilton, 1822)	Reba Carp	LC		+			
Cyprinus carpio (Linnaeus, 1758)	Common Carp	Non-native / Invasive	+				GF
Garra mullya (Sykes, 1839)	Sucker Fish	LC				+	
Labeo catla (Hamilton, 1822)	Catla	LC	+	+			
Labeo calbasu (Hamilton, 1822)	Orangefin Labeo	LC		+			
Labeo rohita (Hamilton, 1822)	Rohu	LC	+	+			
Parapsilorhynchus sp.	-	-			+		
Pethia conchonius (Hamilton, 1822)	Rosy Barb	LC			+	+	
Pethia ticto (Hamilton, 1822)	Ticto Barb	LC			+	+	
Puntius chola (Hamilton, 1822)	Chola Barb	LC	+		+	+	
Puntius sophore (Hamilton, 1822)	Swamp Barb	LC			+	+	
Systomus sarana (Hamilton, 1822)	Olive Barb	LC	+			+	GF
Tor sp.	-	-	+				GF
Family- Danionidae							
Barilius vagra (Hamilton, 1822)	Vagra Baril	LC			+		
Danio dangila (Hamilton, 1822)	Moustached Danio	LC				+	
Danio rerio (Hamilton, 1822)	Zebra Fish	LC				+	
Devario aequipinnatus (McClelland, 1839)	Giant Danio	LC				+	
Opsarius bendelisis (Hamilton, 1807)	Hamilton's Barila	LC	+				
Rasbora daniconius (Hamilton, 1822)	Slender Rasbora	LC	+		+	+	
Family- Nemacheilidae							
Schistura denisoni (Day, 1867)	Sand Loach	LC				+	
Schistura sp.	-	-				+	

Name of the species	Common name	IUCN Red List status	Economic importance				
			Commercial	Fine food	Coarse food	Aquarium fish	Others
Order- Gobiiformes							
Family- Gobiidae							
Glossogobius giuris (Hamilton, 1822)	Tank Goby	LC	+	+		+	
Order- Siluriformes							
Family- Bagridae							
Mystus tengara (Hamilton, 1822)	Stripped Dwarf Catfish	LC			+		
Family- Heteropneustidae							
Heteropneustes fossilis (Bloch, 1794)	Stinging Catfish	LC		+			PF
Family- Siluridae							
Wallago attu (Bloch & Schneider, 1801)	Helicopter Catfish	VU	+	+			GF,PF
Family- Sisoridae							
Bagarius bagarius (Hamilton, 1822)	Dwarf Goonch	NT	+				GF
Order- Synbranchiformes							
Family- Mastacembelidae							
Mastacembelus armatus (Lacepède, 1800)	Zig-zag Eel	LC	+			+	

+—present | VU—Vulnerable | NT—Near Threatened | LC—Least Concern | GF—Game Fish | PF—Predatory Food Fish | LV—Larvivorous Fish | Bt—Bait.

study sites, i.e., riffles, runs, pools and logs, among which pools were the most preferred habitat, and logs the least preferred habitat for fish. All snakeheads were recorded from the three habitats (riffle, pool, and log) except runs, because they tend to avoid fast flowing waters. Among the snakeheads, *C. marulius* was only found in pool habitat, whereas *C. gachua* was found both in riffles and logs, but absent in pool. *C. punctata* and *C. striata* were observed in riffles, pools and logs. The invasive alien species, *O. mossambicus*, was found both in riffle and pool, but mostly in weedy pools. Two species of cobitid loaches (*L. guntea* and *L. thermalis*) preferred pools with substrate composed of soft mud. Among five major carps, three (*L. calbasu, L. catla* and *L. rohita*) were found in only riffles whereas other two (*C. mrigala* and the non-native *C. carpio*) were only found in pools. One minor carp (*C. reba*) was only recorded in logs. Among the six barbs recorded from the study site, *P. ticto* was only found in runs whereas *P. conchonius* and *S. sarana* were found both in runs and logs, whereas *P. chola*, *P. sophore*, and *Tor* sp. were found in both runs and pools. This suggests that all barbs were found in runs. *Garra mullya* was recorded in riffle and runs, whereas *Parapsilorhynchus* sp. was only found in pools. Among the six minnows recorded from the study sites, *D. dangila* was only found in logs whereas *B. vagra*, *D. aequipinnatus* and *R. daniconius* were found in both riffles and runs. Zebra fish, *D. rerio* was observed in runs as well as pools while Hamilton's Barilla, *O. bendelensis* was recorded in riffles, runs and pools. *Schistura* sp. was observed both in riffles and pools. Goby (*G. giuris*) and freshwater eel (*M. armatus*) were found in runs and riffles respectively. *Badis* sp. was found in both pools and logs. Stinging Catfish, *H. fossilis* and Helicopter Catfish, *W. attu* were recorded both in runs and pools. Striped Dwarf Catfish, *M. tengara* and Dwarf Goonch, *B. bagarius* preferred pool as their habitat.

Economic Importance

Among the fish species recorded from hill streams of Saberi at Gupteswar, 12 species (30% of species recorded) – *Bagarius bagarius, Channa marulius, C. punctata, Mastacembelus armatus, Opsarius bendelisis, Oreochromis mossambicus, Puntius chola, Rasbora daniconius, Systomus sarana, Tor* sp. and *Wallago attu* are commercial fishes. Among the recorded species, 18 species (50% of the species recoded) are ornamental fishes such as *Badis* sp., snakeheads (*C. marulius, C. gachua, C. punctata*, and *C. striata*), minnows (*D. dangila, D. rerio, D. aequipinnatus*, and *R. daniconius*), *Garra mullya, Glossogobius giuris,* loaches *(L. guntea, L. thermalis*), *Mastacembelus armatus , Oreochromis mossambicus,* barbs (*P. conchonius, P. ticto, P. chola, P. sophore*, and *S. sarana*), and sand loaches (*S. denisoni* and *Schistura* sp.*).* These species are preferred for aquariums due to their

Table 3. Relative abundance of fish species at three sites of Saberi River at Gupteswar, Koraput, Odisha during different seasons, and microhabitat preference of fish species.

Scientific names	Relative abundance (%)									Microhabitat			
	Pre-monsoon			Monsoon			Post-monsoon						
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	Riffle	Run	Pool	Log
Order- Anabantiformes													
Family- Channidae													
Channa marulius	-	0.87	-	-	-	-	-	-	-	-	-	+	-
Channa gachua	-	0.58	-	-	-	-	-	1.16	-	+	-	-	+
Channa punctata	-	8.12	-	-	-	-	-	9.25	-	+	-	+	+
Channa striata	-	5.22	-	-	-	-	-	2.89	-	+	-	+	+
Family- Badidae													
Badis sp.	-	11.01	21.24	-	-	-	-	4.05	5.81	-	-	+	+
Order- Cichiliformes **Family- Cichlidae**													
Oreochromis mossambicus	5	3.19	-	-	-	-	-	2.31	-	+	-	+	-
Order- Cypriniformes **Family- Cobitidae**													
Lepidocephalichthys guntea	-	5.22	-	-	-	-	-	3.47	-	-	-	+	-
Lepidocephalichthys thermalis	-	0.58	-	-	-	-	3.51	1.16	1.16	-	-	+	-
Family- Cyprinidae													
Cirrhinus mrigala	5	-	-	-	-	-	1.75	-	-	-	-	+	-
Cirrhinus reba	13.13	1.16	-	-	-	-	7.02	0.58	-	-	-		+
Cyprinus carpio	13.13	-	-	-	-	-	7.02	-	-	-	-	+	-
Garra mullya	0.63	0.29	-	-	-	-	1.75	1.16	-	+	+	-	-
Labeo catla	18.75	-	-	12.5	-	-	29.8	-	-	+	-	-	-
Labeo calbasu	4.38	-	-	12.5	-	-	10.5	-	-	+	-	-	-
Labeo rohita	31.25	-	-	50	-	-	38.6	-	-	+	-	-	-
Parapsilorhynchus sp.	-	-	-	-	-	-	-	1.16	-	-	-	+	-
Pethia conchonius	-	19.71	4.66	-	44.44	9.09	-	24.28	1.16	-	+		+
Pethia ticto	-	0.58	-	-	-	-	-	2.31	2.33	-	+		-
Puntius chola	-	5.8	-	-	-	-	-	4.05	-	-	+	+	-
Puntius sophore	-	1.16	-	-	-	-	-	2.89	-	-	+	+	-
Systomus sarana	-	0.87	-	-	3.7	-	-	1.16	-	-	+		+
Tor sp.	1.88	-	-	12.5	-	-	-	-	-	-	+	+	-
Family- Danionidae													
Barilius vagra	-	3.48	-	-	7.41	-	-	2.31	-	+	+	-	-
Danio dangila	-	-	2.59	-	-	-	-	-		-	-	-	+
Danio rerio	-	-	20.21	-	-	22.73	-	-		-	+	+	-
Devario aequipinnatus	-	-	35.75	-	22.22	68.18	-	-		+	+		-
Opsarius bendelisis	-	1.45	-	-	-	-	-	-	-	+	+	+	-
Rasbora daniconius	-	4.35	6.74	-	-	-	-	1.73	2.33	+	+	-	-
Family- Nemacheilidae													
Schistura denisoni	-	17.97	8.81	-	22.22	-	-	26.01	-	+	-	+	-
Schitura sp.	-	-	-	-	-	-	-	1.16	-	+	-	+	-
Order- Gobiiformes **Family- Gobiidae**													
Glossogobius giuris	-	4.64	-	-	-	-	-	4.62	-	-	-	-	-

Scientific names	Relative abundance (%)									Microhabitat			
	Pre-monsoon			Monsoon			Post-monsoon						
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	Riffle	Run	Pool	Log
Order- Siluriformes Family- Bagridae													
Mystus tengara	1.88	-	-	-	-	-	-	-	-	-	-	+	-
Family- Heteropneustidae													
Heteropneustes fossilis	-	2.32	-	-	-	-	-	0.58		-	-	+	-
Family- Siluridae													
Wallago attu	1.88	-	-	12.5	-	-	-	-	-	-	+	+	-
Family- Sisoridae													
Bagarius bagarius	3.13	-	-	-	-	-	-	-		-	-	+	-
Order- Synbranchiformes Family- Mastacembelidae													
Mastacembelus armatus	-	1.45	-	-	-	-	-	1.73		+	-	-	-

very attractive colour and beautiful banding patterns. The species of *Channa*, *Mastacembelus*, *Heteropneustes* have air breathing organs so it fetches good market value as live fish, as well. In the same way, eight species, namely, *Channa marulius, Oreochromis mossambicus, Cyprinus carpio, Labeo catla, L. rohita, Systomus sarana, Tor* sp., and *Bagarius bagarius* are categorized as game fishes (Froese & Pauly 2019).

Devario aequipinnatus is abundant while *Channa marulius* and *Mystus tengara* were less abundant in the study area during the study period. Though commercially important species are available in the study region, they are not sufficiently abundant to make fishery commercial and economical. Conservation measures such as afforestation in catchment and awareness of illegal fishing and killing of brood fishes and juveniles are required steps in the region. The present study of fish fauna in Gupteswar showed that most of the fish species recorded were widely distributed in the streams and rivers of Eastern Ghats.

Gupteswar being a place of pilgrimage, there is a large tourist footfall. It has been observed in the study sites that people use waterbodies for various purposes like bathing, garbage disposal and religious activities. Activities like construction of roads, building amenities for tourists have also increased in the study sites over the years.

Table 4. Freshwater fish species richness and abundance in hill streams of Saberi River at Gupteswar, Koraput, Odisha across seasons and among study sites.

Season	No. of samples	Species richness (Mean±SD)	Species abundance (Mean±SD)
Pre-monsoon	12	10 ± 4.95	58.17 ± 24.3
Monsoon	12	1.58 ± 0.9	4.75 ± 3.86
Post-monsoon	12	6.17± 3.93	26.33 ± 15.1
SE (N = 12)	-	0.81	3.31
5%LSD	-	2.33	9.58
Site			
S1	12	5.0± 3.72	18.75± 18.17
S2	12	9.17 ± 6.52	45.42± 35.56
S3	12	3.58± 2.19	25.08 ± 19.77
SE (N = 12)	-	0.81	3.31
5% LSD	-	2.33	9.58

SE—Standard Error | LSD—Least Significant Difference.

DISCUSSION

The record of 36 fish species within 24 genera and 13 families and six orders in the present study from hill streams of Saberi at Gupteswar is the first report from Koraput District of Eastern Ghats. Among the four habitats in hill streams documented as part of the present study, pool is the most preferred, followed by riffle, and log the least preferred habitat. Pool is a segment of the stream with reduced current velocity and suspended organic detritus, with depths exceeding other surrounding habitats, usable by fish for resting and covers (Armantrout 1998); thereby is the most preferred habitat than others. Habitat diversity influences the structure and composition of stream fish communities (Golterman 1975; Magnuson et al. 1998). Most fish in small, stable streams are most probably habitat specialists that have evolved various morphological and behavioural adaptations to exploit

specific habitat types (Magnuson et al. 1998). Many tropical stream fish specialize in habitat use and exhibit morphological segregation, with a close relationship between morphological and ecological characteristics. As a result, these ecomorphological specializations may serve to facilitate resource partitioning (Deacon & Mize 1997).

The Satpura hypothesis proposed by Hora (1949) suggests that the central Indian Satpura range of hills acted as a bridge for the gradual migrations of Malayan fauna into the peninsula and the Western Ghats of India. Menon (1951) carried out extensive surveys along Odisha hills (the part lying above Godavari River) and Eastern Ghats to understand if the Malayan elements were present in the fish or not. He collected 93 fish and critically analysed and concluded that the fish fauna of Odisha hills and Eastern Ghats have a very close affinity with that of Satpura-Vindhya mountains and the northern division of the Western Ghats and that there is a conspicuous absence of forms common to the fish fauna of Malayan region and peninsular India (Hora 1944). Dutta et al. (1993) collected 28 species of fish from the undivided Koraput district. Of this, only three species have been recorded from present Koraput district, and the remaining 25 species are from the current-day Nawrangpur District. In view of this, the present record of 36 species from hill streams of Saberi at Gupteswar is the first report from this region. The diversity recorded in the present study will be useful as baseline data for any future assessment though no detail study has been carried out in hill streams of Koraput. Most importantly, our study indicates considerable share in supporting fish biodiversity in the region despite alterations like anthropogenic activities including illegal fishing and habitat degradations.

The threat of exotic species on the indigenous fish species in the present study site was relatively low, but it is cause of concern for future. Sarkar et al. (2010) found that the higher relative abundance and distribution of exotic species indicate threat to the other local species due to their establishment in the River. This may cause difficulty to manage other species of conservation importance in the River, and may become more challenging due to the interaction of climatic changes (Rahel et al. 2008).

CONCLUSION

The different microhabitats in hill streams are home to diverse biotic communities which are threatened by major anthropogenic pressures. Though Gupteswar is a pilgrimage site, high anthropogenic pressure in the form of developmental activities result in quick deterioration of water quality, thereby posing threats to aquatic biodiversity, especially fish fauna. In addition, climate change has also become one of the greatest threats to aquatic systems in the study site. Natural modifications like temperature rise and irregular rainfall also have substantial effects on the stream morphology and hydrology. Assessing biodiversity vulnerability to future clime change is essential for developing conservation strategies in this region. Illegal fishing by locals should be strictly banned. The record of 36 species of freshwater fish in the present study highlights the importance of hill streams as critical fish habitats. Further, our habitat study shows that deep pools and runs are the primary habitats contributing to the maximum diversity of fish species, and therefore protection of these particular habitats is recommended for conservation and management of the region's fish biodiversity.

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COMMUNICATION

Hatching in Coromandel Marsh Dart Damselfly *Ceriagrion coromandelianum* (Fabricius) (Zygoptera: Coenagrionidae): process and influence of the oviposition substrate

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Abstract: Coromandel Marsh Dart Damselfly *Ceriagrion coromandelianum* (Fabricius) breeds in stagnant pools, small garden tanks and ornamental cement ponds containing submerged and/or floating vegetation. Eggs were collected to observe two aspects of larval development: (1) The hatching rate of eggs deposited in different vegetation (*Nymphaea nouchali, Lemna paucicostata, Hydrilla verticillata*). Although *C. coromandelianum* prefers to oviposit in the broad leaves of *N. nouchali*, the highest rate of hatching was found in *H. verticillata* (95.8%) followed by *N. nouchali* (87.6%) and *L. paucicostata* (81.3%). Hatching commenced on Day 5 and was completed by Day 9. Maximum hatching (56%) was recorded on the sixth day of oviposition followed by the seventh day (20%) in all three substrates. (2) To document the process of hatching as follows: Around three minutes prior to hatching, the embryo exhibits cyclic pumping and pushing movements of the head (caused by the peristaltic movement of the mid- and hind- gut) of low intensity followed by high intensity and long pumping movements interspaced with smaller pulsating movements. Swelling of the head forces the apical chorion to split along the micropylar chute and like a lid, the apical tip topples over as a conical cap. This allows the prolarva to exit the egg. As it does so, it twists and the thorax swells breaking the prolarval sheath and releasing the first instar larva.

Keywords: Egg, hatching, *Hydrilla verticillata, Lemna paucicostata, Nymphaea nouchali*, Prolarva.

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Author contributions: PRV studied the hatching rate while NRT documented the hatching process. RJA set up the project and evaluated the findings.

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INTRODUCTION

Female damselflies oviposit on floating plants (epiphytic) or inside plant tissue (endophytic). In endophytic species the choice of ovipositing material depends upon "the initial preference" (Waage 1987), which is a suitable place both for landing and easy deposition of eggs into plant tissue (Mokrushov & Frantesevich 1976; Waage 1987; Martens 1992, 1993, 1994, 2001). According to Mokrushov & Frantsevich (1976), the appearance, morphology and texture of the plant triggers the female to deposit eggs. Some species oviposit in a single or very few species of plant, while others, although perhaps exhibiting distinct preferences, oviposit in a wide variety of plants (Martens 1996). Some species not only show preferences in selecting plant species, but also in parts of plants used for oviposition (Martens 1992; Wildermuth 1993; Grunert 1995). Corbet (1999) summarized that infertility, desiccation, displacement (by flooding) and parasitoid infection are the major cause of egg mortality in Odonata; however, no substantial information is available on the survival and successful hatching of eggs depending upon the ovipositing material selected by the female. Hatching in Odonata depends upon levels of dissolved oxygen (Punzo 1988; Miller 1992), onset of light or darkness (Tennessen & Murray 1978), rainfall (Lempert 1988), pH (Hudson & Berrill 1986), fluctuation in incubation temperature and humidity (Pilon & Masseau 1984; Sawchyn & Gillott 1974; Gillooly & Dodson 2000; Koch 2015; Ichikawa et al. 2017; Mendonca et al. 2018).

A general account of the hatching event of an odonate egg was described for the first time by Pierre (1904) in the Zygoptera *Lestes virides* (now *Chalcolestes viridis*). Later Tillyard (1916) documented this in the Aeshnidae *Anax papuensis*. Degrange (1961, 1974) studied the egg hatching of *Agrion puella, Enallagma cyathigerum* and *Calopteryx virgo* and reported that the endochorion has a pre-existing line of weakness which cracks when the prolarva hatches. He also reported that the micropyle orifices permit the entry of water during hatching.

C. coromandelianum is one of the most common Zygoptera in the Indian subcontinent. It breeds in stagnant pools and small garden tanks, tubs and ornamental cement ponds containing submerged and/or floating vegetation. In continuation of the study on the breeding biology of this species in central India (Andrew et al. 2011; Thaokar et al. 2018a,b), this communication evaluates the variation in the rate of hatching success in different oviposition substrates (*N. nouchali, L. paucicostata, H. verticillata*) and also describes the behaviour of the embryo and events leading to hatching of the egg.

MATERIAL AND METHODS

Leaves of *Nymphaea nouchali, Lemna paucicostata* and stems of *Hydrilla verticillata* bearing eggs of *Ceriagrion coromandelianum* were collected from 1000 h to 1400 h during the third week of February 2015 (Average temp. 34.2 ^{O}C (min. 31 ^{O}C, max. 36 ^{O}C); average humidity 51.4 % (min. 43, max. 66)) from small underground cement tubs at the botanical garden of Hislop College, Nagpur (21°8'51.43''N & 79°4'17.26''E), India. This site is being used to study the breeding and reproductive behaviour of *C. coromandelianum* details of which can be found in Andrew et al. (2011) and Thaokar et al. (2018a,b, 2019).

The samples were brought to the laboratory within two/three hours post oviposition, segregated and carefully cut into smaller pieces (without damaging the eggs). They were then labelled and placed in water from the collecting site in petri dishes to permit observation using–binocular microscopes (Primo Star DV-4 and Magnus- MS 24). The water was replaced daily. The number of eggs in each piece of substrate of each petri dish was counted and constantly monitored for the following 15 days. The record of daily hatching was noted up to 1700 h each day and the final count of eggs hatched was tallied on the 10th day (the eggs were observed for the next five days in case of any late hatching). The process of egg hatching was photo/video-graphed with the help of aim-n-shoot Sony (DSC-W30) and Canon (G11) cameras. Detail of the weather reports for the region were obtained from the website https://www.timeanddate.com.

OBSERVATION

The egg of *C. coromandelianum* is typically endophytic, with an elongate cylindrical shape (980 X 140 µm) bearing a pointed anterior and rounded posterior end. The pointed anterior end or the micropylar region (pedicle) is demarcated by a circular grooved line of hatching on the exochorion, which bears five micropylar orifices (Image 1) at regular intervals (Andrew et al. 2011). The micropylar region is apically brown up to (40 µm) while the remaining area is transparent or opaque. Below this region lies a ring of thicker endochorion, which stops the prolarva from moving upwards. The tip

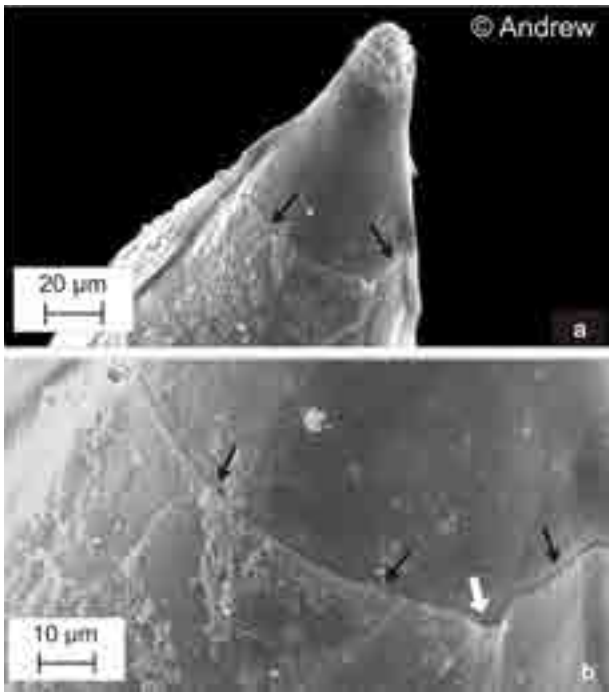

Image 1. *Ceriagrion coromandelianum* – scanning electron micrograph of the apical tip of egg: a, b—showing the circular grooved line of hatching (black arrows) and the micropylar orifice (white arrows) (Andrew et al. 2011).

of the egg is covered with a thick tuft of aquatic debris. The embryo comes to lie just below the micropylar region and is housed below the chorionic rim while the micropylar lumen is empty. At this stage, the embryo is well-formed with a rounded head bearing a pair of conspicuous compound eyes as dark black spots. The thoracic segments cannot be demarcated but abdominal segments are slightly distinct. The process of hatching in *C. coromandelianum* initiates 48±5 minutes before emergence by a corresponding increase in the movement of the embryo, but active movement of the embryo starts about three minutes before the prolarva escapes from the egg.

Table 1. Hatching details of *Ceriagrion coromandelianum* eggs by number of samples.

Vegetation	Number of eggs present	Number of eggs hatched	Hatching percentage
Nymphaea nouchali	88	78	88.63
	60	51	85.00
	102	90	88.23
Total	**250**	**219**	**87.60**
Lemna paucicostata	30	24	80.00
	36	30	83.33
	30	24	80.00
Total	**96**	**78**	**81.25**
Hydrilla verticillata	30	28	93.33
	18	18	100.00
	24	23	95.83
Total	**72**	**69**	**95.83**

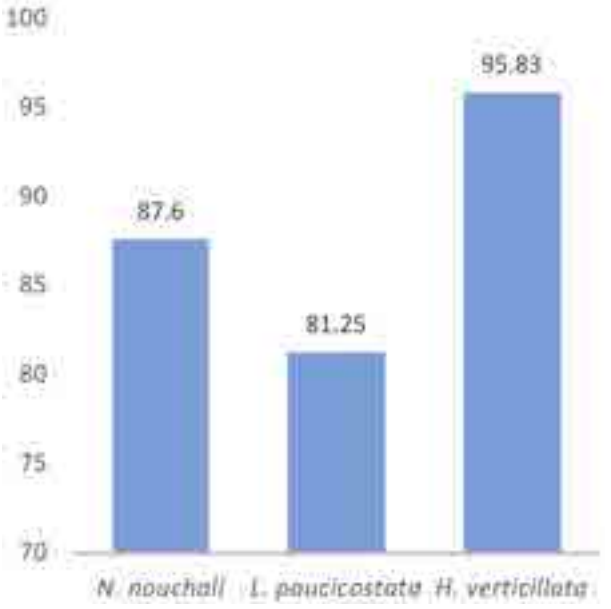

Figure 1. Hatching rate of *Ceriagrion coromandelianum* eggs in all samples.

i) The hatching rate of eggs deposited in different vegetation

Ceriagrion coromandelianum exhibits a hierarchy of preferences for oviposition and chooses floating leaves of *Nymphaea nouchali* over *Lemna paucicostata* and submerged *Hydrilla verticillata* (Thaokar et al. 2018a). Although *Ceriagrion coromandelianum* prefers to oviposit in the broad leaves of *Nymphaea nouchali*, the highest rate of hatching was found in the stems of *Hydrilla verticillata* (95.83%;SD 2.75, SE 1.59) followed by *N. nouchali* (87.60%; SD 1.63, SE 0.94), and *Lemna paucicostata* (81.25%; SD 1.57, SE 0.91) (Table 1, Figure 1). The Student's t-test indicates that there is a significant difference (at p= 0.05) for hatching percentage between *N. nouchali* and *H. verticillata* (3.7808) and between *L. paucicostata* and *H. verticillata* (4.671). Hatching commenced from the fifth day (10%) post oviposition; however, maximum hatching (55.53%) was noticed on the sixth day followed by the seventh day (20.43%) in all three substrates. The process continued up to ninth day in *H. verticillata* and *L. paucicostata* and up to the 10th day in *N. nouchali* (Table 2, Figure 2).

Table 2. Hatching details of *Ceriagrion coromandelianum* eggs by day number (Day 5 first day of emergence).

Day	*Nymphaea nouchali*	*Lemna paucicostata*	*Hydrilla verticillata*	Percentage
5	27	08	14	10.03
6	135	68	72	55.53
7	94	20	15	20.43
8	39	13	05	9.4
9	06	04	07	3.83
10	07	00	00	0.78

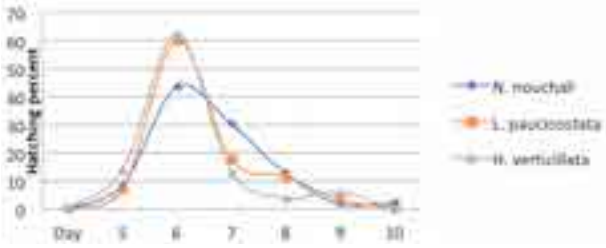

Figure 2. Hatching details of *Ceriagrion coromandelianum* eggs by day number.

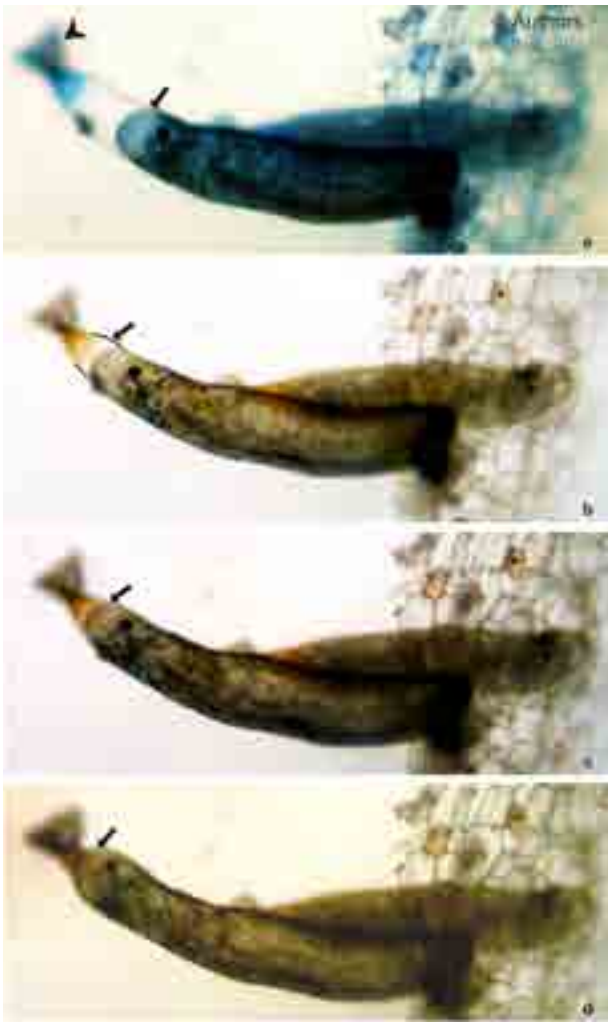

Image 2. *Ceriagrion coromandelianum* – serial photographs of hatching showing movement of the prolarva in the apical conical tip in the egg (arrows). Note the sticky egg tip covered with a tuft of debris (arrowhead).

ii) The process of hatching

This is initiated with constant convulsive pumping movements of the head. Initially, this movement is of low intensity as the head moves to and fro just below the endochorionic ring. At this stage there is a total of 42±8 (N= 5) movements which take 90–115 seconds. These head movements are caused by the peristaltic action of the mid- and hind gut. Hereafter, there is a change in the pumping intensity with 11–15 high pumping cycles alternating with low pumping movements. This continues for 22–36 seconds. These movements bring the head up to the endochorionic ring. After a pause of 8–15 seconds, the pumping movement recommences. At this stage the head pushes upwards with 14–20 long pumping movements interspaced with 3–7 smaller pulses (Image 2). This post-pause cycle of movement takes 18–37 seconds. The head now glides upwards and comes to occupy the complete micropylar region. With a strong final peristaltic motion, the head pushes on the apical tip of the egg. The pressure on the apical tip is further increased by the swelling of the head, which forces the apical chorion to split in a circular manner (along the micropylar chute) and the apical tip (brown area) topples over as a conical cap and the prolarva glides swiftly and easily out of the egg (Image 3).

The prolarva is enveloped by a fine chitinous envelope, 'the pro-larval sheath'. As the prolarva escapes from the egg, the body twists sideway and the thorax swells breaking the pro-larval sheath anteriorly. This allows the first instar larva to emerge.-Whilst the pro larval sheath remains stuck to the fractured edge of the eggshell. The first instar larva wriggles four to six times twisting its body before resting with spread legs and anal cerci. The head is held above the ground with the well-formed mask tucked beneath. The head and compound eyes are prominent, but ocelli are wanting. The body is transparent, devoid of midgut yolk, and a network of tracheoles can easily be traced (Image 4).

DISCUSSION

In *Ceriagrion coromandelianum* completion of embryonic development and hatching are not separated by an interval as found in some odonates (Miller 1992) and therefore most eggs hatch within seven days. Hatching can be triggered by temperature, hypoxia, onset of light/darkness and rainfall. The minimum duration of direct hatching in most non hibernating species (like *C. coromandelianum*) varies from 5–7 days (Corbet 1999). *C. coromandelianum* often oviposits in temporary small ponds and there is strong selection pressure to complete the aquatic phase of the life cycle rapidly. It, therefore, not only exhibits a short hatching period but also rapid larval development within 35 days (Kumar 1980). Although low temperature, desiccation and flooding (Sawchyn & Gillott 1974; Duffy 1994; Bennett & Mill 1995) cause heavy mortality in odonate eggs, such conditions were not observed during the present study.

Egg clutches deposited in submerged vegetative substrate have a higher rate of hatching than those placed along the under-surface of floating vegetation. Many zygopterans exhibit underwater oviposition where the females walk down and lay eggs in submerged vegetation. Some genera descend up to 1 meter and can remain underwater for up to 2.5 hours (Corbet 1999). This high risk underwater oviposition gives free access to the oviposition site (Alcock 1987) and removal of male interference (Waage 1984). Further, when eggs are laid inside completely submerged vegetation, it decreases the risk of desiccation (Corbet 1999) and, as indicated from the present study, increases the rate of hatchability. In Odonata**,** the head of the embryo produces convulsive pumping movements before hatching. Although Tillyard (1916) believed that this action is caused by a special hatching organ the 'cephalic heart', it is now well established that swallowing of the amniotic fluid by the embryo causes these movements (Grieve 1937; Wolfe 1953; Corbet 1965, 1999). As we have observed, in *C. coromandelianum*, the head of the prolarva produces cyclic movements which synchronize with the peristaltic movement of the gut, probably caused by the intake of amniotic fluid by the embryo. In *Anax papuenesis* (Tillyard 1916), the head lies just below the anterior end of the eggshell *i.e*. the micropylar apparatus; however, in *Epiophlebia superstes,* the head is lodged inside the lumen of the pedicel and this region contains the micropylar apparatus (Sahlén 1994; Andrew & Tembhare 1997). In *C. coromandelianum* the head is located below the pedicel and is demarcated by the chorionic ring. The

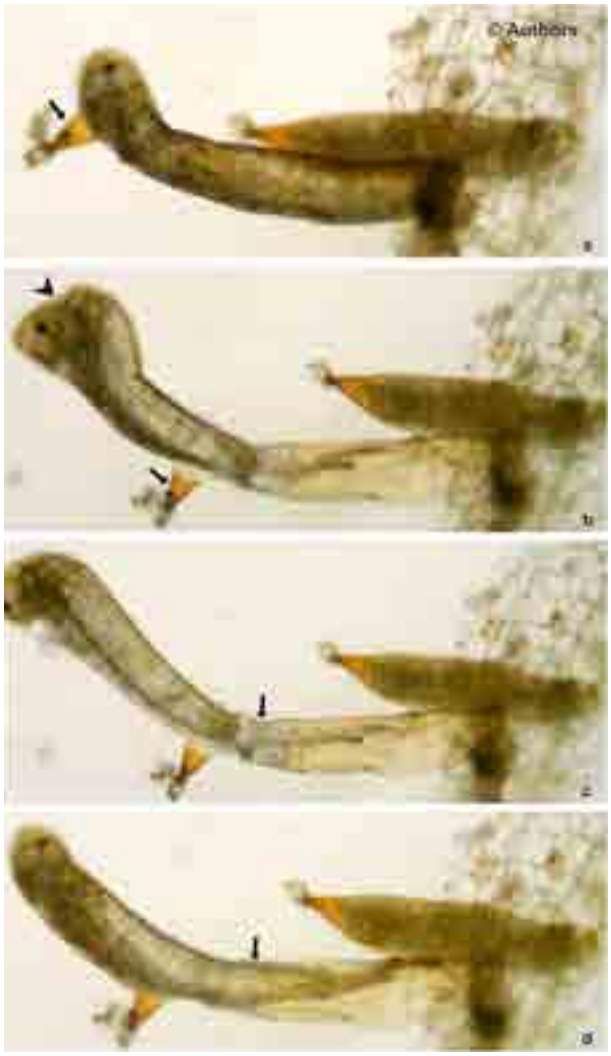

Image 3. *Ceriagrion coromandelianum* – serial photographs of hatching showing breaking of the apical tip of the egg (a & b arrows) followed by swelling of the head (b-arrowhead) and twisting of the prolarva to escape from the prolarval sheath (c & d arrows).

embryo head of *C. coromandelianum* initially passes through the chorionic ring and comes to lie in the lumen of the pedicle, where it commences butting the pedicel with stronger pumping movements and swelling of the head. Similar movements are probably undertaken by the embryo of many odonates before hatching (Corbet 1999).

In dragonflies exhibiting exophytic oviposition, the eggs are mostly spherical and the embryo does not exert a localised upward pressure as found in the endophytic egg. The embryo circulates inside the egg in a spiral manner just before hatching and forms a vertical slit to escape from the egg (Miller 1995). In *C. coromandelianum* the embryo pushes and exerts pressure on the apical pedicel which weakens along the

Image 4. *Ceriagrion coromandelianum* – serial photographs of hatching showing emergence of the first instar larva (arrows) by shedding the prolarva sheath (PLS) which is held by the fractured edge of the eggshell.

rim of micropylar chute around the pedicel of the egg (Andrew et al. 2011). This 'pre-existing line of weakness' (Degrange 1961, 1974) of the egg breaks and the pedicle topples off like a lid. In *Anax papunesis* (Tillyard 1916), *Enallagma cyathigerum, Calopteryx virgo* (Degrange 1961, 1974) and *Anax guttatus* (Andrew & Tembhare 1997), the endophytic egg exhibits both conditions, as the embryo escapes from the egg, the pedicel not only pops out but a vertical slit is also produced, which gives ample space for the embryo to escape.

The duration of the prolarva stage depends upon the ease with which it can free itself from the jelly or detritus around the egg, and also upon the distance it must travel to reach water (Asahina 1950; Robert 1958; Corbet 1999). The duration of the prolarval stage varies from less than a minute as found in *Anax papuensis* (Tillyard 1916) *Brachydiplax sobrina* (Chawdhury & Chakraborty 1988); *Ictinogompus rapax, Rhodothemis rufa* (Begum et al. 1980, 1990), 40 minutes in *Zyxoma petiolatum* (Begum et al. 1982) and about four hours in *Epiophlebia superestes* and *Epitheca bimaculata* (Robert 1958). In *C. coromandelianum* the prolarval stage is almost non-existent because the prolarva does not have to free itself from jelly or aquatic detritus (since the detritus is restricted to the projecting pedicel tip, which detaches during hatching) and the prolarva does not have to travel to find suitable habitat (Thaokar et al. 2018a). In summary, the prolarva discards the prolarval sheath as it leaves the egg allowing the first instar larva to escape. The yolk material as found in the gut of the first instar larva of *Anax papuensis* (Tillyard 1916) is not found in *C. coromandelianum* probably because of its very short prolarval stage and as it emerges from the egg fully equipped with functional mouthparts.

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COMMUNICATION

Distribution of the genus *Pinguicula* (L., 1753) (Lentibulariaceae) in Gunma Prefecture, Japan with new records

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Abstract: We studied the distribution of two *Pinguicula* (Butterwort) species in Gunma Prefecture, Japan, based on our herbarium specimen examinations and field observations. As a result, several localities of *Pinguicula macroceras*, such as Mt. Akagi-yama and the Tanigawa Mountain Range, have been confirmed to exist . In addition, two new localities of *P. macroceras*, Mts. Hotaka-yama and Ojikazawa-no-kashira, which had not been previously recorded, were found. However, only a single locality of *P. ramosa*, a threatened species (Endangered in the Red List of Gunma Prefecture and Vulnerable in the Red List of the Ministry of the Environment of Japan), was confirmed to be present in the prefecture. The two species have extremely narrow environmental preferences and are restricted to specific environmental niches. The population size of both species at each microhabitat is small and there is a potential risk of disappearance of those localities in the future by the impacts of environmental stress or human activities. This study documents the current situation of the genus in Gunma Prefecture and suggests that urgent conservation is necessary to protect both the two species and their habitats in the prefecture.

Keywords: Distribution of *Pinguicula*, flora of Gunma, habitat, Lentibulariaceae, *P. macroceras*, *P. ramosa*

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INTRODUCTION

Species of the genus *Pinguicula* L. (1753) (Lentibulariaceae) are relatively small herbs basically forming basal rosettes with adhesive carnivorous leaves. Over 90 species are distributed in Eurasia, North and South America, the Caribbean Islands, and Morocco (Casper 1966; Fleischmann & Roccia 2018), and approximately half of which are found in Mexico (Zamudio 2005). At least two species, *P. macroceras* Link (1820) and *P. ramosa* Miyoshi (1890), occur in Japan. In general, those species are found in nutrient-poor wet soil where other plant species are scarce.

Pinguicula macroceras was often treated as *P. vulgaris* L. var. *macroceras* (Link) Herder by Japanese botanists, but it is recognized as a distinct species in recent years (after Casper 1962). *P. macroceras* is widely distributed in the northern Pacific region, including Japan, the Kurils, Sakhalin, the Aleutians, Alaska, and western Canada to northern California. The holotype of the species, collected in Unalaska, Alaska and deposited in Botanic Garden and Botanic Museum Berlin (B), was lost during WWII (Casper 1962); however, the lectotype was recently designated by Domínguez et al. (2017).

The southernmost distribution of *P. macroceras* known in Japan is a limestone cliff at Mt. Ishidate-yama on the border of Kochi and Tokushima Prefectures, Shikoku Island (Yamanaka 1953; Komiya & Shibata 1998). In literature, the Botanical Society of Japan (1888) reported that R. Yatabe and some other botanists observed *Pinguicula* sp. (most likely *P. macroceras*) on Mt. Ishizuchi-san in Ehime Prefecture, which could be the westernmost distribution of the species; however, no specimens from the mountain have been found so far. Many localities are known particularly in the Chubu region (central Japan, particularly in Nagano Prefecture) and farther north. *Pinguicula macroceras* in the country are mostly restricted to occurring in higher mountains or deep gorges (Komiya & Shibata 1998). The species exceptionally occurs at low altitudes of 140–200 m along rivers in Gosen, Niigata Prefecture (Katagiri 1980; Komiya & Shibata 1998). The species is often found on limestone or serpentine rocks in the Chubu region and farther south while it is also found in peat or silt in northern Japan. The species is a relic species in the postglacial era (Komiya & Shibata 1998).

