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### Spatial assemblage of shorebirds (Aves: Charadriiformes) in an altered wetland of the southern coast of Sri Lanka

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Abstract: The coastal wetlands along the southern coast of Sri Lanka are home to a diverse array of shorebirds thriving in their natural habitats, classified under the order Charadriiformes. This study examines the impact of land cover changes within the Kalametiva Sanctuary. situated on the southern coast, on the diversity, distribution, and habitat utilization of migrant and breeding resident shorebirds. Three distinct habitat types were selected within the study area: grassland, lagoon, and mixed mangroves. Employing Geographic Information System (GIS) data the land cover changes of these habitats from 2002 to 2023 were analyzed using satellite imagery. Results indicated a substantial decrease in lagoon habitat area by 70% and grassland habitat by 30%, while mixed mangrove habitat saw an increase of >90%. These changes were attributed to anthropogenic interventions and natural events such as the 2004 Tsunami. To determine the shorebird assemblages within the study area thriving in these changing habitats, surveys were conducted from May 2022 to April 2023 at 30 fixed point-count stations along transects in the selected habitat types. A total of 25 shorebird species belonging to six families were recorded. Notable among these were the globally 'Near Threatened' species such as the Black-tailed Godwit Limosa limosa and the Great Thick-knee Esacus recurvirostris, alongside nationally threatened species including Kentish Plover Charadrius alexandrinus, Gull-billed Tern Gelochelidon nilotica, and Common Tern Sterna hirundo species. The mixed-mangrove habitat exhibited the highest shorebird abundance, with 19 species recorded, surpassing the 13 and 11 species recorded in the grassland and lagoon habitats, respectively. Additionally, both grassland and mixed-mangrove habitats demonstrated similar diversity indices and shared more species in common (Shannon's diversity index [H] = 2.17; Jaccard Similarity Index = 0.45) compared to the lagoon habitat (H = 2.09). Despite the notable decline in lagoon habitat cover, during the present study, it was observed that the overall shorebird populations have been sustained within grassland and mixedmangrove habitats utilized for their feeding and nesting. Additionally, over-summering migratory shorebirds were observed utilizing these habitats. Hence, the Kalametiya sanctuary serves as a unique setting to study the ecological resilience of migratory and breeding resident shorebirds amidst human interventions. This research provides valuable insights for biodiversity conservation and habitat management in  $the face of human-induced \ alterations \ within \ ecosystems \ located \ especially \ along \ migratory \ pathways \ of shorebird \ species. \ Both \ grassland$ and mixed-mangrove habitats exhibited similar diversity indices and shared more species (Shannon's diversity index [H] = 2.17; Jaccard Similarity Index = 0.45) compared to the lagoon habitat (H = 2.09). Despite the significant decline in lagoon habitat cover, this study observed that overall shorebird populations have been sustained within the grassland and mixed-mangrove habitats, which they use for feeding and nesting. Additionally, over-summering migratory shorebirds were observed utilizing these habitats. Thus, the Kalametiya sanctuary serves as a unique setting for studying the ecological resilience of migratory and breeding resident shorebirds amidst human interventions. This research offers valuable insights for biodiversity conservation and habitat management, particularly in ecosystems along migratory pathways of shorebird species, in the face of human-induced alterations.

Keywords: Bird migration, Central Asian Flyway, coastal wetland, habitat alteration, habitat utilization, land cover changes, protected areas, wetland conservation.

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#### INTRODUCTION

Sri Lanka serves as a major landmass for migratory birds traversing the Central Asian Flyway (CAF). The coastal wetlands on the island support a rich biodiversity and provide essential ecosystem services such as maintaining ecological balance and nutrient cycling (Basset et al. 2013; Gunatilleke et al. 2017; Newton et al. 2018). They also play a pivotal role in connecting global habitats by serving as a vital stopover, feeding and nesting grounds for migrating and breeding resident avifauna, especially shorebirds of the Order Charadriiformes (Kotagama et al. 2006). Notably, coastal wetlands particularly in southern Asia are known to offer critical habitat for threatened migratory shorebirds with decreasing populations due to the depletion of the coastal environment (Ferreira et al. 2005; Delany et al. 2009). They are indispensable for long-distance migrant shorebirds to replenish energy stores, feed, and rest, and for resident shorebirds to sustain their populations through nesting sites (Pérez-Ruzafa et al. 2011; Aycock & Sims 2015; Duan et al. 2022).

Despite their importance, human exploitation has led to threats and modifications to Sri Lanka's coastal wetlands over the years (Jayathilake & Chandrasekara 2015; Madarasinghe et al. 2020b; Kodikara et al. 2023). However, studies identify that understanding interactions with the ecology and biodiversity of coastal wetlands in developing countries including Sri Lanka show a lack of improvement in comparison to the well-developed regions of the world. Hence, it is important to determine the influence of such interventions on the biodiversity that depends on these habitats, especially for sensitive species like shorebirds, and what measures could be taken to manage and minimize any detrimental effects on the shorebird community.

The Kalametiya Sanctuary, located on the southern coast of Sri Lanka, is renowned for its biodiversity and ecological significance, serving as an avifaunal region (Ekanayake et al. 2005; Bernard et al. 2023). Encompassing over 700 hectares, the sanctuary comprises various habitats including mangrove swamps, lagoons, sandy shores, and grasslands, all of which support shorebird communities (Ekanayake et al. 2005; Bernard et al. 2023). Designated as a sanctuary since 1984, it is governed by the Fauna and Flora Protection Ordinance and the southern Province Hambantota Integrated Coastal Zone Management Project (Jayatissa et al. 2002; Ekanayake et al. 2005; Perera et al. 2013; Madarasinghe et al. 2020a). Scientific research within Kalametiya sanctuary can therefore provide valuable

insights into not only avian ecology but also on coastal wetland dynamics and ecosystem resilience of sustaining faunal communities. Previous studies have documented the sanctuary's floral and fauna diversity and how various interventions, such as the Udawalawe Irrigation and Resettlement Project and the 2004 Tsunami tragedy, have altered the habitat composition within the sanctuary, over the course of the period from 2005 to 2020 (Bambaradeniya et al. 2005; Ekanayake et al. 2005; Madarasinghe et al. 2020a,b).

Based on the literature, one of the main factors for good shorebird habitat is the presence of adequate foraging sites. Effective conservation of shorebird habitats requires a thorough understanding of the patterns of shorebirds' spatial and temporal utilization of habitats. This knowledge can be difficult to attain and may be time-consuming, especially in coastal settings, since shorebirds can show complex local movements, being very specific in their habitat requirements and their capability of traversing long distances between preferred sites (Warnock & Bishop 1998; Pearce-Higgins et al. 2017). Hence, to better understand species-habitat relationships within coastal wetlands, it is imperative to adopt a multispectral and multi-temporal data analysis approach using modern remote sensing and GIS technologies (Albanese et al. 2012; Kaliraj et al. 2017; Vivekananda et al. 2021). Studies such as the present research that incorporates both on-ground field records and remote sensing analysis could serve the purpose of achieving the intended objectives of effective shorebird species conservation and habitat management.

Moreover, since shorebirds serve as biological indicators of ecosystem health (Colwell 2010), conservation efforts must focus on preserving their habitats, including wintering, feeding, and nesting grounds (Thomas et al. 2004; Jumilawaty et al. 2022). Yet none of the research conducted thus far has assessed shorebird diversity, distribution, and habitat utilization in the Kalametiya Sanctuary. This study aims to address this gap by determining shorebird diversity, distribution, and habitat utilization in Kalametiya Sanctuary, considering the effects of recent land cover changes, and proposing management options, including the possibility the Sanctuary be declared as a Ramsar-protected wetland based on identified human and natural threats.

#### **MATERIALS AND METHODS**

#### Study area

This study was conducted within Kalametiya Sanctuary (6.086 N, 80.936 E) situated in the southern province of Sri Lanka. This region falls within the island's dry zone, receiving an average annual rainfall ranging 1,000–1,250 mm. Spanning an area of 700 ha, the sanctuary is designated under the Fauna and Flora Protection Ordinance (FFPO) and governed by the Department of Wildlife Conservation (DWC) (Ekanayake et al. 2005; Perera et al. 2013). It comprises the larger Kalametiya lagoon and a smaller Lunama lagoon, connected by a man-made narrow channel. For the study, three primary habitat types were identified: grassland (G), lagoon (L), and mixed mangrove (MM), and selected (Image 1) based on accessibility and extensive land cover availability (Ekanayake et al. 2005).

#### Field survey and avifaunal sampling

Sampling was conducted from May 2022 to April

2023, employing 30-point count stations fixed along transects (Figure 1; GPS points of point count stations given in Table 4) (Bibby et al. 1998; Ntongani & Andrew 2013) in the three selected habitats. The point counts were fixed such that each station was separated by the other by 50 m or greater to minimize double counting (Bibby et al. 1998; Sutherland et al. 2012; Bernard et al. 2023) and the accessibility to each site was available throughout the study period. ArcGIS version 10.8 (Esri, Redlands, USA) was used to overlay the fixed-point count stations on a satellite map of the selected study area.

Two days of sampling were allocated for each of the three months during both the bird migratory season (October–April) and non-migratory season (May–September). Morning and evening point counts lasting 10 minutes each were conducted within a 2–3 h window of predicted low tide - conducted during daylight, although some shorebird foraging occurs at night (Bibby et al. 1998), on each sampling day at each station by pairs of observers with similar training levels. Surveys were conducted either on foot in the grassland and

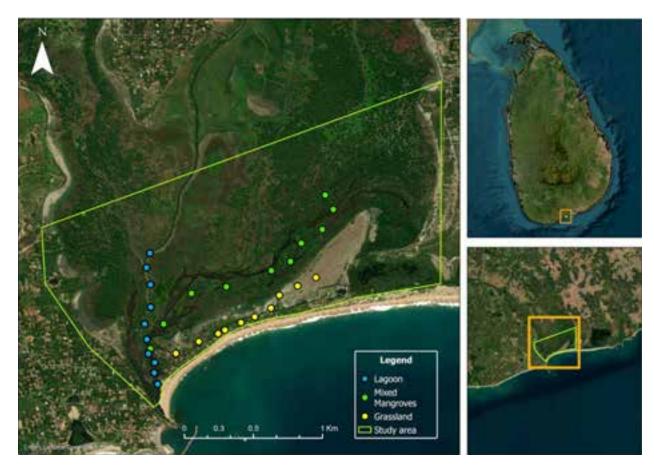


Figure 1. Point count stations along transects within the grassland, lagoon, and mixed-mangrove habitats in the Kalametiya Sanctuary, southern coast of Sri Lanka. Prepared by: E.G.D.P. Jayasekara.

mixed mangrove habitats or using slow paddle boats in the lagoon habitat, taking measures to minimize any disturbances to the species during the survey. Shorebirds that flew over a point were disregarded unless they landed or took off within the point count radius within the observation period. Standard birding equipment such as Nikon 8 × 40 Action Extreme and Nikon Monarch 10 × 42 binoculars, Vanguard Endeavor XF 60A 15–45 x 60 spotting scope, and standard field guides (Harrison & Worfolk 2011; Hayman et al. 2011) were utilized for species identification and recording. The observed shorebird species' common English names were documented, and their conservation status was later determined using the IUCN Red List of Threatened Species and the National Red List of Birds of Sri Lanka

#### Species richness, diversity, and similarity

(Secretariat 2022).

Species richness was recorded as the number of shorebird species present in a particular habitat and season. PAST 4.15 open-source software was used to estimate Shannon's diversity index (H) based on the following equation:

$$H = \Sigma pi \log(ln) pi$$

where, pi is the proportion (n/N) of individuals of a particular species observed (n) divided by the total number of individuals recorded (N), while ln is the natural log, and  $\Sigma$  is the sum of the calculations. Significant differences between the seasonal diversity indices were calculated using the diversity t-test.

The Relative abundance was computed using [(number of individuals per species/total number of individuals)  $\times$  100%] and the Jaccard similarity index [SJ] was calculated between the habitats by using the equation,

$$SJ = S_a / (S_a + S_b + S_c)$$

where  $S_a$  is the number of species unique to the first habitat,  $S_b$  is the number of species unique to the second habitat, and  $S_c$  is the number of species common in both habitats.

#### **Determination of habitat alteration**

Multi-spectral satellite images from Landsat 7 (ETM+/path\_141/row\_56) and Landsat 8 (OLI\_TIRS sensor/path\_141/row\_56) missions were obtained from the United States Geological Survey online database (https://earthexplorer.usgs.gov/). The search aimed to find Landsat datasets with minimal cloud cover during the months of June to August, consistent with previous studies (Jayatissa et al. 2002; Madarasinghe et al. 2020b; Bernard et al. 2023) to avoid months with extreme

rainfall or drought conditions. The selected images for analysis were from 07 July 2002 (Landsat 7), July 18, 2012 (Landsat 7), and 09 July 2023 (Landsat 8). This approach minimized the seasonal impact on habitat spatial variation and allowed an unbiased evaluation of the lagoon's saltwater and freshwater balance, considering historical precipitation levels in July. To address differences in resolution and image quality among the Landsat datasets, Red and NIR (near-infrared) bands were utilized to generate normalized difference vegetation index (NDVI) rasters as the basis for image classification. NDVI values were calculated using the formula: NDVI = (NIR - Red)/(NIR + Red) (Grebner et al. 2013; Pantazi et al. 2020). Habitats were classified based on NDVI pixel values: lagoon <0.15, grassland 0.15-0.2, other vegetation 0.2-0.25, mixed mangrove >0.25 (Drisya & Roshni 2018). An accuracy assessment was performed on the classified images corresponding to the selected years and kappa coefficients were calculated using a confusion matrix (Vivekananda et al. 2021). Ground observations as well as Google Earth images were used for ground truthing the 2023 classified image. Google Earth images alone were used for the ground truthing accuracy assessment of 2002 and 2012 classified images. Therefore, the limited availability of corresponding Google Earth historical data was considered during the Landsat image selection.

#### **RESULTS**

#### **Species Richness and Diversity**

During the survey, 602 individuals of 25 shorebird species belonging to six families were recorded. A similar shorebird species diversity was recorded in the grassland and mixed-mangrove habitats (H = 2.17) and the least diversity was recorded in the lagoon habitat (H = 2.09) (Figures 2 & 3).

The Black-winged Stilt Himantopus himantopus, a breeding resident shorebird with a migrant population was the most abundant species recorded in all three habitats during the study period. The least abundant species recorded were the Gull-billed Tern Gelochelidon nilotica and the Whimbrel Numenius phaeopus species (Table 1). A notable observation was the record of oversummering populations of Common Redshank Tringa totanus and Ruddy Turnstone Arenaria interpres species utilizing the grassland and mixed mangrove habitats during the non-migratory season. The record of the globally 'Near Threatened' species Black-tailed Godwit Limosa limosa and Great Thick-knee Esacus recurvirostris,

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Table 1. Shorebird species of Order Charadriiformes that were recorded during the study period in the selected three habitats.