Pinguicula ramosa, described by Manabu Miyoshi, is endemic to Japan, specifically in Gunma and Tochigi Prefectures. It is confined to altitudes of 1,400–2,300 m in only a few mountains around Nikko City. The authority is often treated as *P. ramosa* 'Miyoshi ex Yatabe' since Casper's (1966) taxonomic monograph of the genus; however, it is correctly *P. ramosa* 'Miyoshi' (Shimai 2016). The voucher specimen stamped as 'TYPUS' in Koishikawa Botanical Garden, University of Tokyo (TI) was designated as the lectotype by Domínguez et al. (2017). The species is restricted to specific mountains in Gunma and Tochigi Prefectures, and the threatened status is Vulnerable (Ministry of the Environment of Japan 2020) and Endangered in Gunma Prefecture (Gunma Prefecture 2018). It is often found on vertical or overhanging cliffs formed by weathered tuff breccia. The habitat in Mt. Koshin-zan in Tochigi Prefecture, where the species were first discovered, is strictly protected as a special rank of the Natural Monument. Regarding the taxonomic rank, Tamura (1953) treated that it was an infraspecific taxon of *P. villosa* L. (1973), i.e., *P. villosa* L. var. *ramosa* (Miyoshi) Tamura (1953). However, *P. ramosa* is a distinct species morphologically (Komiya and Shibata 1998), cytologically (Casper and Stimper 2009), and phylogenetically (Shimai et al. 2021).

Gunma Prefecture (area of 6,362 km^2) is in the northern part of the Kanto region. The prefecture roughly has the Kanto Plain in the south and mountain ranges in the north. It is an inland prefecture without coastlines but there are many sources of the Tone-gawa River System. The climate of the prefecture is affected by both the Pacific Ocean side and the Sea of Japan side. Although the number of localities of *P. macroceras* in Gunma is fewer than that in neighboring Nagano or Niigata Prefecture, Gunma is biogeographically important because it borders the two prefectures, which geologically divide Western and Eastern Japan. Gunma and Tochigi are the only prefectures where the two *Pinguicula* species certainly occur. In Tochigi, *P. macroceras* is recorded from only two mountains, Mt. Nikko-Shirane-san and Mt. Nantai-san. Although more localities of *P. macroceras* are known in Gunma than Tochigi, the population size at each locality in Gunma is small and there is a risk of habitat reduction. Many high mountains and deep gorges prevent the thorough investigation of flora in Gunma, resulting in insufficient research in such inaccessible areas. In this study, we document the distribution of *Pinguicula* including both earlier records as well as newly found ones in Gunma Prefecture by our recent investigations to prevent the disappearance of the species.

MATERIALS AND METHODS

Herbarium specimens housed in museums and botanical gardens in the world were studied (mainly 2013–2020) and specimen data on the labels such as localities, collected years, and collectors were gathered. The localities in Gunma are summarized in Table 1, but detailed locations or coordinates are omitted to protect the habitats. After 1900, it was divided every 10 years, and collection records, if any, at each locality were presented in the table. The elevation and coordinate of each mountain are obtained from the database of the Geospatial Information Authority of Japan (https://www.gsi.go.jp/top.html), and those are of a representative peak of each mountain but do not necessarily indicate the exact location where the species can be found. A distribution map was produced based on the specimen records using GeoCat, Geospatial Conservation Assessment Tool (Royal Botanic Gardens, Kew; http://geocat.kew.org/), and the extent of occurrence (EOO) and the area of occupancy (AOO) were calculated by the program. The AOO was calculated by the default parameter of 2 km × 2 km (= 4 km^2). Two or more microhabitats close to each other (e.g., within 100 m) or two or more collection records from the same area were treated as a single locality. The coordinate datasets on GeoCat were then transferred to QGIS 3.14 to produce a distribution map (Figure 1). Even if other localities were found on literature or internet source, those were not recognized in this study unless specimens from there were found.

RESULTS

Specimens of *Pinguicula* collected in Gunma Prefecture were mostly found at herbaria in Japan, such as GMNHJ, KYO, TI, and TNS. Localities recognized are summarized in Table 1 and are individually discussed below.

***Pinguicula macroceras* Link**

1. Mt. Akagi-yama (1,828 m, 36.560278, 139.193333) (Image 1A, B)

Mt. Akagi-yama (or also called Akagi-san), located ca. 20 km north-east of Maebashi, the prefectural capital of Gunma, is one of the symbolic mountains of the prefecture. It is a complex volcano although no volcanic activities have been recorded for ca. 30,000 years (Kobayashi & Nakamura 2001). The somma consists of

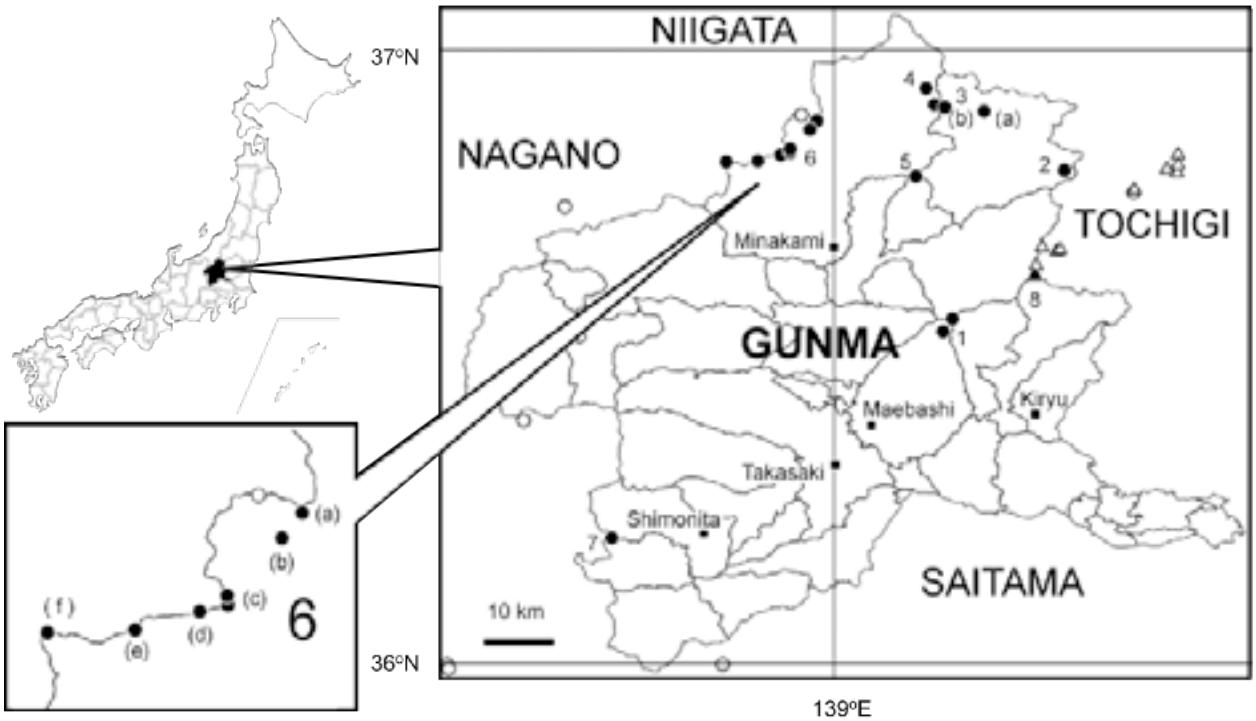

Figure 1. Distribution of two species of *Pinguicula* in Gunma Prefecture and surroundings: Closed circles—*P. macrocears* in Gunma | Open circles—*P. macroceras* in neighboring prefectures | Closed triangles—*P. ramosa* in Gunma | Open triangles—*P. ramosa* in Tochigi | Lines within Gunma Prefecture show municipal borders (as of 2019). Numbers correspond with those in the text: 1. Mt. Akagi-yama, 2. Mt. Nikko-Shirane-san, 3. Oze, 4. Naramata-gawa River, 5. Mt. Hotaka-yama, 6. Tanigawa Mountain Range, 7. Mt. Arafune-yama, 8. Mt. Kesamaru-yama.

Table 1. Observed specimens of *Pinguicula* in Gunma Prefecture. MAC = *P. macroceras*. RAM = *P. ramosa*. Localities are listed in alphabetical order. Numbers correspond with those in the text and Figure 1. [New] = new record reported in this study. X = specimen collected during the period. Voucher specimens are listed in the specimens examined section.

Species	Locality in Gunma	No.	Specimen record											
MAC	Akagi-yama, Mt. (Jizo-dake)	1					X	X	X				X	X
MAC	Akagi-yama, Mt. (Kurobi-san)	1	X		X	X								
MAC	Akagi-yama, Mt. (w/o location)	1						X	X			X	X	
MAC	Arafune-yama, Mt.	7							X					
MAC	Asahi-dake, Mt.	6 (1)								X	X			
MAC	Ayame-daira	3 (1)					X	X?						
MAC	Hotaka-yama, Mt. [New]	5												X
MAC	Kasagatake, Mt.	6 (2)												
RAM	Kesamaru-yama, Mt.	8								X			X	X
MAC	Mantaro-san, Mt.	6 (5)							X					X
MAC	Naramata-gawa River	4						X					X	
MAC	Nikko-Shirane-san, Mt.	2												X
MAC	Ojikazawa-no-kashira, Mt. [New]	6 (4)												X
MAC	Shibutsu-san, Mt. (NE slope)	3 (2)						X					X	
MAC	Shibutsu-san, Mt. (NW slope)	3 (2)											X	
MAC	Shibutsu-san, Mt. (w/o location)	3 (2)			X	X	X	X	X					
MAC	Tairappyo-yama, Mt.	6 (6)						X						
MAC	Tanigawa-dake, Mt.	6 (3)											X	X
MAC	Tanigawa-dake, Mt. (w/o location)	6 (3)			X		X	X	X			X		
			1900-1909	1910-1919	1920-1929	1930-1939	1940-1949	1950-1959	1960-1969	1970-1979	1980-1989	1990-1999	2000-2009	2010-2019

several peaks including Kurobi-san (1,828 m; the highest peak of the mountain), Komagatake (1,685 m), and Jizo-dake (1,674 m). There are a few lakes and ponds, e.g., Lakes Ono (or Onuma) and Kono (or Konuma), in the caldera.

In the mountain, the specimens of *P. macroceras* were collected mainly from Kurobi-san and Jizo-dake Peaks, but the former has no collection records after the 1930s. The specimens have been collected repeatedly from the latter peak and the species is present near the summit; however, the population size is declining rapidly since the soil there is getting dry. It was commonly seen at Jizo-dake Peak in the early 2000s but was only ca. 50 individuals in 2019. At Jizo-dake Peak, the species is found only in eroded wet black soil. The habitat is above the forest line with good sunlight which may result in dry soil and the consequent decline of population size. There are several herbarium specimens that say only 'Mt. Akagi-yama' on the labels (i.e., not specified exact peaks or locations).

Although no specimens have been found so far, Hattori (1908) wrote that numerous plants of *P. macroceras* were seen at Komagatake and Choshichiro-dake Peaks in the mountain. In July, the summit of Kurobi-san was purple in color by the flower of the species (Hattori 1909). Pictures in the book of Hattori (1908) showed that the summit of Kurobi-san was an open grassland at that time while the summit is mostly surrounded by trees, today. No plants were found at these peaks in our recent surveys.

2. Mt. Nikko-Shirane-san (2,578 m, 36.798611, 139.375833) (Image 1C, D)

Mt. Nikko-Shirane-san, a stratovolcano, is located on the border of Gunma and Tochigi Prefectures. It is the highest mountain in the Kanto region and northern Japan. The mountain is officially Shirane-san, but there are some other mountains that have the same name, so it is often called Nikko-Shirane-san to specify. The mountain, characteristically dome-shaped, is located in the western part of Nikko National Park (114,908 ha).

Due to ancient volcanic activities, a few lakes and

ponds have been formed. Relatively large numbers of *P. macroceras* are seen on the Tochigi side of the mountain. There was a record of the species from the Gunma side in literature, but it was thought to be extinct (Moriya 1976). However, we confirmed in 2019 that the species was still present there although less than 30 individuals. It grows among grasses so it may be difficult to find them unless they are in flower. Concerning its small population size, they may disappear from there in the future.

3. Oze National Park

Oze, highland wetlands on the Gunma, Niigata, and Fukushima prefectural borders, was formed by volcanic activities of Mt. Hiuchigatake (2,356 m) ca. 10,000 years before present. It roughly consists of Ozegahara (peatland), Oze-numa Pond, and a few mountains including Mts. Hiuchigatake and Shibutsu-san. Wetlands and ponds in a plateau provide characteristic vegetation with many rare and endemic plant species. Oze and its surroundings have been designated as Oze National Park (37,200 ha). It is an area that symbolizes the conservation of nature in Japan. At least two localities of *P. macroceras*, Ayame-daira and Mt. Shibutsu-san, are known in Oze.

(a) Ayame-daira (1,969 m, 36.900556, 139.244722)

Ayame-daira, with a gentle peak and mountain peatlands, is located in the southern part of the national park. Too many visitors caused erosions and it subsequently became bare ground at the top of Ayame-daira in the 1950s. Since the 1960s, a vegetation recovery program has been attempted by a few organizations and is yet on the way; therefore, the wetland is strictly protected. Some literature occasionally recorded that *P. macroceras* occurred there but only a single herbarium specimen from there has been found so far. We have confirmed in July 2020 that a small population of the species is present there. There is a specimen from Ozegahara, which might be collected at Ayame-daira (probably mislabeled), because the species is not present in Ozegahara.

(b) Mt. Shibutsu-san (2,228 m, 36.903611, 139.173333)

Mt. Shibustu-san, located at the westernmost of the national park, is composed of serpentines (Kawase et al. 2009). Some rare plant species (e.g., *Japonolirion osense* Nakai and *Arenaria katoana* Makino) are seen in the mountain (Tomimatsu et al. 2004; Kawase & Yumoto 2006; Kawase et al. 2009). There are many *P. macroceras* specimens previously collected at the mountain. Some location names on the northeastern slope of the mountain are specified on the specimen labels, but those could be within the same area. There is a specimen from the northwestern slope, but the area is designated as an environmental protection area by Gunma Prefecture and is restricted to access there. There were many specimens that say only 'Mt. Shibutsu-san' on the labels.

4. Naramata-gawa River watershed

Naramata-gawa River, which is upstream of Tone-gawa River, is originated in the Echigo Mountains. The area belongs to Minakami township in Gunma. *P. macroceras* was collected at one of the branch streams of Naramata-gawa River in 2003. There is another collection record from somewhere along Naramata-gawa River in 1955, but it is unclear if this location is identical with the former. No recent information is available.

5. Mt. Hotaka-yama (2,158 m, 36.805278, 139.132500) [New record] (Image 2A)

Mt. Hotaka-yama is often called Joshu-Hotaka-yama to distinguish it from Mt. Hotaka-dake (3,190 m; the third highest mountain in Japan) on the prefectural border of Gifu and Nagano. Mt. Hotaka-yama, an old volcanic mountain, is an independent peak located at the south of Mt. Shibutsu-san.

The specimen of *P. macroceras* was recently collected at the south-facing slope of Mt. Hotaka-yama on 16 August 2016 by T. Ohmori and H. Yoshii which is deposited in Gunma Museum of Natural History (GMNHJ). It is too dangerous to go off the mountain trails and the activities of researchers are restricted as there are many cliffs along streams and above the subalpine zone. Furthermore, there are many areas and mountains nearby, such as Oze, the Tanigawa Mountain Range, and Mt. Nikko-Shirane-san, which are more important in the field of botany, resulting in less exploration by botanists in Mt. Hotaka-yama. Those might be reasons why the species had not been discovered in the mountain until 2016.

6. Tanigawa Mountain Range

Strictly speaking, the Tanigawa Mountain Range is a part of the Mikuni Mountain Range. The Tanigawa Mountain Range, with several mountains, is on the prefectural border of Gunma and Niigata (sometimes called 'jo-etsu kokkyo'), and it depends on literature which mountains are included in the mountain range. In this study, it is defined that the range is from Mts. Asahi-dake and Kasagatake in the east to Mt. Tairappyo-yama in the west. Those mountains are connected by the Gunma ridge trail.

(a) Mt. Asahi-dake (1,945 m, 36.880556, 138.97250)

Mt. Asahi-dake is located in the eastern part of the mountain range. The specimen of *P. macroceras* was collected at the mountain in 1976 and 1981, but the specimen label did not show any exact location. No recent specimens have been found; however, the species is present according to information from climbers who visited the mountain. The species also occurs on Shimizu-toge Pass, on the Niigata side, ca. 2 km north-west of the summit of Mt. Asahi-dake.

(b) Mt. Kasagatake (1,852 m, 36.869444, 138.962778)

Mt. Kasagatake (or Mt. Okura-yama) is located ca. 1.5 km south-west of Mt. Asahi-dake. The specimen of *P. macroceras* was collected in 1965, and it is the only specimen found so far. The specimen label specifies as 'between Okura and Kasagatake', which is probably somewhere on a trail between Okura-one Ridge and the summit of Mt. Kasagatake (the trail is disused today). It is not confirmed whether the species is still present there.

(c) Mt. Tanigawa-dake (1,977 m, 36.837222, 138.930000 (Image 1E, F)

Mt. Tanigawa-dake, a serpentinous mountain, is located on the prefectural border of Gunma and Niigata. The mountain possesses two characteristic peaks, which are Oki-no-mimi (1,977 m) and Toma-no-mimi (1,963 m). A few localities of *P. macroceras* can be found around the peaks. Those microhabitats are covered by grasses; therefore, it is somewhat difficult to find the species unless they are in flower. There are not many individuals, often <50, within each microhabitat. There is a potential risk that they may disappear from there in the future due to ecological succession. Some other populations can be seen on a vertical cliff on the northeast side of the mountain, but it is impossible to approach there without climbing equipment.

(d) Ojikazawa-no-kashira (1,840 m, 36.829167, 138.912500) [New record] (Images 1G, H, 2B)

Ojikazawa-no-kashira is located between Mts. Tanigawa-dake and Mantaro-san (ca. 1.5 km west of the former). The north side of the mountain trail belongs to Niigata Prefecture. *P. macroceras* had not been collected before, but only a few plants were newly discovered in a grassland near the summit of the Gunma side on 07 July 2019, which the specimen collected by H. Shimai has been deposited in the National Museum of Nature and Science (TNS). There is a risk that the species could potentially disappear from there in the future since the locality is densely covered by grasses which is unfavorable for *P. macroceras*.

(e) Mt. Mantaro-san (1,954 m, 36.824167, 138.879167)

Mt. Mantaro-san, ca. 5 km west of Mt. Tanigawa-dake, is located on the Gunma ridge trail to Mt. Tairappyo-yama. Because of the relatively long trail, botanists rarely access the area. Approximately 100 plants were found on the ridge below the peak on 07 July 2019. This locality is about on the prefectural border of Gunma and Niigata. The microhabitat is also covered by grasses due to ecological succession , and the population may disappear in the future. It is unclear whether it is identical to the location below.

There was an earlier collection record of the species in the back of Kawafuru-onsen Spa. Although the exact location is unknown, it is assumed that it may be between a headwater stream of Akaya-gawa River and the ridge on the prefectural border, or near the ridge. The area is on the south slope of Mt. Mantaro-san, but the trail between the spa and the ridge is very long and rugged, requiring crossing streams; therefore, botanists usually do not enter such a tough route.

(f) Mt. Tairappyo-yama (1,984 m, 36.817500, 138.821667)

Mt. Tairappyo-yama, at the western edge of the Tanigawa Mountain Range, stands on the prefectural border of Gunma and Niigata, and it has peatlands near the summit (Sasaki & Kariya 2000). *P. macroceras* was collected from two locations on the Gunma side, but our surveys in 2018 and 2019 failed to find the species along the mountain trail. It may be possible that the species is still present away from the trail. Although we have not accessed the specimens, the species was also collected on the Niigata side of the mountain (Katagiri 1980).

7. Mt. Arafune-yama (1,423 m, 36.203889, 138.637222)

Mt. Arafune-yama, with a massive vertical rock cliff, is in the western part of Gunma (on the prefectural border of Gunma and Nagano). The existence of *P. macroceras*, collected on the Gunma side only once in 1960, was confirmed by the specimen only. No detailed location was mentioned on the specimen label except the name of the mountain. It is unclear whether the species is still present there; however, it is almost impossible to search for plants on the inaccessible massive rock cliff. The locality is not only the westernmost but also the southernmost for the species in Gunma.

***Pinguicula ramosa* Miyoshi**

8. Mt. Kesamaru-yama (1,961 m, 36.649444, 139.327222) (Image 1I, J)

Mt. Kesamaru-yama, a volcanic mountain on the prefectural border of Gunma and Tochigi, is located at the westernmost of the Ashio Massif. Mt. Kesamaru-

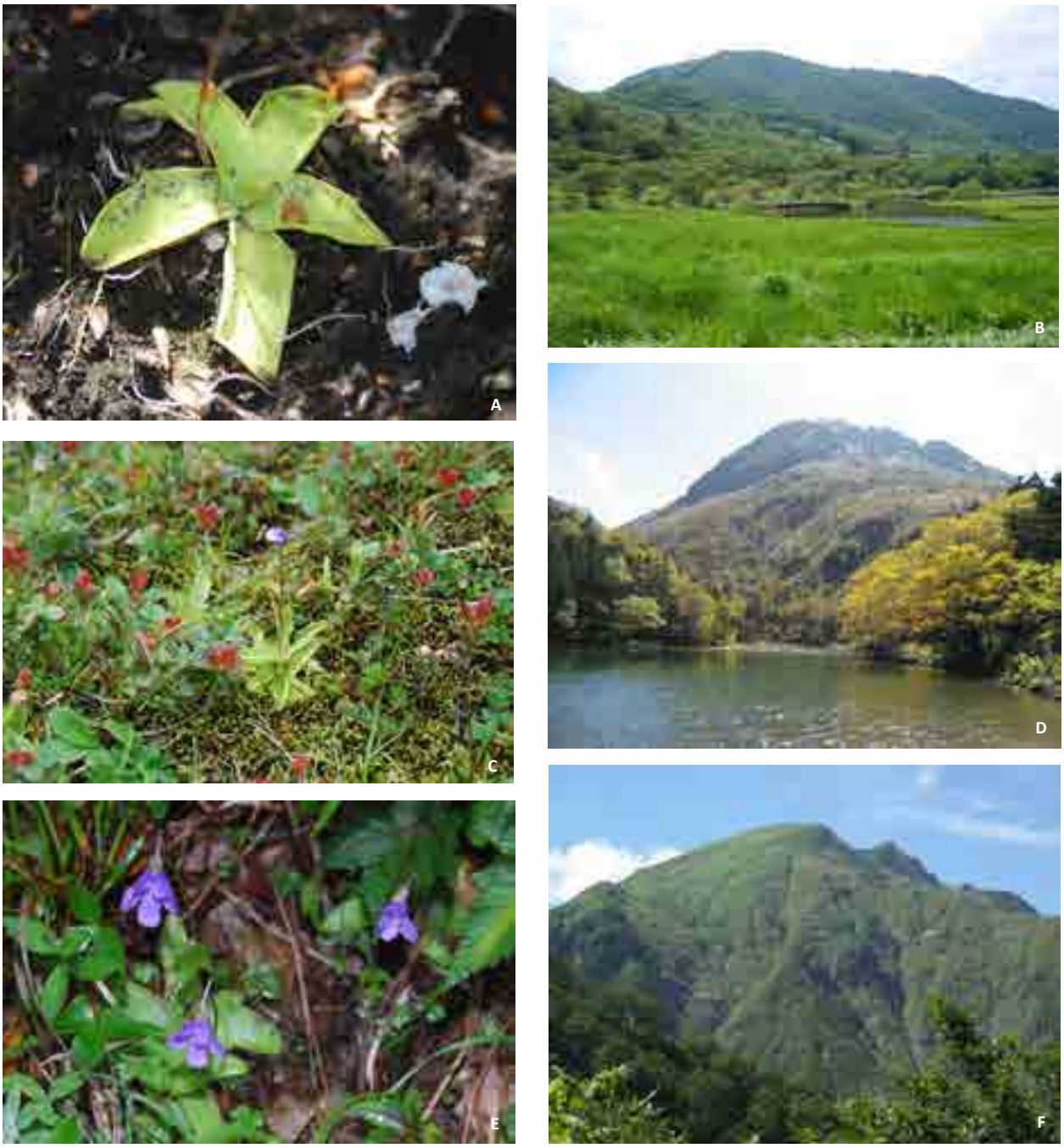

Image 1. *Pinguicula* species and their habitats in Gunma Prefecture: A, B—*P. macroceras* (16.vii.2018) and its habitat, Jizo-dake Peak in Mt. Akagi-yama; view from Kakumambuchi | C, D—*P. macroceras* (02.vii.2019) and its habitat, Mt. Nikko-Shirane-san | E, F—*P. macroceras* (07. vii.2019) and its habitat, Mt. Tanigawa-dake. © H. Shimai.

yama consists of a few peaks. The highest peak is a point at 1,961 m (no name for the peak), north of Ato-Kesamaru Peak (1,908 m). The species occurs on both Gunma and Tochigi sides of the mountain.

Ato-Kesamaru Peak is the only place where *P. ramosa* occurs in Gunma. Only four specimens from the Gunma side were confirmed to exist. Although there were a few other specimens saying only 'Mt. Kesamaru-yama' on the specimen label, no prefecture was specified. The species can be seen only on a vertical cliff of weathered tuff breccia on the mountain as also seen on Mts. Koshin-zan and Nantai-san in Tochigi. The species basically occurs below the tree line, but the habitat on the Gunma side has good sunlight compared with

Image 1. G, H—*P. macroceras* (07.vii.2019) and its habitat, Mt. Ojikazawa-no-kashira | I, J—*P. ramosa* (06.vi.2019) and its habitat, Mt. Kesamaru-yama; view from Tochigi side. © H. Shimai.

localities in Tochigi. The habitat receives direct sunlight in the morning, which may cause dry out the soil of the microhabitat. Minor collapses of the cliff seem to be relatively common, which may affect the habitat of the species. No *P. macroceras* has been found on Mt. Kesamaru-yama.

Distribution areas of *Pinguicula* in Gunma

In *P. macroceras* in Gunma, the EOO calculated by GeoCat was 2,458 km^2 and the AOO was 64.0 km^2, both of which suggested Endangered although the species is not listed in the Red List of Gunma Prefecture. The area of Gunma Prefecture is ca. 6,362 km^2; therefore, the EOO covers one-third of the prefecture. Although the GeoCat instruction suggests that it is possible to evaluate the threatened category at a regional level, it needs further studies whether this method can be applicable for such a widespread species. On the other hand, *P. ramosa* occurs only in a single locality in Gunma Prefecture, which is unable to calculate the EOO by GeoCat since the program requires at least three locations to evaluate the status. However, it is apparent that *P. ramosa* is an endangered species in Gunma.

DISCUSSION

The localities of *Pinguicula macroceras* within Gunma Prefecture are mostly restricted to above the subalpine zone, 1,500 m or higher, in the north (Figure 1). If some other localities outside of Gunma (e.g., Mts. Asama-yama and Iwasuge-yama in Nagano and Mt. Nantai-san in Tochigi) are included, those surround the northern half of Gunma Prefecture. The species is often found in alkaline soil (e.g., limestone or serpentine outcrops) in the Chubu region or farther south. However, the environmental preference of *P. macroceras* in Gunma is similar to that in the Tohoku region (e.g., in Mts. Akita-Komagatake, Nyuto-zan, and Hachimantai). *P. macroceras* in Gunma Prefecture tends to be found in acid peaty soil near snow patches or stream banks rather than alkaline soil, where the species is more often seen in the Chubu region and farther south (particularly at lower altitudes). It is highly possible that the species grows in extreme environments to avoid survival competitions with other plant species, but it may not strictly depend on the soil pH level. It is also probable that increasing pioneering plants, such as grasses, sasa bamboos, and

tall herbaceous plants (i.e., ecological succession) in the habitats, or erosions along mountain trails would be more serious causes for declining populations of *P. macroceras*. Furthermore, natural disasters, including heavy rains or furious typhoons, which tend to be increasing in recent years, may cause the distraction of the locality due to landslides.

Kurobi-san, one of the peaks at Mt. Akagi-yama, used to be famous for *P. macroceras*; however, the species has totally disappeared from there already. There is no recent information whether the species is still present in some localities (e.g., Mt. Arafune-yama) due to remote areas or inaccessible cliffs. Most of the localities of *P. macroceras* are within national parks, which are protected by law. Even within the protected areas, the microhabitats are covered by grasses (e.g., Mt. Tanigawa-dake), which are unfavorable for the species. In the current study, we report two new localities, but many localities in Gunma Prefecture have a small number of the species.

Regarding *Pinguicula ramosa*, large colonies were seen on both the Gunma and Tochigi sides of Mt. Kesamaru-yama before; however, those rapidly shrank in the last 50 years (Hiroshi Masuda pers. comm. vi.2019). On the Gunma side, we recently observed only a small number of *P. ramosa* plants on a vertical rock face. It is unclear how many plants are present there, but it is no doubt that the population size is very small. Dry soil and corruption of the rock face may cause the extinction of species on the Gunma side.

In addition, the localities of *P. macroceras* in other prefectures near the Gunma border are Mts. Asama-yama and Iwasuge-yama in Nagano, Nakatsu-gawa River in Saitama, Mts. Nantai-san and Nikko-Shirane-san in Tochigi. The localities of *P. ramosa* outside of Gunma are Mts. Kesamaru-yama, Nokogiri-yama, Koshin-zan, Nyoho-san, and Nantai-san in Tochigi.

Concerning the EOO in *P. macroceras*, it covers one-third of the area of Gunma Prefecture, but the status of the species including *P. ramosa* is not optimistic. Further studies of both species and their urgent conservation act in Gunma Prefecture are necessary. The current situation of the two species is recorded above, but there may be unknown localities in high mountains or deep gorges.

Image 2. Specimens from new localities: A—Mt. Hotaka-yama, 16.viii.2016, Ohmori and Yoshii 12056 (GMNHJ; as *P. vulgaris* L. var. *macroceras* (Pall. ex Link) Herder: forming hibernacula). B—near Ojikazawa-no-kashira Peak, 07.vii.2019, Shimai s.n. (TNS; as *P. vulgaris* L). Due to environmentally sensitive areas in the national parks, detailed information, such as landmarks and coordinates, on specimen labels has been removed.

Specimens examined

Pinguicula macroceras Link, Jahrb. Gewächsk. 1: 54 (1820).

JAPAN. **Gunma**: Mt. Akagi-yama, 20.vii.1952, Okuyama 10148 (TNS; as *P. vulgaris*); 04.vi.1961, Komiya s.n. (TNS); 01.viii.1962, Tanaka s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 29.vi.1999, Shibata s.n.

(TNS); 18.vi.2000, Komiya & Shibata s.n. (TNS). Mt. Akagi-yama, Jizo-dake, 26.vii.1948, Tanaka s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 30.vii.1950, Komiya s.n. (TNS); vi.1951, Komiya s.n. (TNS); vi.1952, Komiya s.n. (TNS); 13.vi.1954, Higuchi s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 1,400 m, 19.vi.1965, Fukuoka & Naruhashi 20 (KANA, KYO; as *P. vulgaris* var. *macroceras*); ca. 1,650 m, 08.vi.2001, Shimai s.n. (Nippon Dental Univ.); 1,640 m, 05.vi.2004, Yoshii s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 1,660 m, 17.vii.2018, Shimai s.n. (TNS). Mt. Akagi-yama, Kurobi-san, 25.vii.1903, Hayata s.n. (SAPS, TI; as *P. vulgaris*); 09.viii.1927, Kojima s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 12.vii.1928, Hara s.n. (TI; as *P. vulgaris*); 25.vii.1930, Shiobara s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 15.vi.1934, Saito s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 16.vi.1934, Fukushima s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*). Mt. Akagi-yama, between Onuma Lake and Jizo-dake, 1,400–1,700 m, 15.vi.1969, Konta 7707 (TNS; as *P. vulgaris*). Mt. Arafune-yama, 20.ix.1960, Satomi s.n. (TNS; as *P. vulgaris*). Mt. Asahi-dake, 24.vii.1976, Yoshizawa 761 (TNS; as *P. vulgaris*); 14.vii.1981, Haginiwa JH033382 (TNS; as *P. vulgaris* var. *macroceras*). Mt. Hotaka-yama, 16.viii.2016, Ohmori and Yoshii 12056 (GMNHJ; as *P. vulgaris* var. *macroceras*). Mt. Mantaro-san, 07.vii.2019, Shimai s.n. (TNS). Minakami, Upper stream of Kawafuru-onsen Spa; vi.1976, Miyamae s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*). Minakami, branch of Naramata-gawa River, 1,290 m, 25.viii.2003, Ohmori et al. 3540 (GMNHJ; as *P. vulgaris* var. *macroceras*). Naramata-gawa River, upper stream of Tone-gawa River, 20.vi.1955, Matsuda s.n. (TI). Mt. Nikko-Shirane-san, 21.vii.2019, Shimai s.n. (TNS). near Ojikazawa-no-kashira Peak, 07.vii.2019, Shimai s.n. (TNS). between Okura and Mt. Kasagatake, 29.vii.1965, Miyamae s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*). Oze, Ayamedaira, 23.vii.1947, Tanaka s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*). Ozegahara, 23.vii.1951, Ikoshi s.n. (TNS; as *P. vulgaris*). Mt. Shibutsu-san, 15.vii.1924, Takeda & Tatewaki s.n. (SAPS); 16.vi.1929, Hara s.n. (TI; as *P. vulgaris*); 13.viii.1931, Hoshi s.n. (KYO); 09.viii.1935, Ohwi & Tagawa 301 (KYO); 04.vii.1941, Hurusawa s.n. (TI; as *P. vulgaris*); 20.vii.1942, Satomi s.n. (KANA; as *P. vulgaris*); 19.vii.1946, Nakai 2694 (KYO; as *P. vulgaris*); 2,100 m, 05.viii.1949, Kawasaki 4363 (TNS; as *P. vulgaris*); 20.vii.1950, Tobe s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 27.vii.1951, Komiya s.n. (TNS); 30.vii.1952, Tamura s.n. (KYO); vi.1954, Komiya s.n. (TNS); 20.vii.1954, Asai s.n. (TI; as *P. vulgaris* var. *macroceras*); 22.vii.1954, Oda s.n. (Tochigi Pref. Museum; as *P. vulgaris* var. *macroceras*); viii.1954, Sugaya s.n. (TUS; as *P. vulgaris*); 02.viii.1969, Miyamae s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 29.viii.1969, Oze Conservation Center of Gunma Pref. s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*). Northeastern slope of Mt. Shibutsu-san, ca. 2,000 m, 17.vii.1950, Mizushima s.n. (TI; as *P. vulgaris* var. *macroceras*); 30.viii.1950, Komiya s.n. (TNS); 1,900–2,000 m, 04.vii.2003, Shimai s.n. (Nippon Dental Univ.). Northwestern slope of Mt. Shibutsu-san, 1,600 m, 05.viii.2001, Ohmori & Yoshii 1797 (GMNHJ; as *P. vulgaris* var. *macroceras*). Mt. Tairappyo-yama, East side, 1,900 m, 21.vi.1959, Hara s.n. (TI). Mt. Tairappyo-yama, between Tairappyo-goya Hut and summit, 12.vii.1957, Okuyama et al. 13691 (TNS). Mt. Tanigawa-dake, 1,900 m, 14.vii.1928, Hara 9602 (TI; as *P. vulgaris* var. *macroceras*); 06.vii.1948, Yamazaki & Ono 2913 (S, TI; as *P. vulgaris*); 08.viii.1949, Komiya s.n. (TNS); 08.vii.1951, Higuchi s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 24.vii.1951, Tobe s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 02.vii.1953, Kimura & Matsuda s.n. (TI; as *P. vulgaris* var. *macroceras*); 03.vii.1953, Komiya s.n. (TNS); 14.vii.1953, Kanai 382 (TI; as *P. vulgaris* var. *macrocarpa*); vii.1966, Yamazaki s.n. (TI); 01.viii.1961, Suto s.n. (GMNHJ; as *P. vulgaris* var. *macroceras*); 15.vii.1965, Komiya s.n. (TNS); 20.vii.1999, Komiya & Shibata s.n. (TNS); ca. 1,950 m, 23.viii.2004, Shimai s.n. (Nippon Dental Univ.); 03.viii.2018, Shimai s.n. (TNS); 07.vii.2019, Shimai s.n. (TNS). between Mt. Tanigawa-dake and Mt. Asahi-dake, 01.vii.1956, Harazawa s.n. (KANA).

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JAPAN. **Gunma**: Midori, Mt. Kesamaru-yama, 14.vi.1974, Suto s.n. (GMNHJ); 29.vi.2003, Shibata s.n. (Nippon Dental Univ.); 28.vi.2009, Yoshii et al. s.n. (GMNHJ); 06.vi.2019, Shimai s.n. (TNS).

Herbarium code: GMNHJ = Gunma Museum of Natural History | KANA = Kanazawa University | KYO = Kyoto University | S = Swedish Museum of Natural History | SAPS = Hokkaido University Museum | TI = University of Tokyo | TNS = National Museum of Nature and Science | TUS = Tohoku University.

All specimens in Nippon Dental University have been transferred to TNS.

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COMMUNICATION

Reproductive biology of two threatened and highly traded medicinal plants, *Salacia gambleana* and *Salacia oblonga*, from the Western Ghats of India

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Abstract: *Salacia* is a genus of flowering plants in the family Celastraceae, consisting of woody climbers distributed in tropical America, Africa, and Asia. In India it is represented by 21 species, of which 15 occur in peninsular India. Most species of the genus have been used in traditional medicine, mainly the Ayurvedic system. Apart from overexploitation for medicinal purposes, the low fruit set and infestation of seeds have affected natural regeneration, and led to the rarity of *Salacia* species in their natural habitats. The reproductive biology of *Salacia oblonga* and *S. gambleana* was studied for the first time to understand the reproductive constraints of these threatened and medicinally important species. The flowering phenology, pollen viability, germination, stigma receptivity, and insect-pest interaction were analyzed. The obligatory entomophily coupled with insufficient pollinators and seed pest infestation were found to be the main reproductive constraints responsible for the low fruit set and poor natural regeneration of these species.

Keywords: Conservation, health care, management, medicinal genetic resource, reproductive constraints, rarity.

Malayalam: സപുഷ്പികളായ സലേഷ്യവർഗ്ഗം സെലാസ്ട്രേസ്യേ സസ്യകുടുംബാംഗമാണ്. പ്രധാനമായും മരവള്ളികളായ ഇനങ്ങൾ മദ്ധ്യഅമേരിക്ക, ആഫ്രിക്ക, ഏഷ്യ എന്നീ ഭൂഖണ്ഡങ്ങളിലാണ് വിന്യാസം ചെയ്യപ്പെട്ടിട്ടുള്ളത്. ഇന്ത്യ ഉപഭൂഖണ്ഡത്തിൽ പ്രതിനിധാനം ചെയ്യപ്പെട്ടിട്ടുള്ള 21 സസ്യഇനങ്ങളിൽ 15 ഇനങ്ങൾ പശ്ചിമഘട്ടത്തിൽ മാത്രമായി കണ്ടുവരുന്നു. സലേഷ്യ വർഗ്ഗത്തിൽ പെടുന്ന സസ്യങ്ങൾ പരമ്പരാഗത വൈദ്യസമ്പ്രദായത്തിൽ പ്രത്യേകിച്ച് ആയുർവേദചികിത്സയിൽ ഉപയോഗിച്ചുവരുന്നവയാണ്. ഔഷധ ആവശ്യങ്ങൾക്കുവേണ്ടിയുള്ള അമിത ചൂഷണത്തിനു പുറമെ ഫല ഉൽപാദനത്തിലെ കുറവും ഗുണമേന്മയുള്ള വിത്തുകളുടെ അഭാവത്തിലും സസ്യഇനങ്ങൾ ആവാസമേഖലകളിൽ വംശനാശം നേരിട്ടുകൊണ്ടിരിക്കുകയാണ്. ഈ പശ്ചാത്തലത്തിൽ സലേഷ്യ ഒബ്ളോങ്ങ, സലേഷ്യ ഗാബ്ളിയാന (പൊൻകൊരണ്ടി) എന്നീ ഇനങ്ങളുടെ പ്രത്യുൽപാദന-ജീവശാസ്ത്ര ഘടകങ്ങൾ പഠനവിധേയമാക്കുകയും പൂവിടൽ മുതൽ പരാഗണങ്ങളുടേയും സ്റ്റിഗ്മയുടേയും ജീവനക്ഷമത, പ്രാണി - കീട പ്രവർത്തനങ്ങൾ തുടങ്ങിയവ വിശകലനം നടത്തുകയും ചെയ്തു. പ്രാണി ആശ്രിതമായ ഈ സസ്യങ്ങളുടെ പരാഗണത്തിന് പരാഗണകാരികളുടെ അപര്യാപ്തതയും വിത്തുകളിലെ കീടബാധയും സസ്യങ്ങളിലെ ഫലദൗർലഭ്യത്തിനും വിരളമായ സ്വാഭാവിക പുനരുൽപാദനത്തിനും പ്രധാന പരിമിതികളാണെന്ന് കണ്ടെത്തി .

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Author contributions: PSK—carried out the study by her as a part of MSc Dissertation programme. PAJ—Served research supervisor for M.Sc. Dissertation of PSK and manuscript correction and editing. KS—helped in reproductive biological studies in the laboratory and preparation of map, figures and Plate for the article. TVS—helped in field data collection in the medicinal garden.