Family	Scientific name	Common name	GCS (2021)	NCS (2021)	Phenological status	Relative abundance (%)	Habitat
Doublistida	Esacus recurvirostris (Cuvier, 1829)	Great Thick-knee	NT	LC	R	3.99	G/ L/ MM
Burhinidae	Burhinus indicus (Salvadori, 1865)	Indian Thick-knee	LC	LC	R	1.66	G
	Charadrius mongolus (Pallas, 1776)	Lesser Sand Plover	LC	-	М	8.80	G/ MM
	Charadrius leschenaultia (Lesson, 1826)	Greater Sand Plover	LC	-	М	0.83	G/ MM
	Pluvialis fulva (Gmelin, 1789)	Pacific Golden Plover	LC	-	М	2.82	G/ MM
Charadriidae	Charadrius alexandrinus (Linnaeus, 1758)	Kentish Plover	LC	EN	R/M	1.16	G
	Pluvialis squatarola (Linnaeus, 1758)	Grey Plover	LC	-	М	0.83	G/ MM
	Vanellus indicus (Boddaert, 1783)	Red-wattled Lapwing	LC	LC	R	12.62	G/ L/ MM
	Vanellus malabaricus (Boddaert, 1783)	Yellow-wattled Lapwing	LC	LC	R	3.16	G
Jacanidae	Hydrophasianus chirurgus (Scopoli, 1786)	Pheasant-tailed Jacana	LC	LC	R	2.33	L
	Chlidonias hybrida (Pallas, 1811)	Whiskered-Tern	LC	-	М	3.49	L/ MM
	Gelochelidon nilotica (Gmelin, 1789)	Gull-billed Tern	LC	CR	R/M	0.49	L
Laridae	Sterna hirundo (Linnaeus, 1758)	Common Tern	LC	CR	R/M	1.16	L
	Sternula albifrons (Pallas, 1764)	Little Tern	LC	VU	R/M	1.83	L/ MM
Recurvirostridae	Himantopus himantopus (Linnaeus, 1758)	Black Winged Stilt	LC	LC	R/M	24.25	G/ L/ MM
	Tringa totanus (Linnaeus, 1758)	Common Redshank	LC	-	М	16.45	G/ L/ MM
	Tringa nebularia (Gunnerus, 1767)	Common Greenshank	LC	-	М	0.33	MM
	Tringa stagnatilis (Bechstein, 1803)	Marsh Sandpiper	LC	-	М	0.49	L/ MM
	Tringa glareola (Linnaeus, 1758)	Wood Sandpiper	LC	-	М	0.83	MM
Scolopacidae	Actitis hypoleucos (Linnaeus, 1758)	Common Sandpiper	LC	-	М	1.33	L/ MM
	Arenaria interpres (Linnaeus, 1758)	Ruddy Turnstone	LC	-	М	4.98	G/ MM
	Calidris minuta (Leisler, 1812)	Little Stint	LC	-	М	1.66	ММ
	Numenius phaeopus (Linnaeus, 1758)	Whimbrel	LC	-	М	0.49	ММ
	Limosa limosa (Linnaeus, 1758)	Black-tailed Godwit	NT	-	М	0.83	ММ
	Gallinago stenura (Bonaparte, 1830)	Pintail Snipe	LC	-	М	1.16	G/ MM

M—Migrant | R—Resident | GCS—Global Conservation Status | NCS— National Conservation Status.

and nationally critically endangered species Gull-billed Tern *Gelochelidon nilotica* and Common Tern *Sterna hirundo* and the nationally endangered Kentish Plover *Charadrius alexandrinus* within the study area highlights the overall standpoint of Kalametiya Sanctuary to support thriving shorebird communities.

Changes in shorebird species diversity and richness

during the migratory and non-migratory seasons depicted that most shorebirds were observed utilizing mixed mangrove habitats than the grassland and lagoon habitats during the migratory season, while in the non-migratory season, the highest occurrence was recorded in the grassland habitat (Table 2). A t-test comparison of the diversity indices of the migratory season indicates



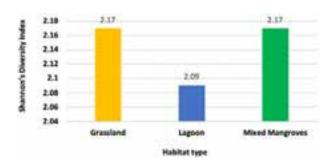


Figure 2. Shannon's diversity (H) indices which were recorded in the three habitats.

that there is a significant difference in the diversity recorded in the mixed mangrove habitat with that of the diversity recorded in the lagoon (t = 2.682, df = 85.52, p = 0.008) and grassland habitats (t = 2.034, df = 287.07, p = 0.0429). During the non-migratory season however, only the diversity of shorebird species recorded in the mixed mangrove and lagoon habitat (t = 3.132, df = 168.94, p = 0.002) significantly differed.

#### Changes in land cover within the study area

The spatio-temporal change in land cover within the study area for the past two decades is depicted in the maps (Figure 4) generated from satellite data. Overall, the lagoon area has decreased by 69.94% and the grassland area has decreased by 30.75%. However, the area of mixed mangroves within the study site has increased by 93% from 2002 to 2023 (Table 3). The accuracy assessment was performed for 2002, 2013, and 2023 land cover maps and an overall kappa statistic of 0.741, 0.754, and 0.736 were computed for each respectively.

#### **DISCUSSION**

The global decline in shorebird populations is of growing concern, highlighting the need for dedicated efforts towards their conservation and sustainable management (Clemens et al. 2010; Aarif et al. 2014). Coastal wetlands, particularly in Asia which support such migratory and breeding resident shorebird species, even though considered the most productive of the many types of wetlands categorized by the Ramsar Convention (https://rsis.ramsar.org/), are affected by exponential population growth and imbalances in demand-supply interactions arising mostly due to urban developments. Shorebirds are considered to play a significant role in maintaining the health of the environment they utilize (Colwell 2010) and therefore, the protection of their stopover resources within coastal wetlands could pave the way for the sustaining of both shorebird communities and the wetland habitats (Myers et al. 1987; Skagen & Knopf 1994). Considering the above concerns, it is evident that Sri Lanka's coastal wetlands present an understudied yet ecologically significant arena for investigating the dynamics of shorebird communities amidst continuing alterations in the southern Asian region. Hence, the present study conducted in the Kalametiya Sanctuary, located in southern Sri Lanka, which assesses the shorebird diversity, distribution, and habitat utilization considering habitat cover changes that have occurred over past decades, provides a reference framework for implementing timely species conservation and habitat management strategies.

During the present research, a total of 25 shorebird

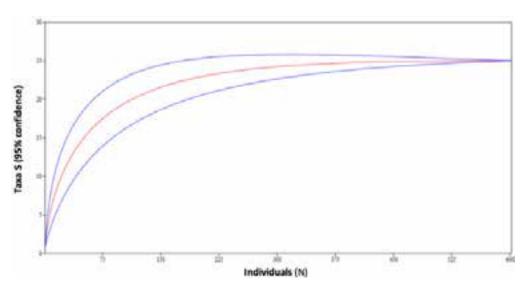


Figure 3. The rarefaction curve for species accumulation with the number of individuals recorded within the study area.



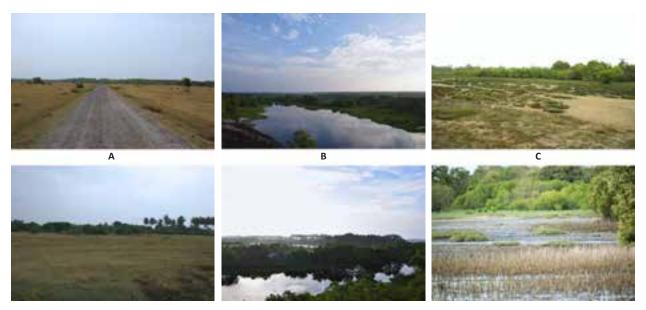


Image 1. A—grassland | B—lagoon | C—mixed mangrove habitats during the study period. © V.N. Mendis.

Table 2. Shorebird species diversity comparison of the migratory and non-migratory seasons in the three habitats.

	Non-migratory season			Migratory season			
	Grassland	Lagoon	Mixed mangrove	Grassland	Lagoon	Mixed mangrove	
Shannon's diversity index (H)	2.055	1.919	1.472	2.207	2.079	2.429	
Evenness	0.7811	0.757	0.436	0.699	0.799	0.630	
Richness	10	9	10	13	10	18	
Total number of individuals (N)	89	68	101	126	48	170	

species belonging to the order Charadriiformes were documented, of which 60% can be classified as migratory species, 20% as breeding residents, and the remainder as breeding resident species with a migrant population. In contrast, a recent study by Bernard et al. (2023) reported only 10 shorebird species, while a previous biodiversity profile by Ekanayake et al. (2005) documented 38 species. However, it is important to note that the 2005 survey considered the entire Kalametiya-Lunama sanctuary area, whereas the present research focused specifically on habitats adjoining the Kalametiya lagoon. Further, the species that were recorded in the 2005 survey that were not recorded in the present research are some of the rare and uncommon migrant shorebird species such as the Ruff Calidris pugnax and Long-toed Stint Calidris subminuta and some of the uncommon migrant tern species such as the Saunders's Tern Sternula saundersi, Great Crested Tern Thalasseus bergii, and Lesser Crested Tern Thalasseus bengalensis, which have been found utilizing mostly the brackish lagoon water

habitat within the entire Kalametiya-Lunama sanctuary area. Consequently, direct comparisons of species richness values between studies are not feasible due to differences in study area delineation. The demarcated study area of the present study was chosen to assess how changes in land cover within the Kalametiya lagoon and surrounding habitats have impacted the diversity and habitat utilization of shorebirds, considering the proportional area of the sanctuary. Further studies in the Lunama lagoon area to supplement the present study could be suggested to follow a comparative approach in identifying the shorebird habitat utilization within the larger sanctuary area post two decades since the last published biodiversity profile (Ekanayake et al. 2005).

Sanctuaries play a crucial role in supporting biodiversity, albeit often subjected to regulated human interventions (Green 1990). Over the past decades, Kalametiya Sanctuary has undergone alterations, necessitating an understanding of the degree of habitat cover change and its impact on long-distance migrant



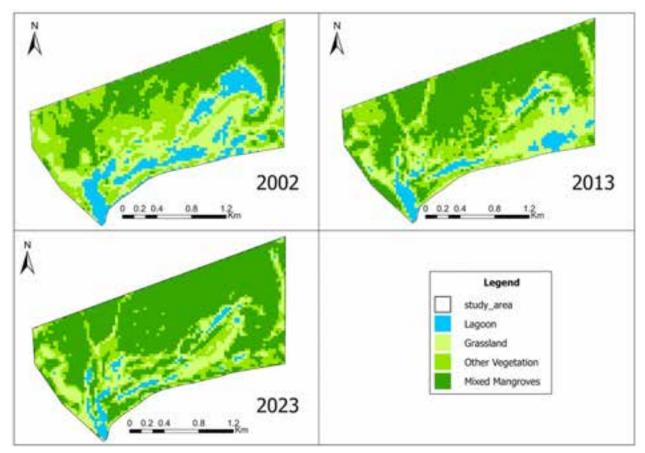


Figure 4. Spatio-temporal change of land cover within the study area depicted using satellite imagery. Prepared by E.G.D.P. Jayasekara.

and breeding resident shorebird species. Hence, the study analyzed satellite imagery data alongside spatial assemblage data of shorebirds utilizing the Kalametiya Lagoon and surrounding habitats with the intention of assessing the present conditions within the sanctuary. The findings revealed a significant decrease of over 69% in the lagoon area since 2002, a notable alteration that could potentially change the species composition within the sanctuary. Yet, this decrease in the lagoon area hasn't led to a drastic decline or a total displacement of shorebird communities. Based on the results of the present study, several reasons for this phenomenon can be proposed.

Studies identify that shorebirds feed and roost within the shallows of a wetland having water depths of less than 30cm, where prey availability and accessibility are mediated by their leg and bill lengths, or on the shores of wetland clusters during low tides, providing them chances with abundant prey (Zwarts & Wanink 1993; Bellio & Kingsford 2013). Hence, shorebirds continuing to sustain within the selected study area is reflected by the study results which show that their most utilized feeding grounds were the mixed mangrove and grassland

Table 3. Land cover change (%) of the habitats in the study area over the period of 2002–2023.

	2002	2013	2023	Change (2002– 2023)	% change
Lagoon	13.7	6.5	4.1	-9.6	-69.9373
Grasslands	24.6	22.7	17.0	-7.6	-30.7483
Other vegetation	30.0	24.0	17.7	-12.3	-40.9414
Mixed mangroves	31.6	46.8	61.1	29.5	93.19583

habitats, recording shallower water depths (less than 30 cm) which match their bill length and leg heights, making these areas preferable over deeper lagoon waters.

Additionally, breeding resident shorebirds such as the Black-winged Stilts (Ashoori 2011) and Red-wattled Lapwings (Hart et al. 2002; Arya et al. 2023) construct their nests in grassy areas or abandoned agricultural lands using dung, mud, and decayed plant materials, unlike waterbirds that prefer aquatic vegetation for nesting. Therefore, the decrease in the lagoon area doesn't significantly impact the nesting behaviors of these shorebirds. The nesting sites also contribute to



Table 4. Point count stations fixed along transects in the three selected habitats within the study area.

Grassland T1			Lagoon T2			Mixed mangrove T3		
Point count station	Longitude	Latitude	Point count station	Longitude	Latitude	Point count station	Longitude	Latitude
P1i	80.937	6.082	P1ii	80.936	6.080	P1iii	80.935	6.083
P2i	80.938	6.083	P2ii	80.935	6.081	P2iii	80.936	6.084
P3i	80.940	6.084	P3ii	80.935	6.081	P3iii	80.938	6.086
P4i	80.940	6.084	P4ii	80.935	6.082	P4iii	80.940	6.087
P5i	80.941	6.084	P5ii	80.935	6.083	P5iii	80.943	6.088
P6i	80.942	6.085	P6ii	80.935	6.084	P6iii	80.944	6.088
P7i	80.943	6.085	P7ii	80.935	6.085	P7iii	80.945	6.090
P8i	80.944	6.086	P8ii	80.935	6.087	P8iii	80.947	6.090
P9i	80.945	6.087	P9ii	80.935	6.088	P9iii	80.947	6.092
P10i	80.946	6.087	P10ii	80.935	6.089	P10iii	80.947	6.093

the notable species diversity observed in the grassland habitat during the non-migratory season. Furthermore, during the non-migratory season, coinciding with the dry spell in the study area, the drying up of mixed mangrove habitats reduces invertebrate food sources for shorebirds. In the grassland habitat where cattle grazing activities occur, the soil is disturbed, exposing land invertebrates such as snails and soft annelids, which serve as a vital food source for nesting parents and newly hatched shorebirds. However, such agricultural interventions contribute both positively and negatively to shorebird habitat utilization, and if not managed can lead to detrimental impacts.

Despite past recommendations for cattle grazing to be limited to demarcated buffer zones (Ekanayake et al. 2005), herds were observed in sensitive mixed mangrove and grassland habitats, risking nest trampling (Hart et al. 2002) and disturbing shorebird feeding grounds. A trampled nest of each of Red-wattled Lapwing and Blackwinged Stilt were recorded during the present study. It is encouraged to conduct further research to actively monitor and quantify trampling rates of shorebird nests and highlight the need for the demarcation of grazing buffer zones within the Sanctuary.

The loss of wintering grounds in southern Asia for long distant migratory shorebirds due to coastal developments and habitat loss has posed a significant threat over the past years (Aarif et al. 2014; Byju et al 2023), especially to over-summering populations of shorebird species including the Common Redshank and Ruddy Turnstone (Aarif et al. 2020) which were recorded during the present study in the Kalametiya Sanctuary. Conservation of their over-summering habitats becomes crucial for successful population recruitment of these

species into migratory counterparts in a consequent season or provides conditions supporting them to adapt and withstand changes beyond their home grounds. Moreover, habitat alterations following the 2004 Tsunami (Bambaradeniya et al. 2005; Perera et al. 2013; Madarasinghe et al. 2020a), the irrigation project bringing in more siltation into the lagoon (Madarasinghe et al. 2020b; Kodikara et al. 2023) and the recent breakwaters built in the Kalametiya fishing harbor, have impacted the ecosystem. Effective management actions are essential to regulate the environment for wildlife and humans. Despite past recommendations (Ekanayake et al. 2005) the present study notes continued habitat depletion due to inadequate intervention. This underscores the urgent need for enhanced conservation efforts to protect these vital habitats and their biodiversity.

Kalametiya Sanctuary sustains local livelihoods through fisheries and cattle farming (Ekanayake et al. 2005) and promotes eco-tourism with peaceful paddle boat rides dedicated to birdwatching. However, despite the numerous services the coastal wetland provides to maintain a healthy and productive ecosystem, it is evident that human interventions like habitat mismanagement, over-exploitation of resources, negligence, and uncontrolled waste disposal have disrupted the human-wildlife coexistence, especially in terms of the sustainability of thriving populations like shorebirds that bring not only ecological but also economical values using eco-tourism, habitat upliftment and food web balances within their utilized habitats. Therefore, advocating for heightened public awareness and community engagement in sustainable practices of living is recommended to safeguard the sanctuary for future generations. Awareness campaigns

among the local fisheries and farming communities on the effects of mismanaged waste disposal affecting both human health utilizing contaminants, and the health of the wetland mangroves that prevent coastal erosion, effective ways of reducing the risk of nest trampling and disturbances to the mixed mangroves by adhering to demarcated grazing buffer zones are some of the ground level initiatives that can be proposed to assist shorebird species conservation and sustainable coastal wetland habitat management implications. Further studies that identify the impacts of microplastic contamination similar to the study conducted by Luna et al. (2022) which found microplastics in nests of Black-winged Stilts

Since the shorebird ecology and causes and drivers for population decline in the CAF are less explored (Mundkur & Selvaraj 2023) research such as the present study supplements to identification of human interactions that affect the shorebird assemblage and their habitat utilization within coastal wetlands along the CAF and what are the timely conservation strategies that can be newly implemented and what existing strategies can be improved for better results in future. The present study, alongside previous research, suggests the potential declaration of the sanctuary as a Ramsar site due to its global conservation significance, providing crucial habitats for long- distant migrant shorebirds, threatened shorebirds, and over-summering shorebird species. Moreover, it sheds light on the impacts of land cover and land use changes on biodiversity in this altered coastal wetland, providing valuable insights for authorities and communities to recognize Kalametiya Sanctuary as a vital hub for biodiversity conservation and environmental sustainability in Sri Lanka.

in a Biosphere Reserve in Spain could comprehend to

the present study in enhancing shorebird nesting habitat

conservation. The year 2024 World Wetland Day theme, "Wetlands and Human Wellbeing," offers an opportune

moment for initiating conservation efforts in the coastal

wetland habitat of Kalametiya Sanctuary.

#### **CONCLUSION**

The present study highlights the resilience of Kalametiya Sanctuary in supporting both migratory and breeding resident shorebird species despite ongoing habitat alterations over the past two decades. A survey conducted covering both migratory and non-migratory seasons recorded 25 shorebird species from six families, including globally and nationally threatened species. Land cover changes were assessed using satellite

imagery, achieving acceptable accuracy rates exceeding 70% for each assessment year. Encouragingly, the study found that the present status of the habitats did not adversely affect migratory and breeding resident shorebirds, avoiding a wipe out of the species from the habitats. Over-summering species were also found utilizing the habitats. However, proactive management and conservation efforts are crucial to ensure the continued thriving of shorebird populations and to prevent further habitat depletion. Overall, this research underscores the significance of conserving altered coastal wetlands to maintain vital stopover and oversummering sites, especially in the southern Asian region along the CAF, while promoting sustainable livelihoods within and surrounding the sanctuary.

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# Current conservation status of Bengal Florican *Houbaropsis bengalensis* in Manas National Park, Assam, India

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Abstract: This study aimed to examine the current conservation status of the 'Critically Endangered' bird species *Houbaropsis bengalensis* (Gmelin, 1789), commonly known as the Bengal Florican or Bustard, in Manas National Park in Assam. The grasslands of this park, once home to the largest population of Bengal Floricans in India, are undergoing natural and anthropogenic changes. Field surveys conducted from February to April 2021 to assess florican populations recorded 50 males and 17 females, while a 2009 survey estimated 24 males in Manas NP (Brahma et al. 2009), indicating long-term population increase. Land use and land cover change maps of florican habitats were created using supervised classification. Grassland habitats face several threats, including uncontrolled natural succession coupled with livestock grazing, growth of invasive alien plants and collection of non-timber forest products (NTFPs) by local people. These disturbances reflect the condition of grasslands that are home to a wide variety of wildlife, including grassland specialists like Pygmy Hog *Porcula salvania*, Hispid Hare *Caprolagus hispidus*, Hog Deer *Axis porcinus*, and mega herbivores like Indian Rhino *Rhinoceros unicornis*, wild Water Buffalo *Bubalus arnee*, Eastern Swamp Deer *Rucervus duvaucelii ranjitsinhi*, Gaur *Bos gaurus*, and Asian Elephant *Elephas maximus*. These findings indicate an urgent need to protect and restore grasslands for conservation of the Bengal Florican and other grassland-dependent species.

**Keywords:** Aves, conservation threats, grassland specialists, habitat supervised classification, habitat disturbance, Otididae, sub-Himalayan grassland.

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#### INTRODUCTION

Bengal Florican, Houbaropsis bengalensis (Gmelin, 1789), is a 'Critically Endangered' grassland bird (IUCN 2022) belonging to the family Otididae. In India, it is protected under Schedule I of the Wildlife Protection Act, 1972. The population of Bengal Florican is 350-400 in India, <100 in Nepal (Collar et al. 2017; Baral et al. 2020) and <500 individuals in Cambodia (BirdLife International 2022). In India, the population of Bengal Florican is restricted to the sub-Himalayan region of the Indo-Gangetic floodplains and the Brahmaputra floodplains (Rahmani et al. 2017). They are extremely habitat specialists and are restricted to grasslands (Prasai et al. 2021). Bengal Floricans were once distributed from Uttar Pradesh, Bihar, and West Bengal to the foothills of Assam and Arunachal Pradesh in India. However, at present, it is confined to a few protected areas in India including Manas National Park (MNP), Kaziranga National Park, Orang National Park, Dibru-Saikhowa National Park, Burachapori Wildlife Sanctuary in Assam, D'Ering Wildlife Sanctuary in Arunachal Pradesh, and Dudhwa National Park in Uttar Pradesh. The species is confined to only these few protected areas and appears nowhere outside these areas (BirdLife International 2022).

In 1989–90, the Bombay Natural History Society (BNHS) conducted a survey in India and found that Manas National Park had the highest Bengal Florican population. Around 80 birds were estimated during the study, which is perhaps the largest known population of Bengal Florican in any single locality. However, the population of Bengal Floricans declined from 1990–2000 because of the socio-political unrest created during Bodo agitation in the areas adjoining Manas. However, according to reports, the population has shown an increasing trend from 2003-2012 (Brahma et al. 2009). The population of Bengal Florican was estimated to be around 50 individuals in Manas National Park (Brahma et al. 2009). Since 1989-1990, approximately 40% of the Bengal Florican population has been lost, and grass height was the main factor. It is assumed that these birds are site-selective in establishing their territory, and prefer wet alluvial grassland for breeding, which is reduced by 47% and succeeding towards savanna grassland (Brahma 2013). The global population of the species has been rapidly declining, and the IUCN Red List for Threatened Species has upgraded Bengal Florican from 'Endangered' to 'Critically Endangered' (BirdLife International 2010).

The highest number of Bengal Floricans was reported from Kokilabari Agriculture Farm (KAF) (Brahma et al.

2009). The KAF is 9 km², which is a large grassland site. The KAF is under extensive agricultural practice and leased to local communities for paddy cultivation. Paddy cultivation began in June and harvested in December. It has been reported that the Bengal Florican population was established during 2000–2001, the period when the farm was not under cultivation. One reason for the selection of these sites by Bengal Floricans is the lack of waterlogging (Brahma et al. 2009).

The aim of this study is to address the lack of information on the populations and habitats of the Bengal florican in Manas National Park through a systematic approach, thereby contributing to the development of long-term conservation strategies. This research serves as a stepping stone for further research and education aimed at preserving the Bengal florican and its habitat.

#### **METHODS**

#### Study site

#### **Manas National Park**

Manas National Park (MNP) is a World Heritage Site, a Tiger Reserve, a Biosphere Reserve, and an Elephant Reserve located in the state of Assam (Das et al. 2022). MNP ((26.583-26.833 °N, 90.750-91.250 °E) is the core area of Manas Tiger Reserve with an area of 500 km<sup>2</sup>, located in Baksa and Chirang districts of the Bodoland Territorial Council, Assam (Figure 1). Apart from Bengal Floricans the grasslands are home to a wide variety of wildlife including grassland specialist like Pygmy Hog Porcula salvania, Hispid Hare Caprolagus hispidus, Hog Deer Axis porcinus and other mega herbivorous like Indian Rhino Rhinoceros unicornis, Brahma et al. 2009 Wild Water Buffalo Bubalus arnee, Eastern Swamp Deer Rucervus duvaucelii ranjitsinhi, Gaur Bos gaurus, and Asian Elephant Elephas maximus. It is a regional priority site for the conservation of charismatic species, including 55 mammals, 50 reptiles, and more than 450 bird species (Sarma et al. 2008; Das et al. 2022). A total of 462 plant species have been reported from within the national park, but the region is rich in plant life with about 622 plant species reported from the larger Manas Biosphere region. This includes 429 dicotyledons, 162 monocotyledons, 30 pteridophytes, and one gymnosperm (Hajra & Baishya 2002). It lies at an altitude of 57-280 m. The temperature ranges from 6-37 °C. The rainfall lies between 3,000 mm to 4,000 mm per year. The rainfall reaches its peak in July and

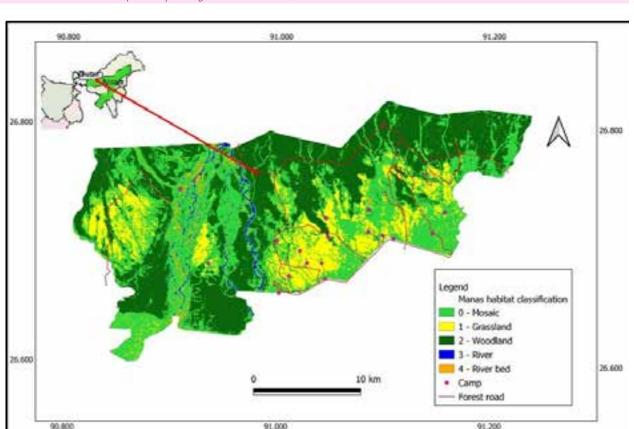


Figure 1. Map of Manas National Park in Assam.

August. The vegetation of the park is moist deciduous forest, early successional woodland grassland, and drywet alluvial grassland (Sarma et al. 2008; Banerjee et al. 2021). Due to resource extraction by local communities and livestock grazing, MNP experiences biotic pressure. Local people may start fires to encourage the growth of fresh grass, but processes are mostly initiated by park managers to control woody plant encroachment into grassland areas. However, the dynamics of fires after ignition are not controlled by forest managers, and the spatial patterns and extent of fires are determined by habitat factors (Banerjee et al. 2021).

#### Methods for population assessment

The survey was conducted based on habitat maps and past records of the complete population census of Bengal Florican. The surveys were carried out in the three ranges, namely, Bansbari, Bhuyanpara, and Panbari of Manas National Park during the peak breeding season of the Bengal Florican from 20 February–22 April 2021. Birds were observed using vehicles, walking on foot, or from elevated forest towers. Sightings and GPS locations were recorded. Field surveys were carried

out during the early morning (0600–0930 h) and late evening (1500–1800 h). On certain days, the survey was conducted until noon. The number of Bengal Floricans spotted, the number of males and females sighted, the time of sightings, along with the location of the species were recorded. Since, the species is territorial in nature during the breeding season, the display site of a male, was considered as an individual male territory and each displaying male was recorded as an individual male and the females are difficult to locate (Images 1 & 2). The breeding territories were identified and marked using handheld GPS (Garmin etrex 30x) and GIS-based map of identified Bengal Florican territories was prepared using QGIS (QGIS Development Team 2020).

The methodologies employed in this survey were consistent with those used in the studies by Brahma (2013) and Narayan (1992). Similar techniques and area coverage were utilized to ensure comparability between the current and previous surveys. No higher conservation technologies were employed during this survey, maintaining the same level of effort and methodological approach as in the earlier studies.



#### Method for land cover change

Satellite images from 2000 and 2020 were analyzed to detect the change in land cover using remote sensing and geographic information system tools. The open-source satellite images were obtained from Landsat 7 and Landsat 8 through https://earthexplorer.usgs.gov. Landsat 7 and Landsat 8 images were used for classification at 30 x 30 m resolution and I, II III, IV, V, VII and II, III, IV, V, VII bands were used respectively. Open-source QGIS software (QGIS Development Team 2020) was used to classify the image objects. Using supervised classification, Bengal Florican record sites were classified and land cover changes were analyzed and the classified habitat into three classes: grassland, woodland, and grassland woodland mosaic.

#### Method for vegetation survey

First, a grid map of Florican locations was prepared using GIS. The standardized grid size was  $1 \times 1$  km. The total area of grassland was divided into 10;  $1 \times 1$  km blocks. Within each  $1 \times 1$  km grid area, 10 quadrats of  $\times 1$  m were placed centrally. To fulfill the objectives, quadrat sampling method (Stohlgren et al. 1995) was used for the quantitative study of the vegetation of Bengal Florican.

#### Method for disturbance study

To study the disturbance drivers of the Bengal florican and their habitats, the same 1 x 1 km grids were used for data collection. Cattle grazing, the presence of invasive alien plant species, problematic native species, and other human-induced disturbances were recorded.

#### **RESULT**

#### Distribution and population status

Houbaropsis bengalensis were recorded from Bansbari, Bhuyanpara, and Panbari grasslands. (Figure 3, Table 1). 50 males and 17 females were observed in Kuribeel, Rupahi, Sidajhar, Kokilabari, Bhumuk, Abiwidora, Agrang, and Murabari (Table 1). Eighteen males were sighted displaying: nine in Kokilabari, four in Rupahi, three in Agrang, and two in Kuribeel. The highest number of individuals were sighted in Kokilabari located in the Bhuyanpara range of the National Park, followed by Rupahi and Kuribeel. Seven males were observed during flight, looking at their flight direction. Females are difficult to spot, and direct observations estimate the total count of Bengal Floricans to be 74 (Table 2). No floricans could be sighted in areas such as Kasindaha, Mahout Camp Fields, Palsiguri, Uchila-

Bongali Hathdhowa, Bura Buri Jhar, Dighlatari, and Pohu Field, where floricans had been previously reported (Narayan 1992; Brahma 2013).

Table 3 list the names of grassland blocks located in Manas National Park where Bengal Floricans have been reported in the past and in the current study. The map was generated using QGIS to study and compare Bengal Florican locations in grasslands over the last few decades. The results of past surveys carried out in 1989 (Narayan 1992), 2011 (Brahma 2013), and 2021 were used to compare the data with those of the current study (Figures 2 & 3).

#### Land cover change

The results indicated habitat changes in vegetation within the recorded site. In the year 2000; 11.7 km² area of grassland represent 79%, 3.03 km² area of grassland woodland mosaic represent 21%, and 0.036 km² area of woodland are represent 0.24 % (Table 4, Figures 4 & 6). In the year 2020; 7.3 km² area of grassland represent 49.6%, 0.75 km² area of woodland represent 5.1%, and 6.67 km² area of grassland woodland mosaic represent 45.3% (Table 5, Figures 5 & 6). From 2000–2020; 4.7 km² of grassland area was converted to mosaic representing 32%, 0.49 km² of grassland area was converted to woodland representing 3.4%, 0.0009 km² of the mosaic area was converted to a waterbody representing 0.01%,

Table 1. Bengal Florican sightings along with GPS locations.

	Locations	Area	Number of males sighted	Number of territories established (Female)
1	N26.74857, E91.17289	Kokilabari	20	7
2	N26.68549, E91.02865	Kuribeel	5	2
3	N26.68469, E91.04027	Bhumuk	2	0
4	N26.71303, E91.08633	Rupahi	7	4
5	N26.72930, E91.08080	Abiwidora	3	3
6	N26.71128, E91.15022	Agrang	7	0
7	N26.68044, E90.91007	Murabari	2	1
8	N26.69722, E90.92653	Sidhajhar	4	0

Table 2. Total count of Bengal Florican population.

	Bengal Florican	Population
1	Male	50
2	Female	17
3	Additional male sighted in flight	7
	Total	74

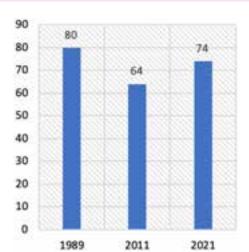


Figure 2. Bengal Florican recorded in 1989, 2011, and 2021.

Table 4. Classification of L7 Satellite imageries of 2000.

Class ID	Class name	Pixel sum	Area [km²]	Percentage [%]	
0	Mosaic	3368	3.031	20.57	
1	Grassland	12960	11.664	79.17	
2	Woodland	40	0.036	0.244	

0.81 km² of the mosaic area was converted to grassland representing 5.5%, 0.24 km² mosaic area converted to woodland representing 1.63%, and 0.02 km² woodland area converted to mosaic representing 0.13% (Table 6).

#### Vegetation survey

The grasslands were burned in the month of February and a fresh flush of grasses emerged soon after establishing a suitable habitat for the Bengal Floricans to breed. The following plant species were observed during the survey (Table 7). And most of the Bengal Florican habitats where they are recorded in the grassland have short grass *Imperata cylindrica* and moderately tall and dense grasses like *Saccharum spontaneum* and *Saccharum narenga*.