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INTRODUCTION

The genus *Salacia* contains important medicinal plants with a wide range of therapeutic properties. In India 21 species are reported; 15 occur in peninsular India including the Western Ghats. The majority of *Salacia* species are over-harvested for their roots, which are used in the Ayurveda and Unani systems of medicine for treating diabetes, asthma, leukemia and ear infections. *Salacia* species mainly contain salacinol, kotalanol, neosalacinol, several phenolic compounds, sesquiterpenes, and triterpenes (Kirtikar & Basu 1975; Prakash et al. 2008; Wang et al. 2012). These components have medicinal properties and significant applications in modern medicine. Besides their overexploitation, these species show a low fruit set and poor natural regeneration leading to low population size and rarity. Therefore, the existing populations of *Salacia* species and their natural habitats warrant urgent conservation and management measures.

The rarity of a plant often stems from its biological functions, particularly its reproductive biology, which has a significant contribution to the sexual reproduction of flowering plants. Reproductive biology studies help in the conservation of genetic resources. The reproductive patterns are some of the key factors leading to the abundance, distribution and genetic diversity of the species. There is a widespread consensus that reproductive biology studies of threatened species can help determine strategies for in situ and ex situ conservation. Several levels of problems can be seen in the reproduction of threatened plants, such as infrequent flowering, flower bud fall, flower infestations, lack of pollinators and low fruit set. Knowledge on anthesis, pollen viability and germination, stigma receptivity, pollen-ovule ratio, breeding behavior, and pollinators are prerequisites to unravel the biological constraints leading to endangerment of the species.

MATERIALS AND METHODS

Study area

The study was conducted at the herbal garden of Kerala Forest Research Institute (KFRI) Peechi, Kerala, South India. KFRI is situated about 20 km east of Thrissur district, spread over a 28 ha reserve forest area adjacent to Peechi-Vazhani Wildlife Sanctuary at 10.530 N latitude and 76.347 E longitude with an altitude of 186 m (Figure 1). The study was carried out from January to June 2019.

Figure 1. Western Ghats of Kerala showing the study area.

Salacia gambleana Whiting & Kaul (Syn. *Salacia talbotii* Gamble)

Scandent shrubs, endemic to the southern Western Ghats, occur in evergreen and semi-evergreen forests. This species has been assessed as threatened by Sasidharan (2017). Flowering and fruiting were recorded from January to July.

Salacia oblonga Wall. ex Wight & Arn. (Syn. *Comocladia serrata* Blanco)

Stout climbers, found in the evergreen and semi-evergreen forests up to an altitude of 1,500 m. The species has a global distribution in the Western Ghats and Sri Lanka. It has been assessed as Vulnerable based on the IUCN Criteria A2cd (Ved et al. 2015). Flowering and fruiting were recorded from March–May. The root-bark is used to treat rheumatism, gonorrhea, and skin diseases (Kirtikar & Basu 1975). This species has shown antiperoxidative properties, and is also used to treat renal complications (Krishnakumar et al. 2000).

Methods

The study covered day-to-day monitoring and recording of flowering phenology, such as bud initiation, development, anthesis, stigma receptivity, pollen viability, pollen-ovule ratio, pollination, pollinators,

blooming period, pest incidence, and fruit set. The data presented as the average values of each trial (Sreekala et al. 2008; Jose & Pandurangan 2012, 2013; Swarupanandan et al. 2013; Gopalakrishnan & Thomas 2014).

Reproductive phenology

Data on reproductive phenology, including the number of inflorescence per branch, number of flowers per inflorescence, flower/ inflorescence development, blooming period, fruit initiation and development,were recorded daily. Five inflorescences per plant for both species were subjected for data collection. Each inflorescence was tagged and monitored for the flower development from bud to full bloom. The average days taken for each bud to bloom were calculated and recorded. The number of flower buds with pest incidence was also recorded. Each flower was tagged to observe fruit formation.

Pollen viability

Pollen grains from fully matured flower buds were dusted into a cavity slide containing a solution of acetocarmine, kept for one hour and then observed under a compound microscope. The pollen grains stained red were treated as viable and others as non-viable. A viability test was carried out in two-hour intervals.

Pollen germination

Pollen grains from fully matured flower buds were transferred to a cavity slide containing germination medium (Sucrose 10%). Pollen germination was counted after one hour using a compound microscope. The pollen tubes with a longer length than pollen diameter were treated as germinated. The experiment was repeated in two-hour intervals from the anthesis.

Stigma receptivity

Visual observations using a hand lens and chemical methods using hydrogen peroxide (H_2O_2) were conducted. In the visual method, the stigma with wetness, turgidity and oily nature was considered receptive and the rest as non-receptive. In the chemical test, a drop of hydrogen peroxide was added to the stigma of a freshly opened flower. The effervescence resulting from the peroxidase enzyme activity was observed in the receptive stigma, and the duration of stigma receptivity was calculated (Dafni et al. 2005).

Pollen-Ovule ratio

The number of pollen grains in anthers per flower was counted using a haemocytometer (Shivanna & Rangaswamy 1992). The number of ovules per ovary was counted by the cross-section of the ovary (Cruden 1977). The pollen-ovule ratio was calculated using the following formula:

$$\text{Pollen-ovule ratio} = \frac{\text{Pollen count per anther x No. of anthers per flower}}{\text{No. of ovules per flower}}$$

Pollination and insect interaction

Bagging experiments were carried out to understand the mode of pollination. The physical observation was made throughout the flowering period, and the insect interactions were recorded day and night. Adhesive tapes were kept on flowering branches to collect insects for identification. The taxonomic identification of insects was made with the relevant literature.

Fruit phenology

Fruit phenology such as fruiting primordia, period of development including premature abscission and pest incidence was monitored and recorded.

RESULTS

Salacia gambleana

Reproductive phenology

Bulbous and light green flower buds were observed during the second week of January. It took about 25 days for the bud to develop into full bloom (Figure 2). The flower started opening from 0430 h to 0500 h and was fully opened by 0930 h. Anther dehisced through the horizontal slit from 0500 h and 0530 h. Stigma found receptive prior to the anther dehiscence (protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains (on anthesis) showed 100% viability, and gradual reduction was noticed to 98, 86, 80% after 6, 10, and 12 hours, respectively. A drastic decline to 52% was noted after 28 hours (Table 1). The hydrogen peroxide application followed by effervescence formation confirmed the stigma receptivity up to 58 hours. The stigma then turned brown, lost turgidity, and became non-receptive.

Pollen germination

At the time of anthesis, 96% of pollen grains were germinated. A gradual decrease in pollen germination was observed in succeeding hours, and 24% pollen

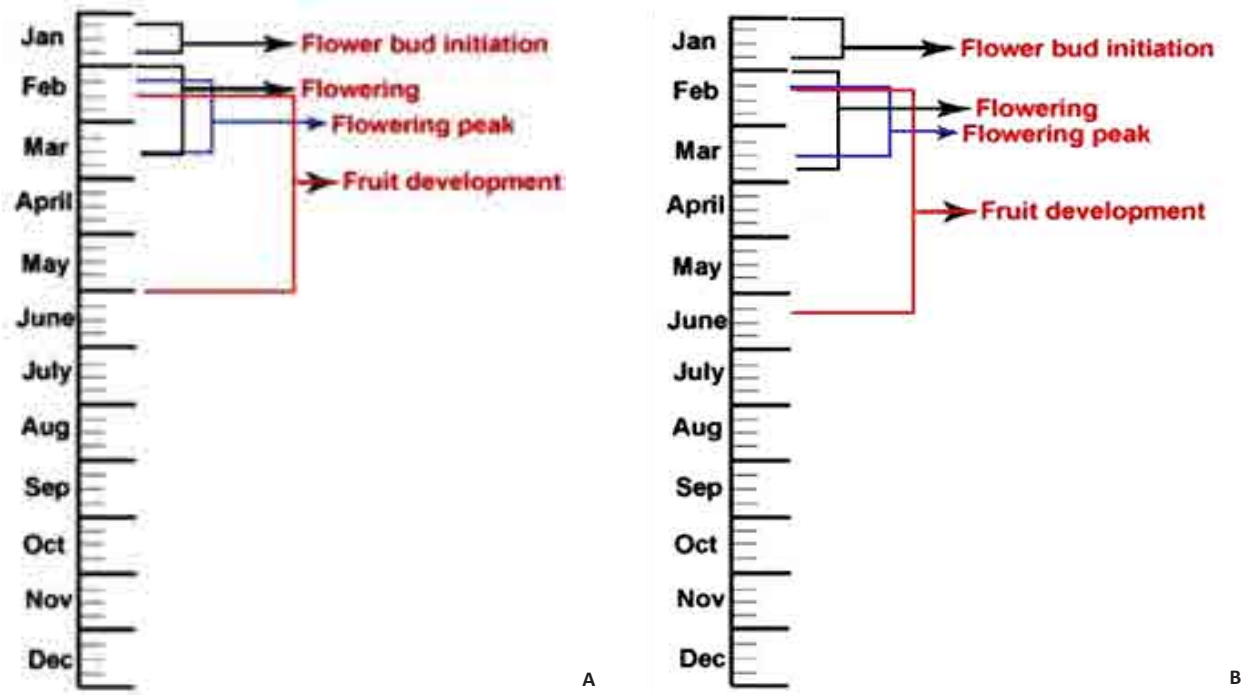

Figure 2. Phenology calendar of *Salacia gambleana* (A) and *S. oblonga* (B).

germination was recorded at 11 hours after anthesis.

Pollen-Ovule ratio

A flower contains three to four anthers, and approximately 277.1±165.7 pollen grains are present per anther. Hence, the pollen count per flower was calculated as 834.56±510.5. A flower has 4.45±1.5 ovules, and pollen-ovule ratio thus estimated to 187.54:1.

Mode of pollination and insect interaction

Flowers are not fragrant. Ants such as *Tetraponera* sp., *Ocecophylla smaragdina*, and *Anoploilepis gracilipes* were usually found foraging during the flowering period. *O. smaragdina* is an arboreal ant that forms colonies on the host plant using leaves stitched together. The maximum incidence of ants was observed in the peak of anthesis hours 0830–0930 h. Bagging experiments resulted in no fruit set; however, floral arrangements per se facilitated self-pollination (Anthers placed over stigma). Further, the incidence of ants during flowering was also found promoting cross-pollination. A larval infestation was found in developing fruits, which later caused the abscission of young fruits. The adult ants were collected for identification.

Salacia oblonga

Reproductive phenology

Flower bud initiation was noted from the first week of January. The development of bud to bloom was observed for 30 days (Figure 2). Flower opening initiated from 0130 h to 0330 h and fully opened by 0830 h. Anthers dehisced through the longitudinal slit from 0430 h and 0530 h. The stigma was receptive prior to anther maturity (protogynous condition).

Pollen viability and stigma receptivity

Fresh pollen grains showed 100% viability for up to six hours from anthesis. A gradual reduction in pollen viability was recorded, viz., 97, 85, 64% after 8, 12, 37 hours, respectively (Table 1). Stigma was found receptive up to 39 hours and later turned brown, lost turgidity, and became non-receptive.

Pollen germination

At the time of anthesis, 90% of the tested pollen grains were germinated. A sudden decline in germination percentage was noted after 6 and 12 hours with 58 and 38% germination, respectively (Image 1).

Pollen-Ovule ratio

A flower contains three to four anthers, and approximately 4,929.05 ±1829.18 pollen grains are present per anther. Hence, the pollen count per flower was calculated as 14,757.27 ±5487.38. Each flower has 9.4±2.95 ovules and an estimated pollen-ovule ratio of 1,573:1.

Table 1. Reproductive biology of *Salacia gambleana* and *Salacia oblonga*.

Floral characters	*Salacia gambleana*	*Salacia oblonga*
Flowering period	February–March	Throughout
Inflorescence type	Axillary umbel	Axillary umbel
No. of inflorescence per branch (n=26)	42.69±25.68	15.34±8.69
No. of flowers per inflorescence (n=26)	11.5±5.33	5.5±1.87
Flower type	Actinomorphic, Hermaphrodite	Actinomorphic, Hermaphrodite
Flower nature	Protogynous	Protogynous
Flower colour	Green	Greenish-yellow
Flower opening time	0430–0530 h	0130–03.30 h
Anther dehiscence time	0500–0515 h	0430–0530 h
Anther dehiscence mode	Horizontal slit	Longitudinal slit
Odour	Present	Absent
Nectar	Present	Present
Number of anther per flower (n= 18)	3 or 4	3 or 4
Pollen per anther (n= 18)	277.10±165.70	4929.05±1829.18
Mean number of pollen grain per flower (n= 18)	834.56±510.50	14787.27±5487.38
Mean number of ovule per flower (n= 30)	4.45±1.36	9.4±2.95
Pollen:Ovule ratio	187.54:1	1573.11:1
Pollen shape	Triangular	Triangular
Pollen diameter (n= 15)	21.264µm±2.27µm	20.41µm±4.92µm
Pollen tube length (after 2 hours)	83.77±38.68µm	103.37±62.11µm

Mode of pollination and insect interaction

Flowers are dull in appearance and are not fragrant; however, the floral nectars attract the ants such as *Tetraponera* sp., *O. smaragdina*, and *Anoploilepis gracilipes* during flowering. Ants forage 18±6 minutes during a visit. Maximum foraging was observed at 1030–1230 h. Ant movement caused pollen deposition on to own stigma, facilitating self-pollination (Image 1). The developing fruits were often damaged, and the seeds were foraged by caterpillars. The adult could not be collected for identification.

DISCUSSION AND CONCLUSION

As plant rarity is often directly related to the ecology and biology of the species, knowledge on reproductive phenological and biological functions is a prerequisite to unravel the complexities of rarity and for effective conservation and management of the species (Reveal 1981; Rathcke & Lacey 1985; Kempel et al. 2020). The absence of an efficient pollination mechanism has been determined as the main disadvantage in both the species studied. The low pollen-ovule ratio promoted cross-pollination through insects; however, insect visits during flowering were extremely low. Floral characteristics such as petal size, colour and nectar production have a significant role in the reproductive success of plants (Kudon & Whiegham 1998). *Salacia* species generally have small and dull flowers (*S. oblonga* 0.36±0.05 cm; *S. gambleana* 0.55±0.09 cm) and comparatively less nectar production.

The role of ants as pollinators was assumed by their relative abundance compared to other insects (Gómez et al. 1996). Various floral signals, especially nectar characteristics and floral scent, play a crucial role in attracting ants. Ants were the common pollinators, facilitating facultative autogamy in the species. The ants foraging in different flowers of the same plant enabled geitonogamy (Rostás & Tautz 2010). Among the ant species, the weaver ant (*O. smaragdina)* was found to be a pollination limiting factor as it acts as a key predator of some pollinators. It affects the behavior of other flower visitors and thus the plant's reproductive success. Observations of Tsuji et al. (2004) on the fruit orchard of *Nephelium lappaceum* suggested that the presence of *O. smaragdina* nest on the plant lowered flower-visiting rates of flying insects involved in pollination. The presence of weaver ants might be one of the reasons for the absence of other pollinators. In this study, an abundance of *O. smaragdina* ants and the absence of other floral visitors were also observed. Subin et al. (2018) reported that in *Salacia fruticosa*, 70–80% of mature fruits were found infested, and the seeds were consumed by the caterpillars of the butterfly *Bindahara moorei.* A similar infestation was also recorded in *Salacia gambleana* and *S. oblonga* where 40–50% of immature fruits were damaged by caterpillars of *B. moorei.* Insect infestation of fruits and seeds and its impact on seedling bank and subsequent rarity have been reported in many threatened plants in the Western Ghats (Jose et al. 2004, 2016). The observations and results of the present study are expected to aid future studies involving the populations of the *Salacia* species and develop suitable measures for their in situ and ex situ conservation.

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Image 1. A–E—*Salacia gambleana*: A—Flowering twig, single flower in inset | B—Oecophylla smaragdina colonies | C—*O. smaragdina* in flower | D—Pollen germination | E—Fruit showing larvae. F–J—*Salacia oblonga*: F—Flowering twig, single flower in inset | G—Pollen germination | H—Insect incidence in flower bud | I—*O. smaragdina* in flower | J—Cut opened fruit showing larval incidence. © Subin K. & Sarath T.V.

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Appendix 1. Floral part measurements.

Floral parts	*Salacia gambleana*	*Salacia oblonga*
Bud	0.55±0.09 mm	0.36±0.05 mm
Pedicel	0.40±0.07 mm	0.05±0.008 mm
Petal	0.19±0.002 mm	0.26±0.04 mm
Sepal	0.09±0.005 mm	0.17±0.025 mm
Pistil	0.14±0.05 mm	0.19±0.006 mm
Stamen	0.28±0.07 mm	0.20±0.01 mm
Anther	0.08±0.02 mm	0.06±0.025 mm
Filament	0.24±0.07 mm	0.14±0.029 mm

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COMMUNICATION

Cytotaxonomy and palynology study of some weed species from the state of Punjab, India

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Abstract: The present study was conducted in Malwa region of Punjab, India in 2019–2020. A total of 10 weed species belonging to seven genera and four families were recorded from different crops. Meiotic analysis has revealed the chromosome numbers in different weed species as *Datura innoxia* (n= 12), *Erigeron bonariensis* (n= 13), *Nicotiana plumbaginifolia* (n= 10), *Physalis angulata* (n= 24), *Sesbania bispinosa* (n= 6), *Sida cordifolia* (n= 8), *Solanum americanum* (n= 12), *Solanum nigrum* (n= 36), *Solanum villosum* (n= 24), and *Solanum virginianum* (n= 12). Chromosome numbers of *Solanum americanum* and *S. villosum* have been worked out for the first time from the state of Punjab, India. Morphological features along with the chromosome numbers have authenticated the identity of weed species. Similarly, pollen fertility analysis has suggested the potential of seed production by the weed species and their subsequent invasiveness.

Keywords: Angiosperm, invasion, morphology, meiosis, pollen, taxonomy.

Punjabi: ਮੌਜੂਦਾ ਅਧਿਐਨ 2019-2020 ਦੌਰਾਨ ਪੰਜਾਬ, ਭਾਰਤ ਦੇ ਮਾਲਵਾ ਖੇਤਰ ਵਿੱਚ ਕੀਤਾ ਗਿਆ ਸੀ। ਵੱਖ-ਵੱਖ ਫਸਲਾਂ ਤੋਂ 7 ਪੀੜ੍ਹੀਆਂ ਅਤੇ 4 ਪਰਿਵਾਰਾਂ ਨਾਲ ਸਬੰਧਤ ਕੁੱਲ 10 ਨਦੀਨਾਂ ਦੀਆਂ ਕਿਸਮਾਂ ਦਰਜ ਕੀਤੀਆਂ ਗਈਆਂ ਸਨ। ਮੀਓਟਿਕ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਵੱਖ-ਵੱਖ ਨਦੀਨ ਪ੍ਰਜਾਤੀਆਂ ਵਿੱਚ ਕ੍ਰੋਮੋਸੋਮ ਨੰਬਰਾਂ ਦਾ ਖੁਲਾਸਾ ਕੀਤਾ ਹੈ ਜਿਵੇਂ ਕਿ ਦਤੂਰਾ ਇਨੋਕਸੀਆ (n=12); ਐਰੀਗੇਰੋਨ ਬੋਨਾਰੀਅਨਸਿਸ (n=13); ਨਿਕੋਟਿਆਨਾ ਪਲੰਬੈਜੀਨੀਫੋਲੀਆ (n=10); ਫਿਸਾਲਿਸ ਅੰਗੁਲਤਾ (n=24); ਸੇਸਬਾਨੀਆ ਬਿਸਪੀਨੋਸਾ (n=6); ਸਿਡਾ ਕੋਰਡੀਫੋਲੀਆ (n=8); ਸੋਲਨਮ ਅਮਰੀਕਨਮ (n=12); ਸੋਲਨਮ ਨਿਗਮ (n=36); ਸੋਲਨਮ ਵਿਲੋਸਮ (n=24) ਅਤੇ ਸੋਲਨਮ ਵਰਜੀਨੀਆਨਮ (n=12)। ਸੋਲਨਮ ਅਮਰੀਕਨਮ ਅਤੇ ਸੋਲਨਮ ਵਿਲੋਸਮ ਦੇ ਕ੍ਰੋਮੋਸੋਮ ਨੰਬਰ ਪਹਿਲੀ ਵਾਰ ਭਾਰਤ ਦੇ ਪੰਜਾਬ ਰਾਜ ਤੋਂ ਤਿਆਰ ਕੀਤੇ ਗਏ ਹਨ। ਕ੍ਰੋਮੋਸੋਮ ਨੰਬਰਾਂ ਦੇ ਨਾਲ ਰੂਪ-ਵਿਗਿਆਨਕ ਵਿਸ਼ੇਸ਼ਤਾਵਾਂ ਨੇ ਨਦੀਨ ਪ੍ਰਜਾਤੀਆਂ ਦੀ ਪਛਾਣ ਨੂੰ ਪ੍ਰਮਾਣਿਤ ਕੀਤਾ ਹੈ। ਇਸੇ ਤਰ੍ਹਾਂ ਪਰਾਗ ਕਣਾਂ ਦੀ ਉਪਜਾਊ ਸ਼ਕਤੀ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਨਦੀਨਾਂ ਦੀਆਂ ਕਿਸਮਾਂ ਦੁਆਰਾ ਬੀਜ ਦੇ ਉਤਪਾਦਨ ਦੀ ਸੰਭਾਵਨਾ ਅਤੇ ਉਹਨਾਂ ਦੇ ਬਾਅਦ ਦੇ ਧਾੜਵੀਪਣ ਦਾ ਸੁਝਾਅ ਦਿੱਤਾ ਹੈ।

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Author contributions: RS—field survey, collection, laboratory practice and prepared a manuscript. MCS—review the manuscript and suggested some corrections. After corrections, the present manuscript is finalized by both the authors.

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INTRODUCTION

Angiosperms are one of the major group of plants with around 2,95,383 species in the world and 18,666 in India (Christenhusz & Byng 2016; Dash & Kumar 2019). Flowering plants supported various life forms including human for various basic needs including food, fodder, shelter, and medicine. Various angiosperm species are growing as weeds both in rabi and kharif crops. According to Dangwal et al. (2010), weed is a plant that competes with crop plants for nutrition, space, and light. Subsequently, they affect the quality and yield of the concerned crops. Weed species have high potential to grow even under unfavorable conditions. They develop some special modifications such as thick cuticle, sunken stomata, and waxy coating to retain water during drought conditions (Ram & Gupta 1997).

Identity of weed species can be established using different tools including morphology, cytology, palynology, phytochemistry, and molecular. Being simple and cost effective, morphological study is still commonly practiced for species identification. Morphological features like leaf shape and color, flower type and color, number and position of stamens, number and structure of gynoecium, shape and type of fruit and seeds are used for identification of species (Rieseberg 1992; Singh & Dey 2005).

Meiotic study has provided information about the number and arrangement of chromosomes which can be used for identification of species. According to Raven (1975) different species of the same genus may show variation in chromosome numbers. The chromosomal changes are also useful in taxonomic and evolutionary studies of plants (Jones 1979; Kaur & Sidhu 2014). Palynology deals with study of pollens and spores. The pollen fertility is directly related to seed production and yield. The more pollen fertility, the more chances of pollination and production of seeds. Acetocarmine staining method is simple and the most suitable to study the pollen fertility (Gaaliche et al. 2013).

The present study was conducted in Malwa region of Punjab. Earlier, Sharma & Bir (1978), Meenakshi & Sharma (1985), Sharma et al. (1987), Sharma (1990), Sidhu (1991), and Singh & Singh (2019) documented the angiosperm diversity in general and weed diversity in particular. Keeping this in view, the present study was planned to establish the identity of selected weed species based on morphological, cytological, and palynological studies.

MATERIALS AND METHODS

Study area

The state of Punjab covers an area of 50,362 km^2 and located in northern India. It is divided into Majha, Malwa, and Doaba zones. During present study, weeds were recorded from kharif and rabi crops growing in Malwa region including Bathinda, Faridkot, Fazilka, Ferozpur, Moga, and Muktsar in the years 2019 and 2020. Malwa is the largest zone, where temperature varies from 3°C to 47°C and average rainfall ranges 480–960 mm. Weed species were recorded from kharif crops (Cotton, Maize, Sorghum, Sugarcane, Rice) and rabi crops (Berseem, Gram, Mustard, Sugarcane, Wheat). Sugarcane crop was considered both in rabi and kharif season.

Morphological study

Various leaf morphological features, like arrangement, shape, type, color, stem, flower colour, shape of stamens and shape of stigma were analyzed for identification of species. The collected weed species were identified on the basis of available literature such as Hooker (1872–1897), Bamber (1916), Nair (1978), and Singh & Singh (2019). Specimens from Herbarium, Botany Department, Panjab University Chandigarh and online herbaria were also consulted for identification of weed species.

Meiotic study

A meiotic study was carried out to determine the numbers and behavior of different weed species chromsomes. Young flower buds of different growth stages were collected from the study area and fixed (ethanol 3: glacial acetic acid 1) for 1–2 days then transferred to 70% ethanol till further use. Anthers were excised and squashed in drop of acetocarmine (1%) on the glass slide. Debris was removed and rest of the material was covered with a micro cover slip. After gently heating, the slide was pressed in two folds of filter paper by putting thumb pressure for spreading of the cells and to remove air bubbles. Slides were observed under the microscope and photographs of the cells with countable chromosomes were taken.

Pollen study

Pollen fertility of 100–150 pollen grains of each species was analyzed by stainability tests. Mature anthers were taken on the glass slide and squashed in glycerol acetocarmine (1:1) mixture. The slide was observed under the microscope after 24 hours. The stained pollen was considered fertile whereas poorly

stained/ unstained pollens were considered as sterile. (Pollen fertility= (Fertile pollen / Total pollens observed) x 100).

Photography and herbarium preparation

Field photographs of the weed species were clicked in natural habitats preferably during flowering or fruiting season. After collection, different plant materials were processed for herbarium preparation. After complete drying, the plant specimens were mounted on the herbarium sheets. Herbarium specimens were deposited in the Herbarium, Department of Botany, Panjab University Chandigarh (PAN No. 21986–21995).

RESULTS AND DISCUSSION

A total of 10 weed species belonging to seven genera and four families were documented and collected from different kharif and rabi crops in Malwa region of Punjab, India in 2019–2020. Morphological, cytological, and palynological details were worked out. These plant species were identified primarily based on their morphological features. Different plant groups or families or genera may have a unique basic chromosome number. Keeping this in view, species have been analyzed for their chromosome number to further strengthen the morphological identification. According to Sidhu et al. (2011) basic chromosome number informs about polyploidy and evolution of species. Palynological study has been conducted to record the percentage of fertile pollens. The seed setting percentage depends upon the pollination which is related to the amount of fertile pollen.

***Erigeron bonariensis* Linn. (Syn. *Conyza bonariensis* (L.) Cronquist) … Asteraceae (Image 1b)**

It is a perennial tall herb. The hairy stem is erect and rarely branched. Leaves are green, linear, narrow, lanceolate, hairy, and green. Inflorescence shows profuse branching and bear numerous heads (Capitulum). Each head consists of outer ray florets and central disc florets. Fruit is papose with linear and yellowish-brown. The course of meiosis was regular anaphase-II (13-13-13-13) (Image 2b). Gupta & Gill (1983) also worked out similar number of chromosomes (n= 13). Pollen size range 18.75 x 16.25–23.75 x 21.25 µm with pollen fertility of 89.36% (Image 3b).

***Sesbania bispinosa* (Jacq.) W. Wight … Fabaceae (Image 1e)**

It is an erect annual plant having green, branched stem bearing spines. Leaves are pinnately compound, alternate, green; leaflets many, narrow, linear, and oblong with round apex. Racemes with bisexual and complete 4–8 flowers. Sepals are 5, green, hairy; Petals are 5, yellow, unequal; Stamens 10, diadelphous; Stigma 1, style long and curved. Pods are long, narrow linear with numerous seeds. Seeds are linear long, shining and brown to black. Meiotic study has witnessed six bivalents at metaphase-I (Image 2f). The chromosome count is similar to the earlier reports of Parihar & Zadoo (1987) and Jahan et al. (1994). Pollen size varies between 20 x 18.75 µm to 22.5 x 21.25 µm (Image 3e) and pollen fertility 91.66%.

***Sida cordifolia* Linn … Malvaceae (Image 2f)**

The plant body erect, branched, annual to perennial herb with woody base. Hairy leaves are alternate, ovate oblong to cordate with long petiole. Flowers are bisexual, axillary with small pedicle. Sepals 5, green and hairy, Petals 5, light yellow. Fruit with 5–8 mericarps with long awns. Seeds are ovoid or trigonous and brown to black in color. Chromosomal segregation was normal at anaphase-I (8-8) (Image 2g). Similar chromosome number (2n= 2x= 16) was also studied by Kumar et al. (2012) that supported the present chromosome analysis. Pollen size (66.25 x 62.5–73.75 x 71.25 µm) (Image 3f) of this species is maximum but pollen fertility comparatively less (61.16%) than the other recorded species.

***Datura innoxia* Mill … Solanaceae (Image 1a)**

The herbaceous plant has dichotomous branches. Leaves are ovate lanceolate to broad ovate, unequal at the base. Flowers large, white or dirty white, funnel-shaped with equal number of sepals and petals (5). Stamens 5, epipetalous; stigma 1 with long style. Capsule nodding, covered with green spines. Numerous, small seeds are brown to black in colour. Meiotic preparations have shown the presence of 12 bivalents at metaphase-I (Image 2a). El-Twab et al. (2010) reported 2n= 2x= 24 chromosomes in this species from Egypt which tallies with the present study. It has shown the genetic stability of this species across the boundaries in term of chromosome number. Pollen size of this species is 48.75 x 46.25–51.25 x 47.5 µm (Image 3a). Pollen fertility of this species is 87.05%.

Image 1a–j. Habitat of weed species: a—*Datura innoxia* | b—*Erigeron bonariensis* | c—*Nicotiana plumbaginifolia* | d—*Physalis angulata* | e—*Sesbania bispinosa* | f—*Sida cordifolia* | g—*Solanum americanum* | h—*Solanum nigrum* | i—*Solanum villosum* | j—*Solanum virginianum*. © Rai Singh.

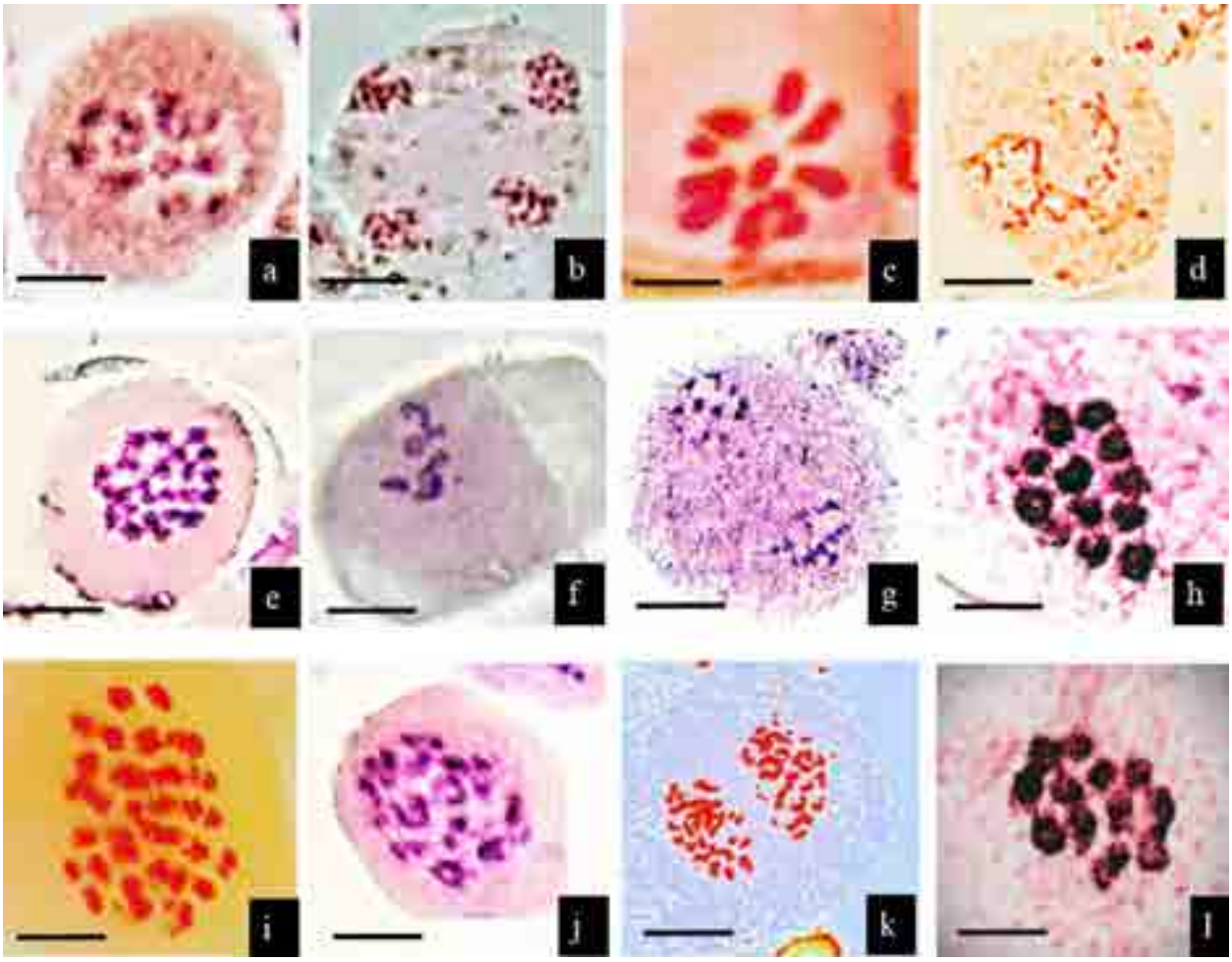

Image 2a–l. Chromosomal details of weed species: a—*Datura innoxia* (n= 12) | b—*Erigeron bonariensis* (n= 13) | c–d—*Nicotiana plumbaginifolia* (n= 10) | e—*Physalis angulata* (n= 24) | f—*Sesbania bispinosa* (n= 6) | g—*Sida cordifolia* (n= 8) | h—*Solanum americanum* (n= 12) | i—*Solanum nigrum* (n= 36) | j–k—*Solanum villosum* (n= 24) | l—*Solanum virginianum* (n= 12). (Scale= 10 µm). © Rai Singh.

***Nicotiana plumbaginifolia* Viv … Solanaceae (Image 1c)**

It is an annual erect herb. Stem is branched, slender, herbaceous at young stage then turns woody at maturity. Leaves are simple, alternate, and ovate to elliptic or lanceolate. Lower leaves form a rosette at the base and these leaves are comparatively larger than the upper ones. Flowers are bisexual, tubular, complete, creamy white, pale yellow to purplish in colour. Fruits are small, round berries. Seeds are small, round, many, and brown to black in colour. Meiotic analysis has revealed the presence of 10 bivalents at diakinesis and metaphase-I (Image 2c,d). Chromosomal stickiness has also been observed in some pollen mother cells. Kaur et al. (2015) also reported similar chromosome number (2n= 20) for this species from Rajasthan, India. Size of pollen grains of this species ranges 20 x 18.7–23.75 x 21.25 µm (Image 3c) and pollen fertility 92.85%.

***Physalis angulata* Linn … Solanaceae (Image 1d)**

It is a common herbaceous prostrate or erect weed of paddy fields. The stem is woody green possessing petiolate, ovate to lanceolate and green leaves. Flowers yellow, bisexual, complete and solitary. Fruit is berry, globose, green and enclosed within the enlarged, reticulate veined calyx. Seeds are numerous, small, and yellowish-white. The meiotic analysis has described 24 bivalents at metaphase-I (Image 2e). Similar chromosome number (2n= 4x= 48) were also reported by Azeez et al. (2019) from Nigeria. Their study has supported the presently studied chromosome number and suggested the conservation of chromosome number of this species in different parts of the world. Pollen size vary from 17.5 x 16.25 µm to 20 x 18.75 µm (Image 3d) and pollen fertility is 75.24%.

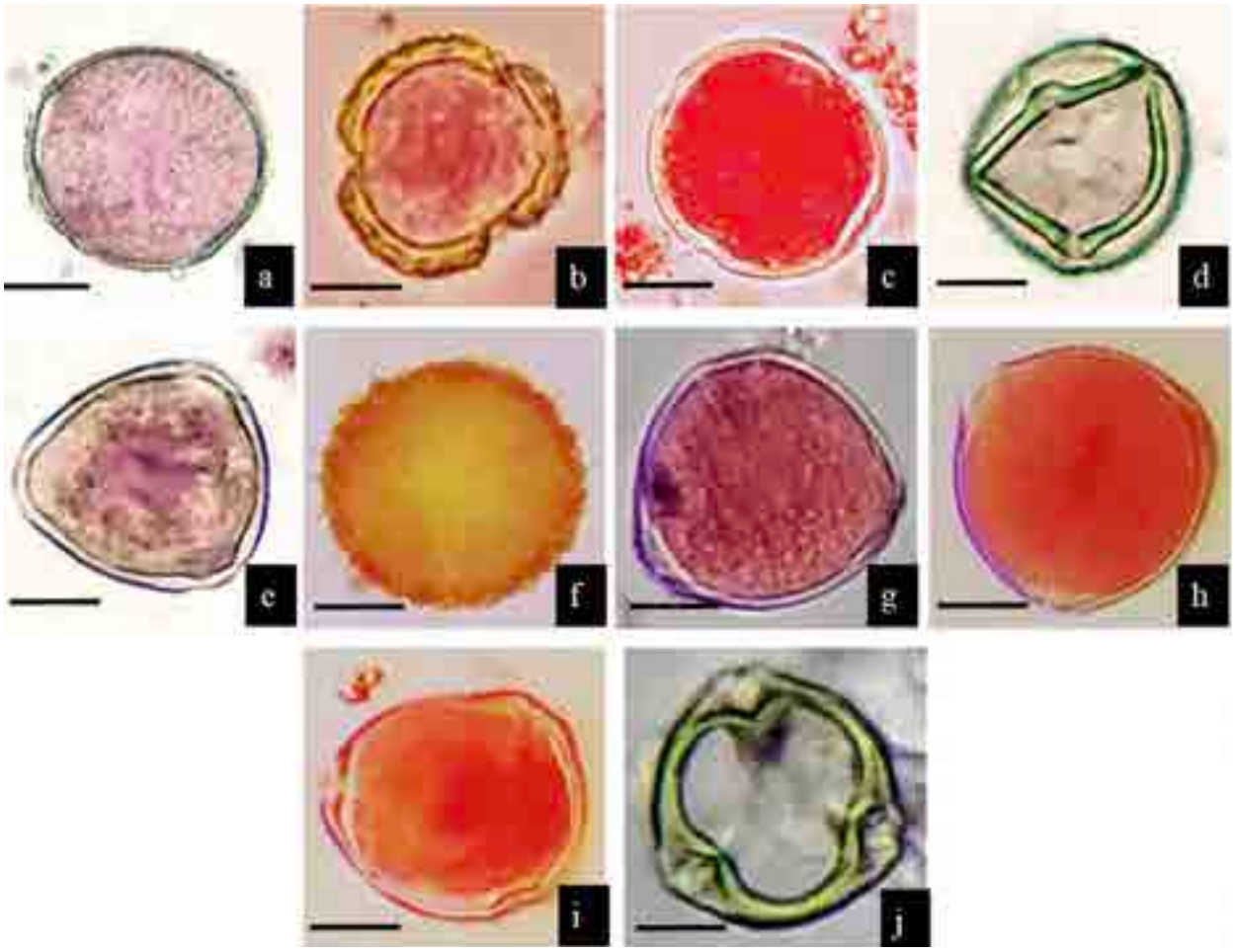

Image 3a–j. Pollen size and viability: a—*Datura innoxia* | b—*Erigeron bonariensis* | c—*Nicotiana plumbaginifolia* | d—*Physalis angulata* | e—*Sesbania bispinosa* | f—*Sida cordifolia* | g—*Solanum americanum* | h—*Solanum nigrum* | i—*Solanum villosum* | j—*Solanum virginianum*. (Scale 10 µm). © Rai Singh.

***Solanum americanum* Mill ... Solanaceae (Image 1g)**

The plant is an annual erect, branched and glabrous herb. Stem is herbaceous during early stages and becomes woody in mature plants. Leaves are alternate, ovate or ovate-lanceolate, glabrous, thin, apex sub-acute or acuminate and light green. White flowers possess equal number of sepals and petals (5); stamens 5; stigma 1. Fruits are berries globose, green, and purplish-black at maturity. Seeds are numerous, disc shaped and yellow. Pollen mother cells contain 12 bivalents at metaphase-I (Image 2h). Ganapathi & Rao (1986) suggested that *Solanum americanum* Mill possess small flowers; globose, bluish-black fruit, and chromosome number 2n= 24. Size of ranges 21.25 x 20–23.75 x 21.25 µm (Image 3g) and pollen viability is 93.65%.

***Solanum nigrum* L ... Solanaceae (Image 1h)**

It is an erect, branched, glabrous annual herb. The stem bear alternate, ovate-lanceolate, glabrous, apex sub-acute or acuminate and dark green. Flowers are white, sepals (5) and petals (5), stamens 5; stigma 1. Fruits are berries globose, green, and purplish-black at maturity. Seeds are numerous and yellowish-white. Meiotic analysis has revealed 36 bivalents at metaphase-I (Image 2i). Similar chromosome numbers (2n= 6x= 72) were also studied by Ganapathi & Rao (1986) and Sidhu & Sharma (2016). Pollen size of this taxa varies 22.5 x 21.25–28.75 x 27.5 µm (Image 3h) and pollen viability 91.51%.