#### **Threats**

The grasslands of the park are subjected to a number of natural and anthropogenic disturbances. The conversion of grasslands into woodlands is a serious threat to grassland species. *Bombax ceiba* (Simul), a tree species was found growing extensively in the grasslands. Cattle grazing is one such disturbance that has been observed in the National Park. The grazing of livestock by communities living in the fringes of the National

Table 3. Male Bengal Florican sightings over the last four decades (Narayan 1992; Brahma 2013).

	Grassland Block	1989	2011	2021
1	Kasindaha	7	NA	NA
2	Mahout Camp Fields	4	NA	NA
3	Palsiguri	3	NA	NA
4	Kuribeel	6	5	5
5	Uchila-Bongali Hathdhowa	1	NA	NA
6	Bura Buri Jhar	1	NA	NA
7	Lafasari	NA	4	NA
8	Kapur-pura/ Sidhajhar	8	5	4
9	Pohu Field	NA	2	NA
10	Dighlatari	NA	4	NA
11	Agrang	NA	4	7
12	Kokilabari	NA	8	20
13	Murabari	NA	NA	2
14	Abiwidora	NA	NA	3
15	Rupahi	NA	NA	7
16	Bhumuk	NA	NA	2
Total		30	32	50

Table 5. Classification of L8 Satellite imageries of 2020.

Class ID	Class name	Pixel sum	Area [km²]	Percentage [%]
0	Mosaic	7421	6.6789	45.33
1	Water	1	0.0009	0.0061
2	Grassland	8110	7.299	49.54
3	Woodland	836	0.7524	5.10

Park is one of the major causes of habitat degradation. *Premna herbacea*, a medicinal herb that grows profusely in the grasslands of Manas is a major non-timber forest products (NTFPs). Local communities depend on these products for their livelihoods. The presence of humans in these grasslands inhabited by Bengal Floricans is another cause of disturbance to the species. The presence of invasive alien plants is another major disturbance to the habitat. *Chromaelaena odorata* a shrub was found growing extensively in the grasslands of the national park occupied by Bengal Floricans.



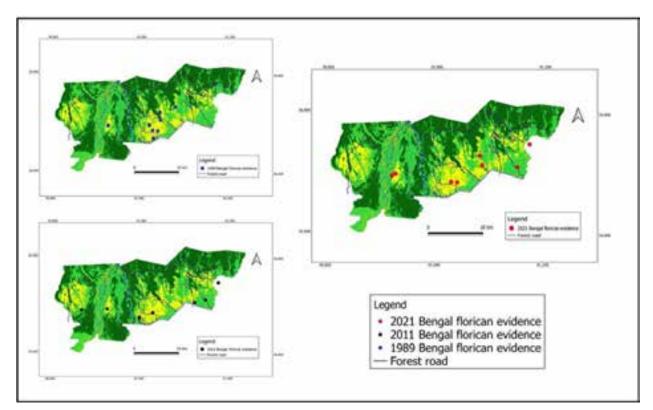


Figure 3. The distribution of Bengal Florican in the surveys carried out in 1989, 2011, and in the current study 2021.

Table 6. Land cover change between 2000 and 2020.

Cross class code	Reference class (L7)	New class (L8)	Pixel sum	Area [km²]	Percentage of change (%)
6	Mosaic	Mosaic	2202	1.98	13.45
10	Mosaic	Water	1	0.0009	0.01
14	Mosaic	Grassland	898	0.8082	5.49
18	Mosaic	Woodland	267	0.2403	1.63
7	Grassland	Mosaic	5197	4.6773	31.78
15	Grassland	Grassland	7212	6.4908	44.10
19	Grassland	Woodland	551	0.4959	3.37
8	Woodland	Mosaic	22	0.0198	0.13
20	Woodland	Woodland	18	0.0162	0.11

#### **DISCUSSION**

Our survey estimated 74 Bengal Floricans in Manas, which compared to 2011 represents an increase (Figure 3). The Florican number 74 is higher than the result of the last survey conducted in 2011 (Brahma 2013), but lower than the result of a previous survey conducted in 1989 (Narayan 1992). Interestingly, at least 20 male floricans and seven female floricans were recorded at the Kokilabari Agriculture Farm (KAF). The highest

number of floricans was recorded in KAF. Thus, there is a long term need to conserve such sites such as the KAF for long term conservation of the species.

The survey for Bengal Floricans in Manas National Park provides important evidence for the presence of Bengal Floricans at the study site. It also indicates an increasing trend since the last survey carried out in 2011 which is encouraging (Brahma 2013). This survey also provided new information from previously surveyed areas such as Murabari, Abiwidora, and Bhumuk grassland areas,



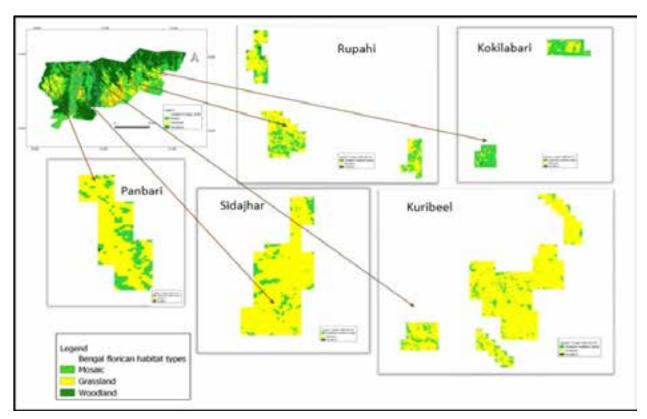


Figure 4. Land use map of Bengal Florican habitat as in 2000.

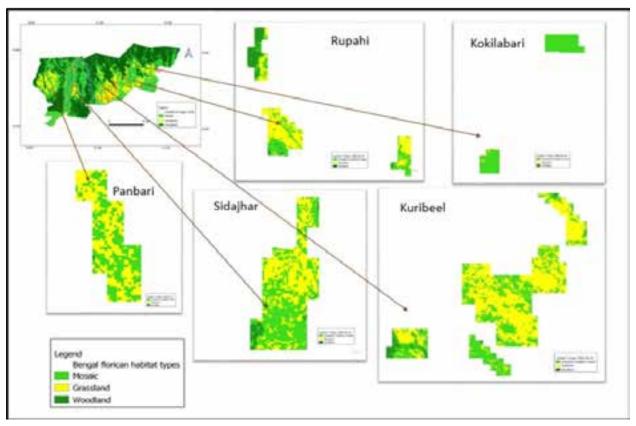


Figure 5. Land use map of Bengal Florican habitat as in 2020.



Table 7. List of plants observed in the habitats occupied by Bengal Florican.

	Scientific name	Local name	Family
1	Saccharum narenga	Barenga	Poaceae
2	Saccharum spontanum	Kohua	Poaceae
3	Leea asiatica	Athu bhanga	Vitaceae
4	Cymbopogon flexuosus	Gondho birina	Poaceae
5	Imperata cylindrica	Ulu kher	Poaceae
6	Phragmites karka	Khagori	Poaceae
7	Premna herbacea	Mati kaldhab	Lamiaceae
8	Commelina sp.	Konaximolu	Commelinaceae
9	Bombax ceiba	Simul	Bombacaceae
10	Croton bonplandianus	Bon tulsi	Euphorbiaceae
11	Scleria terrestris	Har kata	Cyperaceae
12	Cyperus sp.	NA	Cyperaceae
13	Grewia sapida	Mati phehura	Tiliaceae
14	Senecio madagascariensis	NA	Asteraceae
15	Plectranthus ternifolius	NA	Lamiaceae

where Floricans have been recorded. The survey also clearly indicated the success of the method wherein the collaboration between forest frontline staff, invited experts and local guides was instrumental in obtaining a quick result.

The Bengal florican population in Manas has faced severe problems due to habitat loss and degradation, with grassland habitats decreasing alarmingly. Key factors contributing to this conservation challenge include the indiscriminate conversion of grasslands, domestic cattle grazing, unauthorized burning, opportunistic hunting, and egg collection. Invasive species such as Chromolaena and Mikania further exacerbate the problem by outcompeting native flora crucial for Bengal florican survival. Despite these challenges, our data shows a paradoxical increase in the Bengal florican population. This can be attributed to the concentrated conservation efforts by stakeholders, particularly within the KAF areas, which host sizable populations contributing to the overall increase in the park. However, the persistence of habitat issues necessitates robust management interventions to ensure sustainable population growth.

In Assam, most grasslands are confined to protected areas and a few in the Brahmaputra floodplains. Thus, conserving these grasslands is imperative to protect grassland-obligate avifauna (Brahma 2013). Scientific studies are essential to manage these remaining grasslands effectively. Our study involved mapping the habitat and assessing the current Bengal Florican

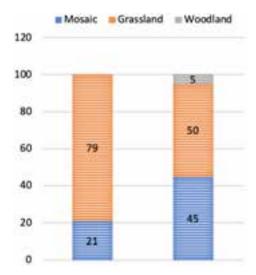


Figure 6. Percentage of land cover change in 2000 and 2020.

population in Manas. While it is encouraging that the BF population is increasing in Manas, the concurrent decrease in grassland habitat is concerning. While an increase in vegetation cover is often beneficial for forests, in Manas, the increase in specific land cover types such as woodland and woody plant infested mosaic forest poses a serious threat to the unique grassland habitat (Das et al. 2022). Burning of grasslands conducted in January aimed to regenerate grasses and control invasive species. However, this practice is insufficient to reduce the encroachment of alien invasive plants and problematic native plants (Das et al. 2019). Therefore, appropriate habitat management actions must be undertaken to restore and conserve grassland habitats.

#### **RECOMMENDATIONS**

- Maintenance of mosaic grassland: Which include both tall and short grasslands.
- Implement comprehensive habitat restoration programs to remove invasive species and native woody species.
  - Strategic implementation of prescribed burning.
- Enforce stricter regulations against unauthorized land conversion, grazing, and burning.
- Promote conservation friendly agricultural practices adjacent to grassland areas, especially in KAF.
- Engage local communities in conservation efforts through education and incentivizing sustainable practices.
- Conduct long-term scientific studies to monitor the impact of management practices in BF population.



Image 1. Male Bengal Florican in flight.



Image 2. Male Bengal Florican in the grassland.

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### Assemblages of frugivorous butterflies in two urban parks in Quezon City, **Philippines**

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Abstract: Urban parks play a crucial role in supporting biodiversity, yet limited research on urban insect diversity poses challenges for conservation. Comprehensive biodiversity records are essential for monitoring insect population trends. Despite their significance as bioindicators, many urban parks lack baseline data on butterfly populations. This study utilized bait traps to assess butterfly diversity in two Quezon City parks: La Mesa Ecopark (LME) and Ninoy Aquino Parks and Wildlife Center (NAPWC). Bait trapping facilitates species identification and population trend monitoring without harming local butterfly populations. From April to August 2023, two bait traps equipped with fermented bananas and rum as lures were deployed in each park. A total of 145 individuals representing nine morphospecies of the Nymphalidae family were recorded. Differences in butterfly diversity were noted between LME and NAPWC, with LME showing greater diversity. However, sampling efforts at NAPWC may need expansion to ensure exhaustiveness, potentially affecting comparison accuracy. Notably, four species observed in both parks are endemic to the Philippines, while data on the IUCN Red llst status of the remaining species are unavailable.

Keywords: Bait trap, biodiversity, bioindicators, La Mesa Ecopark, Lepidoptera, Ninoy Aquino Parks and Wildlife Center, Nymphalidae.

Filipino: Ang mga parke sa lungsod ay mayroong mahalagang papel sa pagsuporta sa iba't ibang uri ng buhay, ngunit ang limitadong pananaliksik sa dami ng mga insekto sa lungsod ay nagsisilbing hamon sa pangangalaga ng kapaligiran. Ang kumpletong tala ng iba't ibang uri ng buhay ay mahalaga para sa pagsubaybay sa mga pagbabago ng populasyon ng mga insekto. Sa kabila ng kanilang kahalagahan bilang mga bioindicator, maraming parke sa lungsod ang kulang sa pangunahing datos tungkol sa populasyon ng mga paru-paro. Gumamit ang pag-aaral na ito ng mga bitag na may pain upang masuri ang dami ng mga paru-paro sa dalawang parke sa Lungsod ng Quezon: La Mesa Ecopark (LME) at Ninoy Aquino Parks and Wildlife Center (NAPWC). Ang paggamit ng pain ay tumutulong sa pagkilala ng mga sarihay at pagsubaybay sa pagbabago ng populasyon nang hindi nakapipinsala sa mga lokal na populasyon ng paru-paro. Mula Abril hanggang Agosto 2023, dalawang bitag na may pain na naglalaman ng binurong saging at rum bilang panghalina ang inilatag sa bawat parke. Nakapagtala ng 145 na indibidwal na kumakatawan sa siyam na sarihay na kabilang sa pamilyang Nymphalidae. Kapansin-pansin ang pagkakaiba sa uri ng mga paru-paro sa pagitan ng LME at NAPWC, kung saan mas mataas ang baryedad ng paru-paro sa LME. Gayunpaman, maaaring kailanganing palawakin pa ang pagsisiyasat sa NAPWC upang matiyak ang kasakupan nito, na maaaring makaapekto sa kawastuhan ng paghahambing. Kapansin-pansin, apat na sarihay ng paru-paro na nakita sa parehong parke ay endemiko sa Pilipinas, habang wala namang datos sa katayuan ng mga natitirang sarihay ang naitala sa IUCN Red List.

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#### INTRODUCTION

#### Importance of studying butterfly species in urban parks

Urban parks play a crucial role in cities by providing a range of ecosystem services, such as biodiversity preservation and urban climate regulation (Sadeghian & Vardanyan 2013; Mexia et al. 2018; Taylor et al. 2020; Sarı & Bayraktar 2023). Traditional efforts to combat global biodiversity decline have focused mainly on conserving natural environments, yet various flora and fauna persist in urban refuges (Gentili et al. 2023). Promoting biodiversity in urban ecosystems enhances the wellbeing of urban residents (Carrus et al. 2015; Cameron et al. 2020; Marselle et al. 2021) and contributes to conserving biodiversity in natural ecosystems (Savard et al. 2000).

While the literature extensively covers the impact of urbanization on prominent animal like birds and mammals (Seress & Liker 2015; Isaksson 2018; Schmidt et al. 2020), research focusing on invertebrates remains notably scarce, creating a concerning knowledge deficit in biodiversity conservation. Despite evidence of insect sensitivity to environmental change (Kellermann & van Heerwaarden 2019; Harvey et al. 2023), comprehensive data is still lacking. Butterflies are an exception, with extensive research covering many species (Essens et al. 2017). The conservation status of butterfly species is primarily assessed by analyzing population trends and changes in range, relying on extensive and systematic monitoring efforts spanning several decades.

#### Role of urban parks as crucial habitats for butterflies

Concomitant with economic growth engendered by urban development are significant alterations to human-environmental interactions (Haase 2021). Urban expansion, a hallmark of this expansion, demonstrably deteriorates biodiversity, disrupts vital ecosystem functions, and alters microclimates (Frank et al. 2017). However, recent research suggests that even seemingly inconsequential urban greenspaces, such as parks, can possess significant ecological value (Loures et al. 2007). Despite their relatively small size and artificial composition, these urban parks play a crucial role within the intricate network of the urban ecosystem, providing essential ecosystem services (Davies et al. 2011).

Butterflies face a multitude of challenges, such as habitat degradation (Geyle et al. 2021; Warren et al. 2021), climate change (Davies 2019; Crossley et al. 2021) and pollution ( Shephard et al. 2020; Liu et al. 2021; Parlin et al. 2022), resulting in a worldwide decrease in butterfly populations. Assessing changes

in butterfly populations is challenging because natural fluctuations (e.g., short-term weather changes (Oliver et al. 2015)) make it difficult to confirm actual decline (Van Strien et al. 1997). This raises serious concerns about ecosystem function as well as human food security, since some species are pollinators or otherwise agriculturally important (van der Sluijs 2020). Studies of butterfly populations underscore the need to assess trends in insect populations, identify vulnerable species and potential pest species (Badon et al. 2023; Eastwood et al. 2006), and determine the underlying causes of their decline. The majority of data so far has come from Europe (Warren et al. 2021), United States (Wepprich et al. 2019; Crossley et al. 2021; Grant et al. 2021), and Australia (Geyle et al. 2021; Sanderson et al. 2021).

The Philippines features a remarkable diversity of butterflies, with a documented total of 927 species, of which >300 are endemic (Treadaway 2012). The archipelagic nature of the Philippines significantly contributes to shaping its biological diversity (Brown et al. 2013). While it poses challenges for conservation, it also provides opportunities for understanding unique ecosystems. While species isolation can lead to speciation, some species are at risk of extinction if their habitat becomes too fragmented. Moreover, anthropogenic environmental changes provide novel ecological niches, which modify selection in many ways to stimulate diversification-however, these changes also frequently eliminate niches and result in extirpations (Ålund et al. 2023).

Understanding how increasing urban sprawl affects biodiversity is imperative in conserving biodiversity in urban green areas (Kuussaari et al. 2021). Among the numerous threats to butterflies in the Philippines is habitat fragmentation brought about by anthropogenic activities (Posa & Sodhi 2006). However, despite the extensive effects of urbanization on natural ecosystems, butterflies remain resilient components within the urban landscape (Pignataro et al. 2023). Moreover, there is a noticeable scarcity of data that looks into the butterfly diversity in urban parks; thus, this research sought to compile a list of butterflies found in two major urban parks in Quezon City. This study primarily focuses on the efficacy of bait traps for capturing frugivorous butterflies, aiming to gather data representative of the broader butterfly population in urban areas.

#### **METHODS**

#### STUDY SITES

#### La Mesa Ecopark

The La Mesa Ecopark, established in 1929, is an essential ecological reserve that serves as the primary water source for Metro Manila. This 700-hectare reserve in Quezon City includes a dam and an ecological reserve spanning 2,000 ha of contiguous forest (Image 1). The La Mesa Ecopark is characterized by its dense tree canopies, which provide ample shade, and the paved main trails, which accommodate bicycles. Visitors can access the park via public transportation, and sufficient parking is available (Masangkay et al. 2016; Estoque et al. 2018).

The La Mesa Dam Reservoir, the only major watershed in the metropolitan area, is protected and located adjacent to the park. The park's biodiversity surveys have revealed a diverse range of species, including ants (Pag-Ong et al. 2022), slime molds (Macabago et al. 2010), trees (Malabrigo et al. 2016), and vertebrates (Estoque et al. 2018). The park used to have a butterfly sanctuary, but it was closed during the 2020 pandemic. The sanctuary, managed by a concessionaire, was intended to house butterflies bred in captivity. There has yet to be a study on butterfly diversity within the park, making it an appropriate study site to evaluate butterfly diversity in urban areas.

#### **Ninoy Aquino Parks and Wildlife Center**

The Ninoy Aquino Parks and Wildlife Center (NAPWC) was established in 1954 as part of the Quezon Memorial Park. It spans over an area of 197.28 ha and is located at 14.6522°, 121.0453° (Image 2). Despite being located beside a busy highway the park has a tranquil atmosphere. It features an artificial lagoon that is surrounded by lush, cultivated plants. Visitors can access the park through paved pathways and can find shaded areas to relax and have picnics.

The NAPWC is a protected area that is home to diverse tree species. It also has a rescue center that houses various animals, including tigers, monkeys, birds, and snakes. Research conducted within the park has primarily focused on animal diseases (Maluping et al. 2007; Lumabas et al. 2018; Sioson et al. 2018; Gamalo et al. 2019), bird surveys (Vallejo et al. 2009), and freshwater invertebrates (de Leon et al. 2023) in the Philippines. However, there has been no study on butterfly diversity in this park to date.

#### **SAMPLING**

## Duration of the Study, Trap Placement, and Monitoring Scheme

The investigation, conducted over five months of April–August 2023, comprised systematic weekly observations throughout both dry (April–May) and wet seasons (June–August). La Mesa Ecopark (LME) facilitated 13 bait trapping sessions, while Ninoy Aquino Parks and Wildlife Center (NAPWC) hosted ten sessions. Bait trapping sessions were subject to postponement during inclement weather, and the frequency of sessions was overseen by the regulatory constraints imposed by the respective management authorities of the urban parks.

Traps were set up between 0800 h and 1000 h in sunny conditions, equipped with rainproof plastic coverings to keep captured butterflies dry in case of sudden rain. After a minimum of 24 h placement, traps were retrieved, and captured butterflies and bycatches were released before deploying fresh traps for subsequent sessions. Each urban park had two traps, at least 200 m away from each other and positioned on sunlit trees less frequented by park visitors' areas to prevent disturbance and theft, in strict adherence to the regulations stipulated in permits issued by the respected administrative bodies responsible for park management.

Bait trapping facilitated the evaluation of specimens caught and subsequent bait replacement. After identifying and recording, butterflies and other insect bycatches were released. The identification of butterflies at the species level and endemicity in the Philippines was accomplished by consulting a wide range of relevant taxonomic literature, including comprehensive publications by Page & Treadaway (2004), Schroeder & Treadaway (2005), Treadaway & Schroeder (2012), Hardy & Lawrence (2017), and Badon (2023). Additionally, the website authored by Badon et al. (2013) entitled "Philippine Lepidoptera" was employed as a resource for conducting image comparisons and species identification, ensuring the research's thoroughness and reliability.

#### **Bait trap Specifications and Observation Method**

This study employed modified Van Someren-Rydon traps (see Image 2), initially proposed by DeVries et al. (1997). These traps, constructed from white nylon netting, are cylindrical with dimensions measuring 38 cm in diameter and 100 cm in height. The choice of these traps was based on their proven effectiveness in capturing butterflies, as demonstrated in previous studies. To protect captured specimens from dew and



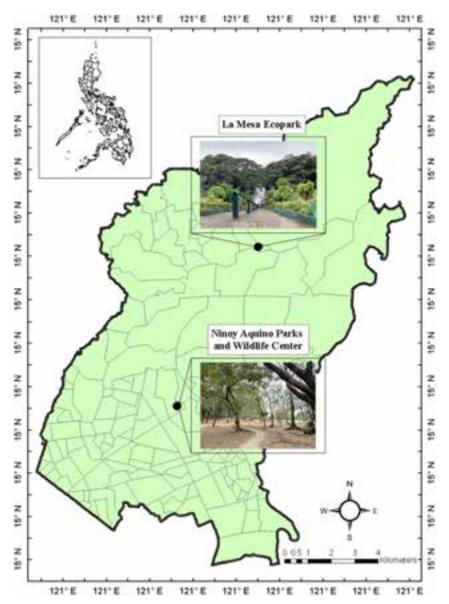


Image 1. This study was conducted in the confines of two urban parks: the Ninoy Aquino Parks and Wildlife Center (NAPWC), located in Diliman, Quezon City, and La Mesa Ecopark (LME), located in Greater Lagro, Quezon City.

rain, two wire hoops, enclosed within plastic casings, are affixed to the top end of each trap. A zippered aperture on the side was employed for ease of insect removal, replacing the use of Velcro. Additionally, a 25 cm diameter plywood sheet was affixed to the lower portion of the netting to serve as an entrance for insects. Beneath this entrance, another plywood sheet of identical dimensions was suspended using hooks, allowing for a five-inch clearance for butterflies. Positioned centrally on the suspended plywood sheet was a reusable plastic plate, 15 cm in diameter, intended for precise bait placement.

The bait selection process relied on prior research

from the Philippines, selecting fermented banana as the bait—a mixture of Tanduay Rhum, with a 40% alcohol content by volume, and mature, fermented bananas. The preparation of this bait mixture occurred two days before its use to ensure thorough fermentation. The researchers deposit generous quantity of the bait mixture at each trap's base and left it undisturbed for a minimum of 24 hours to effectively monitor captures.

Permits issued for both parks stipulated minimal to no direct contact with wildlife, including butterflies. Accordingly, captured butterflies were visually observed, photographed using a smartphone camera, and documented. All butterflies and incidental catches were

released from the traps by gently tapping the exterior of the bait trap to encourage flight. This tapping was done with hand to minimize any potential harm to the butterflies. The zippered access was used to facilitate this process. Additionally, bait replenishment occurred at the commencement of each baiting session.

#### **Diversity analyses**

All ecological data analyses were conducted using R version 3.6.0 (Team 2013) through RStudio version 1.1.453 (Team 2016). Firstly, species accumulation curves (SAC) to assess the adequacy of the sampling effort in this study and estimate species diversity. SAC is a useful tool for evaluating the effectiveness of a fauna survey in accurately representing the fauna population within a geographic area (Thompson & Withers 2003; Ugland et al. 2003; Colwell et al. 2004). The curve shows the cumulative species count in relation to sampling effort and indicates the rate of new species discovery. A steep initial slope suggests rich species diversity or limited sampling, while a flattening curve indicates diminishing returns in species identification. This study calculated SAC using R packages ggplot2 (Wickham & Wickham 2016) and iNEXT (Hsieh et al. 2016). Next, species diversity was calculated using the Hill series of diversity indices (Hill 1973; Jost 2007). This approach

considers species richness and evenness based on the occurrence of butterfly species gathered during the rapid assessment. The researchers used the R package iNEXT (Hsieh et al. 2016) for these calculations as well.

#### **RESULTS**

#### **Species richness**

One-hundred-and-forty-five individuals representing nine species of butterflies were recorded in La Mesa Ecopark and Ninoy Aquino Parks and Wildlife Center. All were fruit-feeding nymphalids of the subfamilies Charaxinae, Nymphalinae, and Satyrinae. The subfamily Satyrinae presented the highest abundance and number of species, followed by Nymphalinae in terms of abundance. The most dominant species were Amathusia phidippus pollicaris Butler, 1870 (N = 38, 26%), Hypolimnas bolina philippensis Butler, 1874 (N = 30, 22%) and Melanitis leda leda (Linnaeus, 1758) (N = 26, 17%).

#### **Species diversity**

The quantified alpha diversity, which measures species richness and diversity within local habitats, is essential for understanding the ecological dynamics of

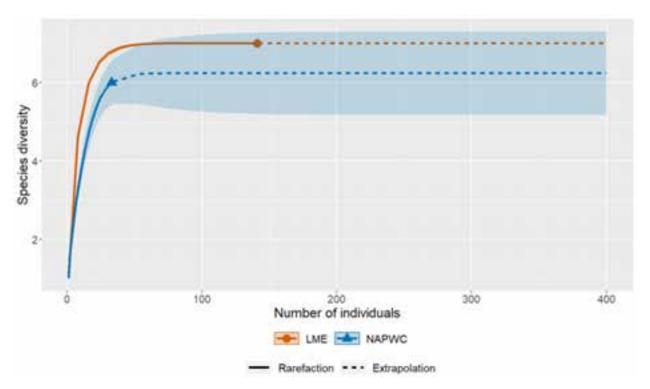


Figure 1. Species accumulation curves for La Mesa Ecopark (LME) and Ninoy Aquino Parks and Wildlife Center (NAPWC). (NOTE: data: solid lines; extrapolation: dashed line). Shaded areas indicate 95% confidence intervals.



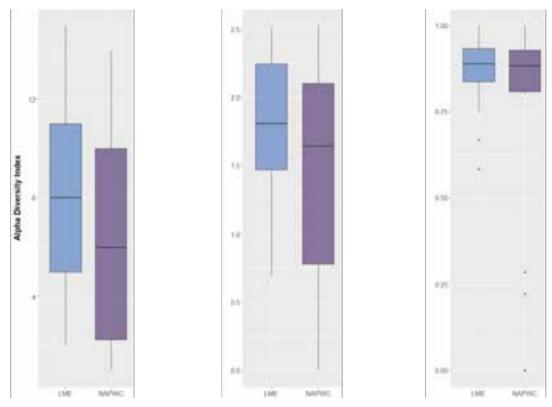


Figure 2. Boxplots illustrating alpha diversity in the two urban parks, highlighting variations in species richness, Shannon diversity, and Simpson diversity in La Mesa Ecopark (LME) and Ninoy Aquino Parks and Wildlife Center (NAPWC).

Table 1. Geographical coordinates for bait traps at La Mesa Ecopark (LME) and Ninoy Aquino Parks and Wildlife Center (NAPWC).

Canada Cian		Bait Trap One			Bait Trap Two		
Study Site	Latitude	Longitude	Elevation	Latitude	Longitude	Elevation	
LME	14.711944	121.072778	60 m	14.711389	121.077222	70 m	
NAPWC	14.649167	121.043889	40 m	14.6525	121.043333	50 m	



Image 2. Details of the bait trap: A—Researcher descending down the bait trap, documenting the captured butterflies and bycatches | B—Details of the top portion of the trap with Amathusia phidippus and Hulodes caranea on resting positions | C—Detail of the narrowed portion of the trap base where the mixture of fermented banana is placed with Discophora ogina feeding. © Micael Gabriel A. Itliong.

Table 2. List of nymphalids recorded in La Mesa Ecopark (LME) and Ninoy Aquino Parks and Wildlife Center (NAPWC). The species' endemicity is based on whether they have only been recorded in the Philippines, as indicated in the relevant taxonomic literature.

Subfamily	Scientific name	Common name	Endemicity in the Philippines	IUCN Status
Charaxinae	Charaxes solon (Fabricius, 1793)	Black Rajah	Native	Not Available
Nymphalinae	Hypolimnas bolina philippensis Butler, 1874	Great Egg-fly	Non-endemic	Not Available
Nymphalinae	Junonia hedonia ida (Cramer, [1775])	Brown Pansy	Non-endemic	Not Available
Nymphalinae	Athyma gutama gutama (Moore, 1858)	Sergeant	Endemic	Not Available
Satyrinae	Amathusia phidippus pollicaris Butler, 1870	Palm King	Non-endemic	Not Available
Satyrinae	Discophora ogina ogina (Godart, [1824])	Duffer	Endemic	Not Available
Satyrinae	Melanitis atrax atrax (C. & R. Felder, 1863)	Evening Brown	Endemic	Not Available
Satyrinae	Melanitis leda leda (Linnaeus, 1758)	Common Evening Brown	Non-endemic	Least Concern
Satyrinae	Mycalesis igoleta C. & R. Felder, 1863	Igoleta Bush Brown	Endemic	Not Available

Table 3. Occurrence and number of individual butterflies recorded in LME (La Mesa Ecopark) and NAPWC (Ninoy Aquino Parks and Wildlife Center), recorded from April to August 2023.

	Species	LME	NAPWC
1	Charaxes solon (Fabricius, 1793)	0	3
2	Hypolimnas bolina philippensis Butler, 1874	9	23
3	Junonia hedonia ida (Cramer, [1775])	0	3
4	Athyma gutama gutama (Moore, 1858)	0	1
5	Amathusia phidippus pollicaris Butler, 1870	38	0
6	Discophora ogina ogina (Godart, [1824])	9	0
7	Melanitis atrax atrax (C. & R. Felder, 1863)	13	2
8	Melanitis leda leda (Linnaeus, 1758)	24	2
9	Mycalesis igoleta C. & R. Felder, 1863	18	0

butterfly populations in urban parks. Figure 2 presents box plots illustrating alpha diversity metrics between two urban parks: La Mesa Ecopark (LME) and Ninoy Aquino Parks and Wildlife Center (NAPWC). LME emerges to be more diverse in terms of species richness and Shannon diversity.

DISCUSSION

Before this study, there was no available data on what butterfly species occur in both parks; therefore, inferring diversity and population changes over time is impossible. All of the butterfly species recorded in both of the parks belong to the Nymphalidae family, which consists of around 7,200 species that are distributed throughout all continents except Antarctica (Zhang et al. 2008; Yan et al. 2023) and are mostly known to be frugivorous. Although alternative bait lures could

Table 4. Number of species recorded in each Nymphalidae subfamily in the two urban parks: LME (La Mesa Ecopark) and NAPWC (Ninoy Aquino Parks and Wildlife Center). Dry season—April–May | rainy season—June–August.

	LME		NAPWC	
Nymphalidae Subfamily	Dry	Wet	Dry	Wet
Charaxinae	0	0	0	1
Danainae	1	1	0	1
Morphinae	2	2	0	0
Nymphalinae	3	4	1	7
Satyrinae	3	4	1	3
Number of species	9	11	2	12

Table 5. Comparison of Shannon diversity index between LME (La Mesa Ecopark) and NAPWC (Ninoy Aquino Parks and Wildlife Center) using Hutcheson t-Test.