***Solanum villosum* Mill ... Solanaceae (Image 1i)**

It is an annual, erect, branched, and glabrous herb. The glabrous stem possesses leaves which are alternate with reticulate venation, hairy, ovate with lobed margin and acute apex and dark green. Flowers white, bisexual; sepals and petals 5; stamens 5; stigma 1. Fruits are berries, globose, green, and orange red when mature. Seeds are numerous, yellowish-white in colour. Meiotic

preparations have shown possess 24 bivalents at metaphase-I and equal distribution of chromosomes at anaphase-I (24-24) in some PMCs (Image 2j,k). Ganapathi & Rao (1986) also recorded 2n= 4x= 48 chromosome numbers in this tetraploid cytotype having orange red berries. Presently studied chromosome count is also to the tune of the findings of Sidhu & Sharma (2016) in this species. Pollen size ranges 25 x 22.5–26.25 x 25 µm (Image 3i) and pollen viability 97.34%.

***Solanum virginianum* L. (Syn. *Solanum xanthocarpum* Schrad. & Wendl.) (Image 1j)**

It is a prostrate herb. Stem is woody at the base, branched and bear spines. Leaves are alternate, elliptic oblong, deeply lobed, dark green and covered with spines. Flowers are bluish-purple, few, in extra-axillary cymes. Berries globose with white lines; green when young and yellow at maturity. Seeds are smooth and brown to black in colour. This species has shown the presence of 12 bivalents at metaphase-I (Image 2l). Pollens are smaller than other studied species (13.75 x 12.5–16.25 x 13.77 µm) (Image 3j) and pollen viability is 77.66%.

CONCLUSION

Morphology is one of the most preferred and commonly used tool for identification of plant species. During present investigations, 10 weed species have been described based on morphological features. The morphological characterization of the weed species has been further strengthen by the chromosome study. Morphological characterizations will be useful for researchers in general and taxonomists and plant breeders. Similarly, the chromosomal study recorded species will be useful to understand genetic variations and stability of these species. The pollen fertility related information of the weed species has pointed out towards the productivity, seed setting, and dominance of the weed species.

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COMMUNICATION

Philately of mangroves: local to global reflection

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Abstract: Philately is one of the most popular hobbies since its inception and there are millions of known philatelists across the globe. Other than just a hobby, it can also be seen as a tool for reflecting social, political, natural specialities of the country for internal and external demonstration. It is an effective medium to display the richness of natural diversity, customs, and traditions and also the man-made wonders for its users. Stamps and other philatelic items can be viewed as conservation tool. They not only sensitize the public to the fate of the threatened environment or biota but also help in raising funds. Mangroves represent one of the most diverse coastal ecotonal wetlands of tropics and subtropics. They are the ecosystems inhabited by diverse group of floral, faunal, and microbial elements. Though neglected initially, these forests have attracted the global attention after understanding their role in coastal ecology and economy. The current study is a modest attempt to use themed philately to investigate the unique representation of the world's most diversified mangrove ecosystem. With this goal in mind, a systematic spatio-temporal review of philatelic publications was conducted, and the results are presented along with the available data and alternative interpretations.

Keywords: Awareness, diversity, educational tool, mangrove forests, philatelic material, postal stamps and covers, public awareness.

Marathi: खारफुटीवरील टपाल तिकिटांच्या संग्रहाचा अभ्यास: स्थानिक ते जागतिक प्रतिबिंब

टपाल तिकिटांचा संग्रह हा सर्वात लोकप्रिय छंद आहे आणि जगभरात लाखो प्रसिद्ध तिकीट संग्रहक आहेत. केवळ छंदाशिवाय, देशाच्या सामाजिक, राजकीय, नैसर्गिक वैशिष्ट्यांचे प्रतिबिंबित करण्याचे साधन म्हणून देखील याकडे पाहिले जाऊ शकते. नैसर्गिक विविधता, चालीरीती आणि परंपरा यांची समृद्धता तसेच मानवनिर्मित स्थळे प्रदर्शित करण्यासाठी हे एक प्रभावी माध्यम आहे. तिकिट आणि इतर छायाचित्रण वस्तू संवर्धनाचे साधन म्हणूनही पाहिल्या जाऊ शकतात. ते केवळ लोकांना पर्यावरणाच्या भवितव्याबद्दल संवेदनशील करत नाहीत तर निधी उभारण्यातही मदत करतात विविध नैसर्गिक स्रोतांचे अंकन टपाल तिकिटांवर आवर्जून केलेले दिसते. खारफुटी ही जगातील उष्ण आणि समशीतोष्ण कटीबंधातल्या किनारी भागात आढळणारी विविधतेने नटलेली पाणथळ भूमी म्हणून परिचित आहेत. खारफुटी ही वनस्पती, प्राणी आणि सूक्ष्मजीव यांनी समृद्ध अशी परिसंस्था आहे. पूर्वापार दुर्लक्षित झालेली ही वने आज मात्र किनारी भागातील त्यांच्या पारीस्थितीकीय आणि आर्थिक लाभांचा विचार करता जगाच्या आकर्षणाचा विषय बनला आहे. प्रस्तुत शोधनिबंधाचा उद्देश हा जगातील अतिशय वैविध्यतापूर्ण म्हणून नावाजलेल्या खारफुटीची परिसंस्था व त्यातील घटकांचे टपाल तिकिटांवरील प्रदर्शन कसे झाले आहे याचा धांडोळा घेण्याचा एक प्रयत्न आहे. जगभरात आणि विविध कालक्रमात खारफुटी परिसंस्था आणि त्यातील वनस्पती, आकर्षक फुले, जलचर प्राणी, स्थानिक किंवा स्थलांतर करणारे पक्षी यांचे सुरेख मुद्रण या तिकिटांवर झालेले दिसते. प्रकाशित टपाल तिकिटे आणि साहित्याच्या माध्यमातून त्याचा शास्त्रीय अभ्यास करणे हा या अभ्यासाचा मुख्य उद्देश आहे.

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MANGROVES ON POSTAGE STAMPS

Mangroves are significant occupants of the ecotonal zone that separates the marine and terrestrial worlds. A group of salt-tolerant plants constructed the entire ecosystem. Through the fisheries and tourism sectors, mangrove forests play an important role in coastal ecology and resilience, as well as a sustainable living and a thriving economy. Mangrove forests are perceived as land builders, coast protectors, nutrient sources, sources of food and fodder, homes for the aquatic organisms and migratory birds. Recent studies have highlighted their contribution to carbon sequestration, nutrient cycling, and wastewater treatment, which motivates them to safeguard their coastal lifestyle. As a result, understanding, protecting, and conserving this valuable ecosystem with all of its components is critical for long-term growth. Since awareness is the first step in conservation, it is believed that a number of proactive efforts in this direction should be made on a priority basis in order to preserve the diversity and utility of this environment.

This paper reviews a total of 172 postage stamps and four covers (1 first day cover, 1 special commemorative cover, and 2 special covers) and associated postal publications from 37 countries. This study considers the representation of mangroves that reflects the diversity of flora and fauna with social and economic perceptions of each country. These are Angola, Bahamas, Bangladesh, Bermuda, Brazil, Brunei, Cayman Island, Cuba, El Salvador, Federated States of Micronesia, Fiji, Grenada Grenadines, India, Indonesia, Japan, Kirbati, Macao, Malaysia, Mauritius, Mexico, New Caledonia, Palau, Papua New Guinea, Peru, Republic Togolaise, Samoa, Senegal, Solomon Island, Sri Lanka, St. Lucia, St. Vincent, Suriname, Swaziland, Taiwan (ROC), Tanzania, Tonga, and Wallis-Futuna.

Although plants and animals have appeared frequently on stamps since 1939, it was only in 1970 that St. Vincent, a southern Caribbean nation published a stamp showing mangrove associated Green Heron *Butorides virescens*, similarly another one showing a mangrove twig and mangrove Cuckoo *Coccyzus minor* as associated avifaunal member. The same stamp was reproduced by Grenadines of St. Vincent in 1974.

In the 1980s, eight countries, namely, Cayman Islands, Macao, Grenada Grenadines, Swaziland, Saint Lucia, Sri Lanka, Solomon Island, and Palau, published stamps related to mangroves.

The Cayman Islands published two stamps in 1980, one on seed of the Red Mangrove *Rhizophora apiculata* and the other one on the Mangrove Crab *Goniopsis cruentata*, which were depicted in sheltered habitats from dense network of aerial roots of the Rhizophora tree. Later in 1982, a stamp was published on the blooming of Red Mangrove *Rhizophora mucronata.*

Macao in 1983 published a stamp showing the mangrove under the theme of medicinal plants. A flowering twig of *Acanthus ilicifolius* L. commonly known as Holy Mangrove is shown in this stamp. It has been used for the treatment of asthma, diabetes, dyspepsia, leprosy, hepatitis, paralysis, snake bite, rheumatoid arthritis and a diuretic. (Bandaranayake 1998).

In the 1985 Grenada Grenadines, Swaziland, & Saint Lucia published a first day cover (as a miniature sheet) and stamps.

Grenada Grenadines published a miniature sheet showing the bird Mangrove Cuckoo on 200th anniversary of the birth of John James Audubon. Swaziland on the same occasion published a set of four stamps showing Ground Hornbills *Bucorvus abyssinicus* and a first day

cover on Mangrove Cuckoo *Coccyzus minor.*

Saint Lucia in 1985 published a stamp showing the association of the Mangrove Cuckoo with the Savannes Bay Nature Area, Scorpion Island, Saint Lucia.

Cayman Island in 1986 issued a set of 12 stamps of marine fauna showing Rhynchocinetes rigens, Nemaster rubiginosa, Calcinus tibicen, Rhodactis sanctithomae, Spirobranchus gigantea, Diodon holocanthus, Pseudocorynactis caribbeorum, Astrophyton muricatum, Cyphoma gibbosum, Condylactis gigantea, Malacoctenus boehlkei, *and Lima scabra.*

Sri Lanka issued a set of four stamps in 1986 showing a sheltered riverine mangrove habitat having a mixed strand of species of the genera *Rhizophora*, *Avicennia*, and *Sonneratia*; a habit of medium-sized tree of *Rhizophora apiculata*; flowering and fruiting of *Bruguiera gymnorhiza* and a fiddler crab *Uca lactea* representing species of economic significance from mangroves and mudflats.

Solomon Island published four stamps in 1987 showing the Mangrove associated Little Kingfisher *Ceyx pusillus* catching food in a mangrove thicket.

Palau, a Pacific Ocean island has published 20 stamps featuring a red mangrove and associated species (*Rhizophora stylosa* and *Hibiscus tiliaceus*) and the associated fauna of Palau. It was exclusively produced for World Stamp Expo' 1989 and therefore had limited postal use. As a consequence, a philatelic mini-sheet featuring an artistic version of the mangroves and their associated fauna was released. Each individual stamp has a feature of one species of floral or faunal element whose scientific name appears on the back of the sheet. The stamp pane features Bridled Tern, Sulphur Butterfly, Mangrove Flycatcher, Collared Kingfisher, Fruit Bat, Estuarine Crocodile, Rufous Night Heron, Stilt mangrove, Bird's Nest Fern, Beach Hibiscus tree, Common Egg fly, Jingle Shell, Palau Bark Cricket, Common Periwinkle, Mangrove Oyster, Jellyfish, Striped Mullet, Mussels, Aea Anemones, Algae, Snapper, and Cardinal fish.

In the 1990s, twelve countries, namely, Suriname, Mauritius, Fiji, Peru, Malaysia, Solomon Island, Cuba, Federated States Micronesia, El Salvador, Samoa, and Saint Vincent, published stamps on mangroves. Suriname is a small country on the northeastern coast of South America. In 1990 it published stamps indicating mangrove sites by Postal Union of America, Spain, and Portugal (UPAEP).

Mauritius, an Indian Ocean island nation, is known for its beaches, lagoons, and reefs. Mauritius in 1990 issued stamp on *Rhizophora mucronata* on eastern Africa and Indo-Pacific region under the theme Protection of Environment.

Fiji in 1991 issued a set of four stamps of mangrove crabs showing *Scylla serrata, Metopograpsus messor, Parasesarma erythrodactyla*, and *Cardisoma carnifex.*

Peru is a country on the central western coast of South America facing the Pacific Ocean. In 1991, Peru issued a stamp of riverine mangrove swamps of Tumbes National Sanctuary.

Likewise in 1992, Malaysia published a stamp on the Mangrove Swamp forest of the Hutan Paya Laut area with a drawing of a tree port of *Rhizophora apiculata* as a dominant species. Solomon Island in 1993 published 15 stamps on crabs. One of the stamps pertains to Mangrove Fiddler Crab *Uca tetragonon.*

Cuba in 1993 published a stamp on endangered fauna from mangrove habitats: Roseate Spoonbill *Platalea ajaja*. Federated States Micronesia in 1994 published four stamps under native flowers. *Sonneratia caseolaris*, commonly known as Mangrove Apple is a species of plant in the family Lythraceae. Flower of the mangrove apple is seen on one stamp.

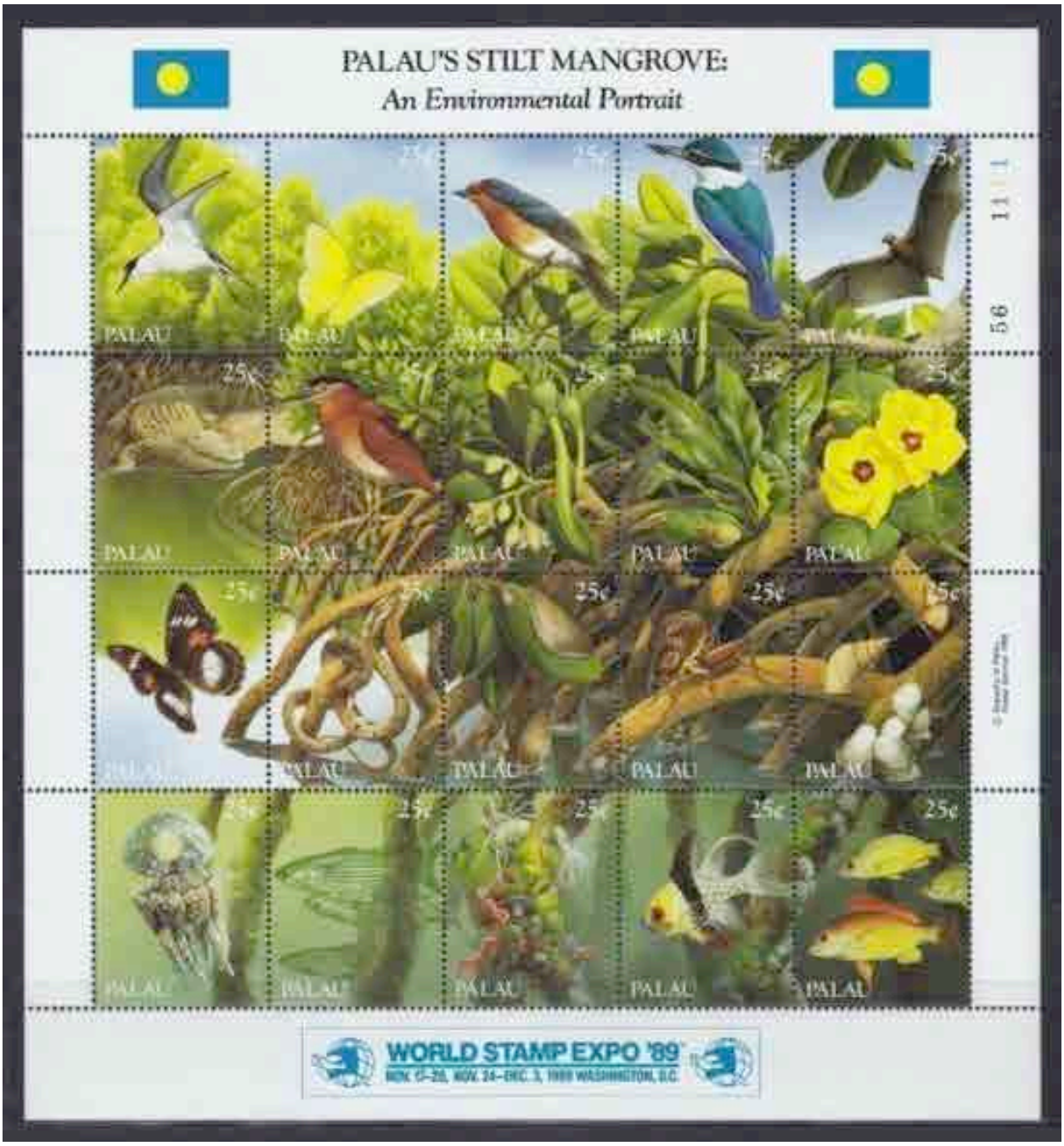
In 1995, El Salvador published two stamps on mangroves of Pacific coast with American Pygmy Kingfisher and Green Kingfisher (*Chloroceryle aenae* and *Chloroceryle americana*).

Brunei in 1997 published three stamps on mangroves flowers of *Acanthus ebracteatus*, *Lumnitzera littorea*, and *Nypa fruticans*.

In 1998, Samoa published a series of four stamps under the theme 'Environment-Mangroves' showing various stages of life cycle (habit, sapling, aerial root network and viviparous seedlings ready for the dispersal) of a mangrove species *Bruguiera gymnorrhiza.* Moreover, Saint Vincent in 1999 published a stamp showing the mangrove tree, a species of *Rhizophora* and the bird White Ibis *Eudocimus albus.*

In the 2000s, 16 countries, namely, New Caledonia, Tonga, Mexico, Palau, India, Senegal, Tanzania, Brazil, Malaysia, Fiji, Taiwan, Bermuda, Indonesia, Angola, Japan, and Sri Lanka published stamps on mangroves. Likewise, New Caledonia in 2000 published a stamp depicting the aerial view of Namelle Caledonie mangrove reserve forest that represents a heart shape.

In 2001, Tonga published five stamps on animals of mangrove swamps on World Environment Day. The animals include fidder crab *Uca* sp., Motuku Reef Heron



Egretta sacra, Black Duck *Anas superciliosa*, Grey Mullet *Mugil cephalus*, Mangrove Crab *Aratus pisoni*, and Emperor Fish *Lutjanus seba).*

Mexico in 2002 published definitive issue covering 24 stamps of which two depict mangroves and lake-lagoon conservation. Out of 18 flower stamps published by Palau in 2002, one depicts flower of *Sonneratia alba.*

India, which hosted the 8th Conference of Parties (COP) in 2002 has published a commemorative set of stamps on mangroves. These stamps depict four common Indian mangrove species *Rhizophora mucronata*, *Sonneratia alba*, *Nypa fruticans*, and *Bruguiera gymnorhiza* on the background of various mangrove forest habitats like rivers, rocks, intertidal islands, back mangroves and a cover carrying an abstract art of work on the need for action on climate change by Kamaleshwar Singh with a conference logo (https://www.istampgallery.com/mangroves/).

In same year of 2002, Senegal published a stamp on mangroves on ecotourism theme. Tanzania in 2003,

published two stamps on Rufiji Delta view and mangroves of coast.

Brazil in 2004 published a set of five stamps under the theme of preservation of mangroves and marine zones. It depicts a Roseate Spoonbill *Ajaja ajaja*, Great Kiskadee *Pitangus sulphuratus*, Burrowing Crab *Chasmagnathus granulata*, Little Wood Rail *Aramides mangle*, and Red Mangrove Crab *Goniopsis cruentata*.

Mexico in 2004 again published definitive issue covering 24 stamps of which two depict mangroves and lake-lagoon conservation.

In 2004, Malaysia published total five stamps showing the mangroves on the occasion of 100 years of 'Matang Mangroves Perak'. Mangrove afforestation program and economically important shrimp species is represented on these stamps.

The Matang Mangrove Forest Reserve which covers an area of 400 km^2, is recognized as the best managed sustainable mangrove ecosystem in the world. This mangrove reserve is rich and diverse in wildlife that encourages nature lovers to visit and explore it. There are 28 true mangrove species and 13 associate mangrove species, 19 mammals, at least 155 species of birds, a species of river dolphin (Chinese White Dolphin), 112 species of modern bony fishes and three species of stingrays; and approximately 50 species of crabs and 20 species of prawns and shrimps. Like other mangrove swamps, the Matang Mangrove Forest Reserve has also been proven to act as a natural barrier against Tsunami. This is undoubtedly an important site for coastal migratory water birds and part of migrant forest birds. As many as 43,000 to 85,000 birds may be seen using the forest during the migration.

Fiji in 2005 issued a set of four stamps on Little Green-backed Heron *Butorised striatus*, Great White Egret *Egretta alba*, White-faced Heron *Ardea novaehollandiae*, and Pacific Reef Heron *Egretta sacra* in mangrove habitat.

Taiwan (Republic of China) (2005) published a series of special stamps illustrating 'Mangrove Plants of Taiwan' to raise public awareness and understanding of mangroves. These are *Kandelia obovata*, *Rhizophora stylosa*, *Avicennia marina*, and *Lumnitzera racemosa*. In the background of each stamp, there is an example of the plant species in a strand while a close up view of fruits or the viviparous propagules of the plant and the flowers are shown in inset view.

In recent years, the total area of mangrove forests in Taiwan has declined drastically. After harbour's development, few species became extinct or endangered. In view of the growing understanding about the importance of conservation, and strong efforts

from government and environmentalists to promote conservation, a systematic attention has been paid to conserving Taiwan's mangroves (Wang et al. 2020).

Indonesia in 2005 on the occasion of World Environment Day issued two stamps on Save Mangrove Forest. A mangrove vegetation strand of *Rhizophora* with mangrove ecosystem dependent bird and aquatic life is depicted on these stamps. Meanwhile, the Indonesian Postal Administration issued an air waybill on the first day.

'Mangrove Forest' is the theme used to design the stamp. The purpose was to socialize and make Indonesians aware of the importance of the mangrove forest for the protection of ecosystems, mainly for the coastal areas. If the society is aware of the benefits, they will actively take part in the efforts of rehabilitation and conservation of the Mangrove Forest.

Bermuda in 2005 published six stamps. Out of these, Yellow Crown Night Heron*(Nycticorax violaceus*


AMERICA
PERU CORREOS
1/m.0.50


MALAYSIA 50c


Solomon Islands
MANGROVE FIDDLER CRAB
$10


CUBA
50
AMERICA


Federated States of Micronesia
29


CORREOS DE EL SALVADOR
80c.
AMERICA


CORREOS DE EL SALVADOR
¢2.20
AMERICA


20
30


SAMOA
SAMOA
SAMOA
SAMOA


Nouvelle-Calédonie
100
RF


Kingdom of Tonga
ATA'ATA
10
Kingdom of Tonga
MOTUKU
$1.00
55
Kingdom of Tonga
Kingdom of Tonga
$2.70
80
Kingdom of Tonga


$0.50
MÉXICO
conserva
manglares


$30.00
MÉXICO
conserva


PALAU
5c


MANGROVES


300Fr
LES MANGROVES
SENEGAL


WATER FALLS AND LANDSCAPES
RUFIJI DELTA VIEW
Tanzania


WATER FALLS AND LANDSCAPES
MANGROVES OF COAST
Tanzania
1000/-


PRESERVAÇÃO DOS MANGUEZAIS
E ZONAS DE MARÉ
Pró-Brasileira


Malaysia
Malaysia
Malaysia
Malaysia


FIJI
FIJI
FIJI
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$1
$1
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$1


350
5
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5


1500
1500
INDONESIA


BERMUDA


FIJI
54
PostBeeld


Palau
Mangrove Fly
50¢


SITES TOURISTIQUES
Singe rouge (patas)
Flamants roses et nids
Mangrove
75 Frs
Postes 2007
SINE SALOUM
SENEGAL


ANGOLA
37
ANGOLA
37
ANGOLA


80
SRI LANKA
5.00
Madu Ganga Ramsar Wetland
SRI LANKA
25.00
Madu Ganga Ramsar Wetland


BAHAMAS
FRIENDS OF THE ENVIRONMENT
70c
PAPUA NEW GUINEA
K5.00


Magnificent Birds of The Sundarbans World Heritage
BANGLADESH
Bubo coromandus
Halcyon pileata
Pitta megarhyncha
Haliaeetus leucogaster
Alcedo meninting
Gorsachius melanolophus
Leptoptilos javanicus
Halcyon coromanda
Treron bicincta
Haliopais personata
Pelargopsis amauroptera
Todiramphus chloris
2011


Fiji
100


5000


KIRIBATI
45c
KIRIBATI
75c
KIRIBATI
$1.00
KIRIBATI
$3.00


-2019


WALLIS & FUTUNA
WALLIS & FUTUNA
WALLIS & FUTUNA
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15.00
World Wetlands Day
2 February 2020
Wetlands and biodiversity

in mangroves is showed on one of the stamps. It also represents the root diversity, plantlets and mangrove associated crabs from the forest.

Fiji in 2007 published stamp on Mangrove Lobster. Palau in the same year published a stamp showing the bird Mangrove Flycatcher *Myiagra erythrops*. Senegal in 2008 issued a stamp showing mangrove associated fauna, flamingo and monkey.

Angola in 2008 published three stamps on Mangrove along river Chilango: stilt roots of *Rhizophora.*

Japan in 2009 published a stamp on Tourism Okinawa Prefecture II showing the mangrove scrub vegetation probably an afforestation site of *Rhizhophora* plantation. Sri Lanka in the same year published two stamps on Madi-Ganga shallow water body in south west Sri Lanka which is a Ramsar Wetland Site.

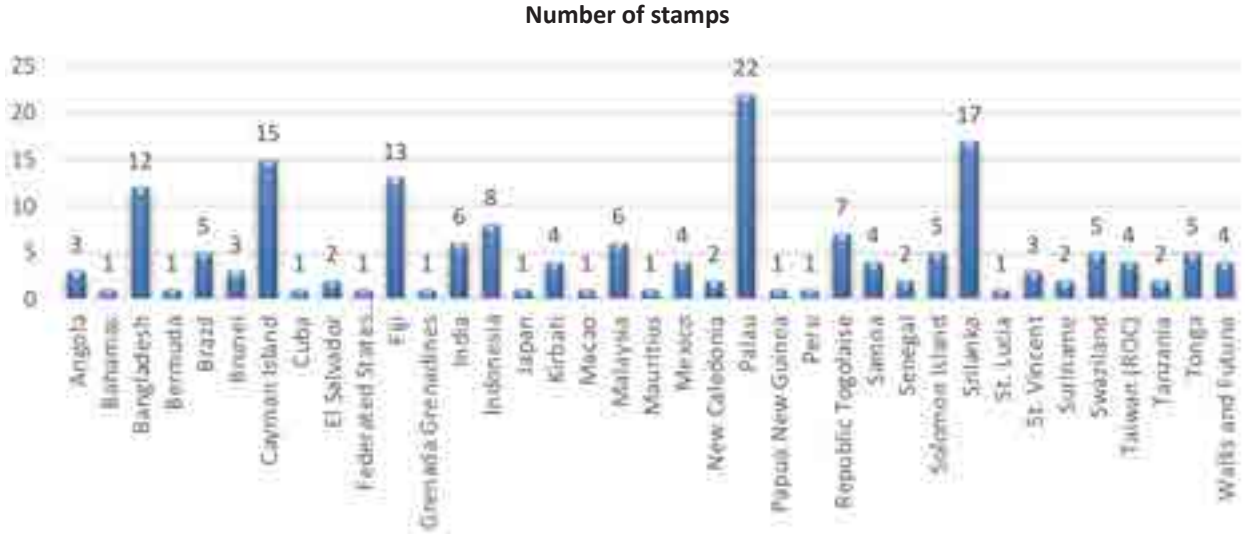
In 2010 decade 11 countries namely Bahamas, Papua New Guinea, Bangladesh, Republic of Togolaise, New Caledonia, Fiji, Indonesia, Kirbati, India, Wallis & Futuna Islands, and Sri Lanka issued the stamps related to mangroves.

The Bahamas issued the stamp 'Friends of the Environment' on 3 March 2010, showing the egret, a bird and a mangrove tree. Papua New Guinea in 2011 published stamp showing mangrove associated lizard *Varanus indicus*.

In 2011, Bangladesh released a sheet of 12 stamps entitled Magnificent Sundarban Birds. The 'Sundarbans' is the largest mangrove in the world located along the Ganges Delta. It is the Tiger Reserve as well as on the UNESCO World Network of the Biosphere Reserve. The 10 km² 'Sundarbans' of land and water have about 5.98 km² in India and the rest in Bangladesh. It is densely covered with mangrove forests and is also a suitable habitat to a variety of birds, reptiles and invertebrate species.

Four beautiful souvenir sheets containing seven stamps were issued by the Republic of Togolaise in 2011, as part of the ecosystems of Africa series, depicting the fauna of the mangroves forests of South Africa. It includes Kingfisher, crocodile, tortoise, and crabs as mangrove inhabitants.

The mangrove stamps were published by the Fiji government during the Manage Mangrove campaign in 2013. This was coordinated jointly by the WWF-South Pacific and the MESCAL Fiji project of the Ministry of Environment and supported by the Department of Lands and Post Fiji. The theme, 'My Mangrove My Livelihood' for these stamps showed the importance of mangrove ecosystems for Fiji and Pacific Island countries to carry message on a global scale. The MESCAL logo on the



Graphical representation showing country wise stamps related to mangroves

Table 1. Mangrove ecosystem elements on stamps and other philatelic material: a summary.

	Country	Theme / Feature of mangrove ecosystem	No. of Stamp	Date of issue
1	St. Vincent	Mangrove associated bird Green Heron (*Butorides virescens*) and mangrove cuckoo (*Coccyzus minor*)	2	12.i.1970 & 24.iv.1974
2	Cayman Island	seed of the 'Red Mangrove (*Rhizophora apiculata*)' and Mangrove species and associated crab	2	09.xii.1980
3	Cayman Island	Red Mangrove Flower blooming of *Rhizophora mucronata*.	1	09.xii.1982
4	Macao	flowering twig of *Acanthus ilicifolius* L under the theme of medicinal plants	1	14.vii.1983
5	Grenada Grenadines	200th Anniversary of the Birth of John James Audubon showing the bird 'Mangrove Cuckoo'.	1	11.ii.1985
6	Swaziland	A set of four stamps showing Ground hornbills (Bucorvus abyssinicus) and a first day cover on Mangrove cuckoo (Coccyzus minor).	5	15.v.1985
7	St. Lucia	Mangrove Cuckoo to the Savannes Bay Nature Area, Scorpion Island, Saint Lucia.	1	20.vi.1985
8	Cayman Island	Marine Fauna	12	15.ix.1986
9	Sri Lanka	Set of 4 stamps depicting mangrove habitat, species, flowering & fruiting and associated crab	4	11.xi.1986
10	Solomon Island	Mangrove associated Little Kingfisher (*Ceyx pusillus*) catching food in mangrove thicket	4	15.vii.1987
11	Palau	The environmental portrait set of 20 stamps depicting mangrove flora and associated fauna	20	20.xi.1989
12	Suriname	Mangrove site	2	10.x.1990
13	Mauritius	Protection of Mangroves: *Rhizophora mucronata* on East Africa and Indo-Pacific region.	1	29.xi.1990
14	Fiji	Mangrove Crabs	4	26.ix.1991
15	Peru	Stamp of riverine mangrove swamps of Tumbes National Sanctuary	1	23.xii.1991
16	Malaysia	Mangrove Swamp forest of the Hutan Paya Laut area with a drawing of a tree port of *Rhizophora apiculata*	1	23.iii.1992
17	Solomon Island	Mangrove associated crab - *Uca tetragonon*	1	15.i.1993
18	Cuba	Endangered fauna from mangrove habitats: Roseate spoonbill (*Platalea ajaja*)	1	12.x.1993
19	Federated States Micronesia	Flower of the mangrove apple *Sonneratia caseolaris*	1	06.vi.1994
20	El Salvador	Mangrove of Pacific coast with American Pygmy Kingfisher and green Kingfisher (*Chloroceryle aenae* and *C. americana*)	2	11.x.1995
21	Brunei	Mangrove Flowers	3	29.v.1997
22	Samoa	Mangrove diversity	4	26.ii.1998
23	St. Vincent	Mangrove tree a species of *Rhizophora* and the bird 'White Ibis' *Eudocimus albus*	1	12.iv.1999
24	New Caledonia	Aerial view of mangrove forest	1	10.viii.2000
25	Tonga	Five stamps on animals of mangrove swamps on world Environment Day.	5	05.vi.2001
26	Mexico	Definitive issue covering 24 stamps of which 2 depicts mangroves and lake-lagoon conservation	2	18.ii.2002
27	Palau	18 stamps of flowers which 1 depicts flower of *Sonneratia alba*	1	04.iii.2002
28	India	8th COP meeting: Four mangrove species	4	30.x.2002
29	Senegal	Mangrove fauna	1	20.xii.2002
30	Tanzania	Rufiji river mangrove and delta	2	22.vii.2003
31	Brazil	Stamp sheet on Preservation of the Mangrove Swamps and Tidal Zones.	5	05.vi.2004
32	Mexico	Definitive issue covering 24 stamps of which 2 depicts mangroves and lake-lagoon conservation	2	01.viii.2004
33	Malaysia	Matang mangrove forest	5	04.x.2004
34	Fiji	Herons & Egrets in mangrove habitat	4	26.i.2005
35	Taiwan (ROC)	Four mangrove species *Kandelia obovata*, *Rhizophora stylosa*, *Avicennia marina* and *Lumnitzera racemosa*.	4	10.iii.2005
36	Indonesia	Save Mangrove Forest	2	05.vi.2005
37	Bermuda	Out of 6 stamps Yellow Crown Night Heron (*Nycticorax violaceus*) in mangroves	1	18.viii.2005
38	Indonesia	Milky stork (*Mycteria cinerea*)	2	05.vi.2005

	Country	Theme / Feature of mangrove ecosystem	No. of Stamp	Date of issue
39	Fiji	Mangrove Lobster	1	24.i.2007
40	Palau	A flycatcher in mangrove forest	1	01.iii.2007
41	Senegal	Mangrove associated fauna: Fmaingo & Monkey	1	27.ii.2008
42	Angola	Mangrove along river Chilango: stilt roots of *Rhizophora*	3	30.viii.2008
43	Japan	Tourism Okinawa Prefecture II	1	02.ii.2009
44	Sri Lanka	Madi-Ganga shallow water body in south west Sri Lanka which is a Ramsar Wetland Site	2	02.ii.2009
45	Bahamas	Egret bird with mangrove tree background	1	03.iii.2010
46	Papua New Guinea	Mangrove associated Lizard *Varanus indicus*	1	05.i.2011
47	Bangladesh	Magnificent avifauna of Sundarbans World Heritage	12	17.vii.2011
48	Republic Togolaise	Four sheet with the themes of mangrove associated faunal members	7	28.ix.2011
49	New Caledonia	Mangrove Forests	1	08.xi.2012
50	Fiji	My mangrove – My Livelihood	4	30.x.2013
51	Indonesia	Environmental Care	4	04.v.2014
52	Kirbati	Mangrove ecosystem and conservation	4	16.vi.2014
53	India	a special cover on Wild Life Sanctuary (Mangroves, Coringa).	1	25.vii.2014
54	India	a special cover Mangroves	1	01.xii.2015
55	Wallis and Futuna	Mangrove forest conservation	4	08.xi.2018
56	Sri Lanka	a special cover commemorative cover on mangroves	1	26.vii.2019
57	Sri Lanka	Set of 10 mangrove plant species	10	03.ii.2020
		Total	176	

Decade	1970s	1980s	1990s	2000s	2010s (till date)	Total
No. of countries that published stamps related to the mangroves	1	8	12	16	11	48
No. of stamps	2	51	22	50	51	176

stamp indicates a collective effort and makes it a product owned by everyone in the country.

Indonesia in 2014 on the occasion of World Environment Day published four stamps in two sets, one of them shows a mangrove bird *Leptoptilos javanicus* and the other set has Mangrove Crab *Scylla serrata.* New Caledonia in 2014 published stamp on mangroves forest.

Kirbati has published four stamps related to mangrove ecosystem and conservation with tagline of mangroves helping to save our community.

Similarly, in 2014, the Postmaster General of Vijaywada, Andhra Pradesh, India, published a special cover on 'Coringa Wildlife Sanctuary and estuary'. It is the second area of mangrove forests in India with 24 mangrove tree species and over 120 bird species. It is home to the Critically Endangered White-backed Vulture and Long-billed Vulture. In 2015 during Navi Mumbai Festival of Stamps, the postal department issued a special cover showing mangrove vegetation.

In 2018 Wallis and Futuna Islands published four stamps on conservation of mangrove ecosystem.

In 2019 Sri Lanka published a special commemorative cover on mangroves on World Mangrove Day.

Recently, in 2020, on the occasion of World Wetland Day, Government of Sri Lanka published a set of 10 mangrove species, viz.: 01. *Bruguiera exangular*, 02. *Bruguiera cylindrical*, 03. *Bruguiera gymnorrhiza*, 04. *Nipa* Palm, 05. *Stemanoporus moonii*, 06. *Mesuastylosa–Suwanda*, 07. *Xylocarpus granatum*, 08. *Scyphiphora hydrophyllacea*, 09. *Sonneratia alba*, and 10. *Aegiceras corniculatum*.

For philatelic purposes, the term mangrove is fortunate enough to have a post office known as Mangrove Bay Post office. It is situated in Somerset Village of Bermuda with an official address as 55 Mangrove Bay Road, Sandys MA 02.

DISCUSSION AND CONCLUSION

. The ecological and economic importance of mangroves is recognized throughout the world. They are also depicted on stamps and other philatelic products around the globe. Though the world is adopting 'paperless' strategy today and surface mails have limited scope in future, philately would continue to be a passion and thus a medium of awareness.

· In the stamp records, mangroves are depicted in several ways such as panoramic views of forests, waterways bordered by stilt roots and plant thickets, floral elements and associated fauna, habits of plants and animals, flowers and fruits, fishing also covering the conservation and tourism aspects of mangrove habitats. However, as observed in philatelic records, mangrove fauna (especially avifaunal diversity) is favoured and predominantly expressed by all the countries.

· Mangrove habitats are reflected as major coastal wetlands and protected areas in the philatelic material. They are also specifically used as tools for national public awareness and global communication through visual/ pictorial language.

· It is observed that since 1970, the mangrove forest or its components have appeared regularly on the postal stamps and other documents. Out of 112 known countries hosting mangroves at their coasts along the tropical and subtropical regions, it is interesting to note that around 37 countries have published them on their national stamps.

The trend of stamps related to mangroves is presented below in a tabular form. It clearly shows a trend of increasing number of philatelic products added by various countries across the globe. This can be correlated with the level of awareness at Global scenario and at the scale of local government. This could be due to direct or indirect impacts of climate change or sea level rise or empathy for natural resources and biodiversity.

. Palau, an island in the Pacific Ocean, published the maximum (22) of mangrove related postal displays while Sri Lanka ranks second with the thematic publication of 17 stamps including a special cover.

· To conclude, the mangrove ecosystem which is respected for its diversity, is represented in a diverse way in philately. A thematic philatelic approach underlines the possibility of the use of philately (as a combination of art and science) to represent the mangrove environment, which can indirectly help in understanding, protecting and conserving mangrove ecosystem.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMMUNICATION

Amanitaceous fungi of central Western Ghats: taxonomy, phylogeny, and six new reports to Indian mycobiota

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Abstract: The study presents nine species from the family Amanitaceae collected during field work in Western Ghats forests of Karnataka State, of which six species (*Amanita ballerina*, *A. franzii*, *A. griseofusca*, *A. lignitincta*, *Saproamanita manicata*, and *S. praeclara*) are newly recorded from India. Descriptions, illustrations, molecular phylogenetics of all species, and brief discussions on distinguishing characters, ecology, & distribution are provided.

Keywords: Agaricales, Agaricomycetes, *Amanita*, Amanitaceae, Basidiomycetes, molecular phylogeny, nrLSU.

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Author contributions: RK carried out the research work, wrote the article. MK guided in every step and corrected mistakes in the article.

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INTRODUCTION

Amanitaceae E.–J. Gilbert (Agaricales, Agaricomycetes) is a family of cosmopolitan fungi. Most species of the family Amanitaceae are ecologically important as ectomycorrhizal associations with forest plants of more than 10 families and some are saprotrophic (e.g., *Amanita vittadinii* (Moretti) Vittad., *Catatrama costaricensis* Franco-Mol.).

Amanita Pers. is a genus with vast taxonomic exposure and harbors about 95% of the species in the family Amanitaceae. Since the establishment of the genus by Persoon (1794), mycologists have contributed to the taxonomy of *Amanita*. Redhead et al. (2016) coined a new generic name *Saproamanita* to replace the saprotrophic amanitas from *Aspidella* based on molecular phylogenetics. In a recent study to construct a higher rank phylogeny using multiple gene sequences, Cui et al. (2018) suggested that, *Amanita* includes three subgenera (subgen. *Amanita*, subgen. *Amanitina*, and subgen. *Lepidella*) and 11 sections. The multi-locus phylogeny treated these newly formed genera under sect. *Lepidella* due to moderate support for a monophyletic group and the closest relation with the remaining clades of *Amanita* which support monophyly.

The tropical belt of India with its rich biodiversity is a hub of different fungal species and about 83 species of the family Amanitaceae, including 75 species of *Amanita* reported from different parts of the country (Bhatt et al. 1999; 2003, 2017; Vrinda et al. 2005a,b; Shridhar 2018; Verma & Pandro 2018; Verma et al. 2020). The present study presents nine species of Amanitaceous fungi from Western Ghats forests of Karnataka, of which six species are newly recorded from the Indian sub-continent.

MATERIALS AND METHODS

Study area

Central Western Ghats encompasses the districts of Uttara Kannada, Shivamogga, Chikkamagaluru, Hassan, Kodagu, and parts of Dakshina Kannada in Karnataka state. The region covers two sub-clusters (Talakaveri and Kuduremukh) of UNESCO world Heritage sites. The region includes different types of forest patches from dry-deciduous forest to evergreen forest. The dry-deciduous forest patches dominated by the members of Combretaceae (Mainly *Terminalia* spp.), moist deciduous forest regions composed of tree species belonging to Fabaceae (*Xylia* spp.), and some species of Apocyanaceae (e.g., *Alstonia scholaris*). The semi-evergreen forest patches mainly dominated by tree species of Rubiaceae, Moraceae, and Myrtaceae. In the present study, the specimens of Amanitaceae were collected from the different sites during our fieldwork conducted in the rainy seasons of 2019 and 2020.