	LME	NAPWC	
Abundance	111	34	
Species Richness	6	6	
Shannon Diversity	0.002647	0.036779	
t value	2.627909081		
Degree of freedom	39		

have been employed, potentially leading to different results, the choice was guided by previous butterfly trapping research conducted in the Philippines (Toledo & Mohagan 2011; Gestiada et al. 2014; Mohagan et al. 2018; Reeves & Daniels 2020). Nevertheless, the species accumulation curve (see Figure 1) indicates adequate sampling was conducted in LME. Conversely, the curve has yet to reach its asymptote in NAPWC, implying that further sampling efforts could reveal additional



Image 3. Butterfly species caught in bait traps. A—Charaxes solon (Fabricius, 1793) ♂ | B—Hypolimnas bolina philippensis Butler, 1874 ♂ | C—Junonia hedonia ida (Cramer, [1775]) | D—Athyma gutama gutama (Moore, 1858) | E—Amathusia phidippus pollicaris Butler, 1870 ♂ | F—Discophora ogina ogina (Godart, [1824]) | G—Melanitis atrax atrax (C. & R. Felder, 1863) | H—Melanitis leda leda (Linnaeus, 1758) dry season form | I—Mycalesis igoleta C. & R. Felder, 1863. © © Micael Gabriel A. Itliong.

species. Various factors could contribute to the species accumulation curve failing to reach the asymptote. The most evident explanation is the possibility that the sampling effort has yet to achieve full exhaustiveness. Another plausible scenario is that the baits used might not be effective in attracting butterflies. However, it is more probable that the constraints of time imposed by park authorities impeded the optimal number of bait trapping, and increasing the sampling effort beyond the confines stipulated by the park might have facilitated the capture of additional butterfly species.

Among the butterfly species, only three occur in both parks (see Table 3), namely, *Hypolimnas bolina philippensis* Butler, 1874, *Melanitis atrax atrax* (C. & R. Felder, 1863), and *Melanitis leda leda* (Linnaeus, 1758).

Both parks have recorded three unique butterfly species (see Table 2). NAPWC exclusively recorded *Charaxes solon* (Fabricius, 1793), *Junonia hedonia ida* (Cramer, 1775), and an individual *Athyma gutama gutama* (Moore, 1858). La Mesa Ecopark, on the other hand, recorded three satyrine species: *Amathusia phidippus pollicaris* Butler, 1870, *Discophora ogina ogina* (Godart, 1824), and *Mycalesis igoleta* C. & R. Felder, 1863.

As indicated by the abundance data presented in Table 4, it is anticipated that a greater number of species would be observed during the wet season. This trend is commonly associated with the wet season's propensity to foster lush vegetation and abundant flowering plants, consequently offering substantial food sources for both butterfly larvae and adults.

It is worth discussing the presence of *D. ogina* in LME. According to Schroeder & Treadaway (2005), species under this genus can be found in forests. They may be attracted to lights and ripened fruits such as pineapple, sometimes flying towards lowland areas. This occurrence and behavior were observed in the Balinsasayao Twin Lakes Natural Park (a Montane Forest). The species were attracted to the bait trap (bananas with Tanduay rhum). It was also observed near the Sierra Madre in Baler, Aurora, where it got attracted to household lights. The presence of *D. ogina* in LME may indicate isolation caused by urbanization, or there may be habitat corridors that connect LME to the mountains of Sierra Madre on the east.

The findings depicted in Figure 2 highlight a contrast between the LME and NAPWC in terms of species richness and diversity. Notably, the LME site demonstrates a considerably higher level of Shannon and Simpson diversity than NAPWC. However, it is crucial to reiterate the caution when interpreting this discrepancy, given the ongoing nature of sampling efforts at NAPWC, as indicated by the species accumulation curve depicted in Figure 1. This curve underscores that the sampling conducted at NAPWC may still need to be exhaustive, potentially impacting the accuracy of the comparison. Therefore, it's essential to approach these findings with caution. Nevertheless, the T-test results presented in Table 5 underscore a statistically significant difference in the number or diversity of observed butterflies between the two urban parks.

It is worth noting that LME is situated adjacent to a semi-natural landscape, suggesting that preserving natural habitats surrounding the city will be crucial for successfully preserving urban butterfly species (Koh et al. 2004). This result is consistent with previous studies conducted in Singapore (Koh & Sodhi 2004), southern China (Sing et al. 2016), and Brazil (Brown & Freitas 2002), which found that urban parks connected to forests had a greater diversity of butterfly species than standalone parks with limited space or lacking diverse flora. It is also expected that LME would have the most butterfly species, as Mohagan et al. (2011) have emphasized that butterflies prefer forested habitats over disturbed areas.

#### Challenges and opportunities in conservation

Utilizing bait traps for butterfly diversity assessment presents several advantages over the conventional insect net sampling technique. This approach allows researchers to target a more specific subset of local butterfly populations. The presence or absence of butterfly species in both study sites may offer insights

into the type of habitat these species inhabit. Notably, the occurrence of endemic species in urban parks carries significant implications for public awareness and biodiversity conservation (Padrón et al. 2020; Koethe et al. 2023) The presence of endemic butterfly species in these urban parks, as evidenced in Table 2, serves as an indicator of the overall ecological health and habitat integrity (Pe'er & Settele 2008; Miller III et al. 2011) -a trend observed among invertebrates in general (Paoletti 1999; Gerlach et al. 2013). Thus, the presence of these species within urban parks underscores the critical importance of preserving natural habitats within urban environments. Habitat alterations, as noted by de Carvalho (Santos et al. 2020) and Uehara-Prado et al. (2007), can influence the diversity of frugivorous butterflies, potentially explaining the disparities in abundances and species presence or absence between LME and NAPWC. Consequently, long-term monitoring of butterflies in these parks is essential to establish baseline data regarding their occurrence and abundance.

#### **CONCLUSION**

The findings of this study emphasize the importance of urban parks in sustaining butterfly diversity, including the presence of endemic species. To ensure the survival of butterfly populations, it is important to focus on preserving and restoring interconnected natural forests and facilitating gene flow among butterfly populations. Monitoring schemes should be implemented to track butterfly populations in these parks as they are sensitive to environmental changes. Bait trapping is an effective method for monitoring butterfly populations in urban parks. However, due to urban management protocols, the use of fermented bananas as bait is limited. Therefore, future research should explore the efficacy of alternative lure bait traps in urban park settings. Butterflies are considered umbrella species and can serve as vital conservation indicators for these remaining refuges. Protecting these species is vital in the face of increasing urbanization risks. Urgent measures must be taken to safeguard these unique habitats and ensure the preservation of butterfly populations within urban parks. Integrating scientific data into urban planning and management processes is essential to understand the ecological significance of these habitats and devise effective conservation strategies.

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# Assessment of the status of *Spodoptera* species (Lepidoptera: Noctuidae: Armyworm) in India through DNA barcoding technique

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Abstract: Insects constitute the majority of animal fauna worldwide, but quantifying their species diversity is still incomplete. A few recent studies indicate a marked decrease in the population of insects which calls for urgent efforts to document and understand insect diversity to get a complete picture of Earth's ecosystems. Modern technology can accelerate species identification beyond traditional methods' limitations. Hence, a focused and expedited approach through DNA barcoding coupled with morphological identification is necessary. This present research highlights the gaps that exist and it examines the current status of *Spodoptera* species barcode in India. Six *Spodoptera* species were studied confirming their presence in India including two invasive species. That means less than 50% of taxa or described *Spodoptera* species are covered by genetic data from barcoded specimens after analysis. Therefore, comprehensive DNA barcoding should be achieved from all insect species occurring on the Indian subcontinent to speed up the discovery and documentation of new species by involving both traditional taxonomists and molecular biologists working towards a common goal.

Keywords: Biodiversity in India, conservation, current status, insect, identification, molecular biology, species, taxa.

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**Author contributions:** DN, IC, TS and YG conducted the investigation, collected, and curated the data, and wrote the original draft of the manuscript. SK, YA and PK contributed to the development of the initial concept of the study and was responsible for the design and implementation of the methodology also he was responsible for reviewing and editing the manuscript, creating visualizations, and supervising the overall progress of the study. All authors have read and approved the final manuscript.

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#### dib

#### **INTRODUCTION**

Identifying insect species is crucial for understanding ecological, evolutionary, and conservation-related queries. Properly diagnosing these species is vital for monitoring biodiversity and utilizing it effectively (Khedkar et al. 2016). Despite the contributions of the long-standing Linnaean classification system to taxonomy, its reliance on morphology has limitations. These limitations, like difficulties in resolving cryptic species and identifying immature stages, hinder progress. Furthermore, the scarcity of experts in morphotaxonomy restricts this approach (Shashank et al. 2022), leaving many species undiscovered or known only through descriptions and lost type specimens. The backlog of unidentified specimens in museum collections has existed for decades. After its introduction in 2003, DNA barcoding has evolved as a complementing technique to conventional taxonomy (Hebert et al. 2003). By characterizing species using standardized DNA regions, DNA barcoding aids in identifying cryptic species, and immature stages, and rapidly distinguishing species in various contexts, such as identification food stuff (Khilare et al. 2019; Tiknaik et al. 2019; Suryawanshi et al. 2020). However, creating high-quality reference libraries based on voucher specimens remains crucial for its applications. Despite challenges due to the vast diversity of life forms and limited taxonomic expertise, several countries, including India, have created massive DNA barcode reference collections for certain creature categories, such as insects. India, known for its rich insect diversity, houses a significant portion of the world's insect fauna.

Major biotic stress on crops is insect pests. Hundreds of insects can cause severe crop damage (Mahmood-ur-Rahman et al. 2014; Nalage et al. 2023). The Spodoptera (Lepidoptera: Noctuidae) genus comprises a few of the world's most important crop predators. They are commonly referred to as 'armyworms'. Thirty-one species have been described with members present on six continents (Kergoat et al. 2021). These species feed on a wide range of vegetable, grain, row, forage, and ornamental crops. While young larvae burn leaf tissue and skeletonize into leaves, advanced stages on all leaves are roughly and brutally fed and transported from leaf to leaf (Chandel et al. 2013). The group Spodoptera includes species closely related to a similar ecology, difficult to identify at the level of the species (Henaish & Elmetwaly 2020). It is also referred to as the caterpillar cluster, cotton leaf worm, tropical armyworm, and tobacco cutworm (Meagher et al. 2008).

So far, DNA barcoding in Lepidoptera has shown mixed success in determining species. There are several examples of fake DNA barcodes that determine the potential limitations of the methodology (Dasmahapatra et al. 2010; Goergen et al. 2016). This is because current diversity may be difficult to quantify due to missing barcode scopes, absence of uniform barcode spaces in some taxa, and perhaps confounding consequences of an incomplete pedigree (Rubinoff et al. 2006; Silva-Brandão et al. 2009). However, the approach was effectively employed in a variety of investigations, where 150 insect specimens were appropriately assigned and used a barcode information of 200 closely related species (Hebert et al. 2003). To adequately document India's diverse insect population across various ecological zones, efficient methods like DNA barcoding are essential. However, as of 2024, the Barcode of Life Data (BOLD) system contained only a small fraction of Indian insect species barcodes, highlighting the need for more comprehensive data. The paper aimed to analyze DNA barcode data of the Spodoptera (Lepidoptera: Noctuidae) genus from India on BOLD to assess the current status and discuss future steps.

#### **MATERIAL AND METHODS**

All sequences and data were collected from The Barcode of Life Data System (BOLD) (Ratnasingham & Hebert 2007) and the National Center for Biotechnology Information (NCBI) (Benson et al. 2012). Specifically, from public data sources we retrieved genetic data of the Spodoptera genus dated 19/12/2023, filtering by country ("India"), gene ("COI"), and length (">500bp"). With these settings, we created a dataset named "DS-SPODOPTERA" on BOLD (https://v3.boldsystems.org/index.php/MAS\_ Management\_OpenDataSet?datasetcode=DS-SPOD). Additionally, data for Spodotera mauritia, S. littoralis, and S. exempta were obtained using similar filtering criteria for gene and sequence length, adding them to the same dataset. Two outgroup sequences, Lymantria dispar dispar (NCBI ID: XAG005-05) and Hyphantria cunea (NCBI ID: XAB076-04), were also included.

Following alignment, all DNA sequences were translated into amino acid sequences, guaranteeing the absence of stop codons. The aligned files were then utilized for phylogenetic analysis and distance matrix computation using Mega 10.2. The phylogenetic tree was constructed using the neighbor-joining method (Saitou & Nei 1987) with bootstrap analysis (1,000 replicates) to assess the reliability of the branches. Genetic distances

were computed using the Kimura 2-parameter model (Kimura 1980).

#### **Single GYMC Analysis**

The Generalized Mixed Yule Coalescent (GYMC) model was applied to delineate species boundaries using the COI gene sequences. This approach integrates both yule processes (modeling species diversification) and coalescent processes (modeling intraspecific variations). We implemented the GYMC method using the 'GMYC' package in R, setting the MCMC chain to run for 100,000 generations with a burn-in of 10,000 generations to ensure robust and accurate delineations (Pons et al. 2006).

#### **BPP Analysis**

Bayesian phylogenetics and phylogeography (BPP) analysis was employed to confirm the species boundaries suggested by the GYMC model. We used the BPP v4.0 software, incorporating multi-locus sequence data. The analysis involved specifying a guide tree based on prior phylogenetic knowledge and running the MCMC for 200,000 generations, sampling every 20 generations, and discarding the first 10% as burn-in. Priors were set as theta  $^{\sim}$  G(2, 2000) and tau0  $^{\sim}$  G(2, 1000), reflecting prior expectations of population size and divergence time, respectively (Yang & Rannala 2010).

#### mPTP Analysis

The multi-rate poisson tree processes (mPTP) model was utilized to further validate species delimitation results. This method accounts for rate variation among branches, providing a more flexible framework compared to traditional PTP models. The analysis was conducted using the mPTP web server, with default parameters and a bootstrap analysis (1,000 replicates) to assess confidence in species boundaries (Kapli et al. 2017).

By integrating these methods, our analysis aims to provide a comprehensive and robust species delimitation for the *Spodoptera* genus in India, contributing to the accurate identification and understanding of both native and invasive species.

#### **RESULTS**

We analyzed the COI region DNA sequences of six *Spodoptera* species, totaling 817 sequences. For the four species found in India, we obtained COI region sequences for only two species, *S. litura* and *S. exigua*,

from the BOLD database of the 817 sequences, 365 were from outside India, including S. littoralis (51 sequences), S. mauritia (190 sequences), and S. exempta (124 sequences). The remaining 450 sequences were from India, comprising S. frugiperda (265 sequences), S. exempta (1 sequence), S. exigua (58 sequences), and S. litura (126 sequences) (Table 1). These were contrasted with barcode sequences from S. frugiperda and S. exempta, two possible invasive species, since its confirmed status based on the literature. No deletions, insertions, or no stop codons were found when the COI sequences were aligned, suggesting that the amplified DNA originated from functional COI genes. The sequences' total mean GC content is 29-30 %. The mean GC content on codon pos 1 is 39-41 % (except S. litura, which has a mean GC% content of 41.07%), the mean GC% content on codon pos 2 is 42-43 %, and the mean GC% content on codon pos 3 is 6-7 %. There was no discernible variation in the overall GC% for Codon Pos 1, Pos 2, and Pos 3.

With the exception of S. frugiperda species, which has the largest nucleotide divergence among species at 5.38%, the dataset has no considerable barcode gap. The maximum nucleotide difference within species is ≤2.2% (Table 2). The minimal nucleotide difference between species S. littoralis and S. litura was 2.9%, which was quite near to the cut off (≤3.0%). Apart from this, there was ≥4.2% minimal nucleotide difference between species. The two host strains of S. frugiperda, S. mauritia & S. exigua, and S. litura & S. littoralis showed the closest similarities, however even these pairings separated at >95% bootstrap values. Neighbor-joining phenetic analysis, which distinguished at > 75% bootstrap scores among the predicted species and showed that S. exigua was the most divergent, supported this. The phylogeny based on morphological and phenetic connections was typically in agreement (Pogue 2002). According to those cladistic analyses, S. exigua is the most plesiomorphic species in the Spodoptera group, whereas S. littoralis and S. litura are closely related sister species (Figure 1). Comparisons of adult genital morphology are the only way to distinguish between S. littoralis and S. litura (Mochida 1973; Ellis 2004). The morphological study of the male and female genitalia of Spodoptera species have been provided to identify the species from India (Supplementary Tables 1 & 2).

# Comparative Morphological Analysis of *Spodoptera* Species

This section provides a comparative morphological analysis of key Spodoptera species found in India. By

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Table 1. Current genetic and morphological reports, number of COI gene sequences from India and outside of India and mean GC% content, mean GC% content on codon pos. 1, mean GC% content on codon pos. 2 and mean GC% content on codon pos. 3 sequences on the BOLD status of *Spodoptera*.

	Native species name in India	Genetically reported till date in India	Morpholo- gically reported to date in India	Genetically reported till date outside of India	No. of sequences public on BOLD from India	No. of sequences public on BOLD from Outside of India	Total no. of sequences public on BOLD	Mean GC % content of sequences public on BOLD	Mean GC % content on codon pos 1 of sequences on BOLD	Mean GC % content on codon pos 2 of sequences on BOLD	Mean GC% content on codon pos 3 of sequences on BOLD
1	S. littoralis	No	Yes	Yes	0	51	51	29.32	39.22	41.78	6.97
2	S. mauritia	No	Yes	Yes	0	190	190	29.94	40.90	42.14	6.85
3	S. exigua	Yes	Yes	Yes	58	626	684	29.43	40.40	41.77	6.05
4	S. litura	Yes	Yes	Yes	126	250	376	29.72	41.07	41.71	6.39
	Invasive Species Name in India										
5	S. exempta	Yes	Yes	Yes	1	124	125	29.51	39.57	42.52	6.45
6	S. frugiperda	Yes	Yes	Yes	265	1088	1353	29.77	40	42.07	7.25

Table 2. Genetic distance between the Spodoptera species (indicated by green color) and within the species (indicated by yellow color).

	S. exempta	S. exigua	S. frugiperda	S. littoralis	S. litura	S. mauritia
S. exempta	1.59					
S. exigua	6.3	1.15				
S. frugiperda	4.8	8.6	5.38			
S. littoralis	4.2	6.0	5.3	2.14		
S. litura	4.5	7.1	5.3	2.9	2.18	
S. mauritia	6.0	9.1	8.3	8.3	9.3	1.91

highlighting differences and similarities in the male and female genitalia, this comparison facilitates accurate identification crucial for pest management.

#### Male Genitalia Comparison

#### Valve

S. exigua: Broad elongate ovalS. exempta: Narrow rectangularS. mauritia: Narrow taperingS. frugiperda: Very broad, quadrate

S. littoralis: Broad quadrate with dentate ventral

S. litura: Broad

S. eridania: Not specified

Juxta

margin

S. exigua: Narrow elliptical band

S. exempta: Narrow elliptical band with triangular median process

S. mauritia: Narrow elliptical band with triangular median process

S. frugiperda: Narrow rectangular band

S. littoralis: Broad quadrate

S. litura: Triangular

S. eridania: Narrow rectangular band

#### Coremata

S. exigua: Moderately elongate, no distinct lobes

S. exempta: Single lobe S. mauritia: Single lobe

S. frugiperda: Single lobe, elongate

S. littoralis: Two lobes S. litura: Two lobes S. eridania: One lobe

#### Ampulla

S. exigua: Elongate, slightly curved apex

S. exempta: Elongate, bent in the middle

S. mauritia: Elongate, slightly curved downwards
S. frugiperda: Elongate, curved with decurved apex

S. littoralis: Short, curved with decurved apex

S. litura: Short, curved

S. eridania: Straight clasper proper

#### **Female Genitalia Comparison**

## Corpus Bursae

S. exigua: Elongate
S. exempta: Bulbous

S. mauritia: Bulbous, constricted caudally

S. frugiperda: Bulbous S. littoralis: Bulbous S. litura: Bulbous S. eridania: Elongate

#### **Ductus Bursae**

S. exigua: Short, sclerotized

S. exempta: Medium length, sclerotized

S. mauritia: Short, sclerotized S. frugiperda: Short, sclerotized S. littoralis: Short, sclerotized S. litura: Elongate, sclerotized S. eridania: Short, sclerotized

#### Signum

S. exigua: Elongate, <30° angle S. exempta: Elongate, almost vertical S. mauritia: Medium elongate S. frugiperda: Short, >30° angle

S. littoralis: Short S. litura: Short

S. eridania: Elongate, >30° angle

#### **Key Distinguishing Features**

*S. exigua* vs. *S. frugiperda: S. exigua* has a broad elongate oval valve and elongate corpus bursae, while *S. frugiperda* has a very broad quadrate valve and bulbous corpus bursae.

*S. exempta* vs. *S. mauritia*: Both have a narrow rectangular valve, but *S. exempta's* coremata is a single lobe, while *S. mauritia's* is also a single lobe but with a constricted caudal end in the corpus bursae.

*S. littoralis* vs. *S. litura*: Both have broad quadrate valves, but *S. littoralis* has a dentate ventral margin and two lobes in the coremata, while *S. litura* has a triangular juxta and two lobes.

## Species Delimitation using Single GYMC, BPP, and mPTP Single GYMC Analysis:

The Generalized Mixed Yule Coalescent (GYMC) model identified six distinct species within the *Spodoptera* genus using COI gene sequences. The species boundaries had posterior probabilities exceeding 0.95, demonstrating strong support for the classifications. This analysis differentiated the closely related species *S. littoralis* and *S. litura*, which were previously difficult to distinguish based on morphology alone.

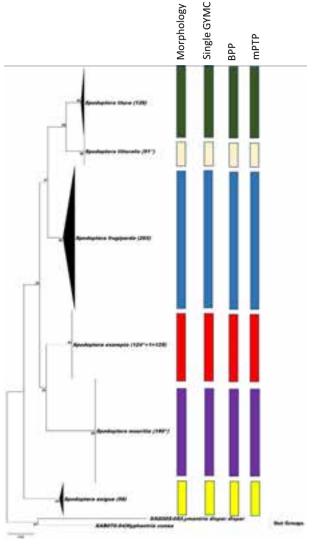


Figure 1. Phylogenetic analysis of *Spodoptera* species (\*indicates genetic data taken from outside of India).

#### **BPP Analysis**

The Bayesian phylogenetics and phylogeography (BPP) analysis further validated the species boundaries suggested by the GYMC model. The results showed high posterior probabilities (>0.90) for all nodes representing species splits, reinforcing the delineation of six species within the dataset. The BPP analysis confirmed the presence of distinct evolutionary lineages corresponding to the species identified by morphological and genetic data.

#### mPTP Analysis:

The multi-rate poisson tree processes (mPTP) model analysis supported the species boundaries identified by both GYMC and BPP methods. The mPTP analysis revealed the same six species with high confidence,

and bootstrap support values were above 95% for all species delimitations. This method effectively accounted for rate variation among branches, providing additional robustness to our species delimitation results.

#### **Comparative Analysis**

Comparative analysis across the three methods showed a high level of congruence, with all methods consistently identifying the same six species: *S. littoralis*, *S. mauritia*, *S. exigua*, *S. litura*, *S. exempta*, and *S. frugiperda*. The use of multiple methods provided a comprehensive framework for species delimitation, ensuring that the results were robust and reliable.

#### **Genetic Distances and Phylogenetic Relationships**

Genetic distance analysis revealed minimal within-species variation (≤2.2%) and clear between-species differences (≥4.2%), except the difference between species *S. littoralis* and *S. litura* was 2.9%, The phylogenetic tree constructed using the neighbor-joining method showed distinct clades for each species with high bootstrap support (>75%), consistent with the species boundaries identified by GYMC, BPP, and mPTP analyses. *S. exigua* was identified as the most divergent species within the genus, while *S. littoralis* and *S. litura* were confirmed as closely related sister species.

#### **DISCUSSION**

In the Indian subcontinent, four Spodoptera species were previously identified as native: S. litura (Muthusamy et al. 2024), S. exigua (Ramaiah et al. 2022), S. littoralis, and S. mauritia (Madhu et al. 2023). Additionally, one invasive species, S. frugiperda (fall armyworm or FAW), was reported (Ganiger et al. 2018), originating from North and South America (Jing et al. 2020). Recent comprehensive genomic analyses suggest that S. frugiperda likely consists of two closely related sister species, known as the corn-preferred and rice-preferred strains. These findings are supported by multiple studies (Pashley 1986; Meagher et al. 2004; Kergoat et al. 2012; Dumas et al. 2015; Gouin et al. 2017; Le Ru et al. 2018). Both sister species are present in India, but the manner of their introduction, whether together or separately, remains uncertain. Additionally, it is unclear if they have spread as a unified population since their introduction.

We observed that all four native *Spodoptera* species were reported through morphological methods, but genetic data is available for only two species on BOLD to date (Table 1). On BOLD/NCBI, only one sequence of *S. exempta* was submitted from India. This is very surprising

that commonly found species' genetic data was lacking. The same observation was noted by Shashank et al. (2022). They also highlighted the present state of insect species barcoding in India. They pointed out the existing gaps which must be addressed soon. Their examination indicates that barcoded specimens encompass a minimal percentage, specifically less than 3.73%, of the recognized taxa or described species. The most predominant orders include Lepidoptera and Hemiptera, followed by Diptera and Coleoptera. It is imperative to accelerate the discovery and documentation of insect species through collaborative efforts between traditional taxonomists and molecular biologists. This collaborative approach aims to achieve comprehensive DNA barcoding for all identified insect taxa in India.

The genus Spodoptera presents challenges for morphological identification across all species due to variability and shared characteristics. The complexity arises due to overlapping rib numbers between species, and the morphology of eggs in many Spodoptera species remains unknown. Therefore, molecular methods become essential for accurate species-level identification during this developmental stage (European and Mediterranean Plant Protection Organization (OEPP/ EPPO) 2015). While fully grown larvae of quarantine Spodoptera species can be distinguished, molecular identification is recommended for early stages, especially when the larva's origin is unknown or expertise is lacking. Distinguishing between younger larvae of S. littoralis, S. litura, and S. frugiperda is possible, but molecular identification is advised for early stages, offering reliability in cases where experience is limited or larval origin is uncertain. For S. eridania, S. frugiperda, S. littoralis, and S. litura, a practical approach involves using four simplex real-time PCR tests based on TaqMan® chemistry (Van de Vossenberg & Van der Straten 2014). To address geographical distribution overlap, tests for S. eridania and S. frugiperda, as well as S. littoralis and S. litura, are combined into single tests, providing an effective means of identification (European and Mediterranean Plant Protection Organization (OEPP/EPPO) 2015).

Biodiversity-rich nations like India, grappling with burgeoning populations, confront significant challenges in harmonizing economic progress, ensuring food security, and preserving biodiversity (Shashank et al. 2022). The foundational field of systematics, crucial for biodiversity research, is under considerable strain. Traditional taxonomy has historically played a pivotal role in identifying over 1.4 million global insect species for the past two centuries. However, the pace of this progress falls short of documenting the entire biota before it faces

extinction. Consequently, novel technologies (Patil et al. 2023; Sontakke et al. 2023), notably DNA barcoding, have gained traction for rapid and cost-effective biodiversity documentation.

As one of the mega-diverse countries, India aspires to make substantial contributions toward achieving the United Nations Sustainable Development Goals (SDGs) (Nalage et al. 2023) and targets (Shashank et al. 2022). However, this review unveils a disconcerting scenario concerning the status of DNA barcoding in India, which described very less insect species. There is apprehension that in the genomics era, the delayed establishment of DNA barcode reference libraries for insects may hinder our ability to comprehensively document India's abundant biodiversity.

#### **CONCLUSION**

This study has left a remarkable footprint in understanding *Spodoptera* species in India. It confirms the presence of four native species—*S. litura, S. exigua, S. littoralis,* and *S. mauritia*—along with two invasive species—*S. frugiperda* and *S. exempta*—in the country. The confirmation of the presence of *S. eridania* in India awaits the reporting of its mature larva or molecular data.

The study underscores the importance of a combined approach, emphasizing that both morphological and genetic studies must complement each other to accurately identify invasive and native species in the country. It highlights the integration of DNA barcoding and molecular analysis as indispensable for improving the precision and comprehensiveness of *Spodoptera* species identification.

The combined use of Single GYMC, BPP, and mPTP methods provided a robust and comprehensive approach to species delimitation in the *Spodoptera* genus. The results confirmed the presence of six distinct species within India, highlighting the importance of integrating multiple analytical methods to accurately delineate species boundaries in taxonomically challenging groups. This study contributes valuable genetic data and methodological insights for the improved identification and management of *Spodoptera* species in India.

This approach not only tackles challenges associated with morphological identification positively but also contributes valuable data for the development of more targeted and efficient strategies in pest management and conservation efforts.

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OPEN ACCESS

## Taxonomy and distribution of some orthopteran species (Orthoptera: Gryllidae, Trigonidiidae, Acrididae) from northwestern Morocco

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Abstract: Orthopterans have emerged as a crucial group of invertebrates for environmental monitoring and assessment. According to available literature the study of Moroccan orthopteran species remains limited in comparison to other countries. In recent years, the field of orthopteran classification has witnessed significant progress thanks to groundbreaking research in taxonomy and phylogeny that have shed new light on relationships and evolutionary history. In Morocco, there are many types of Orthoptera, including grasshoppers, crickets, and locusts, and different regions of the country have not been equally well sampled and studied. Notably the northwestern, particularly the Sidi Kacem region, are little studied. Here we present a taxonomic update of the most abundant orthopterans in Morocco based on field visits between spring and summer 2019. Five species were identified: Dociostaurus maroccanus, Aiolopus strepens, Gryllus bimaculatus, Gryllus campestris, and Nemobius sylvestris. We aim to use this publication as a baseline for future work on Orthopterans from northwestern Morocco.

**Keywords:** Caelifera, Ensifera, grasshopper, invertebrates, taxonomic update.

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Author contributions: El Harche Hanae—conducted surveys, collected specimens, identified the species, and writing - review and editing the manuscript. Mansouri Dalale & Kajoua Samiha—assisted with data collection.

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### **(D)**

#### **INTRODUCTION**

The order Orthoptera, comprising a vast array of species, holds a prominent position among insect orders (Bidau 2014). With approximately 28,000 species worldwide, it ranks as the sixth largest order, trailing only Hemiptera (Cigliano et al. 2022). These insects, commonly known as grasshoppers, locusts and crickets, are present in many terrestrial environments and exhibit remarkable diversity (Yadav & Kumar 2017). The composition of grasshopper communities serves as a valuable indicator, as their structure is highly responsive to environmental changes (O'Neill et al. 2003). Moreover, these insects play a crucial role in the functioning of ecosystems and can potentially serve as useful bioindicators for land disturbance (Saha et al. 2011). This order can be classified into two distinct suborders: the Ensifera, which includes crickets, katydids, and their relatives, characterized by their long antennae (longer than their bodies and consisting of more than 30 segments); and the Caelifera, which encompasses grasshoppers and their allies, distinguished by their short antennae (shorter than their bodies and comprising less than 28 segments) (Song et al. 2018).

Grasshoppers play a crucial role as ecological and biological indicators, providing valuable insights into ecosystem qualities and the effectiveness of ecological networks (Zhang et al. 2019). However, they have also garnered significant attention due to the extensive damage they inflict on crops and various forms of green vegetation (Dakhel et al. 2020). Despite the rich biodiversity of grasshoppers, Morocco has received little attention in terms of research, with only a few localities being studied. The first significant contribution to our understanding of Moroccan grasshoppers was made by Chopard (1936, 1943, 1949). Subsequently, several taxonomic studies were conducted such as: Badih & Pascual (1998), Latchininsky (1998), and Faucheux et al. (2013). More recently, some faunistic surveys have been carried out, including those by Defaut & Francois (2018, 2020, 2021), Mabrouki et al. (2021), Defaut (2022), and Aziz et al. (2023). Despite these efforts, our knowledge of the grasshopper fauna in many regions of Morocco remains insufficient, and further research is needed. There is a lack of published work or reports on the grasshopper fauna in the northwestern region of Morocco. This region remains poorly studied, presenting an opportunity for future research and exploration. The aim of this study was to improve our comprehension to the grasshopper fauna in Morocco, with a specific focus on the taxonomy, ecology, and distribution of the most

prevalent orthopteran species found in a poorly studied northwestern region. By conducting this research, we aimed to contribute valuable insights to the existing knowledge in this field.

#### **MATERIAL AND METHODS**

#### Study area

The study was conducted in three stations in the region of Sidi Kacem (34.13.00 N, 5.42.00 E) located in the northwest of Morocco (Figure 1). The climate of the region is classified as semi-arid, the temperature in autumn goes down to 6 °C while in summer it can exceed 40 °C, with a probability of daily precipitation above 13%.