Sampling and morphological characterization

The sporocarps encountered during the field studies were photographed and described macroscopically *in situ* (Atri et al. 2017), and collected specimens were dried and subsequently utilized for further characterizations. The micro-morphological characters were studied by mounting the sections in 5% KOH stained with Phloxine B using Olympus CH20i binocular light microscope with oil immersion objectives of about a maximum of 1000X magnification. Around 50 measurements for basidiospores and at least 20 measurements for basidia and cheilocystidia were derived from each specimen and the range of spore length by width x, the mean value of all spores with standard deviation (SD). Q, the range of spore length to width ratio of all basidiospores measured and the mean value (Q^m) and their SD were calculated (Zhang et al. 2017). The specimens then identified by matching the descriptions given by (Vizzini et al. 2016; Thongbai et al. 2017; Cui et al. 2018).

DNA extraction, PCR, and sequencing

DNA of every fresh specimen was extracted using CTAB method (Doyle & Doyle 1987) with some modifications (Kantharaja & Krishnappa 2020). The extracted DNA was analyzed for purity by 0.8% agarose gel electrophoresis and bio-photometer (Eppendorf India Pvt. Ltd., Chennai, India) for absorbance ratio calculation under 240 and 260 nm.

PCR reactions were carried out to amplify ITS and LSU regions of the nuclear ribosomal RNA using Eppendorf Mastercycler nexus GX2 (Eppendorf India Pvt. Ltd.) in 0.2 ml PCR tubes following the protocols given by Kantharaja et al. (2020). The amplified PCR products were examined by 1% agarose gel stained with ethidium bromide and visualized under gel image documentation system (BioRad Laboratories, Inc. India) followed by purification and sequencing at Eurofins Genomics India Pvt. Ltd.

Sequence alignment, dataset assembly, and phylogenetic analysis

The obtained sequences were aligned using Clustal W multiple sequence alignment with default parameters (Madeira et al. 2019) in BioEdit sequence alignment editor v. 7.2.5 (Hall 1999). The obtained consensus sequences were used for the BLAST search analysis

on NCBI database to identify the percent similarity and alignment patterns. Based on the percentage of similarity, a distance tree was drawn for each specimen sequences on NCBI BLAST search to confirm the species identification. The identified specimen sequences were deposited on NCBI GenBank database.

Phylogenetic reconstruction was conducted using 101 sequences (both nrITS and nrLSU sequences), retrieved based on (Cai et al. 2014; Cui et al. 2018; Fraiture et al. 2019) including the sequences derived during the present study (Table 1). A dataset of combined sequences of nrITS and nrLSU was prepared and aligned using MAFFT v7.450 (Katoh et al. 2017). The alignment output was used to test the nucleotide substitution model to conduct phylogenetic reconstruction on jModelTest v.2.1.10 (Darriba et al. 2012) for both maximum likelihood analysis and Bayesian analysis. The maximum likelihood analysis was carried on raxmlGUI 2.0 (Edler et al. 2020) which works on the program RAxML v.8.2.10 (Stamatakis 2014) and Bayesian inference of posterior probability was drawn using MrBayes v3.2.7a (Ronquist et al. 2012). The tree obtained was viewed on FigTree v.1.4.4 (Rambaut 2018) and arranged accordingly (Figure 1).

RESULTS

Phylogenetic analysis

The maximum likelihood analysis of dataset comprising 46 combined sequences of 51 species of Amanitaceae and an outgroup *Limacella roseicremea* consisted 1,419 distinct alignment patterns during RAxML analysis using GTRGAMMA substitution model suggested by jModelTest v. 2.1.10. The best tree (Figure 1) found with ML optimization score of -18540.374143. The newly generated specimen sequences (indicated in bold characters in Table 1) appeared to form respective clades with significant bootstrap support and Bayesian probability values.

The phylogenetic analysis depicted that the Indian collections of *Amanita* spp. belonging to 4 sections (*Phalloideae*, *Roanokenses*, *Vaginatae*, and *Lepidella*) with significant ML bootstrap support (>70%) and Bayesian probability values (>0.7). The newly recorded collections of *Amanita ballerina*, *A. franzii*, *A. griseofusca, A. lignitincta*, *Saproamanita manicata*, and *S. praeclara* are well recovered in clades representing respective sections according to previous studies (Thongbai et al. 2017; Cui et al. 2018; Fraiture et al. 2019) and each of them clustered with respective specimens retrieved from GenBank database.

TAXONOMY

Amanita ballerina Raspe, Thongbai & K.D. Hyde, PLoS One 12 (8): e0182131, 8 (2017).

MycoBank MB 552936

Basidiomata small to medium. Pileus 35–48 (–55) mm wide, hemispheric when young, broadly convex towards maturity, dry to sticky mucilage when moist, floccose universal veil remnants on surface becoming smooth in age, buff white to milky white (Image 1a,b), margin non-striate or plane when young, then striate, context 2–4 mm thick at center, dull white, soft. Lamellae 5–8 mm broad, narrow, sinuate attachment to the stipe, buff white to yellowish white, 2–3 length of lamellulae (Image 1c), truncate. Stipe 45–95 × 12–25 mm above bulb, cylindrical, bulbous, dull white, floccose with fine white floccules, context thin, white to yellowish creamy white. Bulb marginate-compressed, sometimes clefted, 10–15 mm wide, sub globose to elongate napiform at maturity, dull white to yellowish white, context stuffed, white. Universal veil a volval limb, 4–5 mm high, cottony-felted, white to dirty white (Image 1a). Partial veil 5–8 mm broad from stipe surface, median, persistant, white, cottony, skirt like (Image 1c), thick and split edge, striate inside. Odor and taste not observed. Spore print white.

Basidiospores (6.5-) 7.5–8.5 (-9.5) × (5.0-) 5.5–7.5 (-8.0) µm (x= 8.1 ± 0.2 × 6.5 ± 0.4, Q= 1.1–1.4, Q^m= 1.2 ± 0.1) globose to broadly ellipsoid, hyaline, thin-walled, smooth (Image 1d,g), amyloid, apiculate. Basidia 40–55 × 10–15 µm, clavate, tetrasporate (Image 1e, h), sterigmata up to 7 µm long, no clamp connections. Cheilocystidia 25–40 × 15–35 µm, subfusiform to subglobose (Image 1i), colorless, thin walled, hyaline cells. Lamellar trama bilateral, divergent, up to 70–85 µm wide, filamentous hyphae. Pileipellis 85–130 µm thick, bi-layered, upper layer up to 45–75 µm thick filamentous hyphae, 3–8 µm wide, ixocutis with hyaline, colorless, thin-walled, terminal cells ellipsoid; lower layer up to 45–60 µm thick, non-gelatinous filamentous hyphae, 2–7 µm broad, hyaline, branched, clamp connections not observed.

Habitat: Solitary or scattered on the ground in moist deciduous forest.

Specimens examined: India, Karnataka, Shivamogga district, Thirthahalli taluk, Near Kesare village (13.698472, 75.275500), 26 June 2019, Kantharaja R, KUBOT-KRMK-2019-06; Chikmagaluru district, Sringeri taluk, Near Kigga village (13.417194, 75.214722), 07 June 2020, Kantharaja R, KUBOT-KRMK-2020-19.

Table 1. Details of the specimens used in phylogenetic analysis.

	Species	Voucher / Isolate no.	GenBank accession no.	
			ITS	LSU
1	***Amanita ballerina***	**KUBOT-KRMK-2020-19**	**MW029919**	**MW029941**
2	*Amanita ballerina*	MFLU 16-2559	NR_151656	NG_058607
3	***Amanita bisporigera***	**KUBOT-KRMK-2020-24**	**MW031861**	**MW031169**
4	*Amanita bisporigera*	RET 628-4	MG968868	MG968375
5	*Amanita bisporigera*	RET 639-1	KR919763	
6	*Amanita bisporigera*	RET 632-7		KX827615
7	***Amanita eriophora***	**KUBOT-KRMK-2020-51**		**MW040076**
8	*Amanita eriophora*	RET 350-4		HQ539672
9	*Amanita farinosa*	HKAS67958	MH508341	MH486498
10	*Amanita farinosa*	HKAS100578	MH508340	MH486496
11	*Amanita flavofloccosa*	HKAS90174	MH508352	
12	*Amanita flavofloccosa*	HKAS92006		MH486516
13	***Amanita franzii***	**KUBOT-KRMK-2020-25**	**MW032434**	**MW032660**
14	***Amanita franzii***	**KUBOT-KRMK-2020-50**	**MW036452**	**MW036453**
15	*Amanita franzii*	HKAS91231	MH508358	MH486525
16	*Amanita griseofarinosa*	HKAS80017	MH508374	
17	*Amanita griseofarinosa*	HKAS83447		MH486561
18	*Amanita griseofarinosa*	HKAS80926	MH508375	MH486559
19	***Amanita griseofusca***	**KUBOT-KRMK-2020-78**	**MZ452030**	**MZ452031**
20	*Amanita griseofusca*	SWAT000137	MH241057	MH241058
21	*Amanita griseofusca*	LAH35366	MH241055	MH241056
22	***Amanita lignitincta***	**KUBOT-KRMK-2020-76**	**MW145007**	**MW145006**
23	*Amanita lignitincta*	HKAS69411	MH508424	MH486625
24	*Amanita lignitincta*	HKAS69408	MH508423	MH486624
25	***Saproamanita manicata***	**KUBOT-KRMK-2019-16**	**MN447235**	**MW147220**
26	*Amanita manicata*	RET 387-4	HQ625014	HQ539708
27	*Amanita manicata*	PDD 88301	MT863750	
28	*Amanita manicata*	Hemmes 2008		HQ593115
29	***Amanita ovalispora***	**KUBOT-KRMK-2020-77**	**MZ453080**	**MZ453085**
30	*Amanita ovalispora*	HKAS79625	MH508479	MH486722
31	*Amanita ovalispora*	HKAS101406	MH508478	MH486720
32	*Amanita ovalispora*	HKAS101394	MH508477	MH486719
33	*Amanita pallidocarnea*	HKAS97678	MH508482	MH486728
34	*Amanita pallidocarnea*	HKAS97689	MH508483	MH486729
35	*Amanita phalloides*	Berch0167	KX449211	KX449231
36	*Amanita phalloides*	Berch0154	KX449212	KX449230
37	*Amanita phalloides*	RET 053-2	KF561975	KF561979
38	*Amanita populiphila*	RET 506-5	KX270317	KX270336
39	*Amanita populiphila*	RET 266-9	KP224323	KP224346
40	***Saproamanita praeclara***	**KUBOT-KRMK-2020-02**	**MW031170**	**MW029933**
41	*Amanita praeclara*	RET 726-7	MK351812	MK351833
42	*Amanita praeclara*	RET 822-1	MT073021	
43	*Amanita praeclara*	RET 387-6	MH806862	MH806864
44	*Amanita subcaligata*	RET 266-6	MN963590	HQ539746
45	*Amanita subjunquillea*	HKAS100622	MH508624	MH486910
46	*Amanita subjunquillea*	HKAS100581	MH508622	MH486908
47	*Amanita subjunquillea*	HKAS100597	MH508623	MH486909
48	*Amanita thiersii*	SKay4041 het	HQ625010	HQ593114
49	*Amanita thiersii*	SKay4041		HQ619205
50	*Amanita thiersii*	NEthiersii	MN481407	
51	*Amanita vaginata*	KA12-1190	KF017949	KF021688
52	*Amanita vaginata*	CUB:Microbiology MN18	AB458889	AF024482
53	*Amanita vestita*	HKAS77277	MH508646	KC429044
54	*Amanita virosa*	CNV106	MT345282	
55	*Amanita virosa*	NL-2767		MK277592
56	*Amanita vittadinii*	HKAS101430	MH508651	MH486950
57	*Amanita vittadinii*	ML711142AV	MH603603	
58	*Amanita vittadinii*	CBS 168.46		MH867677
59	*Limacella roseicremea*	RET 136-9	MT883671	MT883670

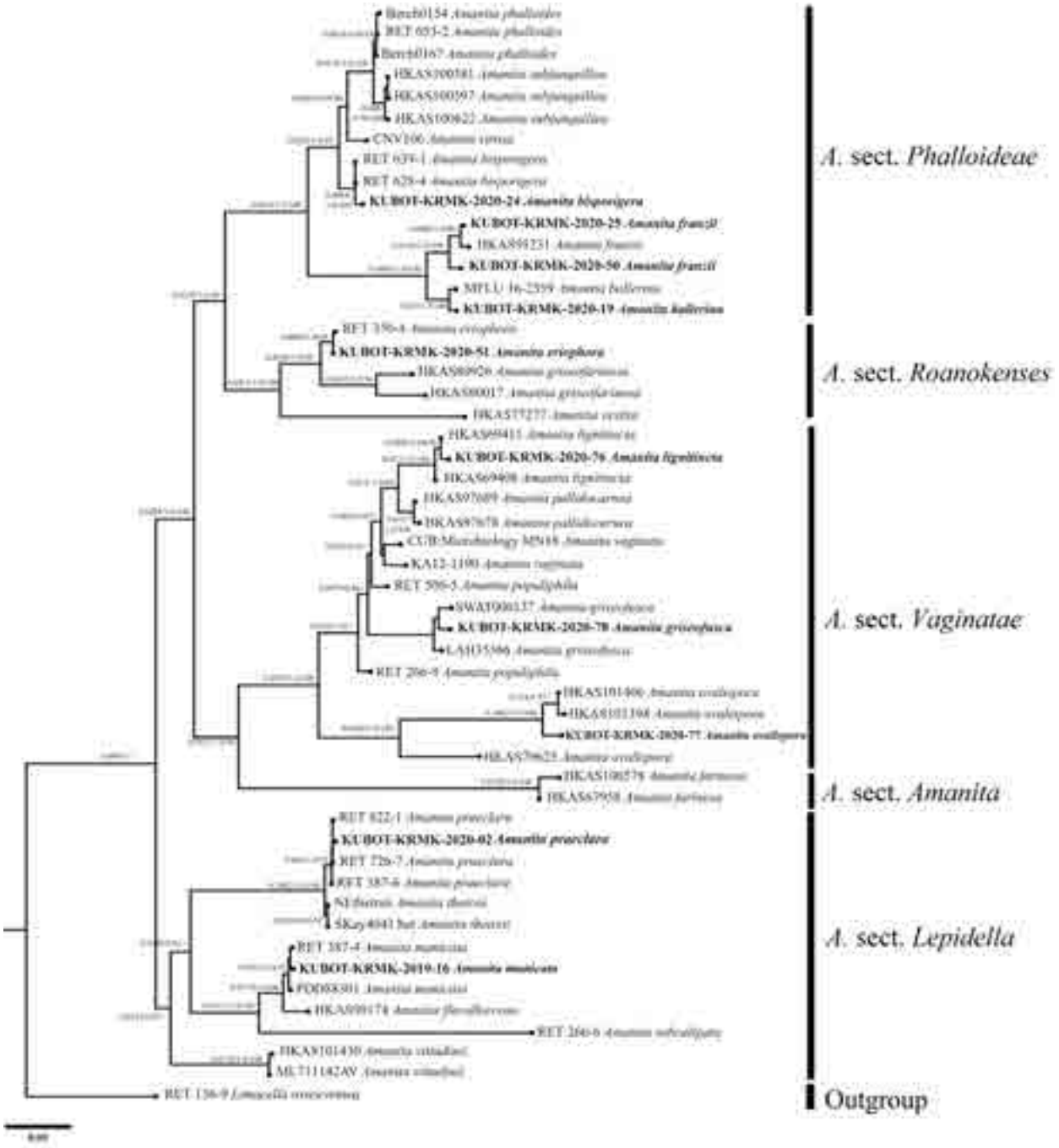

Figure 1. RAxML tree of *Amanita* spp. based on maximum likelihood analysis of nrITS+nrLSU sequences by GTR+G model with *Pluteus pantherinus* as an outgroup showing branch Length (BL), Bayesian posterior probability values (PP>0.5), and bootstrap support values (BS>50%) as (BL/PP/BS).

Amanita bisporigera G.F. Atk., Botanical Gazette Crawfordsville 41(3): 348 (1906).

MycoBank MB208433

Basidiomata medium to large. Pileus 55–120 (-140) mm wide, ovoid to subglobose when young, convex to horizontally flat at maturity (Image 2a,b), dry to vicid with slightly sticky mucilage when moist, without universal veil remnants, pink to brown warts near center in age, white, smooth surface, margin plane with white floccules to finely striate near maturity, context 4–5 mm thick at center, white, soft, not stuffed, unchanging. Lamellae 6–8 mm broad, free to close or barely adnate, crowded to sub-distant, narrow, white to dull white (Image 2c), unchanging on bruising, subelliptical, entire margin,

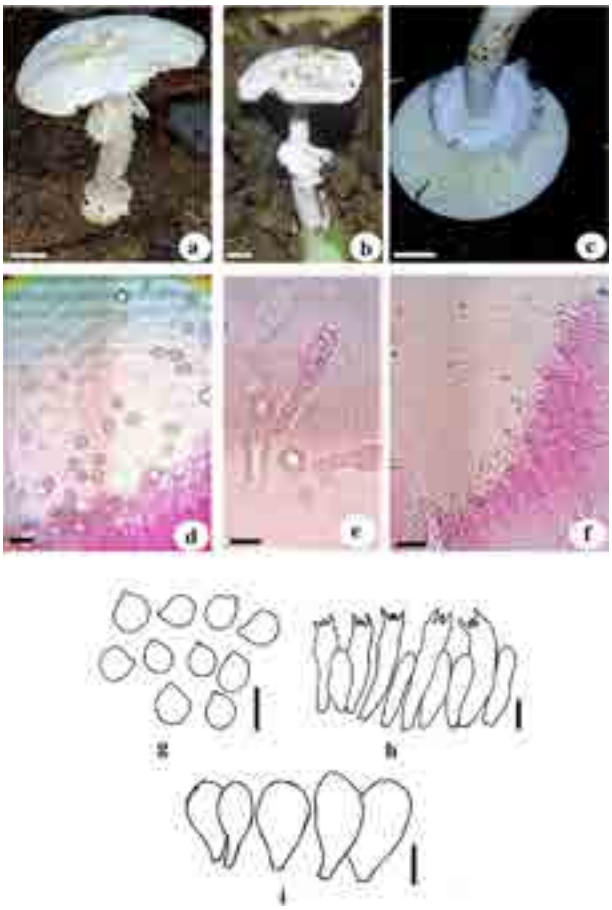

Image 1. *Amanita ballerina*: a,b—Sporocarp in habitat (a—KUBOT-KRMK-2019-06; b—KUBOT-KRMK-2020-19) | c—Lamellae and partial veil | d,g—Basidiospores | e,h—Basidia | f—Lamellar edge | i—Cheilocystidia. Scale bars: a–c—10 mm | d–e—10 µm | f—20 µm | g–i—10 µm. © Kantharaja. R.

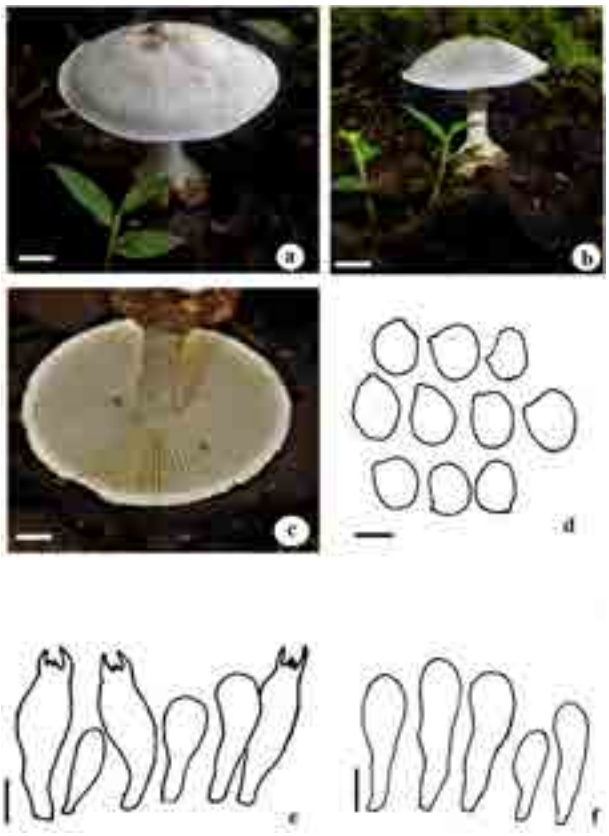

Image 2. *Amanita bisporigera*: a,b—Sporocarp in habitat | c—Lamellae | d—Basidiospores | e—Basidia | f—Cheilocystidia| Scale bars: a–c—20 mm | d—10 µm | e-f—15 µm. © Kantharaja. R.

sometimes with decurrent tooth on the stipe, lamellulae of 3 different length, attenuate to truncate, numerous. Stipe 50–110 × 8–15 mm above bulb, white, narrowly tapering upward with frequent floccose to fibrillose-squamose becoming glabrous with age, bulbous, context white, stuffed, unchanging on bruising, yellowish white pith. Bulb 12–28 mm broad, white, globose to subglobose, tuning subelliptical or irregularly ellipsoid, context white, solid, stuffed. Universal veil a volval limb with 2–3 lobes, membranous, white, appressed. Partial veil superior to subapical, membranous, delicate, thin, skirt-like, slightly striate, fragile, shred with age. Odor pleasant to sweet. Taste not observed (this is a deadly poisonous species). Spore print white.

Basidiospores (4.5-) 6.5–8.9 (-11.5) × (4.0-) 5.9–8.5 (-10.5) µm (x= 7.4 ± 0.3 × 6.5 ± 0.4, Q= 1.06–1.23, Q^m= 1.15 ± 0.02) globose to subglobose, occasionally broadly ellipsoid to ellipsoid (Image 2d), hyaline, amyloid, smooth, apiculate. Basidia 50–65 × 25–35 µm, large, clavate to cylindrical (Image 2e), bi-sporate, occasionally 4-spored, sterigmata 6–9 µm long, clamp connections absent. Cheilocystidia 50–60 × 25–30 µm, clavate, hyaline, thin-walled (Image 2f). Lamellar trama 60–80 µm wide, bilateral, divergent filamentous hyphae, subhymenial region branched. Pileipellis 3–8 µm broad, a cutis or ixocutis, thin-walled, clamp connections absent.

Habitat: Solitary or distantly colonized on soil of moist deciduous forest region.

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Near Kumsi village (14.051278, 75.401222), 12 June 2020, Kantharaja R, KUBOT-KRMK-2020-24.

Amanita eriophora (Berk.) E.J. Gilbert, Iconographia Mycologica 27 (Suppl. 1): 230 (1941).

MycoBank MB517341

Basidiomata medium to large. Pileus 85–180 mm wide, hemispherical to convex turning broadly convex with flat center or concave in age, slightly vicid, ornamented, appendiculate, margin non-striate, grey

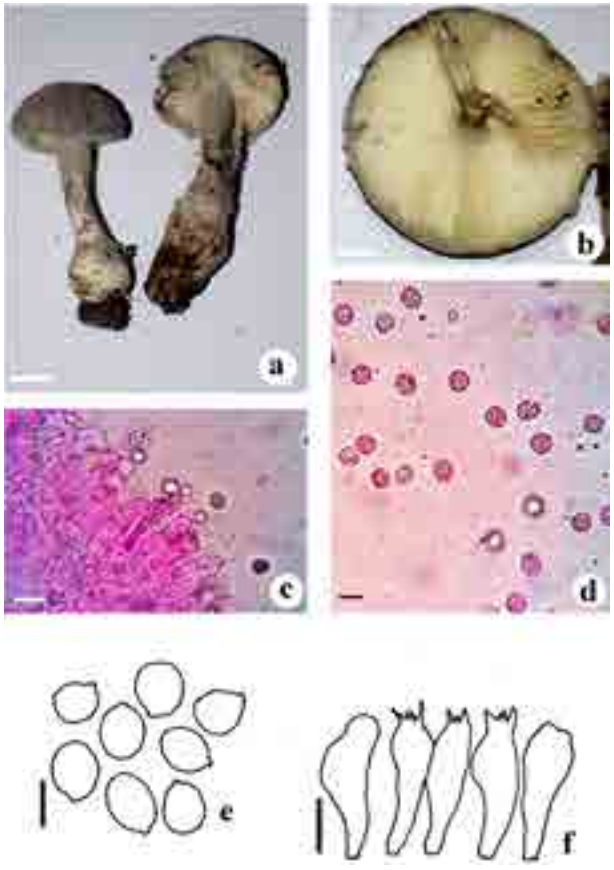

Image 3. *Amanita eriophora*: a—Sporocarps | b—Lamellae | c,f—Basidia | d,e—Basidiospores. Scale bars: a,b—50 mm | c,f—10 µm | d,e—5 µm. © Kantharaja. R.

Image 4. *Amanita franzii*: a-b—Sporocarp in habitat | c,e—Basidiospores | d,f—Basidia | g—Cheilocyctidia. Scale bars: a–b—10 mm | c–e—10 µm | d,f,g—20 µm. © Kantharaja. R.

at first, then greyish brown to pale brown (Image 3a), context 5-8 mm thick at center, white to pinkish white, stuffed, slightly red on bruising, Lamellae 8–10 mm broad, free, crowded with slight decurrent lines on stipe apex, white to cream near maturity (Image 3b). Stipe 110–135 × 15–20 mm solid, firm, white with greyish brown remnants of partial veil, bulbous, contest, white, stuffed. Bulb 15–25 mm broad, roughly napiform, covered with greyish brown, floccose volva, context white, solid, stuffed. Universal veil a volval limb, fragile, greyish brown, felted-floccose, as a rim around bulb in mature sporocarps. Partial veil fragile or friable, absent in mature sporocarps. Odor and taste not observed. Spore print whitish cream.

Basidiospores (7.5-) 8.5–11.0 (-12.5) × (7.0-) 8.0–10.0 (-10.5) µm (x = 9.6 ± 0.4 × 8.7 ± 0.2, Q = 1.08–1.23, Q^m = 1.14 ± 0.02) globose to broadly ellipsoid (Image 3d,e), amyloid, colourless, thin-walled, smooth, apiculate, with large oily contents. Basidia (35-) 40–45 (-50) × (10-) 12–15 (-18) µm, clavate (Image 3f), tetrasporate, sterigmata up to 4 µm long, colourless, clamp connections absent. Lamellar trama bilateral, divergent, composed of hardly inflated hyphae about 20 µm wide. Pileipellis ixocutis, 2–10 µm wide, thin, consisting of somewhat radially interwoven greyish-brown hyphae, clamp connections absent.

Habitat: Solitary or scattered on leaf litter rich soil in moist deciduous forest region.

Specimens examined: INDIA, Karnataka, Shivamogga district, Sagar taluk, Near Somashetti Koppa village (14.050750, 75.401722), 18 June 2020, Kantharaja R, KUBOT-KRMK-2020-51.

Amanita franzii Zhu L. Yang, Y.Y. Cui & Q. Cai, Fungal Diversity 91: 120 (2018).

MycoBank MB825038

Basidiomata small to medium sized. Pileus 40–85 mm diam, convex to broadly convex and finally flat or applanate (Image 4a), brownish white to yellowish white, whitish towards margin, universal veil remnants are like unilateral malformation, sometimes with fine particles, margin slightly striate, non-appendiculate,

context 5–8 mm in the center, stuffed. Lamellae 5–10 mm broad, free, crowded, white to creamy white (Image 4b), lamellulae of 2–3 lengths, plentiful. Stipe 80–120 × 5–15 mm, subcylindrical, tapering towards apex and expanded near pileus attachment, white to brownish white, covered with greyish brown squamules below annulus, milky white above partial veil, bulbous, context white, stuffed. Bulb 25–30 mm broad, marginate, brownish white to grey. Universal veil limbate on upper edge of bulb, brownish white to grey. Partial veil superior, membranous, white to greyish white, covers stipe at later stage. Odor and taste indistinct. Spore print not observed.

Basidiospores (8.0-) 8.5–11.0 (-12.0) × (6.0-) 6.5–8.5 (9.5) µm (x = 9.7 ± 0.3 × 7.9 ± 0.2, Q = 1.19–1.35, Q^m = 1.25 ± 0.02) broadly ellipsoid to ellipsoid, occasionally subglobose (Image 4 c,e), slightly amyloid, colourless, thin-walled, smooth, apiculate, apiculus small. Basidia 40–60 × 10–15 µm, clavate, tetrasporate (Image 4 d,f), sterigmata up to 5 µm long, clamp connections absent. Cheilocystidia 25–40 × 15–30 µm, sterile, globose to subglobose at first, fusiform to elongated later (Image 4g), filamentous hyphae. Lamellar trama 25–55 µm wide, bilateral, divergent, composed of ellipsoid to clavate cells with abundant filamentous hyphae. Pileipellis 110–130 µm thick, bi-layered, upper layer cutis to ixocutis, 30–50 µm thick, composed of interwoven, thin-walled, colourless, filamentous, hyphae, 2–9 µm broad; lower layer 50–70 µm thick, composed of radially arranged compact colourless, filamentous hyphae, up to 12 µm broad, clamp connections absent.

Habitat: Solitary or in groups on soil in moist deciduous forest region**.**

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Near Jog Falls (14.231722, 74.820944), 12 June 2020, Kantharaja R, KUBOT-KRMK-2020-25.

Amanita griseofusca J. Khan & M. Kiran, Phytotaxa 364 (2): 186 (2018)

MycoBank MB825012

Basidiomata medium sized. Pileus 60–130 mm in diam., hemispherical to oval when young, broadly convex to almost flat with a broad depression at the disc, dark brown at center, greyish brown to light brown towards margin, striated all over except the central dark disc (Image 5a), volval remnants in young basidiomata as white to greyish warts, membranous, context 2–3 mm near disc, thinner towards margin, creamy white, fragile. Lamellae 4 -6 mm broad at center, free, close, creamy white to pinkish white, fragile, lamellulae short, rare, mostly single length, rarely 2 or 3 lengths (Image 5b). Stipe 95–130 × 6–10 mm, central, subcylindric, slightly tapering upwards, without bulb, creamy white, with white to greyish white fibrils when young, later fibrils turn brownish white especially at base. Partial veil absent. Universal veil saccate, white when young to pinkish white at maturity. Odour and taste not observed.

Basidiospores (9.5-) 10.2–11.5 (-12.5) × (8.2-) 8.5–10.0 (-10.5) µm, (x = 11.2 ± 0.2 × 9.3 ± 0.4, Q = 1.1–1.3, Q^m = 1.2 ± 0.03) broadly ellipsoid, sometimes ellipsoid (Image 5 c,e), colourless, thin-walled, inamyloid. Basidia 35–45 × 12–15 µm, clavate, tetrasporate (Image 5 d,f), sometimes 2–spored, without clamp connections. Cheilocystidia 25–50 × 15–33 µm, subglobose to ovoid (Image 5g), hyaline, thin-walled. Lamellar trama 30–60 µm wide, bilateral, divergent, composed of closely interwoven filamentous hyphae with ellipsoid to sub-fusiform inflated cells. Pileipellis 40–70 µm thick, upper layer ixocutis, up to 30 µm thick, composed of radially arranged, thin-walled, colourless to pigmented, interwoven hyphae, 2–6 µm wide, lower layer up to 40

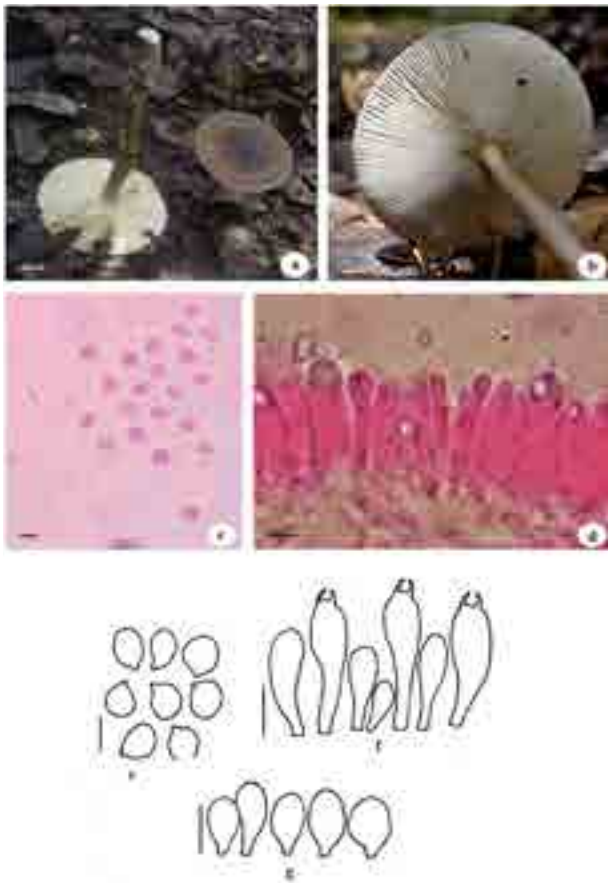

Image 5. ***Amanita griseofusca*****: a-b—Sporocarp in habitat | c-e—Basidiospores | d,f—Basidia | g—Cheilocystidia. Scale bars: a–b—10 mm | c–d—10 µm | e-f—15 µm. © Kantharaja. R.**

µm thick, composed of radially arranged, thin-walled, brownish hyphae, non-gelatinized.

Habitat: Solitary or scattered on soil in semi-evergreen forest region.

Specimens examined: INDIA, Karnataka, Shivamogga district, Sagar taluk, Agumbe rain forest (13.499000, 75.088417), 23 June 2020, Kantharaja R, KUBOT-KRMK-2020-78.

Amanita lignitincta Zhu L. Yang ex Y.Y. Cui, Q. Cai & Zhu L. Yang, Fungal Diversity 91: 82 (2018).

MycoBank MB825009

Basidiomata medium sized. Pileus 40–75 mm wide, convex to irregularly flat, slightly depressed in center with age, smooth, pale brown, greyish brown to brown, transparent when wet towards margin, striate, non-appendiculate, without universal veil remnants (Image 6a), context 3-5 mm thick at center, white, stuffed. Lamellae 5–8 mm broad, free, crowded, white (Image 6b), lamellulae of 2–3 lengths, plentiful, truncate. Stipe 80–130 × 6–13 mm, subcylindrical or slightly tapering towards apex, slightly expanded near attachment to the pileus, white to rarely pale brown, glaborous, sometimes with white to colourless fibrils towards base, context white, hollow pith. Bulb absent. Universal veil sac like, 20–30 mm in height, greyish white to brownish, membranous, persistent. Partial veil absent. Odor and taste indistinct. Spore print not observed.

Basidiospores (9.0-) 10.0–13.5 (-14.5) × (8.5-) 9.5–11.5 (-12.5) µm, (x = 12.6 ± 0.3 × 10.9 ± 0.4, Q = 1.09–1.21, Q^m = 1.12 ± 0.03), globose to subglobose, sometimes broadly ellipsoid (Image 6 c,d), colourless, thin-walled, inamyloid, smooth, apiculate, apiculus small. Basidia 35–65 × 10–22 µm, clavate, tetrasporate (Image 6e), sterigmata up to 5 µm long, clamp connections absent. Cheilocystidia 25–45 × 20–30 µm, subglobose to fusiform, sterile, inflated, single or abundant in strips (Image 6f), thin-walled, colourless. Lamellar trama 30–45 µm wide, bilateral, divergent, composed of abundant fisiform, elongated, clavate cells with filamentous hyphae, without clamp connections. Pileipellis 25–65 µm thick, upper layer ixocutis, composed of radially arranged thin walled cells, colourless, filamentous hyphae, 2–4 µm wide; lower layer with radially arranged filamentous hyphae, 2–12 µm wide, pale brown to brown, non-gelatinized.

Habitat: Solitary or scattered on soil in semi-evergreen forest region.

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Kundadri hills (13.551778, 75.171139), 23 June 2020, Kantharaja R, KUBOT-KRMK-2020-76.

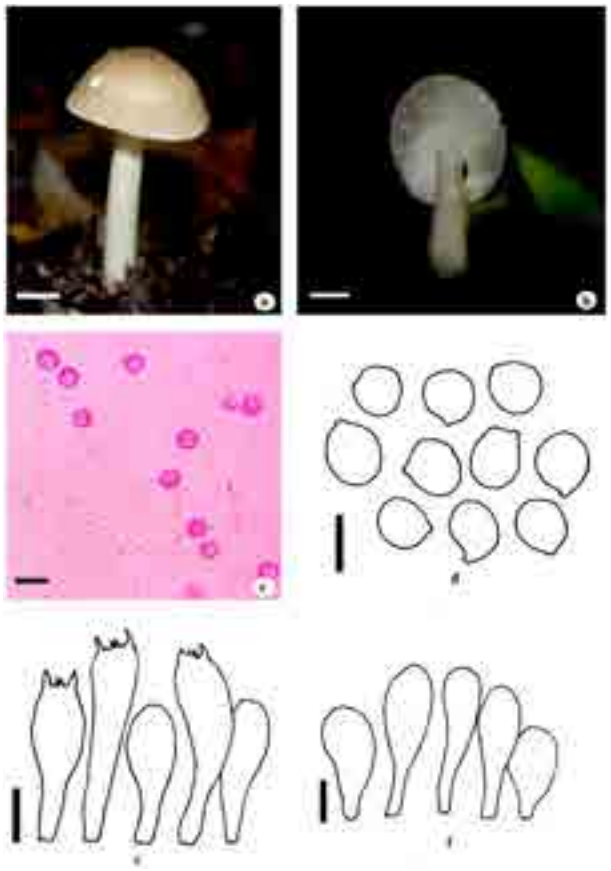

Image 6. ***Amanita lignitincta*****: a-b—Sporocarp in habitat | c-d—Basidiospores | e—Basidia | f—Cheilocystidia. Scale bars: a–b—10 mm | c–d—10 µm | e-f—15 µm. © Kantharaja. R.**

Amanita ovalispora Boedijn, Sydowia 5 (3-6): 320 (1951).

MycoBank MB14685

Basidiomata small to medium sized. Pileus 50–90 mm in diam, campanulate to plano-convex and finally applanate, sometimes with slightly depressed center, greyish brown, dark grey towards center, without universal veil remnants (Image 7 a,b), viscid when moist, margin striate, marked with parallel grooves, regular, incurved in some cases, context white, stuffed. Lamellae 4–6 mm broad, free, pure white to creamy, thin, moderately crowded (Image 7c), no colour change on bruising. Stipe 85–110 × 7–14 mm, white to greyish white, fleshy, central, slightly tapering upwards, smooth to fibrillose, hollow, context white, fleshy. Universal veil white, membranous, saccate, free, sheathing, persistent. Partial veil absent. Odor and taste indistinct. Spore print white.

Basidiospores (7.5-) 8.5–12.5 (-13.5) × (6.5-) 7.8–

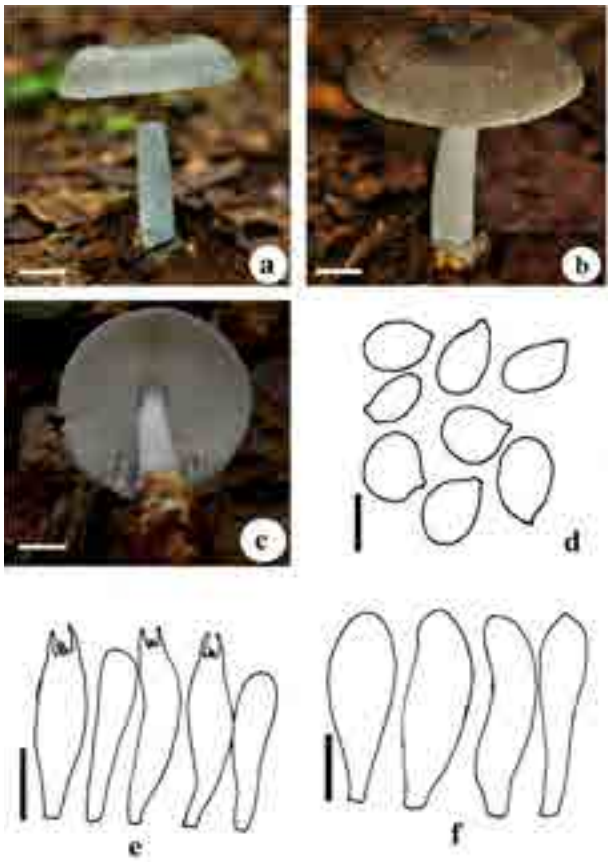

Image 7. *Amanita ovalispora*: a-b—Sporocarp in habitat | c–Lamellae and stipe | d— Basidiospores | e—Basidia | f—Cheilocystidia. Scale bars: a–c—10 mm | d—10 µm | e-f—15 µm. © Kantharaja. R.

11.0 (-12.0) µm (x = 10.9 ± 0.3 × 9.8 ± 0.3, Q = 1.06–1.21, Q^m = 1.13 ± 0.02), globose to broadly ellipsoid (Image 7d), colourless, thin walled, smooth, apiculate, inamyloid. Basidia 47.0–65.5 × 11.0–19.5 µm, clavate, tetrasporate (Image 7e), sterigmata 2-6 µm long, thin walled, guttules present, clamp connections absent. Cheilocystidia 25.0–32.5 × 6.5–15.5 µm, pyriform (Image 7f), thin walled, colourless. Subhymenium 8.5–15.0 µm wide, distinct, thin walled, pseudoparanchymatous cells. Lamellar trama 5.5–28.5 µm broad, bilateral, divergent, hyaline, thin-walled, septate hyphae. Pileipellis 20–35 µm thick, upper layer trichodermium, ixocutis, hyphae colourless, 2–7 µm long; lower layer with radially arranged filamentous, septate hyphae, 4–10 µm wide, greyish brown, non-gelatinized.

Habitat: Solitary or scattered on soil in semi-evergreen forest region.

Specimens examined: India, Karnataka, Shivamogga district, Sagar taluk, Agumbe rain forest (13.499000, 75.088417), 23 June 2020, Kantharaja R, KUBOT-KRMK-2020-77.

Saproamanita manicata (Berk. & Broome) Redhead, Vizzini, Drehmel & Contu, IMA Fungus 7 (1): 123 (2016).

MycoBank MB816358

≡ *Amanita manicata* (Berk. & Broome) Pegler, Kew Bulletin Additional Series 12:216 (1986)

Basidiomata medium to large sized. Pileus 80–140 mm wide, fleshy, hemispherical initially, broadly convex to completely flat near maturity, rarely depressed in the center, whitish to creamy white, ochre-orange grainy remnants cover the surface completely when young, eventually forms triangular patches leaving uncovered surface appear orange-white in colour (Image 8a), margin non striate, strongly appendiculate, appendage triangular veilar residues of partial veil, whitish but covered with ocher-orange flakes, appendage fragile, leaving margin naked towards maturity. Lamellae 40–55 mm, slightly ventricose, adnate to somewhat free, close to crowded, eroded, white to pale pinkish (Image 8b), lamellulae of 2–3 different lengths, truncate. Stipe 80–150 × 10–18 mm, cylindrical, medially sinuous, base rounded to sub-clavate, smooth and whitish above partial veil, covered with white to ocher-orange, flaky residues below, context whitish, stuffed. Universal veil absent. Partial veil pendant or hanging, fragile, white to ocher orange, lower surface with concolorous flakes. Odor intense, unpleasant, aromatic. taste indistinct. Spore print not observed.

Basidiospores (4.5-) 5.5–8.0 (-9.5) × (4.5-) 5.0–7.5 (-8.0) µm (x = 7.3 ± 0.3 × 6.8 ± 0.1, Q = 1.03–1.18, Q^m

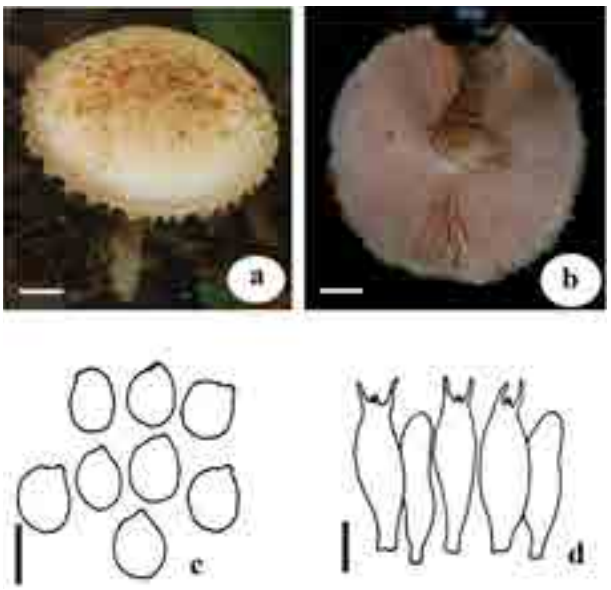

Image 8. *Saproamanita manicata*: a—Sporocarp in habitat | b–Lamellae | c— Basidiospores | d—Basidia. Scale bars: a–c—10 mm | d—10 µm | e-f—15 µm. © Kantharaja. R.

= 1.13 ± 0.2), globose, rarely subglobose to broadly ellipsoid, slightly amyloid, smooth (Image 8c). Basidia 20–35 × 8–15 µm, clavate, tetrasporate (Image 8d), sterigmata up to 4 µm long. Lamellar trama 25–30 µm wide, bilateral, divergent, hyphae 3.5–6.0 µm wide, subhymenium well developed pseudoparanchymatous. Pileipellis a cutis, with extended, interwoven hyphae, 4–8 µm wide.

Habitat: Solitary or scattered on soil in dry deciduous forest region.

Specimens examined: India, Karnataka, Chikmagalur district, Narasimharajapura taluk, near Bakrihalla irrigation project (13.641000, 75.507000), 08 July 2019, Kantharaja R, KUBOT-KRMK-2019-16.

Saproamanita praeclara (A. Pearson) Redhead, Vizzini, Drehmel & Contu, IMA Fungus 7(1): 123 (2016).

MycoBank MB816480

≡*Amanita praeclara* (A. Pearson) Bas, Persoonia 5 (3): 380 (1969)

Basidiomata medium to large sized. Pileus 65–180 (-220) mm in diam, globose to plano-convex, white, covered with pale yellow to orange yellow lanose-floccose covering when young (Image 9a, b), staining pale yellow afterwards, appendiculate, margin non-sulcate, entire, context white, thick, up to 12 mm thick at center. Lamellae 12–15 mm broad, adnexed to free, crowded to close, thin, mostly broad, sometimes narrow to ventricose, white, pale yellow on bruising. Stipe 80–150 × 10–30 mm, cylindrical, base clavate, yellowish white, covered with orange yellow to pale yellow wooly floccules, context white, solid, stuffed. Universal veil absent, Partial veil pendant or hanging, fragile, upper surface white, smooth, lower surface covered with wooly floccules. Odor intense, strongly unpleasant. Taste indistinct. Spore print not observed.

Basidiospores (7.0-) 8.5–9.5 (-10.5) × (6.5-) 7.5–9.0 (-10.5) µm (x = 8.7 ± 0.2 × 7.9 ± 0.2, Q = 1.01–1.11, Q^m = 1.04 ± 0.1), globose, smooth (Image 9c), amyloid. Basidia 25–35 × 8–20 µm, clavate, tetrasporate (Image 9d), sterigmata up to 5 µm long, clamp connections absent. Lamellar trama 15–25 µm wide, bilateral, divergent, hyphae 2–3 µm wide. Subhymenium with pseudoparanchymatous cells. Pileipellis a cutis, compact, interwoven hyphae, 3–10 µm broad.

Habitat: Solitary on soil under in dry deciduous forest region.

Specimens examined: India, Karnataka, Shivamogga district, Bhadravathi taluk, near Koppa (13.968000, 75.709000), 19 May 2020, Kantharaja R, KUBOT-KRMK-2020-02.

Image 9. ***Saproamanita praeclara*****: a-b—Sporocarp in habitat | c—Basidiospores | d—Basidia. Scale bars: a–b—15 mm | c—5 µm | d—10 µm. © Kantharaja. R.**

DISCUSSIONS

In India a total of 83 species of fungi belonging to the family Amanitaceae are recorded (Bhatt et al. 2003; Verma & Pandro 2018; Verma et al. 2020). As a cosmopolitan group the members of the family are distributed among different habitats of the country. The species found are either ectomycorrhizal or growing on humic soil. The Western Ghats of India being cool and humid, supports the growth of macrofungi. Especially, the central Western Ghats region of Karnataka includes differential habitat structures from dry deciduous forests to evergreen forests. The exploration of diversity and distribution of Agaricales in this region resulted in identification of nine Amanitaceous fungi, of which five species are newly recorded in India (*Amanita ballerina, A. franzii, A. griseofusca, A. lignitincta, Saproamanita manicata* and *S. praeclara*).

Amanita bisporigera is previously reported growing on soil in Wayanad, Kerala (Mohanan 2011), and as ectomycorrhizal association with trees of Sal forest from Madhya Pradesh (Verma & Pandro 2018). The specimen identified in this study also habited on soil of moist-deciduous forest growing individually or in scattered structure. *Amanita ovalispora* is common in tropical areas and originally described from Indonesia. In India

the species is reported from several locations of the state of Odisha (Dancholia 1989) and the present study identifies the first specimen from Western Ghats region based on the original description (Boedijin 1951) and the comparison distinguishes the collected specimen by having slightly depressed center, which accordingly considered in one of the reports from China (Yang 1997)

Amanita eriophora a rare species of mushroom described originally from West Bengal, India (Berkley 1850). Also reported from Singapore, Malaya (Corner & Bas 1962) and has little known literature since then. Except some citations of Kaur & Atri (2002), reporting the species from Punjab plains. The specimen collected in this study was identified based on morphological characters and confirmed by molecular phylogenetics where it was clustered with a collection from Cambodia (RET 350-4) with considerable statistical values. The sample could be a first ever report from the Western Ghats of India.

Six species of *Amanita* collected in this study are reported for the first time from India. *Amanita ballerina*, a recently described species from Chiang Mai Province of Thailand (Thongbai et al. 2017). The species is reported growing under Dipterocarp- or Fagaceae-dominant forest covers and the key identification characters like small, white basidiomata with floccose pileus, skirty partial veil and basal cottony-felted, dirty white volval limb are completely accurate with the Indian collection which phylogenetically well recovered in *Amanita* sect. *phalloideae* clade along with *Amanita franzii* another new record to Indian mycobiota. The species is characterized by its bran-like grey-brown universal veil remnants on yellow brown to pale brown pileus surface with a marginate basal bulb and slightly amyloid basidiospores. The Indian collection KUBOT-KRMK-2020-50 is almost similar to the type species recently described from Southwestern China (Cui et al. 2018).

Amanita lignitincta is a grey-brown to pale brown capped mushroom species with striate margins and lacking partial veil structure. The species is primarily described from the southwestern China on soil, growing solitary or scattered in subalpine forest region (Cui et al. 2018). The Indian collection of the species appeared in a forest with canopy of semi-evergreen trees in Kundadri Hills situated near Agumbe Rain forest region. *Amanita griseofusca* described originally from Pakistan (Kiran et al. 2018) and the specimen KUBOT-KRMK-2020-78 in the present collection shows distinguishing characters; medium sized basidiomata, greyish brown pileus with dark brown disc, pale, thinner and striated towards margin, universal veil remnants present only on young basidiomes, creamy lamellale, broadly ellipsoid to globose basidiospores and the absence of clamp connections in all tissues. The characters are completely similar to the original description and the phylogenetic analysis of the species using a combined dataset of nrITS and nrLSU regions also shows similarity with the source sequences with good statistical support. The species are well recovered in *Amanita* sect. *vaginatae* clade and both are recorded for the first time in India.

Saprotrophic amanitaceous fungi are very distinct in their morphology, with respect to the available nutritional profile; the appearance of the sporocarps often varies. The genus *Saproamanita* is a group of saprotrophic amanitas, which is coined to replace the generic name *Aspidella* (Redhead et al. 2016). Some molecularly characterized species and closely classified grassland species are transferred to the genus *Saproamanita*. The present study illustrates two new records of *Saproamanita* with morphological and molecular phylogenetic relationship. *S. manicata* characterized by the creamy white pileus surface completely covered with ocher-orange grain-like remnants when young, which form triangular patches on maturity and strongly appendiculate-margin showing triangular appendages. The molecular characterization of the Indian collection revealed more than 80% bootstrap support and clustered with the collection from USA (RET 387-4) confirming the identity of species.

Saproamanita praeclara collected in India (Image 9) is unique with its thick pale-yellow to orange-yellow lanose-floccose cover all around the fruiting body of mushroom while the context being purely white and pileus is non-sulcate, appendiculate margin. The descriptions of the previously reported collections illustrate the dense shaggy white wool like covering on the surface of the sporocarp (Pearson 1950; Reid & Eicker 1991). There are some discussions over the years regarding the colour of fruiting body covering wooly substance (Tullos 2020), which often tend to be considered for describing the collection as a new species. However, the molecular characterization using nuclear gene sequences completes the discussion by solving the ambiguity in the identity of the species. The nrITS and nrLSU sequences of Indian collection KUBOT-KRMK-2020-02 shows more than 99% similarity with the collection RET 822-1 from the Herbarium Amanitarum Rooseveltensis and well recovered in the *Amanita* sect. *Lepidella* clade with considerable statistical support.

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SHORT COMMUNICATION

Distribution records of Dormer's Bat *Scotozous dormeri* (Dobson, 1875) (Mammalia: Chiroptera: Vespertilionidae) in Nepal

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Abstract: Dormer's Bat is endemic to southern Asia and distributed in tropical, semi-arid, or arid climatic zones in India, Pakistan, Bangladesh, and Nepal (Srinivasulu & Srinivasulu 2019). It is insectivorous in nature and plays an important role in the natural control of insects. Roost search and mist netting were conducted during early evening to 2200 h in three sites and the species was identified by field-based morphology including medium body size, pale gray brown color on the dorsal side, buffy white ventral surface, and face is necked and uniform mid-brown in color. A total of five individuals of *Scotozous dormeri* were recorded from east to west Nepal and the forearm ranged 34.4–36.00 mm in length. An individual was observed in a cavity in a pillar in a wooden house at Ramjhoda, Sunsari District. Two individuals each were trapped at the Morange River, Morang District in the east and Hattikhauwa, Dang District in the west. Three localities of the species' record lie in the dry and arid sub-tropical areas. This study records the second to fourth locality records of the species distribution to Nepal.

Keywords: Dry and arid sub-tropical area, endemic, mist netting, Morange River, roost.

Dobson (1875) reported a new species *Scotozous dormeri* (Dobson 1875) from Bellary hill, Karnataka, India. This species is medium in size (32.7–36.3 mm, n= 25) and tail is shorter than the head and body. The dorsal surface is greyish-brown with silvery hair tips with brown or black roots. The ventral surface is contrastingly paler with all the hair tips white or pale buffy white (Bates & Harrison 1997). Cavities and holes in buildings, trees, under the roof tiles of old constructions are major habitat of the species. It occurs in tropical, semi-arid or arid climatic zone and is found near to or within human settlements (Advani 1981; Sinha 1981; Bates & Harrison 1997; Molur et al. 2002; Srinivasulu & Srinivasulu 2019). However, the species has been observed in arid deciduous forest in Hyderabad, India (Molur et al. 2002). It is a solitary or colonial bat, the size of its colonies varies from two to 24 individuals (Agrawal 1973; Bates & Harrison 1997). Dormer's Bat is fully insectivorous in nature and hunts different species of insects seasonally and plays an importance role in the natural control of pests and other harmful insects (Bates & Harrison 1997; Molur et al. 2002).

It is endemic to southern Asia and is thus far reported from 101 locations in India, two locations each in Bangladesh & Pakistan, and a single location from Nepal (Bates & Harrison 1997; Khan 2001; Molur

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et al. 2002; Thapa et al. 2012; Srinivasulu & Srinivasulu 2019). India has most distribution records of Dormer's Bat and it has been recorded from 25 states; Andhra Pradesh, Assam, Bihar, Goa, Gujarat, Haryana, Jammu & Kashmir, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharastra, Manipur, Megahalaya, Mijoram, Nagaland, Odisha, Punjab, Rajasthan, Tamil Nadu, Telangana, Tripura, Uttarakhand, Uttar Pradesh, and West Bengal (Bates & Harrison 1997; Molur et al. 2002; Srinivasulu & Srinivasulu 2019). The species has been reported from old temples of Rajshahi in western Bangladesh and a railway station at Dinajpur of northern Bangladesh (Khan 2001). Sialkot and Shikarpur were recorded localities in Pakistan (Bates & Harrison 1997). In Nepal, a male individual of Dormer's Bat was reported from southeastern Kusaha of Koshi Tappu wildlife reserve, Nepal (Thapa et al. 2012). Steamer Ghats, Bangladesh, and Sindh Pakistan are eastern- and western-most record of the species. Likewise Murapanadu Tamil Nadu, India and Firozpur, Punjab, India are southern- and northern-most distribution record of Dormer's Bat till now (Bates & Harrison 1997; Khan 2001; Molur et al. 2002; Srinivasulu & Srinivasulu 2019). It is distributed from sea level to 2,000 m (Bates & Harrison 1997; Srinivasulu & Srinivasulu 2019). Here we add three new distribution localities and second, third, and fourth records of the species from Nepal (Thapa et al. 2012).

Materials and methods

Study area

A total of three sites were surveyed in eastern to western regions of Nepal. Morange River, Letang Municipality, Morang District and Ramjhoda, Barahachettra Municipality, Sunsari District are two localities in the eastern region and Hattikhauwa, Tulsipur municipality, Dang District is in the western region of Nepal (Figure 1).

Morange River is the border of Letang Bazaar and Jante village, which is originated from Mahabharat range, however, the river is dry most of the time and is flooded during monsoon season. The surveyed locality in the Morange River lies at an elevation of 243 m. The locality is an arid subtropical area at the foothills of Churia range. Mist netting site is a small water canal and ditches at the eastern side of the river. Small patches of bamboo and paddy fields are in the surroundings. Ramjhoda,

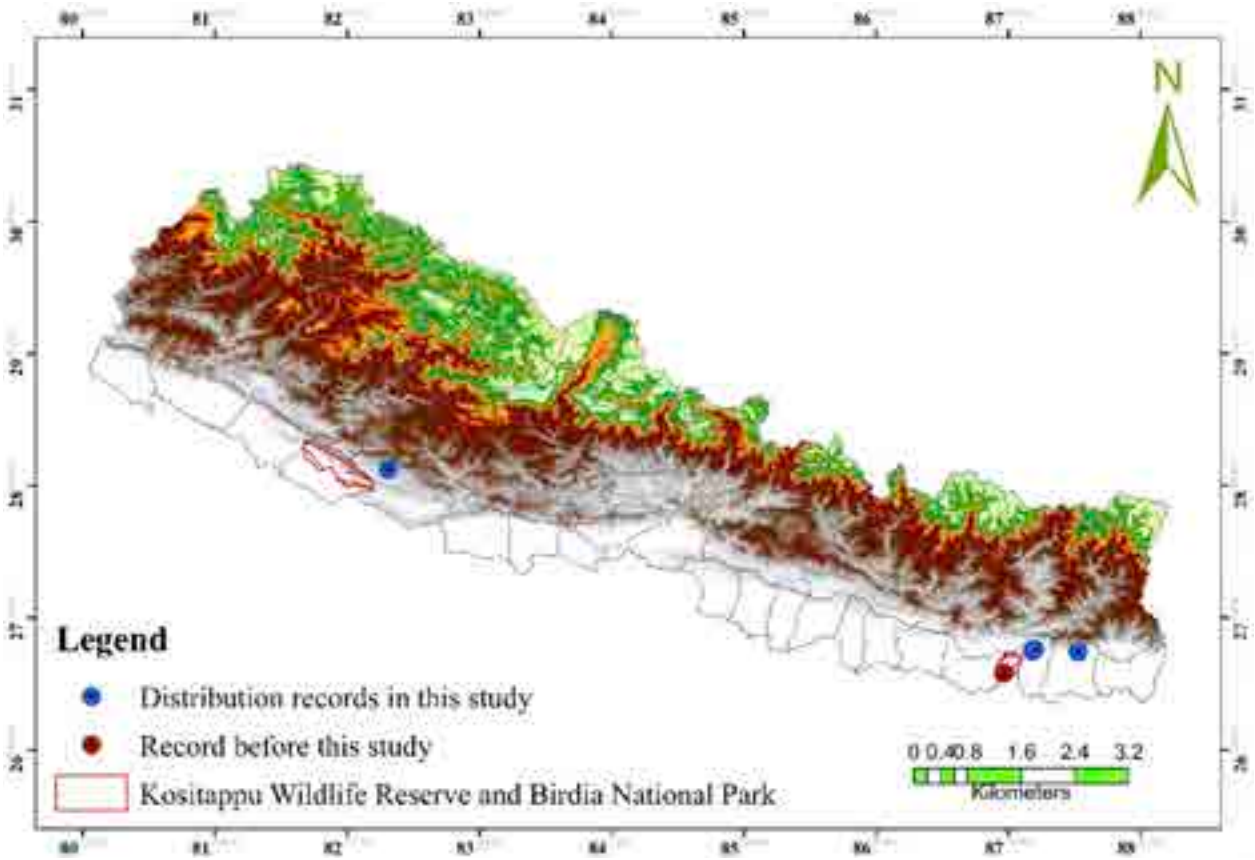

Figure 1. Distribution records of *S. dormeri* in Nepal.

Barahakshetra Municipality ward #5 in Sunsari District lies at an elevation of 119 m. The locality is also an arid area. It is a small village with traditional wooden houses in the human settlement surrounded by small patches of forest, fishponds and paddy croplands with water canals. Tulsipur municipality wad #6, Hattikhauwa is 635 m and in the eastern border of Tulsipur town. This locality is a small stream flowing through an arid area with red soil surrounded by patches of bamboo forest and maize and millet croplands.

Bat survey and identification

Field surveys were conducted during three months of January, October, and November 2020. Roost searches were conducted by direct observation of possible sites during daytime to select the mist netting sites in stream, pounds, walking trails or tree canopies. We searched for bats in cavities of wooden houses and huts, bamboo holes, old buildings, holes, and bark of dead trees. Cavity dwelling individuals were captured by gloved hands and released immediately after taking photographs and measurements of fore arm (FA) (Kunz et al. 2009). Six monofilament mist nets with 14 mm mesh size and of three sizes 6*3 m^2, 9*3 m^2 and 3*2.5 m^2 dimensions were installed over the stream, in the bank of pond, riverbank and edge of forest. At each site, mist-nets were opened at sunset just before the time of emergence of the bats (normally 1730 h) and closed after the bats activities came to an end (normally 2300 h) for a single night (Collins 2016). Captured bats were immediately taken out of mist-net by loose gloved hand without any stress. Morphology characters and morphometrics (forearm length 'FA') were recorded. Measurements of FA were taken by FREEMANS IP54 digital vernier caliper. Captured bats were photographed from dorsal, ventral and lateral views using 18–55 mm and 75–300 mm lens (Cannon EOS 1100D). Bats were carefully released soon after handling. Bat capture and handling methods followed standard procedures and recommendations described in Kunz & Parsons (2009). The species was identified in the field based upon the morphological characters and morphometrics (Bates & Harrison 1997; Molur et al. 2002; Acharya et al. 2010; Srinivasulu et al. 2010; Thapa et al. 2012).

Results

Four species of 18 bats were tapped in Hattikhuwa and Morange River survey stations but no bat was trapped in Ramjhoda (Table 1). Two males of *S. dormeri*, two individuals (male and female each) of *Scotophillus heathii* were trapped at Hatikhauwa and two individuals (male and female each) of *S. dormeri*, one male *Megaderma lyra*, one female *Cynopterus sphinx*, and 11 individuals (four male and seven female) of *S. heathii* were trapped in Morange River site. Densities of flight were extremely high from early evening until about two hours after sun set. All captured bats were released immediately after taking photographs and measurement without any stress.

Two roosts of *Pipistrellus* sp. and a roost of *S. dormeri* were reported from Ramjhoda. A colony of 10 individuals of *C. sphinx* was found in the canopy of *Saracaasoca* in Sikha School, more or less one kilometer west of the mist netting site at Tulsipur Municipality # 4 (Table no. 1). A colony of *Pipistrellus* sp. in a bamboo hollow of a cattle shed near the house was found and two male individuals

Table 1. Bat species identification three survey stations with sex, forearm (FA) length and types of survey.

	Date	Location	Species of bats	Ind. & sex	FA length (mm)	Type of survey	Elevation (meter)
1	22.i.2020	Ramjhoda, Barahachettra, Sunsari	*Pipistrellus* sp.	Male		Roost search	107
2	22.i.2020	Ramjhoda, Barahachettra, Sunsari	*Scotophilus heathii*	1 Ind.		Roost search	119
3	22.i.2020	Ramjhoda, Barahachettra, Sunsari	*Scotozous dormeri*	1 Male	34.31	Roost search	122
4	23.i.2020	Ramjhoda, Barahachettra, Sunsari	*Pipistrellus* sp.	1 Male		Roost search	118
5	4.x.2020	Hattikhauwa, Tulsipur, Dang	*Scotophilus heathii*	1 Male, 1 Female	Male: 63.39 & Female: 64.72	Mist netting	635
6	4.x.2020	Hattikhauwa, Tulsipur, Dang	*Scotozous dormeri*	2 Males	Male1: 36.01 & Male2: 34.64	Mist netting	636
7	5.x.2020	Hattikhauwa, Tulsipur, Dang	*Cynopterus sphinx*	10 Ind.		Roost search	663
8	4.xi.2020	Morange River, Letang, Morang	*Scotophilus heathii*	4 Male, 7 Female	Male1: 61.91 & Female1: 61.67	Mist netting	243
9	4.xi.2020	Morange River, Letang, Morang	*Cynopterus sphinx*	1 Female		Mist netting	243
10	4.xi.2020	Morange River, Letang, Morang	*Megaderma lyra*	1 Male		Mist netting	243
11	4.xi.2020	Morange River, Letang, Morang	*Scotozous dormeri*	1 Male, 1 Female	Male: 34.65 &Female: 34.04	Mist netting	243

Image 1. A—*S. dormeri* captured in Ramjhoda 1.i.2011 | B—*S. dormeri* in Ramjhoda 22.i.2020 | C—*S. dormeri* Morange River 11.iv.2020 | D—*S. dormeri* Hattikhauwa 10.iv.2020. © A—Dibya Raj Dahal | B–D—Sanjan Thapa.

was captured and measurement of FA and close up photographs were taken and then released immediately at Ramjhoda. A male individual of *Pipistrellus* sp. was roosting in a cavity of the wooden ceiling of a house, 500 m east of the first colony. A single individual of the Greater Asiatic Yellow House Bat *S. heathii* was found in a cavity of a bamboo cottage close to the second colony of *Pipistrellus* sp. This was identified by direct observation and photography without capture. Male *S. dormeri* was found in a small cavity in a wooden pillar of an old house in southern Ramjhoda.

Five individuals (four males and one female) of Dormer's Bats were captured in Morange River, Letang, Morang District; Ramjhoda, Barahachetrra, Sunsari

District and Hattikhauwa, Tulsipur, Dang District. Four of them were trapped by mist netting in Morang and Dang districts whereas an individual was found roosting in Sunsari District (Table 1). A male was trapped in early evening and a female was netted in 2100 h at Morange River whereas both were netted in early evening just after sunset in Hattikhauwa. Forearm lengths of the five individuals measured were 34.04–36.01mm (n= 5, Mean= 34.04, Sd= 0.7591) (Table 1). Dorsal pelage was greyish-brown with silvery hair tips and ventral pelage was pale with buffy white hair tips (Image 1 A & B). Ear, face, and membranes of all individuals were brownish in color (Image 1C). Wing and inter femoral membrane were naked (Image 1C,D).

Discussion

After the first national record of *S. dormeri* by Thapa et al. (2012), the second locality record of Dormer's Bats from Nepal was found about 20 km north-east from the first locality record at Paschim Kusaha. Another roost of the species was reported from a wooden house at southern Ramjhoda in 22 January 2020. The latter locality is 300 m east of the second record at Ramjhoda, which is the third report of the species in the country. The species at Ramjhoda was found in narrow cavities of wooden pillar of old wooden houses. A mature male and female of Dormer's bats were trapped in Morange River, Letang, Morang District on 4 November 2020, which is the eastern most record from Nepal. Morange River is around 36 km and 50 km east from Ramjhoda and and Paschimkusaha, respectively. Two male individuals were captured in Hattikhauwa, Dang District in 4 October 2020, which is the fourth locality record and is 540 km west from Morange River and it is also the westernmost record for Nepal (Figure 1). Dormer's bats were roosting in cavities, cracks, holes of old building, temple and huts (Molur et al. 2002; Thapa et al. 2012; Srinivasulu & Srinivasulu 2019). The recent records were also found roosting in cavities of wooden pillars of old wooden houses at Ramjhoda. Thapa et al. (2012) reported the species from the bamboo hollow in a hut at Paschim Kusaha.

S. dormeri is distributed in tropical, semi-arid or arid climatic zone of near or within human settlements (Srinivasulu & Srinivasulu 2019). All reported sites during the current surveys of Dormer's bat were arid and dry red soil. Suburban areas which contain wooden houses and agricultural terraces and croplands surrounded the surveyed sites. *Noelamarkia cadamba*, *Ficus benjamina* and *Pseudosasa japonica* were the dominant vegetation in surveyed sites throughout Nepal. *C. sphinx* and *S. kuhlii* were reported from the vicinity of the Dormer's Bat recorded sites.

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SHORT COMMUNICATION

A report on the butterfly (Lepidoptera: Rhopalocera) diversity of the Upper Ganga River Ramsar site in Uttar Pradesh, India

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Abstract: This study provides a primary inventory of the butterfly diversity of the Upper Ganga River Ramsar site in Uttar Pradesh, India. The study was carried out in two phases, first in March 2019 and then in November 2019. A total of 44 species of butterflies belonging to 34 genera and five families were seen in the area. The species observed in the study site belonged to the families Hesperiidae (4 genera, 4 species), Lycaenidae (4 genera, 4 species), Nymphalidae (18 genera, 24 species), Papilionidae (2 genera, 4 species), and Pieridae (6 genera, 8 species). Three of these species are legally protected under various schedules of the Indian Wildlife Protection Act (1972).

Keywords: Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, scheduled species, wetland.

There are a total of 42 wetlands in India covering a total of 1,081,438 ha of the country's geographic surface area that have been recognised as Ramsar sites (Ramsar Sites Information Service 2021). Since the life cycle of a terrestrial pollinator insect is not dependent on the aquatic ecosystem in most cases, it is assumed that their diversity in wetlands and riparian areas will always be low, but Begosh et al. (2020) observed that there was little difference in abundance and richness of pollinators between uplands and wetlands. However, there have been only a few studies on the butterfly diversity of Indian Ramsar sites. Trigunayat & Singh (1998) reported 35 butterfly species in Keoladeo National Park, Rajasthan and Palot & Soniya (2000; 2001) reported 40 species under seven families. Chowdhury & Soren (2011) reported the presence of 74 species of butterflies under six families from East Calcutta Wetlands, Kolkata. Thakur et al. (2010) reported the presence of 50 species of butterflies under eight families from the Ropar wetland, Punjab. Following that, Sharma et al. (2014) and Narender (2017) observed *Elymnias hypermnestra* and *Megisba malaya* in the Ropar wetland. Sarath et al. (2017) reported the presence of 50 species of butterflies under eight families from Kole Wetlands, Kerala. So far, no studies have been conducted to document the diversity of butterflies in the Upper Ganga River Ramsar

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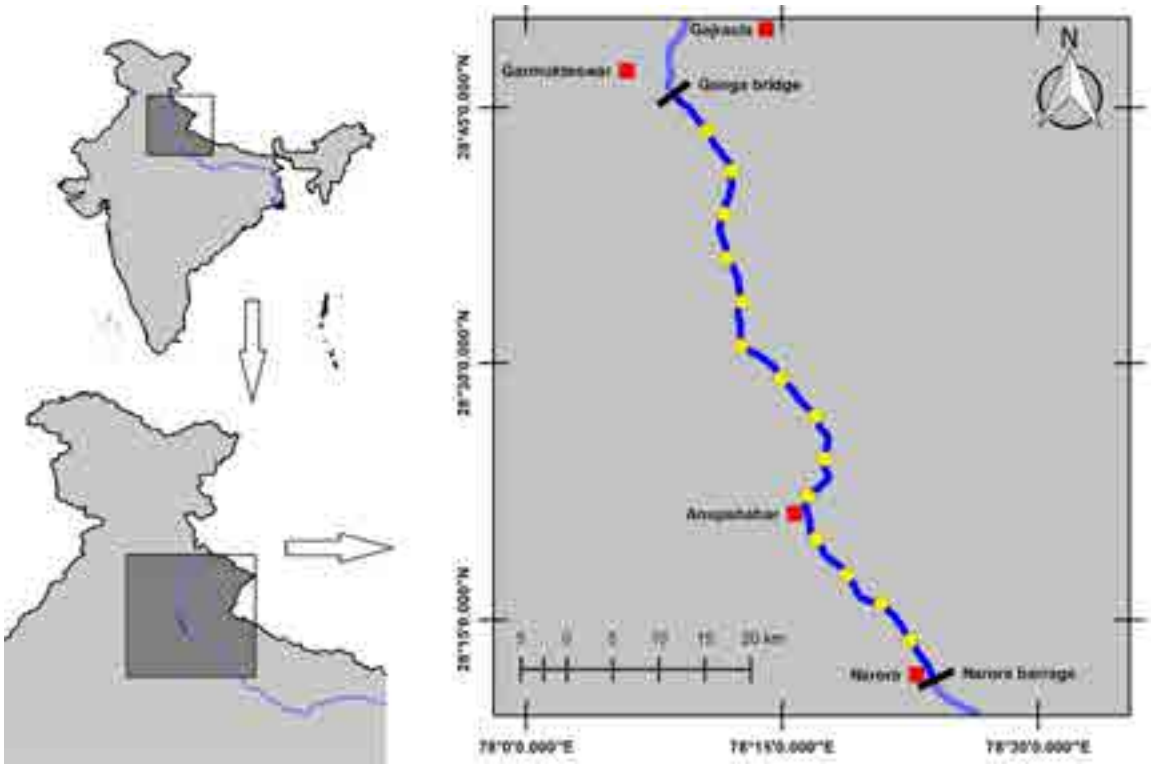

Figure 1. Location of Upper Ganga river Ramsar site (dark blue line) between Ganga bridge at Garmukteswar and Narora barrage. Red squares represent nereast major towns and yellow circles reresent sampling lications.

site. The current study was carried out in order to create a primary inventory of butterflies of the Upper Ganga River Ramsar site.

Materials and Methods

The study was carried out first in March 2019 and then again in November 2019 along an 85-km stretch of the Ganga River in Uttar Pradesh, from Brijghat to Narora (Figure 1). This section of the river was designated as a Ramsar site in 2005 (Ramsar site no. 1574). It is India's only Ramsar site in the lotic (river) wetland category (Murthy et al. 2013). This stretch is shallow with deep water pools that are home to many conservation-worthy species such as Gangetic River Dolphins, Gharials, crocodiles, six species of turtles, otters, 82 species of fish, and hundreds of bird species (Ramsar Sites Information Service 2021). Agriculture is the most important land use in this region (Prasad et al. 2021). A total of 14 study sites were selected at 5-km intervals along the left bank of the river. A 100-m stretch was chosen at each site to record butterfly species. Sampling was conducted at each study site between 0930 h and 1330 h.

The opportunistic sampling method was used to visually record the species with binoculars. Butterflies that could not be identified in flight were captured with a butterfly net, identified, and safely released. The butterflies were identified in the field using field guides (Evans 1932; Wynter–Blyth, 1957; Kunte 2000; Kehimkar 2008). For further confirmation, a photograph of the documented species was taken during the survey using a DSLR camera. Scientific names of butterflies were followed as per Varshne & Smetacek (2015).

Results

A total of 44 species of butterflies belonging to 34 genera and five families (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, and Pieridae) were found in the study area (Table 1). The butterflies under the family Nymphalidae was most abundant with 24 species (54.545 % of total species) and 18 genera (52.941 % of total genera), followed by family Pieridae with eight species (18.182 % of total species) and six genera (17.647 % of total genera), family Lycaenidae with four species (9.091 % of total species) and four genera (11.765 % of total genera), family Hesperiidae with four species (9.091 % of total species) and four genera (11.765 % of total genera), and family Papilionidae with four species (9.091 % of total species) and two genera (5.882 % of total genera) (Figure 2).

Among these butterflies, three species are listed in

Table 1. List of butterflies reported from the Upper Ganga River Ramsar site.

Family	Scientific name	Authority	Common name	WPA status
Hesperiidae	*Matapa aria*	(Moore, [1866])	Common Branded Redeye	
Hesperiidae	*Parnara* sp.			
Hesperiidae	*Pelopidas* sp.			
Hesperiidae	*Udaspes folus*	(Cramer, [1775])	Grass Demon	
Lycaenidae	*Euchrysops cnejus*	(Fabricius, 1798)	Gram Blue	Schedule II (Part II)
Lycaenidae	*Chilades lajus*	(Stoll, [1780])	Lime Blue	
Lycaenidae	*Tarucus balkanica nigra*	Bethune-Baker, [1918]	Black-spotted Pierrot	
Lycaenidae	*Zizeeria karsandra*	(Moore, 1865)	Dark Grass Blue	
Nymphalidae	*Acraea terpsicore*	(Linnaeus, 1758)	Tawny Coster	
Nymphalidae	*Ariadne merione*	(Cramer, [1777])	Common Castor	
Nymphalidae	*Cupha erymanthis*	(Drury, [1773])	Rustic	
Nymphalidae	*Cyrestis thyodamas*	Doyère, [1840]	Map Butterfly	
Nymphalidae	*Danaus chrysippus*	(Linnaeus, 1758)	Plain Tiger	
Nymphalidae	*Danaus genutia*	(Cramer, [1779])	Striped Tiger	
Nymphalidae	*Euploea mulciber*	(Cramer, [1777])	Striped Blue Crow	Schedule IV
Nymphalidae	*Hypolimnas bolina*	(Linnaeus, 1758)	Great Eggfly	
Nymphalidae	*Hypolimnas misippus*	(Linnaeus, 1764)	Danaid Eggfly	Schedule II (Part II)
Nymphalidae	*Junonia almana*	(Linnaeus, 1758)	Peacock Pansy	
Nymphalidae	*Junonia atlites*	(Linnaeus, 1763)	Grey Pansy	
Nymphalidae	*Junonia iphita*	(Cramer, [1779])	Chocolate Pansy	
Nymphalidae	*Junonia lemonias*	(Linnaeus, 1758)	Lemon Pansy	
Nymphalidae	*Junonia orithya*	(Linnaeus, 1758)	Blue Pansy	
Nymphalidae	*Kaniska canace*	(Linnaeus, 1763)	Blue Admiral	
Nymphalidae	*Libythea myrrha*	Godart, 1819	Club Beak	
Nymphalidae	*Melanitis leda*	(Linnaeus, 1758)	Common Evening Brown	
Nymphalidae	*Mycalesis* sp.			
Nymphalidae	*Neptis soma*	Moore, 1858	Creamy Sailer	
Nymphalidae	*Parantica aglea*	(Stoll, [1782])	Glassy Tiger	
Nymphalidae	*Phalanta phalantha*	(Drury, [1773])	Common Leopard	
Nymphalidae	*Symbrenthia lilaea*	(Hewitson, 1864)	Common Jester	
Nymphalidae	*Vanessa cardui*	(Linnaeus, 1758)	Painted Lady	
Nymphalidae	*Ypthima huebneri*	Kirby, 1871	Common Four-ring	
Papilionidae	*Pachliopta aristolochiae*	(Fabricius, 1775)	Common Rose	
Papilionidae	*Papilio demoleus*	(Linnaeus, 1758)	Lime Swallowtail	
Papilionidae	*Papilio polymnestor*	Cramer, 1775	Blue Mormon	
Papilionidae	*Papilio polytes*	Linnaeus, 1758	Common Mormon	
Pieridae	*Cepora nerissa*	(Fabricius, 1775)	Common Gull	
Pieridae	*Catopsilia pyranthe*	(Linnaeus, 1758)	Mottled Emigrant	
Pieridae	*Delias eucharis*	(Drury, 1773)	Common Jezebel	
Pieridae	*Ixias marianne*	(Cramer, [1779])	White Orange Tip	
Pieridae	*Ixias pyrene*	(Linnaeus, 1764)	Yellow Orange Tip	
Pieridae	*Pareronia hippia*	(Fabricius, 1787)	Indian Wanderer	
Pieridae	*Pieris brassicae*	(Linnaeus, 1758)	Large Cabbage White	
Pieridae	*Pieris canidia*	Sparrman, 1768	Asian Cabbage White	

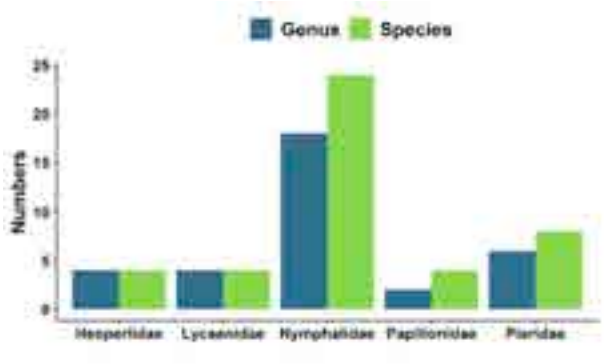

Figure 2. Comparative accountof numbers of genus and species of butterflies under five families found from the Upper Ganga River Ramsar site

Figure 3. Percentage of genus and species of butterflies under five families found from the Upper Ganga River Ramsar site.

the schedules of the Wildlife Protection Act (WPA), 1972. Among these three species one species: *Euchrysops cnejus* (Fabricius, 1798) belongs to the family Lycaenidae and two species: *Euploea mulciber* (Cramer, [1777]) and *Hypolimnas misippus* (Linnaeus, 1764) belong to the family Nymphalidae.

Discussion

In comparison to other ecological elements, the terrestrial arthropod fauna of wetlands has received little attention from researchers (Batzer & Wu 2020). Butterflies are a major herbivore group in terrestrial ecosystems but they are also common in riparian ecosystems because they actively use riparian habitats for nectar and larval food, and they can be used as an indicator group for riparian ecosystem assessment (An & Choi 2021).

Since butterflies are pollinators of their nectar plants as well as indicators of the health and quality of their host plants (Tiple et al. 2006) and the ecosystem as a whole, exploration of butterfly fauna is important in identifying and preserving potential habitats under threat. The presence of the Upper Ganga River Ramsar site, a wetland of international significance and India's only riverine Ramsar site, it is practically equivalent to the presence of a 'spring in a desert'. The river Ganga experiences different anthropogenic dangers throughout its course causing habitat degradation, which makes the preservation of a Ramsar site even more critical for species survival that cause the natural habitat of several aquatic and riparian biota to decline, however this region secures them.

Despite its immense ecological importance, the entomofauna of the Upper Ganga River Ramsar site is poorly documented. From this area, De et al. (2021) reported presence of 29 species of aquatic insects, including three species of Coleoptera, four species of Hemiptera, and 22 species of Odonata. For the first time, this study found 44 species of butterflies from 34 genera and five families in this area. Because the butterfly fauna of Indian Ramsar wetlands is largely unknown, the findings of this study contribute to our understanding of butterfly biodiversity in them.

The current list of butterfly species is non–exhaustive, and further detailed studies encompassing all seasons, variety of host and nectar plants, and other influential factors is recommended for creating favourable environments to sustain butterfly diversity in this wetland ecosystems.

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Image 1. Butterfly reported from the Upper Ganga River Ramsar site: 1—*Matapa aria* (Moore, [1866]) | 2—*Parnara* sp. | 3—*Pelopidas* sp. | 4—*Udaspes folus* (Cramer, [1775]) | 5—*Euchrysops cnejus* (Fabricius, 1798) | 6—*Chilades lajus* (Stoll, [1780]) | 7—*Tarucus balkanica nigra* Bethune-Baker, [1918] | 8—*Zizeeria karsandra* (Moore, 1865) | 9—*Acraea terpsicore* (Linnaeus, 1758).

Image 2. Butterfly reported from the Upper Ganga River Ramsar site: 10—*Ariadne merione* (Cramer, [1777]) | 11—*Cupha erymanthis* (Drury, [1773]) | 12—*Cyrestis thyodamas* Doyère, [1840] | 13—*Danaus chrysippus* (Linnaeus, 1758) | 14—*Danaus genutia* (Cramer, [1779]) | 15—*Euploea mulciber* (Cramer, [1777]) | 16—*Hypolimnas bolina* (Linnaeus, 1758) | 17—*Hypolimnas misippus* (Linnaeus, 1764) | 18—*Junonia almana* (Linnaeus, 1758).

Image 3. Butterfly reported from the Upper Ganga River Ramsar site: 19—*Junonia atlites* (Linnaeus, 1763) | 20—*Junonia iphita* (Cramer, [1779]) | 21—*Junonia lemonias* (Linnaeus, 1758) | 22—*Junonia orithya* (Linnaeus, 1758) | 23—*Kaniska canace* (Linnaeus, 1763) | 24—*Libythea myrrha* Godart, 1819 | 25—*Melanitis leda* (Linnaeus, 1758) | 26—*Mycalesis* sp. | 27—*Neptis soma* Moore, 1858.

Image 4. Butterfly reported from the Upper Ganga river Ramsar site: 28—*Parantica aglea* (Stoll, [1782]) | 29—*Phalanta phalantha* (Drury, [1773]) | 30—*Symbrenthia lilaea* (Hewitson, 1864) | 31—*Vanessa cardui* (Linnaeus, 1758) | 32—*Ypthima huebneri* Kirby, 1871 | 33—*Pachliopta aristolochiae* (Fabricius, 1775) | 34—*Papilio demoleus* (Linnaeus, 1758) | 35—*Papilio polymnestor* Cramer, 1775 | 36—*Papilio polytes* Linnaeus, 1758.

Image 5. Butterfly reported from the Upper Ganga river Ramsar site: 37—*Cepora nerissa* (Fabricius, 1775) | 38—*Catopsilia pyranthe* (Linnaeus, 1758) | 39—*Delias eucharis* (Drury, 1773) | 40—*Ixias marianne* (Cramer, [1779]) | 41—*Ixias pyrene* (Linnaeus, 1764) | 42—*Pareronia hippia* (Fabricius, 1787) | 43—*Pieris brassicae* (Linnaeus, 1758) | 44—*Pieris canidia* Sparrman, 1768.

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SHORT COMMUNICATION

Case report of hook worm *Grammocephalus hybridatus* and stomach bot *Cobboldia elephantis* infections in a free-ranging Asian Elephant *Elephas maximus* in Tamil Nadu, India

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Abstract: Elephants in the wild are susceptible to many gastrointestinal parasites. In the present study, necropsy was conducted on a free-ranging Asian Elephant *Elephas maximus* female aged about 15 years which died at Coimbatore forest range, Tamil Nadu state, India. The necropsy revealed that the liver was infected with round worms and the stomach was heavily infested with dipteran larvae. These round worms and larvae were collected and processed by dehydrating in ascending grades of alcohol and then cleared in carbolic acid. The cleared samples were mounted and examined under light microscopy for species identification. Faecal samples collected from the rectum were analysed by sedimentation for the presence of helminth eggs. On microscopic examination, the head end of the round worms showed a buccal capsule which possessed a pair of semilunar ventral cutting plates. Male worms showed well-developed bursa at the posterior end. The anterior end of the dipteran larvae showed two powerful oral hooks with cephalopharyngeal skeleton. Anterior spiracle appeared as a short club-shaped tube with 12 lobes. The abdominal segments of the larvae had a row of belt-like triangular spines. The posterior spiracles of the larvae had three longitudinal parallel slits in each spiracle with closed peritreme. Based on the above morphological characters, the round worms and larvae were identified as *Grammocephalus hybridatus* and *Cobboldia elephantis*, respectively. Strongyle eggs were identified in the faecal sample based on the morphology of thin shell and segmented yolk. This appears to be the first report of *G. hybridatus* infection in a free-ranging elephant in Tamil Nadu state, India.

Keywords: Dipteran larvae, faecal samples, gastrointestinal parasites, helminth eggs, infection, morphological characters, necropsy, strongyle egg, round worms.

The life of the wild animals is threatened by many factors such as cannibalism, infighting injuries, accidents, habitat loss & fragmentation, poaching & hunting, as well as different diseases (Riddle et al. 2010). Furthermore, parasitism has an impact on host species evolution and ecology through sexual selection (Hamilton & Zuk 1982), and parasite-mediated competition results in reduced population size or extinction (Price et al. 1986). The Asian Elephant *Elephas maximus* is an 'Endangered' species as per the IUCN Red List of Threatened Species (Williams et al. 2020). Free-living wild animals are generally infected with numerous parasites, but adverse effects occur only in the animals that are physiologically or nutritionally stressed (Gaur et al. 1979; Fowler & Mikota 2006). Elephants in the wild are susceptible to many gastrointestinal (GI) parasites (Vidya & Sukumar 2002). According to investigations, many gastrointestinal parasites have been found in Indian and Sri Lankan elephants: *Murshidia murshidia, M. falcifera, M. indica, M. longicaudata, Quilonia renniei, Equinurbia sipunculiformis, Decrusia aditicta, Amira pileata* (Seneviratna 1955; Fernando & Fernando 1961;

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Sundaram et al. 1971), *Choniangium epistomum* (Datta et al. 1972), *Bathmostomum sangeri* (Sathianesan et al. 1979), *Grammocephalus hybridatus* (Islam & Talukdar 2014), and larvae of *Cobboldia elephantis* (Seneviratna & Jayasinghe 1968). Strongyles are the most frequent GI parasites in African (Scott & Dobson 1989) and wild Asian Elephants (Watve 1995; Vidya & Sukumar 2002; Dharmarajan et al. 2005; Nishanth et al. 2012; Abeysekara et al. 2018).

Parasitic infections can cause disease and death in wild animals and they can also infect domestic animals and vice versa. Parasites also alter the host's behaviour, health and reproduction, and make parasite transmission easier (Abhijit et al. 2018). Understanding infections in wild animals is critical because illnesses can cause elephants to die under extremely stressful situations. Hence, it is important to monitor the mortalities of wild elephants and carry out post-mortem sampling to determine whether the cause of death was due to GI parasitic infections (Vidya & Sukumar 2002; Lynsdale et al. 2017). This study reports the occurrence of *Grammocephalus hybridatus* worms in the liver and *Cobboldia elephantis* larvae in the stomach collected during the post mortem of a free-range elephant in Tamil Nadu.

Materials and Methods

During August 2020, the death of a wild female elephant (approximately 15 years of age) was reported in the Coimbatore forest range, Tamil Nadu. Necropsy was carried out by the forest veterinary officer. On detailed examination, the liver was found to be infected with round worms and the stomach was heavily infested with dipteran larvae. These round worms and larvae were collected and sent in 10% formalin to the Department of Veterinary Parasitology, Madras Veterinary College for further species identification. Faecal sample collected directly from the rectum was also sent to analyse the sample for helminth eggs.

Processing of the samples

The round worms were washed in water, dehydrated in ascending grades of alcohol, and then cleared using carbolic acid. Cleared specimens were mounted using DPX and examined under light microscope. The species identification of the round worm was carried out based on van der Westhuysen (1938) and Kumar et al. (2011). The dipteran larvae were boiled in 10% sodium hydroxide solution. They were kept in the same solution for one week. Later, they were dehydrated and cleared. The larvae were identified based on the morphological features described by Zumpt (1965). The faecal sample was analyzed using sedimentation and flotation technique as per the standard protocol (Soulsby 1982) and examined under light microscope for the presence of helminth eggs.

Results

In the present study, the round worms recovered from the elephant liver were identified as *Grammocephalus hybridatus*. The male worms measured 35 mm and 1.5 mm while the females were 37 mm and 1.5 mm in length and breadth, respectively. Both the male and female worms were more or less equal in size (Image 1a,b).

Morphology of female worm

The head end of the worms was characteristically curved towards the dorsum. The buccal capsule was wide anteriorly and narrowed posteriorly and possessed a pair of small semilunar ventral cutting plates (Image 2a). At a depth of the buccal cavity, there was a dorsal cone. The dorsal cone did not reach half the length of the buccal capsule. It was slender and curved (Image 2b). The oesophagus was long with a caecal diverticulum extending towards the pharynx (Image 2c). The vulva was present close to the middle of the body (Image 2d). The width of the worm reached a maximum at the vulvar region, then tapered to both ends. The tail end of the female was slightly bent dorsad and tapered to the posterior extremity with a slight knob-like structure at the end (Image 2e).

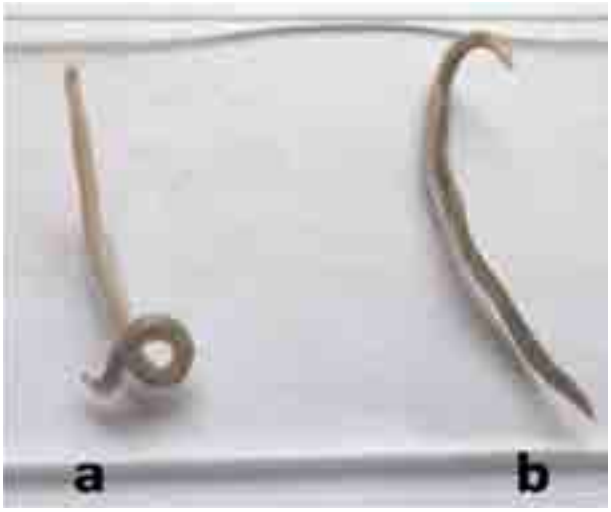

Image 1. Gross appearance of male (a) and female (b) worms of *Grammocephalus hybridatus* isolated from a free-ranging Asian Elephant in Tamil Nadu showing equal size. © Authors

Image 2. Internal morphology of female worm: a—Lateral view of the anterior end of a female worm (10x), arrows indicate cutting plate | b—Lateral view of the buccal cavity (40x), arrow indicates dorsal cone | c—Lateral view of worm showing oesophagus (upper arrow) with caecal diverticulum extending towards pharynx (lower arrow) (10x) | d—Vulvar opening (Arrow) (10x) | e—Lateral view of posterior end female worm (10x) with knob (lower arrow) and anus (upper arrow). © Authors

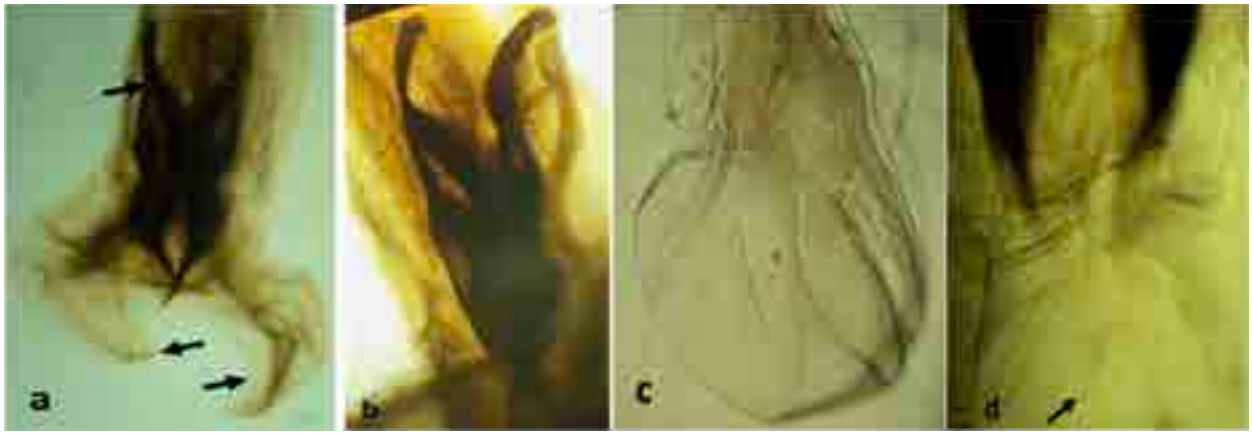

Image 3. Internal morphology of male worm: a—Ventral view of caudal end of worm showing well developed bursa with lateral lobes (opposing arrows) and spicules (upper arrow) (10x) | b—Pair of spicules (40x) | c—Lateral view of bursa (10x) | d—Dorsal view of bursa showing bifid tip of external branch of dorso-dorsal ray (Arrow) (40x). © Authors

Morphology of male worm

Male worms showed well-developed bursa at the posterior end (Image 3a). The spicules were equal in length (1.4 mm). They were stout, wavy, contained a dark core and were alate with ornamentations. Their tips were pointed and placed together (Image 3b). The lateral lobe was elongated when compared to the dorsal lobe; the bursal rays were relatively short and the lateral rays were quite stout (Image 3c). In the dorsal lobe, the external-dorsal rays were elongated; dorso-dorsal rays arose directly from the base of the dorsal lobe. Tip of the dorsal rays were bifurcated with the external branch ending in a cup-like structure (Image 3d).

Faecal examination

Strongyle eggs were identified in the faecal sample based on the morphology of egg showing thin shell and segmented yolk (Image 4); the same has been

Image 4. Strongyle eggs isolated from a free-ranging Asian Elephant in Tamil Nadu showing thin shell and segmented yolk (40x). © Authors

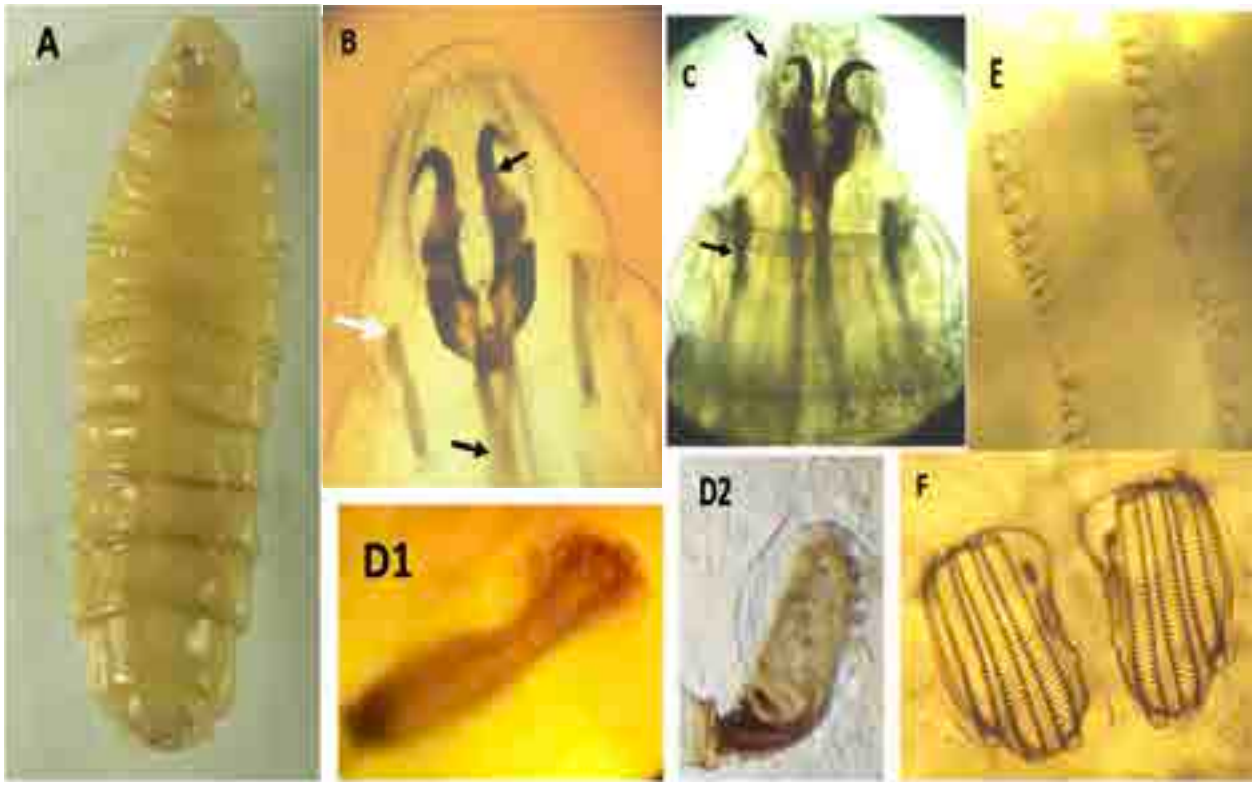

Image 5. A—Larva of *Cobboldia elephantis* (stomach bot) under stereozoom microscope | B—The anterior end showing two powerful oral hooks with cephalopharyngeal skeleton (black arrows) and anterior spiracle (white arrow) (40x) | C—The mouth cavity is surrounded by a crown of teeth (upper arrow) which are somewhat shorter ventrally, the anterior spiracles (lower arrow) are also seen (40x) | D1—Lateral view of anterior spiracle showing a short, club shaped tube (100x) | D2—Ventral view of anterior spiracle with 12 lobes (100x) | E—Abdominal segments of the bot with a row of belt like triangular shaped spines (40x) | F—The posterior spiracles showing three longitudinal parallel slits in each spiracle with completely closed peritreme (40x). © Authors

earlier described by van der Westhuysen (1938). Many investigators made similar observations in Asian Elephants from various places (Nishant et al. 2012; Abeysinghe et al. 2017; Abeysekara et al. 2018; Abhijit et al. 2018).

Morphology of dipteran larvae

In this study, the dipteran larvae collected from the elephant stomach were identified as stomach bot *Cobboldia elephantis* (Cobbold, 1866) based on the morphological characters of the anterior end, body spines and posterior spiracles as per the descriptions of Zumpt (1965). A total of 400 stomach bots (2–2.8 cm long) were collected from the stomach (Image 5A). The anterior end of the larva showed two powerful oral hooks with cephalopharyngeal skeleton (Image 5B). The mouth cavity was surrounded by a crown of teeth which was somewhat shorter ventrally (Image 5C). Anterior spiracle appeared as a short club-shaped tube with 12 lobes (Image 5D1,D2). The abdominal segments of the larvae had a row of belt-like triangular shaped spines (Image 5E). The posterior spiracles of the larvae showed three longitudinal parallel slits in each spiracle with closed peritreme following processing (Image 5F).

Discussion

The present communication records the morphological features with specific characters that assisted in the identification of *G. hybridatus* and *C. elephantis*. *Grammocephalus hybridatus* is a hookworm inhabiting the bile duct of elephants and immature stages are present in the intestine causing nodules which is similar to *Bunostomum* sp. (Fowler & Mikota 2006). Elephants get infected through skin penetration or by direct ingestion of larvae. They are the largest hook worms under the family Ancylostomidae (Sundaram 1966). *G. clathratus, G. hybridatus, G. varedatus*, and *G. intermedius* are the four species in the genus *Grammocephalus*, each of which is unique to a single host (Obanda et al. 2011). *G. clathratus* was observed in the liver and bile duct of African Elephants in Kenya by Obanda et al. (2011). *G. varedatus* and *G. hybridatus* infect Indian Elephants, while *G. intermedius* lives in the large intestine of African Rhinoceros (van der

Westhuysen 1938). The adult parasites are blood suckers which cause anaemia and weakness along with other signs of hepatic insufficiency when occurring as heavy infection (Fowler & Mikota 2006).

The study of the morphology of *G. hybridatus* was in accordance with the description of van der Westhuysen (1938) from Asian Elephant origin, with slight variations in morphometry. Bhalearo (1935) first recorded the hookworm (*G. clathratus*) from India, while Rajasekhariah et al. (1975) reported the occurrence of both the immature and mature *G. hybridatus* from the stomach nodule of a captive Asian Elephant. Previously, Pillay et al. (1976) reported the occurrence of *G. hybridatus* from an elephant in Mysore while Islam & Talukdar (2014) reported *G. hybridatus* infection in a free-ranging Asian Elephant from Assam. Apart from *G. hybridatus*, elephants are affected with a variety of intestinal strongyle worms (Vidya & Sukumar 2002), implying that the strongyle eggs found in the faeces likely represented a diversity of species.

Vidya & Sukumar (2002) analyzed the faecal samples from wild elephants in Nilgiris, southern India and found that 86.8% of the samples were positive for parasite eggs. Strongyles were found in 40%, 16%, and 8% of samples from Mudumalai, Anamalai, and Sathyamangalam forests in southern India, respectively (Nishant et al. 2012). In Sri Lanka, wild elephants (93.3%) had a greater prevalence of parasite infection than semi-captive (55.0%) and captive elephants (25.0%) (Abeysekara et al. 2018). Mixed infections were also reported to be more common (47.1%) than single infections (21.2%) (Abeysekara et al. 2018). The strongyle eggs were found in 100% of wild elephants in Sri Lanka, according to Abeysinghe et al.(2017). The majority of strongylid nematodes have eggs that are morphologically indistinguishable and is referred to as strongyles. Because of their direct life cycle, nematodes are the most common and numerous helminths in elephants, as evidenced by the high species richness (Elsheikha & Obanda 2010). Intestinal parasites in general and strongyles in particular, appear to be common in wild elephants, but the prevalence appears to vary greatly between locations.

The morphology of *C. elephantis* larvae (stomach bot) in this study was in accordance with that observed by Panda et al. (2005), Venu et al. (2015), and Ananda et al. (2017) who reported the larvae of *C. elephantis* from the stomach of free-ranging wild elephants from different parts of country. The anterior spiracle of this larvae, however, has yet to be described. The anterior spiracle and crown of teeth around their mouth were described for the first time in this study. Such characters were identified based on the descriptions of the larva of the subfamily *Platycobboldia loxodontis*, which affects African Elephants (Zumpt 1965).

The pathogenesis of *C. elephantis* larvae in elephants has not been well studied and they appear to be well tolerated by the animals even in large numbers; however, it has been reported that the infested animals show symptoms of gradual emaciation, disinclination to feed and loss of condition (Raquib 1970). In the present case, major area of the stomach was attached with 2–2.8 cm sized larvae of *C. elephantis* similar to the observations by Panda et al. (2005) and Kakkassery et al. (2011). The gastric wall revealed congestion with tiny ulcers and significant inflammation of the gastric mucosa after these larvae was removed. Panda et al. (2005) had previously reported similar findings.

This appears to be the first report of *G. hybridatus* infection in a free-range elephant in the state of Tamil Nadu. The anterior spiracle and crown of teeth around the mouth of *C. elephantis* larvae were morphologically described for the first time in this work, based on descriptions of larvae from the same subfamily. The morphological characteristics, along with supporting figures, will be helpful in diagnosis.

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SHORT COMMUNICATION

Management of traumatic ulcerative keratitis in a Red Serow

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Abstract: Red Serow *Capricornis rubidus* is an elusive herbivore native to the montane forests of the Himalaya. Currently it is categorized as 'Vulnerable' in the IUCN Red List and placed under Schedule I species of the Wildlife Protection Act, 1972. A serow was presented with complaints of mild inappetence, irritability, sporadic scratching of head over the enclosure barrier and serous ocular discharge from the left eye. Based on detail clinical examination, the animal was diagnosed with unilateral conjunctivitis, corneal oedema, and ulcerative keratitis. A combination of Xylazine @ 1.5mg/ kg body weight and Zolatile® (zolazepam and tiletamine) @ 2.5 mg/kg body weight was used to chemically restrain the animal, followed by auriculo-palpebral nerve block using 1 ml of 2 % lignocaine hydrochloride. The affected eye was adequately cleaned with isotonic sterile Normal Saline solution (NSS). Combination of 0.5 ml of ceftriaxone and 0.5 ml of flubiprofen was injected into upper and lower palpebral conjunctiva. The cornea and the third eyelid flap were carefully sutured using 5-0 and 3-0 Vicryl, respectively. Finally, tarsorrhaphy was done using 2-0 nylon. Post-operative care consisted of an antibiotic regimen of Cephalaxin @ 20 mg/ kg body weight b-i.d-twice in a day for seven days along with probiotic supplement (Vizylac®) and Vitamin A capsules (Aquasol A®) orally once daily for the next 30 days. The animal showed complete recovery within 30 days of proper treatment, monitoring, care and management.

Keywords: Capricornis rubidus, Ceftriaxone, Flubiprofen, Vulnerable, Tarsorrhaphy.

The conjunctiva is a mucous membrane that covers the inner aspect of each eyelid (Palpebral conjunctiva) and the sclera of the eye (bulbar conjunctiva). Inflammation of this conjunctival mucous membrane is known as conjunctivitis (Kumari et al. 2016). It can be unilateral or bilateral and can be caused by various etiological factors like virus, bacteria, fungi, parasites, allergens (e.g., foreign proteins, pollen, drugs), irritant chemicals, and trauma (Gelatt 2014). Red Serow *Capricornis rubidus* is an elusive, mostly solitary (Prater 1993) herbivore belonging to the class Mammalia, order Artiodactylia, family Bovidae, and subfamily Caprinae. It is presently categorised as 'Vulnerable' in the IUCN Red List (Shepherd 2021) and as a schedule I species of the Wildlife Protection Act, 1972 (Aryal 2008). The serow is oriental in origin (Schaller 1979) and is found within the geographical boundaries of Jammu & Kashmir in India to Japan in the far east (Shackleton & Lovari 1997). The present paper discuss about a clinical case of traumatic ulcerative keratitis in a male serow of 3.5 years of age belonging to the Assam State Zoo and Botanical Garden, Guwahati, Assam. Surgical correction with tarsorrhaphy

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technique was employed to protect the cornea and to attain faster recovery of the affected cornea.

Case History and Observation

The affected serow was presented with a history of depression, slight inappetence, occasional scratching of the head over the enclosure barrier, and ocular discharge from the left eye.

Chemical restraining was planned in order to conduct better and clear clinical examination of the affected eye. On comprehensive clinical inspection, it was revealed that the serow was suffering from cloudiness of the cornea, redness and swelling of the conjunctiva along with muco-purulent ocular discharge (Image 1). All the physiological parameters were within the normal range. The probable aetiology is believed to be of traumatic origin inflicted by a sharp object or enclosure fence. On the basis of these clinical findings, a diagnosis of ulcerative keratitis, unilateral conjunctivitis, and corneal oedema was made. The serow responded well to te external stimuli, viz., menace reflex, direct papillary light reflex, and dazzle reflex. Fluorescent dye test revealed moderate ulcerated lesions covering partially paraxial and perilimbal location ranging 1.5–2 mm in size. Schirmer tear test was found to be higher (27 mm per minute). Tonometry test revealed no other abnormality or intra-ocular pathology.

Treatment

It was planned to restrain the animal chemically in the early morning hours to reduce sedation stress. For chemical restraining, a combination of Xylazine @ 1.5 mg/kg body weight and Zolatile®(zolazepam and tiletamine) @ 2.5 mg/kg body weight, were loaded into a pneumatic dart and was fired using a pneumatic gun. Prior to anaesthesia, fasting for 20 hours followed by withdrawal of water for the next 12 hours was advised. Upon darting, induction of anaesthesia was observed within 5 minutes evident by the staggering movement, followed by sternal recumbency after 10 minutes. A stage of complete surgical anaesthesia was finally achieved within 20 minutes with lateral recumbency. Auriculo-palpebral nerve block using 1 ml of 2% lignocaine hydrochloride was injected to the affected side soon after complete sedation.

The affected eye was adequately irrigated with isotonic normal saline solution (Image 2) to soothe irritation and discomfort. Normal Saline Solution (NSS) is an isotonic solution that helps in flushing out any loose foreign material in the eye. Mixture of antibiotic and Non-steroidal anti inflammatory drug (NSAID) preparation containing 0.5 ml of ceftriaxone and 0.5 ml of flubiprofen respectively was injected into the conjunctiva (Image 3).

Following corneal suture using vicryl 5-0, the third eyelid flap was carefully sutured using vicryl 3-0 (Image 4). For Tarsorrhaphy, the eyelids were sewn together by simple interrupted technique using 2-0 nylon suture(Image 5). Intravenous injection of Yohimbine (concentration 10 mg/ml) @ 0.5 mg/kg was used to reverse the effects of anaesthesia.

The animal was subsequently shifted to an isolated enclosure and was closely monitored for a period of 20 days. Cephalaxin @ 20 mg/kg body weight twice in a day for seven days along with probiotic supplement (Vizylac®) and Vitamin A capsule (Aquasol A®) orally once daily was continued for the next 30 days. Further, the animal was kept under normal diet during this duration.

On the tenth day, the sutures were opened to assess the recovery of the operated eye. Formation of granulation tissue was observed (Image 6). Subsequently, with proper care and post-operative management, full recovery was achieved by one month, uneventfully (Images 7 & 8).

Discussion

Eye injuries when treated within 24 hours there is higher chances of recovery, delay in same (more than 24 hours) may result in loss of eyesight, prolapse of corneal membrane and severe complications (Rajak et al. 2015).

The animal showed complete recovery within one month of the above mentioned treatment. This suggest that tarsorrhaphy along with sub-conjunctival injection of ceftriaxone and flubiprofen is the best and safest option for the treatment of conjunctivitis complicated with corneal ulceration, especially in wild animals, as it becomes increasingly difficult to restrain them regularly which may result in undue stress. (Fischer et al. 2019). Startup (2008) opined that the probable cause of trauma/injury in case caged animals is mostly inflicted by a sharp object, enclosure fence, grass blade or during infighting. Also, continuous itching, rubbing, and photophobia seen in ulcerative keratitis might have resulted into excessive lacrimation, subsequently may have resulted in getting a higher range of Schirmer tear test results during physical inspection (Senchyna & Wax 2008).

Third eyelid flap provides protection and supports the weakened cornea. It assists corneal healing by decreasing evaporation of tears, warming of cornea, supplying inflammatory cells, fibroblasts, blood and eventually providing better stability by reducing trauma associated with movement of eyelids (Gellat et al.

Image 1. Day of Examination: ulcerative keratitis.

Image 2. Cleaning affected eye with NSS.

Image 3. Administering sub-conjunctival injection into upper and lower palpebral conjunctiva.

Image 4. Nictitating membrane (3rd eye lid) pulled over eye ball and holding with suture through the skin lateral to the eye.

Image 5. Tarsorrhaphy: Sewning of upper and lower eyelids.

Image 6. Appearance of granulation tissue on tenth day of post operation.

Image 7. Thirtieth day of post-operative care.

1994). Tarsorrhaphy was conducted to facilitate healing of corneal ulcer and to prevent corneal exposure to environmental contamination (Acharya et al. 2020). Tarsorrhaphy is a convenient option and advisable in cases where repeated handling of an animal is difficult. Also, in those cases where patient may create sort of animal welfare issues/conflict or augments certain vetero-legal disputes.

The eyelids were sewn by simple interrupted suture instead of vertical mattress suture. This helps in avoiding chances of rupture of sutures during scratching the eye.

Further, specifically Vitamin A supplement was added in the ration. Vitamin A is a fat-soluble vitamin having wound-healing and anti-oxidant properties (Palace et al. 1999). Inclusion of Vitamin A is necessary as it initiates epithelisation that accelerates wound-healing especially, when it comes to perform surgical corrections of eye diseases (Zinder et al. 2019).

Conclusion

Traumatic ulcerative keratitis is often considered to have a good prognosis. However, the same may be challenging for field veterinarian especially, when it is comes to wild fauna. Early identification of causes and prompt diagnosis may be potentially curable with good prognosis in such cases. In the present case, tarsorrhaphy along with sub-conjunctival injection of ceftriaxone and flubiprofen was found to be effective. Constant monitoring over health attributes and other supportive medications has yielded a better response without any complication. Catamnesis revealed that the serow had attained a stable condition with improved appetite and muscle volume.

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Image 8. Showing complete recovery after one month of operation.

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NOTE

Group size pattern and distribution of threatened Sambar *Rusa unicolor* (Artiodactyla: Cervidae) in Moyar River Valley, India

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The Sambar *Rusa unicolor* (Kerr, 1792) is the largest deer in southern Asia and has a wide geographical distribution (Schaller 1967; Leslie 2011; Jhala et al. 2020). The subspecies *R. u. niger* is found throughout India except in the high Himalaya (>3,800 m), desert of Kutch, and the coast. It occurs in a wide range of habitat types that include mixed deciduous forest, thorn forest, arid-dry forest, shola grassland, coniferous forest, and evergreen forest (Varman & Sukumar 1993; Menon 2014). It is adapted to a wider variety of environmental conditions than any other ungulate in India (Schaller 1967). Sambar's readiness to graze/browse is the primary reason for the broad distribution of this species. They mainly graze when fresh green grass is available (Schaller 1967; Eisenburg & Lockhart 1972). Although Sambar is distributed in widespread locations and habitat types, its population has declined in the past few decades. Hence it is classified as 'Vulnerable' on the IUCN Red List (Timmins et al. 2015). In India, Sambar is protected under the Schedule III category of the Wildlife Protection Act 1972 (Jhala et al. 2020).

Sambar is essentially a non-social species (Sankar & Acharya 2004) and is usually seen as solitary or in small groups with fewer than six individuals (Schaller 1967). The characteristic social unit is one hind and one fawn, or one hind, one yearling and one fawn (Schaller 1967). Sambar prefer dense vegetation cover and avoid resting in open areas (Johnsingh 1983). Though it is a widely distributed deer species in India, information on its group size and composition is scanty. We recorded the Sambar distribution and group composition based on opportunistic sightings during the python telemetry project survey between January 2018 and January 2020 in the Moyar River valley that encompasses

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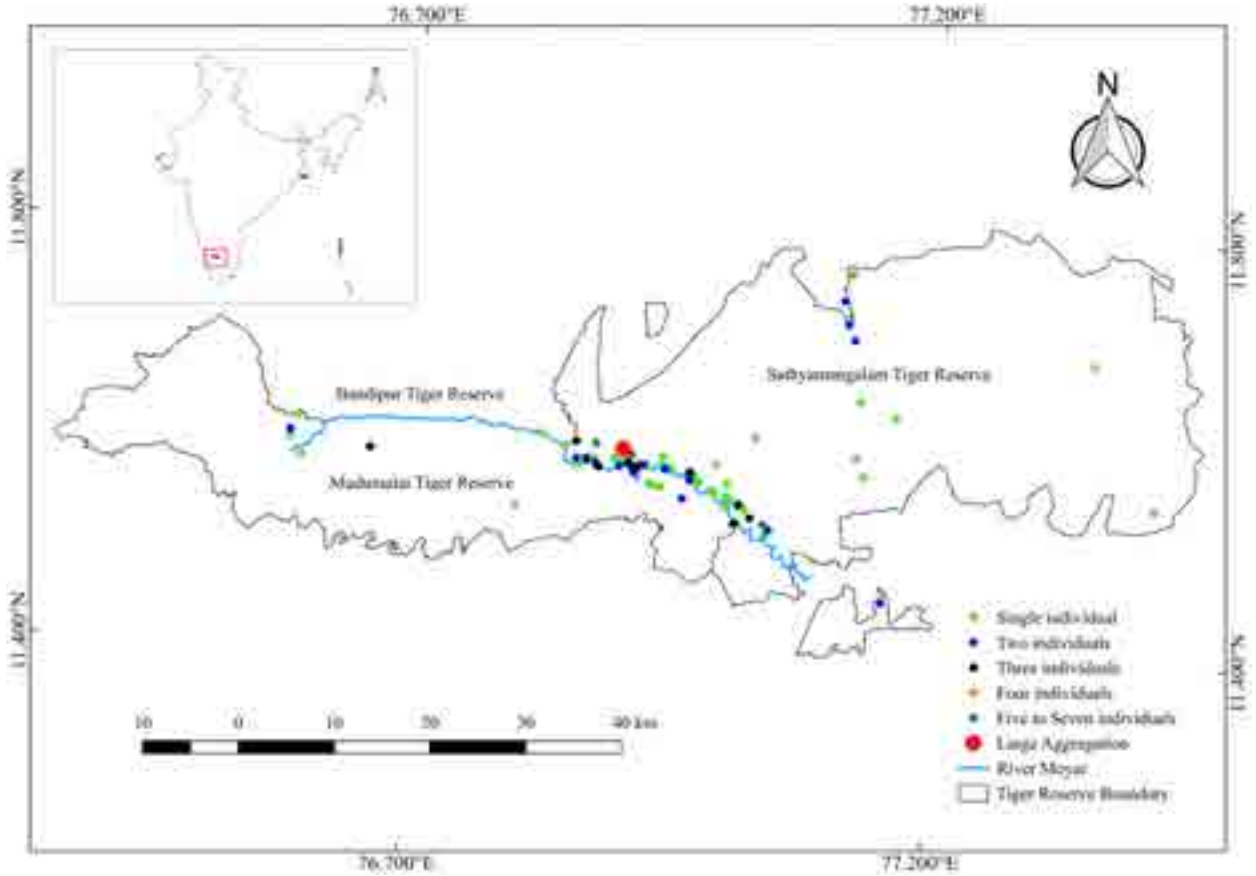

Figure 1. Distribution of Sambar sighting locations in Moyar River valley, Tamil Nadu.

Sathyamangalam and Mudumalai tiger reserves.

Moyar River valley (Figure 1) is a unique landscape situated at the tri-junction of Tamil Nadu, Kerala, and Karnataka states. This landscape is also a juncture of the Western and the Eastern Ghats, and has high diversity of flora and fauna (Thirumurugan et al. 2021). In addition, this landscape supports a large wild population of Asiatic Elephants and high density of carnivores & herbivores, including Sambar (Jhala et al. 2020). During our two years of field work, we recorded a total of 182 Sambar individuals (101 females, 38 males, 11 fawns, and 32 individuals whose sex could not be determined) at 86 different occasions (Figure 1). Among our observations, 65% were solitary individuals, and 17% and 12% were two and three individuals, respectively. Only in 6% of our observations we saw four or more individuals. Solitary Sambar (n= 56) were common in the study area; occasionally, females with a fawn (n= 6) and male/female pairs (n= 5) were recorded (Figure 2). The group size usually varied between one and seven with a mean of 1.62±1.13 (SD). In one instance, we recorded a large aggregation of 44 individuals resting in the grassland on 29 June 2019 at 1510 h, (11.593^{0}N & 76.902^{0}E; 837 m), in Thekkathimalai, Bavanisagar range, Sathyamangalam Tiger Reserve (Image 1). The aggregation site was about 1.5 km away from the perennial river Moyar. This aggregation consisted of four hard-antler adult stags, four velvet stags, 32 females, and four fawns. In addition, small groups of 3–7 individuals were also recorded adjacent to the large group in the adjacent areas on the same day. In all, about 63 individuals were recorded that day in the surrounding locations. The following tree species were observed around the large aggregation site, viz.: *Dicrostacys cinerium*, *Anogeissus latfolia, Givotia rotteniformis*, *Bauhinia racemosa*, *Bridelia feruguinnea*, *Semicarpus anacardium*, *Hardwickia binata*, *Dalbergia latifolia*, *Diospyros* sp., *Grewia tilifolia*, *Flacourtia indica*, *Diospyros montana*, *Pterocarpus marsupium*, *Phyllanthus emblica*, and grasses such as *Cymbopogon flexuosus*, *Apluda mutica*, *Eragrostiella bifaria*, *Digitaria* sp., *Eragrostis tenuifolia*, *Themeda cymbaria*, and two unidentified species.

Earlier studies suggest that Sambar is usually found in small groups or as solitary individuals and prefers dense vegetation (Schaller 1967; Eisenburg & Lockhart 1972; Johnsingh 1983; Karanth & Sunquist 1992; Leslie

Table 1. Maximum number in Sambar groups reported by other authors in India.

	Group Size (N)	Location	Authors
1	9	Kanha Tiger Reserve, Madhya Pradesh	Schaller 1967
2	10	Periyar Tiger Reserve, Kerala	Ramachandran et al. 1986
3	15	Mudumalai Tiger Reserve, Tamil Nadu	Krishnan 1972
4	28	Sariska Tiger Reserve, Rajasthan	Sankar 1994
5	39	Bandipur Tiger Reserve, Karnataka	Johnsingh 1983
6	44	Sathyamangalam Tiger Reserve, Tamil Nadu	Present observation
7	45	Mudumalai Tiger Reserve, Tamil Nadu	Prasanna 1990
8	36–45	Mudumalai Tiger Reserve, Tamil Nadu	Ramesh et al. 2012
9	50	Mudumalai Tiger Reserve, Tamil Nadu	Varman & Sukumar 1993
10	150–200*	Simlipal Tiger Reserve, Odisha	Jammal & Johnsingh 2015

*Aggregate every night in and around salt lick near the Bakmuda Range office, and this is the largest aggregation reported in the native range of Sambar.

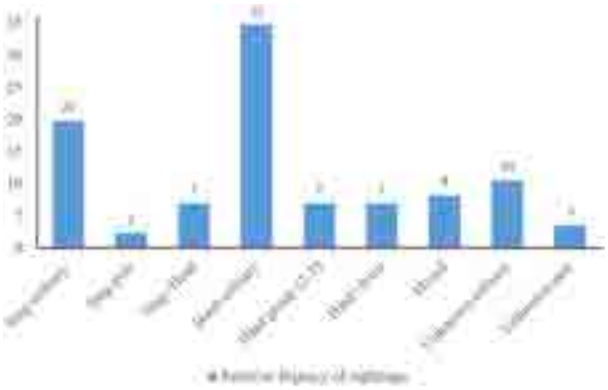

Figure 2. Group composition of Sambar sightings in Moyar River Valley, Tamil Nadu.

Image 1. a—Part of the large aggregation of Sambar sighted in the Sathyamangalam tiger reserve, Tamil Nadu | b—Panoramic view of the woodland savannah habitat along with the large aggregation of Sambar. © Thirumurugan V.

Image 2. The undisturbed woodland savannah habitat in the hills and hill slopes © Thirumurugan V.

2011). Occasionally, Sambar form large groups near water holes, swampy grasslands, open areas, salt licks, and burnt areas (Johnsingh 1983; Eisenburg & Lockhart 1972; Ramesh et al. 2012). In the adjacent landscape at Mudumalai TR, the group size varied between one and 50 (Varman & Sukumar 1993) and group sizes >15 individuals were in 3.7% of 377 sightings from April to October in swampy grasslands by Ramesh et al. (2012). Interestingly, in Mudumalai, the maximum dry-season (March–mid May) group size is recorded as 19 individuals in the deciduous forest, and the maximum wet-season (July–September) group size was between 44 and 50 individuals in the swampy grasslands (Varman & Sukumar 1993; Ramesh et al. 2012). However, though our observation is very close to the wet season, it was made on hilly grassland where the substratum is usually dry, unlike the wet swampy grasslands.

The earlier studies suggest that the increased food availability immediately after rain in Mudumalai may account for such large aggregations (Varman & Sukumar 1993; Ramesh et al. 2012). In Bandipur, an aggregation of 39 individuals was observed near a pond in response to a Dhole pack, indicating antipredator strategy which also contributes to large aggregations (Johnsingh 1983).

Although several speculations are advanced to explain the large aggregations among Sambar individuals, there is no concrete evidence to describe why such infrequent aggregations occur. Based on the previous records it is inferred that resource availability, water holes, feeding sites including swampy grasslands, salt lick areas, predator pressure, reproduction, parental care, rainfall, vegetation cover characteristics, and climate are some of the factors known to influence large aggregations of Sambar. How these factors individually or in combination act upon the large aggregations of Sambar merits further detailed study.

The present observation is the first large gathering of sambar reported from Sathyamangalam TR. The large aggregation of Sambar in the Moyar landscape denotes that the grassland savannah (Image 2) is a preferred habitat on a seasonal basis. Similar reports of large gatherings of Sambar are available primarily from the adjacent contiguous landscapes, viz., Mudumalai and Bandipur TRs (Table 1). Interestingly, all previous large group size reports are from famous tiger reserves of India (Table 1), which denotes the importance of this species as prey to large predators. Sambar is a preferred prey species for carnivores such as Tigers, Leopards, Dholes (Varman & Sukumar 1993; Sankar & Acharya 2004), and pythons (Bhupathy et al. 2014). Recently Jhala et al. (2020) reported that the Sambar density in Sathyamangalam TR was higher (8.97/ km^2) than Mudumalai TR (3.21/ km^2). Considering the Sambar's preference for cover and avoidance of disturbance, its high abundance would be a reliable indication of the health of the forest ecosystem in the study area. The availability of suitable habitat and better habitat management practices implemented by the local forest department could be a reason for such a high Sambar density that can potentially assist in holding high densities of large predators in the Moyar River valley landscape. The grassland (Image 1a,b) where the congregation was observed could be an important habitat for the Sambar. Further, the depleting grassland habitats in the plains due to the rapid spread of invasive species like *Prosopis juliflora* and *Lantana camara* is a cause for concern. Hence, providing special attention to this habitat in the management aspects can be critical for the long-term conservation of this globally threatened species within the study area.

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NOTE

First photographic record of the presence of Smooth-coated Otter *Lutrogale perspicillata* in Ghaghra River, India

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Growing human populations and high resource dependency have led to depletion of ecosystems in rivers and wetlands (Moser et al. 1996; Prigent et al. 2012). Depleted resources and disturbed habitat have made dependent species such as otters increasingly vulnerable (de Silva et al. 2015; Wright et al. 2015). Shy by nature (Gupta et al. 2020), a declining trend has been observed due to anthropogenic pressures (Roos et al. 2015), loss of habitat, exploitation (de Silva et al. 2015; Wright et al. 2015) and poaching (Savage & Shrestha 2018). Otters have, thus, become very rare.

Otters are fish-eating, semi-aquatic carnivora of the order Mustelidae (Pardini 1998). Three species occur in the Indian sub-continent: Eurasian Otter *Lutra lutra*, Smooth-coated Otter *Lutrogale perspicillata*, and Asian Small-clawed Otter *Aonyx cinereus*. The Ganga River basin is home to all three species (Chanda 1991). The Smooth-coated Otter is categorized as 'Vulnerable' in the IUCN Red List (Khoo et al. 2021), and is protected under Schedule II of the Indian Wildlife (Protection) Act, 1972. There have been very few studies on the Smooth-coated Otter in India (Hussain & Choudhury 1997).

The Smooth-coated Otter occurs southwards across the Himalaya. Beyond the Indian subcontinent, its range extends across Myanmar, Laos, Vietnam, Kampuchea, South-Western China, Malaysia, Brunei, and Indonesia with the lone presence of *Lutra perspicillata maxwelli* in southern Iraq marshlands (Macdonald et al. 1986; Hussain & Choudhury 1997).

Despite the wide distribution of Smooth-coated Otters (Hussain & Choudhury 1997), no recorded information was available on their occurrence from the main stem Ghaghra River. Historically, lack of a detailed ecological assessment in Ghaghra River from origin to its confluence with the Ganga has resulted in this knowledge gap. The present sightings are the first photographic records of Smooth-coated Otters from the Ghaghra, a major transboundary tributary of the Ganga.

During the rapid ecological assessment undertaken by the Wildlife Institute of India (WII) in 2019–2020

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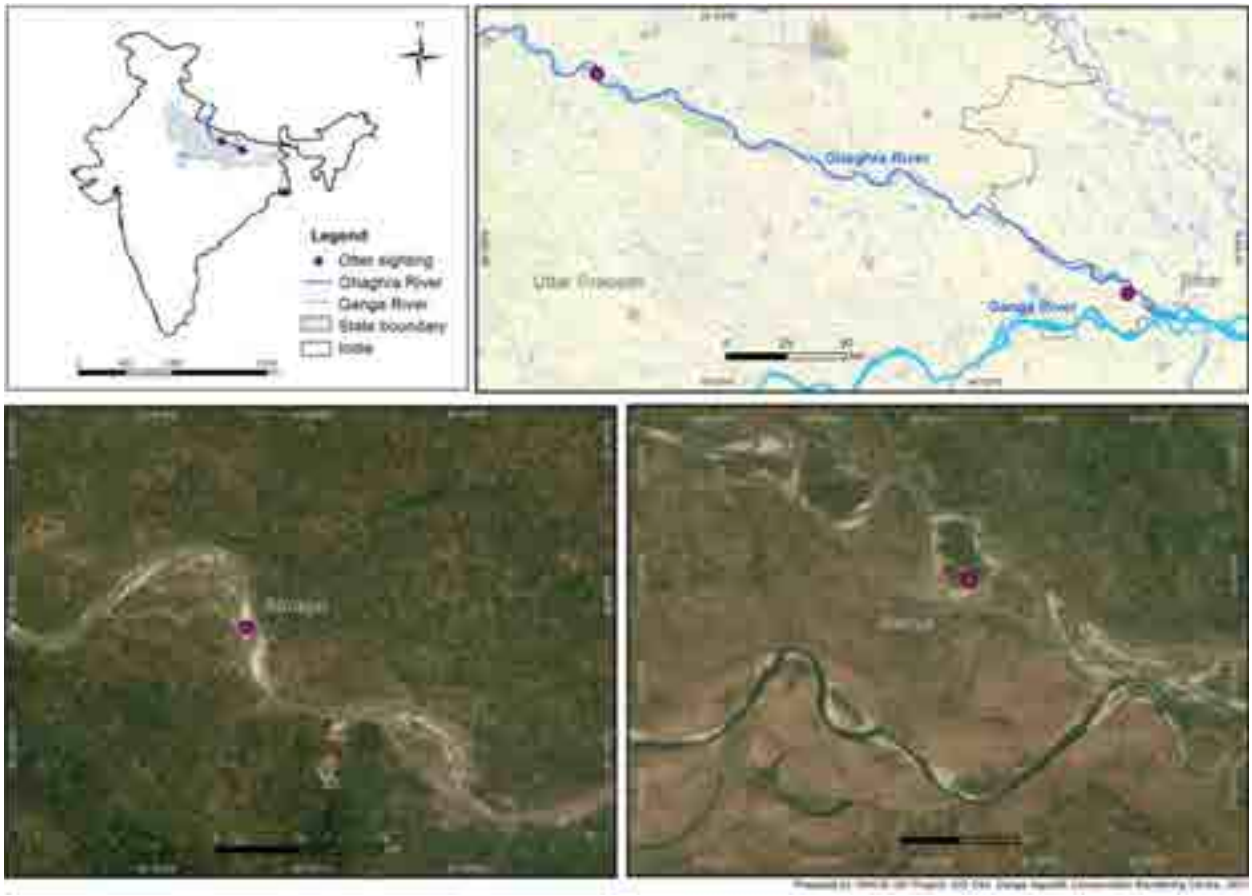

Figure 1. Locations of smooth-coated otter sighted in Ghaghra river during ecological survey 2019–2020.

Table 1. Details of observation site of smooth-coated otter in Ghaghra River sighted during the post-monsoon ecological assessment.

Date	Coordinates	Habitat characteristics	No of Individuals sighted	Record details	Anthropogenic activities	Remarks
17/12/2020	26.649^{0}N 82.547^{0}E	Sandbank with high slopes, water depth range 1.7 to 2.6 m and channel width 380 m.	1	Direct sighting	Riverbed agriculture	Swimming in the river
21/12/2020	25.828^{0}N 84.528^{0}E	High sandbank in close vicinity of tall grasses, water depth range 2 to 3.5 m and channel width 310 m.	1	Direct sighting	Riverbed agriculture	Swimming in the river

under the project "Planning and Management for Aquatic Species Conservation and Maintenance of Ecosystem Services in the Ganga River Basin for a Clean Ganga" funded by the National Mission for Clean Ganga (NMCG), Ministry of Jal Shakti, Government of India, two direct sightings of smooth-coated otter were recorded from two different locations in lower stretch of Ghaghra River.

The first sighting was recorded on 17 December 2020 at 1230 h IST in the waters of Ghaghra River near Bhatia village of Basti district in Uttar Pradesh (26.649°N 82.547°E) during the boat survey (Figure 1). The moment was captured through Panasonic DMC-FZ1000- Lumix digital camera. The animal was observed for a couple of hours while it was searching for food along the bank. Its length was around 1 m with smooth and sleek fur (Image 1). The colour was dark brown on the upper side, and undersides were lighter as stated by Gray (1865).

Small eyes & ear, whitish-coloured upper lip (Image 2) and heavier teeth (Image 3) were observed (Tate 1947). The tail of the animal was flattened dorsoventrally at the tip (Image 4) (Hwang & Larivière 2005). Rhinarium was naked and dark situated anteriorly with a barely convex dorsal border (Image 5), the typical features of a Smooth-coated Otter (Harrison, 1968).

The landscape featured river islands, high sandbanks,

Image 1–8. 1—Smooth-coated otter, *Lutrogale perspicillata* in Ghaghra river | 2—Small eyes, ears and whitish upper lip of the species | 3—Heavier teeth | 4—Flattened tail at the tip | 5—Barely convex shaped naked and dark rhinarium | 6—A view of landscape dominated by high sand bank and *Saccharum* spp. where the species was sighted | 7—Impressions of pugmarks of smooth-coated otter on the sandbank | 8—Body rubbing behaviour of the species.
© 1–7—Saurav Gawan | © 8—Ashish K. Panda.

thick riparian vegetation dominated by Saccharum spp. on both banks of the river (Image 6). The elevated banks in the stretch are prone to erosion with the continuous flowing waters of the Ghaghra River. The water depth at the point ranged 1.7–2.6 m, and the channel width was 380 m.

The second sighting was recorded on 21 December 2020 at 1447 h IST near Raja Tengaraha village in Ballia district of Uttar Pradesh (25.828°N 84.528°E). This was approximately 250 km downstream from the first sighting. The channel depth at sight ranged 2–3.5 m, and the channel width was 310 m; there were high sandbanks on both sides of the river with riparian vegetation dominated by *Saccharum* spp. Here, the otter was sighted swimming in the river near the bank, and later it moved to the sandbanks (Image 7), where it rubbed itself (Image 8) for a while; before jumping back into the water.

Otters lead an amphibious life, which gives them the advantage of disappearing into the wide riverine landscapes and enhances their role in many ecological processes pertaining to the flood plains (Khan et al. 2014). Smooth-coated Otters thus play a critical role in maintaining balance of freshwater ecosystems (Sivasothi 1995; Acharya & Lamsal 2010; Gupta et al. 2016). The otter populations are declining in their ranges due to habitat loss and poaching activities (Hussain 1999; Nawab 2007, 2009; Nawab & Gautam 2008). The situation gets grave as their population is mostly fragmented and sighted in close proximity to protected areas like Corbett Tiger Reserve (Hussain 1993).

Even though the Smooth-coated Otter is distributed throughout the country, there have been very few sighting records from India with occasional notes on their occurrence from different parts of the country (Hinton & Fry 1923; Pocock 1940; Chitampalli 1979). The present sighting gives hope to the survival of this shrinking population. As the Smooth-coated Otter is a threatened species, this can be an opportunity for in-depth study of the population that will further aid in developing conservation measures in the area; far from any protected area (Gupta et al. 2015). Further, regular monitoring of this landscape coupled with community engagement programmes will aid in generating a database on the population status of the species. Detailed studies will assist in taking ahead the research work initiated in the year 1988 by the Wildlife Institute of India under the project in National Chambal Sanctuary (NCS), to study the ecology of the Smooth-coated Otters, one of the top carnivores of the freshwater ecosystem (Gupta et al. 2016).

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NOTE

Back after 40 years: a rare sighting of Eurasian Siskin *Spinus spinus* (Linnaeus, 1758) (Aves: Passeriformes: Fringillidae) in Himachal Pradesh, India

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The Eurasian Siskin *Spinus spinus* (Linnaeus, 1758) is a small and greenish-yellow finch, distributed largely in the westernmost and easternmost parts of Eurasia (Clement 2020). A vagrant species in India, it has been recorded only in four states so far, with the majority of records from Jammu & Kashmir. We report a very recent sighting of this species from Himachal Pradesh.

As part of a research project on flycatcher phenology in the Great Himalayan National Park Conservation Area (GHNPCA), PP (hereafter, 'the observer') conducts regular surveys in the region. During a reconnaissance survey for this research project, on 16 November 2021, he walked a narrow mountain path atop a ridge, adjacent to the village of Gushaini, which is within the ecozone of GHNPCA. He sighted a small passerine on the left side of the trail at 1157 h for ~20 seconds. The bird flew from ~52° north-east direction and perched on a low branch of a royal variety Apple tree *Malus domestica* less than two metres away, slightly below eye level, as a villager walked in the opposite direction of the observer, both near the bird. The bird visited the branch despite having sighted the two humans in the vicinity. This made it very easy to capture high-resolution pictures of the individual (Image 1). The bird may have been using the tree as a vantage point to assess the surroundings (Image 2). Soon, the bird moved to the top of an oak tree *Quercus* sp. ~20 m-tall in the centre of the ridge, approximately 10 m back in the direction the bird came from, after which the observer lost sight of the bird. Coordinates, elevation, and orientation were recorded using a Garmin Etrex 20x GPS with a pre-installed DEM layer for accuracy of elevation, and the photographs were taken using a

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Competing interests: The authors declare no competing interests.

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Image 1. The male *Spinus spinus* seen in Gushaini.

Nikon D5600 with a 300 mm lens.

The observer immediately identified the bird as a male Eurasian Siskin *Spinus spinus* using a field guide (Grimmett et al. 2011). Shortly afterwards, he uploaded the sighting in a 20 minute eBird checklist made during that time. The identity was later confirmed using descriptions given in Clement (2020). No similar finch species has a black bib on the chin (Gaston & Chattopadhyaya 1980), the size of which is linked to their intraspecific dominance status and metabolic rates (Senar et al. 2000) and thus eliminating all other possibilities. Two subsequent visits to the same area (17 and 21 November 2021) did not yield any re-sighting of *S. spinus*. There are pine trees (*Pinus* sp.) nearby, which are known to be used by the species (Clement 2020).

Image 2. Habitat in which the individual was sighted. *Malus domestica* (Apple tree), the perch to the left.

Table 1. All sightings of *Spinus spinus* in India.

Date & time	No.	Sighting duration	Elevation (in m)	Observer	Sighting location	District	State	Sex
12.xi.2019; U	U	U	2,526	Imran Shah	Batura Lake, Passu, Gojal	Hunza	Jammu & Kashmir	F (&M?)
08.xi.2019; U	U	U	2,648	Imran Shah	Borit Lake	Hunza	Jammu & Kashmir	M (&F?)
*11xi.2016; 1000 h	10	U; survey duration- 4 hours	2,648	Imran Shah	Borit Lake	Hunza	Jammu & Kashmir	F (&M?)
27.xi.2016; 0900 h	5	U; survey duration 5 hours	2,658	Imran Shah	Borit Lake	Hunza	Jammu & Kashmir	M(&F?)
01.iv.1995; 0600 h	1	U; survey duration 12 hours	2,309	Matti Rekilä	Karimabad/ Gilgit	Hunza	Jammu & Kashmir	U
01.xi.2021; 1312 h	4	3 min	1,588	Mohammad Arif khan	Srinagar	Srinagar	Jammu & Kashmir	F(&M?)
25.i.1980; 1330 h	1	~15 min	2,681	Tony Gaston & S. Chattopadhyaya	Solang Nalla	Kullu	Himachal Pradesh	M&F
16.xi.2021; 1157 h	1	~20 s	1,687	PP (this report)	Gushaini	Kullu	Himachal Pradesh	M
29.iv.2010–04.v.2010; 0600–0800 h, 1100–1300 h, 1600–1800 h	≥ 1	U; survey duration- 10 min (multiple)	1,606, 1,948	**Utpal Singha Roy(?), Arijit Pal(?), Purbasha Banerjee(?) & some Masters student(s) (?)	Neora Valley National Park	Kalimpong	West Bengal	?
23.iv.2013; 1000 h	1	U; survey duration 8 hours	2,702	Craig Robson	Mandala Road (i.e., road from Dirang junction to Mandala village)	West Kameng	Arunachal Pradesh	M
07.iii.2017; 0530 h	5	U; survey duration 4 hours	2,475	Rofikul Islam	Eaglenest Wildlife Sanctuary	West Kameng	Arunachal Pradesh	M(&F?)

U—Unknown | M—Male | F—Female | ?—Unclear.
All records are from eBird except the current sighting, first sighting in Himachal Pradesh (also available on eBird), and those in West Bengal, which are from published literature. *—Two sightings on the same day (one under the 'Historical' protocol in eBird) in Borit Lake reported by the same person is being considered as one, because they are likely the same individual(s) | **—Roy et al. (2011). Authors were contacted for further details about the sightings, but they are currently too busy to check the raw data or don't have access to the data. To avoid double-counting, it is assumed that the sightings in the two areas surveyed for the study have been during the same day. Hence, this is considered as one record.

We thoroughly searched several online platforms for previous sightings of this species within India. This included the following: published peer-reviewed and grey literature through academic search engines and simple query searches; citizen science platforms eBird, iNaturalist, and India Biodiversity Portal; and social online platforms Facebook (including dedicated bird groups), Instagram, Twitter, and Flikr. Two records were obtained from peer-reviewed publications. All other records were from eBird. The first and the latest sighting of the species within India has been in Himachal Pradesh, the first at Solang Nalla, which is around 109 km away (shortest distance, geodesic) from the current location (Image 3) (Gaston & Chattopadhyaya 1980). As Gaston & Chattopadhyaya (1980) had stated, the sightings of the species may have been overlooked because of the lack of surveys during winter, when they are usually seen. The location of the current sighting was along a trail that is not used by birdwatchers, as it is high above but parallel to the main route to the entry gate of GHNP from Gushaini, which they do take. This trail is almost exclusively used by locals. The combined effect of birdwatchers surveying less in the area during the cold winters, and that too, restricted to some trails, may have resulted in the missing of some sightings between these past 40 years.

This is the second sighting of the species within India in 2021. The previous sighting was further north-west in Srinagar, only 15 days prior. The current sighting is highly significant for several reasons. There are only around 10 other records of this species within the Indian boundary (Table 1). In Himachal Pradesh, this is the first sighting of the species in 40 years, and only the second sighting in recorded history. Of all the sightings in the western

Image 3. Distribution of sightings of *Spinus spinus* in India (yellow and black dots). Himachal Pradesh and its districts are represented in yellow outline; and other states in red outline. Inset images show clustered records in Hunza and West Kameng districts.

Himalaya, this is the most southeastern. Both sightings were in low altitude sites compared to all previous sightings in western Himalaya with the difference in elevation exceeding a kilometre in some cases. *S. spinus* exhibit irruptive migration (variable patterns in north-to-south migrations) based on the availability of food and possibly climate change (Arnaiz-Villena et al. 2009; Kanerva et al. 2020). For this reason, it is important to keep a lookout for this species in the coming years, as it can show if their phenology during irruptive migrations is advancing, which may be correlated with advancing spring arrival dates as a result of climate change.

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NOTE

First record of the jumping spider *Pancorius changricus* Żabka, 1990 from India (Araneae: Salticidae)

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The genus *Pancorius* Simon, 1902 comprises a total of 40 described species mainly distributed in the Oriental region with the exception of *P. crassipes* (Karsch, 1881) which may have been introduced in Poland (WSC 2022). There are six species known from India: *Pancorius daitaricus* (Prószyński, 1992), *P. darjeelingianus* Prószyński, 1992, *P. magnus* Żabka, 1985, *P. nagaland* Caleb, 2019, *P. submontanus* Prószyński, 1992, and *P. tagorei* Prószyński, 1992 (WSC 2022). All these species are distributed in eastern Himalaya or its proximity except for *P. daitaricus* in Odisha (eastern India). The present paper aims to add one more species, *P. changricus* Żabka, 1990 to the Indian list, which is previously known only from its type locality in Bhutan (Żabka 1990).

The specimen was hand collected and studied under a Leica M205 A stereomicroscope and the microphotographic images were taken by Leica DFC2900 digital camera attached to the same stereomicroscope enabled with the software package Leica Application Suite (LAS), version 4.5.0. Drawings were made with the aid of a drawing tube attached to the microscope. All measurements are in millimeters (mm). The examined specimen has been deposited in the reference collection of the Department of Zoology, Sikkim University, India.

Genus *Pancorius* Simon, 1902

Type species: *Ergane dentichelis* Simon, 1899

Diagnosis: For detailed diagnostic features of the genus see Żabka (1985).

***Pancorius changricus* Żabka, 1990**

Images 1–10; Figures 1–2

Pancorius changricus Żabka, 1990: 164, figs. 7–12

Material examined: SU9895A, 22.v.2021, 1 male, India, Sikkim, Labdang (27.390N, 88.286E), 1,798 m,

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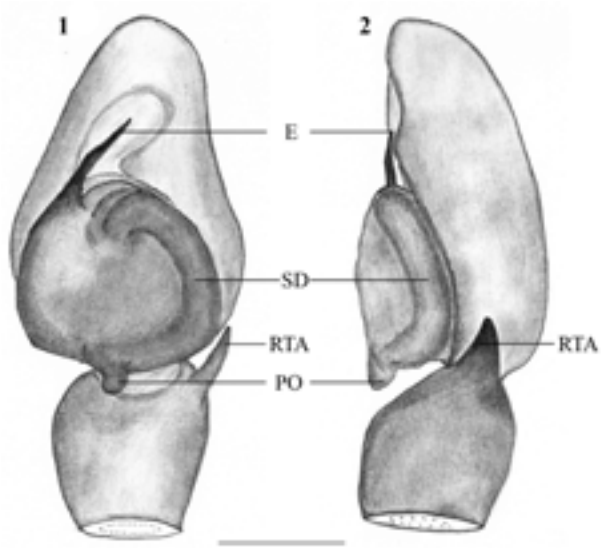
Images 1–9: Pancorius changricus Żabka, 1990: 1—Live photograph of male, dorsal view | 2—Same, frontal view | 3—Male, dorsal view | 4—Same, ventral view | 5—Same, lateral view | 6—Same, frontal view | 7—Left chelicerae, ventral view | 8—Male left pedipalp, ventral view | 9—Same, retrolateral view. Scale bars: 1 mm (3–6); 0.5 mm (7); 0.1 mm (8–9). © 1–2—Anushka Gurung | 3–9—Dhruv Prajapati



Figures 1–2. *Pancorius changricus* Żabka, 1990: 1—Left pedipalp, ventral view | 2—Same, retrolateral view. Scale bars: 0.2 mm (1–2). Abbreviations: E—embolus | PO—posterior tegular outgrowth | RTA—retrolateral tibial apophysis | SD—sperm duct.

from foliage, leg. A. Gurung.

Diagnosis: For detailed diagnostic features see Żabka, 1990.

Description: Male (Images 1–7). Carapace length 2.90, width 2.27, height 1.63. Abdomen length 2.91, width 1.94, height 1.56. Pedipalp (Images 8–9; Figures 1–2): palpal segments brownish; tegulum nearly circular with very small postero-median outgrowth (Image 8; Figure 1); sperm duct encircles the tegulum before reaching the spine-like embolus (Image 8; Figure 1); embolus without any curve, with sharp tip, directed at 1 o'clock position (Image 8; Figure 1); tibia nearly half of cymbium, with small, slightly curved RTA, broad at the base and narrowed at tip, resembles canine teeth (Image 9; Figure 2).

Distribution: India (Sikkim) and Bhutan (Image 10).

Variation: The studied specimen has the following variations when compared to the holotype male from Bhutan: apical and postero-median patches of whitish setae present on abdomen (Image 1) (apico-median light grayish longitudinal belt in holotype; see fig. 9 in Żabka 1990); embolus slightly broader (narrow, spiniform in holotype); posterior tegular outgrowth much pronounced than in holotype (cf. Image 8; Figure 1 with fig. 7 in Żabka 1990); RTA without apical notch (present in holotype) (Image 9; Figure 2) (cf. fig. 8 in Żabka 1990).

Habitat and feeding habit: The specimen was collected from a large cardamom-based agro-forestry

Image 10. Distribution of *Pancorius changricus* Żabka, 1990.

system (LCAS) in West Sikkim located at the temperate zone in the eastern Himalaya. The forest cover in the area consists of broadleaved forests comprising of trees such as oak, birch, maple, chestnut, magnolia, rhododendron, along with several species of shrubs and grasses. The terrain is hilly and remains cool during summer and very cold during the winter. The spider seems to feed mainly on book-lice, hoverfly, black fly, cluster fly, and thrips.

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NOTE

An abandoned nest of *Vespa affinis* (Hymenoptera: Vespidae)

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Although *Vespa affinis* is one of the most common hornets in subtropical and tropical Asia (Archer 1997), very few studies have been made on the taxonomy of this species in Bangladesh (Akter et al. 2020) and no studies have been reported yet on its nest structure in this southern Asian country. Therefore, we present some observations of an abandoned nest of *Vespa affinis* obtained from the southern part of Bangladesh.

The nest was collected from Barishal Sadar Thana (22.698N & 90.363E) of Barishal metropolitan area in Bangladesh (Figure 1) on 8 December 2019 after the whole colony had left the nest. It was built on the outer wall of a house (Image 1) and the nest bottom was approximately 1.6 m above the ground. According to Archer (2008), *Vespa* sp. maintain an annual colony cycle and the cycle length is longer in tropical regions for *Vespa affinis*; on the basis of this statement and our observation, we also found that the colony cycle length of this species was approximately eight months and the wasps vacated the nest just prior to the arrival of winter. On 19 December 2019, the nest was transferred to the Entomology Laboratory of the University of Dhaka, Dhaka.

The nest was found roughly pear-shaped, consisting of seven combs. The upper combs were comparatively larger in diameter than the lower ones, especially the second comb which was found larger in diameter than the other six combs. Each comb was attached to the adjacent combs with the support of a varied number of both flattened and ribbon like pillars. The nest was surrounded by layers of envelopes, which were light in weight, with a single opening to the outside. It was unfortunate that the outer envelope was destroyed due to transportation and dryness (Image 2). The role of the layered envelope is to safeguard the colony against enemies and maintain constant humidity and temperature inside the nest (Klingner et al. 2005, 2006). The experimental nest had a blunt roof cone rather than a pronounced conical roof showed by van der Vecht (in Malaya, 1957). Van der Vecht (1957) had suggested that during heavy rains, the roof cone assists in shedding water from the nest.

We took some measurements of the nest. Maximum diameter of the nest was recorded at 45.5 cm and the total weight and height of the nest structure (without the external envelope) measured 913 g and 27 cm, respectively. The measurement of maximum diameter and height was done by using a measuring tape and weight was measured in a weight balance machine.

To count the total number of cells, Latter's formula was applied to each of the combs and the formula was: N (the total number of cells in one comb)= (3n/2+1)

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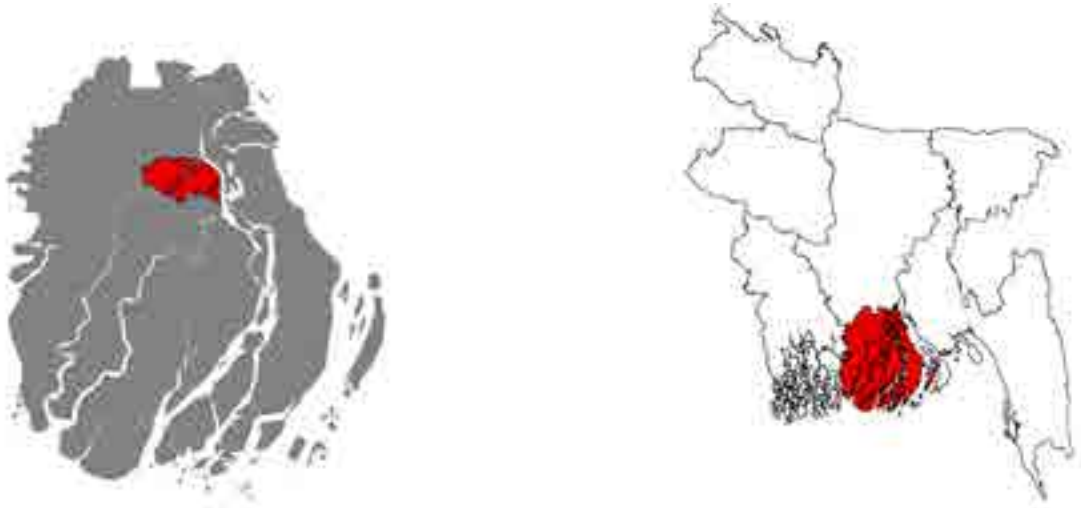

Figure 1. The right image shows the location of Barishal metropolitan in Bangladesh. The left image shows the location of Barishal sadar thana in Barishal metropolitan from where the nest of *Vespa affinis* was collected.

Image 1. Nest of *Vespa affinis*.

Image 2. The same nest without the envelope.

× n/2; where n is the number of cells counted across its maximum diameter (Latter 1935). By applying this formula, in total 10,639.25 cells were estimated from the whole nest. But unfortunately, the cells were found empty (without any eggs, larvae, and pupae). Like Seeley & Seeley (1980), we also assume that before leaving the nest the wasps had stopped the rearing of their offspring.

As wasps play an important role in ecology and some characters of nest structure has importance in taxonomy (i.e., supra-specific classification) (Yamane & Makino 1977), we suggest further research on the nest structure to better understand the taxonomy, distribution, nesting biology, and evolution of nests of this species.

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NOTE

Endemic *Primula xanthopa* Balf.f. & R.E. Cooper: rediscovery after 88 years from Bumdeling Wildlife Sanctuary, Bhutan

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The genus *Primula* L. is one of the largest genera with approximately 430 species distributed in temperate and alpine regions of the northern hemisphere (Richards 2003; Ghalley et al. 2021). About 71 species of *Primula* L. are recorded in Bhutan (Grierson & Long 1999; Ghalley et al. 2021). *P. xanthopa* Balf.f. & R.E. Cooper is endemic to Bhutan, recorded only from a single locality where it was discovered by R.E. Cooper on 10 August 1915 during an expedition in Bhutan (Smith & Fletcher 1943). The species was collected again by Ludlow & Sherriff from the same locality in 1933 (Smith & Fletcher 1943). The present record of *P. xanthopa* Balf.f. & R.E. Cooper was rediscovered from south-west of Bumdeling Wildlife Sanctuary, Bhutan (91.313^{0}E, 27.577^{0}N) on 5 August 2021 at an elevation of 3,785 m. This rediscovery of the species comes after a lapse of 88 years from the time it was last recorded by Ludlow and Sherriff in 1933. *P. xanthopa* was found growing in a Fir-forest with existential threats from grazing and anthropogenic activities. The current findings used Flora of Bhutan manual for identification and morphological description of *P. xanthopa*, along with description on its distribution. Herbarium specimen was prepared, and the specimen is deposited in the herbarium center of Bumdeling Wildlife Sanctuary.

P. xanthopa was collected during a field exploration on 5 August 2021 from Dongla pass, Lhuntse district, Bhutan. Herbarium specimen was prepared following Smith (1971) and submitted to Bumdeling Wildlife Sanctuary herbarium collection center with the herbarium collection number 330. The morphological characteristics were examined for confirmation of the identity of the specimen using the manual *"Flora of Bhutan"* (Grierson & Long 1999). The Geo-coordinates were recorded with Garmin GPS.

Taxonomy: *P. xanthopa* Balf.f. & R.E.Cooper in Notes Royal Botanical Garden Edinburgh (1916); Ward (1930); Smith & Fletcher (1943); Grierson & Long (1999).

Type: Bhutan, Dong La, Kurted (Cooper, 4393-type, 4977); Ludlow & Sherriff, 329, (Syntype-BM, Photo!)

Description: Perennial herb with slender rhizome bearing withered leaves remains. Leaves obovate or elliptic or oblong including petiole 2–9 cm long, 0.8–2 cm broad; apex rounded, margin coarsely sharply toothed, base narrowed to a slender petiole, glabrous and efarinose above, thickly golden farinose beneath.

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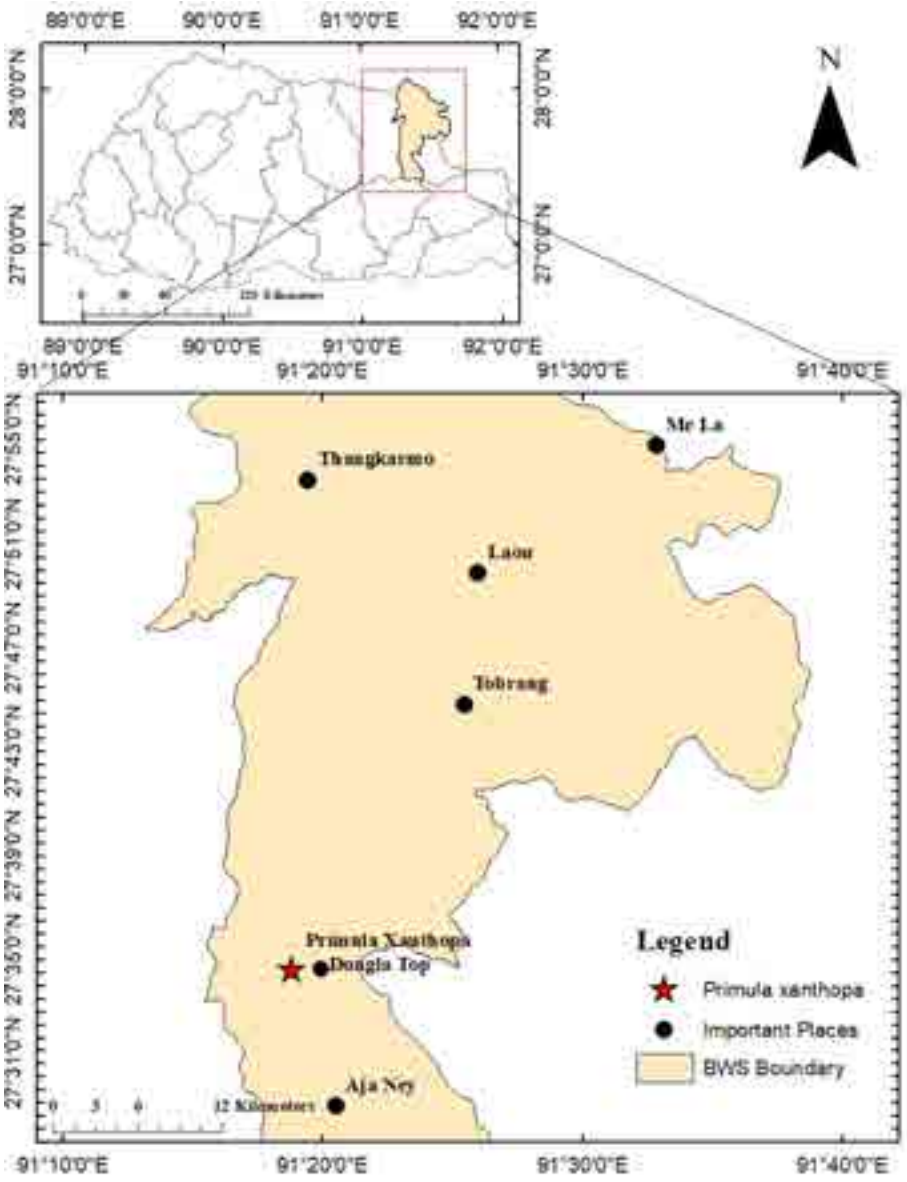

Figure 1. Distribution record of *Primula xanthopa* in Bumdeling Wildlife Sanctuary, Bhutan.

Midrib conspicuous below with very slender lateral nerves; petiole winged, as long as the blade or up to twice as long, broad and sheathing at the base, yellow-farinose. Scape 5–12.7 cm tall, slender, farinose towards the apex, carrying 1–4 flowers. Bracts unequal, oblong to slightly oblanceolate, 7 mm long and nearly 2 mm broad, or linear-lanceolate and about 3.5–4 mm long, in all cases more or less farinose. Pedicels up to 1 cm long, sub-erect, yellow-farinose. Calyx 4–6 mm long, campanulate, farinose within and without, cut to about two-third into oblong-ovate obtuse or acute teeth. Corolla purplish pink with a yellow eye, exannulate, heteromorphic; tube cylindrical, ampliate above the insertion of the stamens, about 1 cm long; limb 1–16 cm across with oborate emarginate lobes. Stamens, Kith anthers barely 1 mm long, inserted near middle of tube in pin-eyed flowers, towards apex in thrum-eyed. Long style almost equaling tube; short style as long as calyx. Capsule oblong, almost 5–6 mm long, included in calyx (Smith & Fletcher 1943; Grierson & Long 1999).

Distribution: Dongla, Bumdeling Wildlife Sanctuary, Bhutan (Figure 1)

Specimens examined: Bhutan, Bumdeling Wildlife Sanctuary, (Dongla, Lhuntse) (91.313^{0}E, 27.577^{0}N; 3,785m) 5 August 2021, R.E Cooper, Dongla, 1915, (Syntype BM, Photo!). The specimen was deposited at Bumdeling Wildlife Sanctuary herbarium collection center, Trashiyangtse with collection number 330, which

Image 1. *Primula xanthopa*: A—Habit and habitat | B & E—Corolla with yellow eye | C—Inflorescence | D—Entire plant | F—Calyx | G—Leaves. © K. Sangay & P. Wangchuk

was collected on 5 August 2021 by Mr. Karma Sangay from Dongla, Lhuntse (Image 2).

Habitat: This species is found only from Dongla in Bumdeling Wildlife Sanctuary, eastern Bhutan, where it was found by Cooper in 1915, growing on mossy rocks/ cliffs under fir forest at 4,000 m (Smith & Fletcher 1943). The actual coordinates of *P. xanthopa* was not reflected by R.E. Copper as well as other plant explorers except the locality name and habitat (Smith & Fletcher 1943) Similarly, in this current observation, the plant was found growing on mossy rock and cliff-ledges in a fir forest in the vicinity of the same locality as found by R.E Copper and Ludlowi & Sherrif, dominated by *Abies densa* Griff, *Rhododendron lepidotum* Wall, associated with *Rosa sericea* Lindl and herbs like *P. gracilipes* Craib and *P. denticulata* Sm. The plants of *P. xanthopa* were found growing in Dongla, Lhuntse at a single locality at an altitude of 3,785 m in the current observation. The plants were inhabiting shady cliffs facing north-west direction.

Flowering: *P. xanthopa* flowers in July–August, Corolla purplish-pink with a yellow eye with long style almost equaling tube; short style as long as calyx and deeply incised leaves and long petiole which distinguish it from similar plants like *P. yunannensis* Franch (Smith & Fletcher 1943).

Conservation status: *P. xanthopa* is endemic to Bumdeling Wildlife Sanctuary, Bhutan. The species is not listed in International Union for Conservation of Nature Red List (IUCN) as the species remained unexplored since 1933 with limited information (Ward 1930). Despite being endemic and rare, it is not included in protected plant species list of Forest and Nature Conservation Rule and Regulation (2017), Bhutan. The habitat of *P. xanthopa* was observed to have existential threats from grazing animals and anthropogenic activities such as timber extraction, which pose major concerns over its distribution. Proper conservation methods are needed before the habitat of this endemic species will deteriorate and vanish leading to extinction of the species. Therefore, concerned authorities and agencies should initiate appropriate strategies to conserve this species from threats and developmental activities.

Conclusion: *P. xanthopa* is recorded from Dongla pass, Bumdeling Wildlife Sanctuary, Bhutan during floristic exploration on 5 August 2021. A single plant was found growing on a mossy rock in a fir forest in the vicinity of the same locality where it was found by R.E Copper, Ludlowi & Sherrif. The habitat of rare *P. xanthopa* was observed to have existential threats from grazing animals and anthropogenic activities such as timber extraction, posing major concerns over its distribution. The plant should be protected at the earliest by all feasible methods before its disappearance.

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Image 2. Herbarium sheet of *Primula xanthopa*.

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