Station 1: 34.2295°N; -5.7013°E. It is a field of *Vicia faba* L. beans (Fabaceae).

Station 2: 34.2402°N; -5.7094°E. This is a field of cereal crops: soft wheat: *Triticum aestivum* L. (Poaceae). Station 3: 34.2302°N; -5.7055°N. This is an uncultivated area. The plant species dominate the area are *Nicotiana glauca* Graham tree tobacco (Solanaceae), *Ferula communis* L. fennel (Apiaceae), *Cynara humilis* L thistle (Asteraceae), and *Ammi visnaga* L. toothpick weed (Apiaceae).

#### Sampling

The orthopteran specimens collected, studied, and documented during faunistic surveys between April and August 2019. The insects were captured by sweeping vegetation using an entomological net, to collect orthopterans from plants and by handpicking, seeking under stones and different substrates. After the collection, insects were transferred into specimen bottles containing 70% alcohol. Specimens were identified using a relevant, published key, and by referring to the Orthoptera collection at the Scientific Institute of Rabat (Morocco) and Data available in the Global Biodiversity Information Facility (GBIF) of Morocco. The nomenclature has been updated using the websites http://orthoptera.speciesfile.org, https://www.gbif.org and MNHN Paris website http://acrinwafrica.mnhn.fr.

Abbreviations used in the examined material are the following: CSIR—Collection at the Scientific Institute of Rabat (Morocco). GBIF—https://www.gbif.org/country/MA.

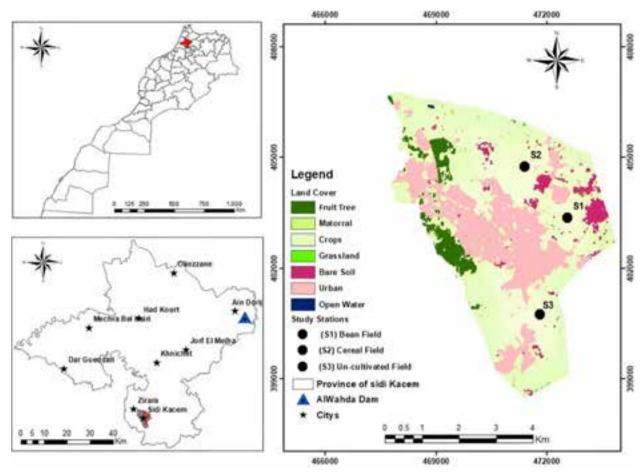


Figure 1. Map of Morocco showing the location of sampling stations.

#### **RESULTS**

Systematic Account

Suborder: Ensifera Chopard, 1922 Family: Gryllidae Laicharting, 1781 Subfamily: Gryllinae Laicharting, 1781 Tribe: Gryllini Laicharting, 1781 Genus: *Gryllus* Linnaeus, 1758

#### Gryllus (Gryllus) bimaculatus De Geer, 1773

**Material examined:** MOROCCO: Sidi Kacem (Station 1), 34.2295°N; -5.7013°E, 191 m; 15.v.2019, H. El Harche leg, 1 adult ( $\sigma$ ), GBIF. Sidi Kacem (Station 3), 34.2302°N; -5.7055°N, 192 m, 20.vi.2019, H. El Harche leg, 2 adults ( $\sigma$ ), CSIR.

**Diagnosis:** 17–23 mm. Black almost all over the body, males have a yellowish area between the pronotum and the elytra and light legs. This species can be differentiated from other cricket species by the two yellow/white spots on the dorsum of its thorax. Females have a tubular organ at the rear, the ovipositor, which is used to lay eggs in the ground.

**Nutrition:** Foliage, seeds, roots, and small insects **Habitat:** Inhabits pastures, shrubs, dunes, grasslands and ruderal terrain (Bent et al. 2018).

Life cycle: Adults appear from June, July to autumn (Gawałek et al. 2014). This species, like other crickets, has an incomplete metamorphosis (hemimetabolous) with an egg, nymph, and adult stage (Donoughe & Extavour 2016; Watanabe et al. 2017). Females lay their eggs in humid soil or sand and hatchlings emerge from the eggs in about two weeks (Donoughe & Extavour 2016).

**General distribution:** A Palearctic species, occurs predominantly in the Mediterranean area (Ferreira & Ferguson 2010; Panagiotopoulou et al. 2016) northern Africa, Madagascar, the Indo-Malayan area, Ethiopia, and Central Asia (Gorochov & Llorente 2001).

**Distribution in Morocco:** Recorded at Oued Cherrat, Korifla, Tafrata, Amizmiz (Chopard 1936), in the cork oak forest, Mamora (El Alami Idrissi 2013), and the Oriental region (Mabrouki et al. 2021).

#### Gryllus (Gryllus) campestris (Linnaeus, 1758)

**Material examined:** MOROCCO: Sidi Kacem (Station 1), 34.12.35N, 5.42.31E, 191 m; 10.vi.2019, H. El Harche leg, 1 adult (♂), CSIR. Sidi Kacem (Station 3), 34.13.50N, 5.40.12E, 192 m, 18.vii.2019, H. El Harche leg, 1 adult (♂), CSIR.

**Diagnosis:** Male: range from 19–23 mm. Female: from 17–22 mm. Body: shiny black to rarely brown of compact cylindrical shape and with strong legs. Head: black, large, wider than the pronotum. Tegmina: brown with yellow spots at base, with rounded end not exceeding the abdomen. Wings not exceeding elytra: Inner side of hind femur red

**Nutrition:** Mostly plants (herbs, grasses), but also occasionally (dead) insects.

**Habitat:** inhabits nutrient-poor grasslands of all kinds, rather dry, extensive hay meadows, large embankments, heathland, and extensive, sunny pastures (Witzenberger & Hochkirch 2008).

Life cycle: The life cycle includes eggs, several instars of nymphs and adults. Nymphs can be found together with adults (Vrenozi & Uchman 2020). During growth, the nymph's males dig out the burrow and hibernate until late April and early May–July (Witzenberger & Hochkirch 2008; Gawałek et al. 2014). Mature females lay hundreds of eggs into the soil around May and June (Rodriguez-Munoz et al. 2010). Only eggs laid fairly early in the season (late May–early June) are robust enough to hibernate successfully.

**General distribution:** A Palearctic species, the distribution of *G. campestris* includes all of Europe, northern Africa (Morocco, Algeria & Tunisia), and western Asia (Gorochov & Llorente 2001; Hochkirch & Adorf 2007; Panagiotopoulou et al. 2016).

**Distribution in Morocco:** Recorded in the cork oak forest, Mamora (El Alami Idrissi 2013).

Family: Trigonidiidae Saussure, 1874 Subfamily: Nemobiinae Saussure, 1877 Tribe: Nemobiini Saussure, 1877

Genus: Nemobius Audinet-Serville, 1838

#### Nemobius (Sylvestris) sylvestris (Bosc, 1792)

Material examined: MOROCCO: Sidi Kacem (Station 3),  $34.2402^{\circ}N$ ;  $-5.7094^{\circ}E$ , 192 m, 28.viii.2019, H. El Harche leg, 1 adult ( $\sigma$ ). GBIF.

**Diagnosis** 7–10 mm. Both adults and nymphs of *N. sylvestris* may be recognized by their unique color pattern golden brown to almost black body. **Head:** black with pale Y-shaped marking attached to the body with very short wings. **Pronotum:** very pale with dark

speckles. Male: Absence of glandular hind tibial spines. **Female:** ovipositor shape and tooth dentation, straight, about as long as the hind femur, and without teeth. Straight ovipositors.

**Nutrition:** Omnivorous, feeding on a wide range of organic matter, including carrion, leaf litter, decaying plant parts, fruits & also fresh plants, and dead or living insects (Martín-Vega et al. 2013).

**Habitat:** It can be found at ground level in meadows, in leaf litter, shrubs, and woodland borders in which it looks for food (Brouwers & Newton 2008; Brouwers et al. 2011). It inhabits also boulders in grasslands, where they are hidden in the pore systems of the stones.

Life cycle: Nemobius sylvestris passes through two to three winters in its life cycle. The first winter is spent as eggs, and the second as nymphs of a medium size. These mature by the middle of the summer, and some of these adults may endure a third winter (Vahed 2020). Adults appear from July–October (Brouwers et al. 2011).

**General distribution:** A Palearctic species, found-in the Iberian Peninsula, covers the southwestern Europe, the south of England, France, the south of Portugal, and Poland (Gorochov & Llorente 2001). Also recorded in North America (Woo 2022).

**Distribution in Morocco:** Recorded in Tangier (Defaut et al. 2016).

Suborder: Caelifera Ander, 1939 Family: Acrididae MacLeay, 1821 Subfamily: Gomphocerinae Fieber, 1853 Tribe: Dociostaurini Mishchenko, 1974 Genus: *Dociostaurus* Fieber, 1853

## Dociostaurus (Dociostaurus) maroccanus (Thunberg, 1815)

**Material examined:** MOROCCO: Sidi Kacem (Station 1), 34.2295°N; -5.7013°E, 191 m; 16.viii.2019, H. El Harche leg, 2 adults (σ), GBIF. Sidi Kacem (Station 2), 34.2402°N; -5.7094°E, 190 m, 12.vi.2019, H. El Harche leg, 1 adult (σ), GBIF. Sidi Kacem city (Station 3), 34.2402°N; -5.7094°E, 192 m, 20.v.2019, H. El Harche leg, 1 adult (σ), GBIF.

**Diagnosis:** Body: 16.5–28.5 mm for males and 20.5–38.0 mm for females. Tegmina: are 17.5–27.0 mm for males and 23.0–36.0 mm for females, nearly transparent that extend far behind the tip of the hind femur can have sporadic, tiny brownish or gray patches. The pronotum: bears a pale cross-shape. Wings: longer than wide. Hind Femurs: femora slender. Hind femur male 13.2–17.4 mm, female 15.5–21.6 mm. Grayish-yellowish in color overall, with dark markings on the body. Depending on

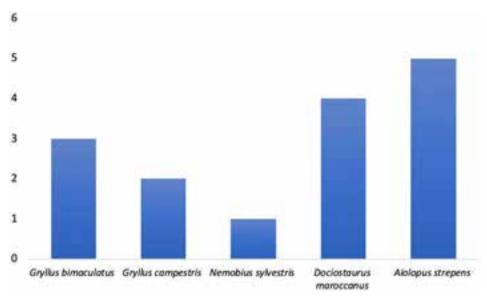


Figure 2. Number of orthoptera individuals caught in the study area.

the phase, the hind femora may or may not have black bands. Tibia: usually red, less often yellow, pinkish, or even whitish, with short striae that don't reach behind the transversal furrow. Pronotum: yellow or pale.

**Nutrition:** Polyphagous insect, mostly plants (herbs, grasses), but also occasionally insects.

**Habitat:** *Dociostaurus maroccanus* is a thermophilous and xerophilous species inhabiting open, well-lit areas (Victorovich & Zlatanov 2020). The species is mainly adapted to valleys and foothills with xerophytic vegetation at an altitude of between 400 m and 800 m (Song 2011).

Life cycle: This cricket species undergoes a single annual generation and exhibits an incomplete metamorphosis, known heterometabolous development. Interestingly, during this process, the nymph closely resembles the adult form, eliminating the presence of a distinct nymphal stage. To ensure successful reproduction, female crickets require firm and exposed ground to lay their eggs. Once laid, these eggs hatch during the subsequent spring and the young crickets migrate towards vegetated areas to find nourishment. It is worth noting that the egg-laying activity primarily occurs in early summer. Each female lays between 18 and 42 eggs. Usually, a single female provides two egg-pods (Quesada-Moraga & Santiago-Álvarez 2001). Nymph development is quite rapid, lasting no more than 25–35 days. The adult stage actively migrates from the hatchling sites to humid depressions where the adults find a sufficient amount of food (Popova & Popov 2009). The breeding season starts in May (Victorovich & Zlatanov 2020).

General distribution: A Palearctic species; in Africa the species occurs in Morocco, Algeria, Tunisia, Libya, and Egypt (Latchininsky & Launois-Luong 1992). It is also present in west and central Europe, i.e., Portugal, Spain, France, Italy, countries of ex- Yugoslavia, Greece, Bulgaria, Moldova and southwestern Ukraine, as well as in the Caucasus, i.e., Armenia, Azerbaijan, and Georgia. The northern limits of its distribution area reached in Hungary and Rumania. The species is also found in countries of the Middle East and Minor Asia, i.e., Turkey, Syria, Lebanon, Jordan, Iraq, and Afghanistan (El Ghadraoui et al. 2003; Guerrero et al. 2017).

**Distribution in Morocco:** recorded in Al-Azaghar of the Middle Atlas (El Ghadraoui et al. 2008) and the Oriental region (Mabrouki et al. 2021).

**Subfamily: Oedipodinae** 

Tribe: Parapleurini Brunner von Wattenwyl, 1893

Genre: Aiolopus Fieber, 1853

#### Aiolopus (Strepens) strepens (Latreille, 1804)

**Material examined:** MOROCCO: Sidi Kacem (Station 2), 34.2402°N; -5.7094°E, 190 m, 26.vi.2019; H. El Harche leg, 2 adults ( $\sigma$ ), GBIF. Sidi Kacem city (Station 3), 34.2302°N; -5.7055°N, 192 m, 19.viii.2019, H. El Harche leg, 3 adults ( $\sigma$ ), GBIF.

**Diagnosis:** Body: 19–24 mm for males while females reach 24–31 mm. *Aiolopus strepens* has a wide range of coloration (green, yellow, and brown). Their strong bodies are typically light brown, though occasionally they might be green, have green patches, or have reddish stripes. Females, can also be totally green in

color. Wings: translucent, slightly bluish, marked by a clear dark spot in the apical region. Antennae: 22–24 segmented, shorter than head and pronotum together. Eyes: ellipsoid, almost twice as long as wide. Pronotum: male 3.9–5.0 mm, female 4.5–7.0mm, usually brown, the disc may occasionally have median longitudinal stripes that reach the vertex. Subtectiform, rather flat. Tegmina: male 16.7–23.6 mm, female 19.6–30.9 mm, relatively short and broad, exceeding end of hind femur. Hind femur: male 11.3–14.8 mm, female 13.4–19.2 mm, brown with testaceous spots, blackish at upper surface, reddish on inner surface. Wings: transparent with darkened brownish apex. Tibia: as long as hind femur, with 10 outer and 11 inner spines.

Nutrition: The insects feed mainly on grasses.

**Habitat:** It inhabits dry meadows, riverbanks, dry to mesophilic grasslands, woodland edges, shrubland with open soil spots, quarries. The species can be found over 1,500 m.

**Life cycle:** The presence of *Aiolopus strepens* adults for most of the year, with only one generation per year (univoltine) in imaginal hibernation. The imagines (= adults) can be found from August-May of the following year. Some of these animals hibernate and reproduce the following spring. There is therefore only one generation per year, but due to the extended lifespan of some individuals, they may still be alive when the nymphs from the eggs laid the previous year are already hatching (Baur et al. 2006). According to the work of Hamdi (1989) in the mid-northern region of Algeria, Benrima (1990) in the Koléa region, Guecioueur (1990) in the Lakhdaria region, Fellaouine (1989) in the Sétif region, Zergoun (1991, 1994), and Douadi (1992) in the Ghardaïa region, Aiolopus strepens is present as an adult throughout most of the year. The larvae begin to appear in April. These authors note that Aiolopus strepens has only one annual generation and spends the winter in the imaginal state.

**Remark:** In southern Spain, *A. strepens* exhibits a fascinating life cycle, characterized by two distinct generations per year. The first generation commences in March, when larvae make their initial appearance, followed by the emergence of adults in April. The second generation, on the other hand, commences from July and extends until December, during which a dense population is observed. Between these two periods, the species enters a state of hibernation. Notably, the two generations overlap, resulting in the presence of adult individuals throughout the year (Hernández et al. 1985).

**General distribution:** A west Palearctic species-widespread throughout the Mediterranean region until

Asia Minor, often reported throughout southern Europe and North Africa (Algeria, Morocco & Tunisia) (Defaut 1999).

**Distribution in Morocco:** Observed in Moroccan Middle Atlas (Sefrou at an altitude altitude of 800 m and Mazdou at an altitude of 1,200 m) (Essakhi et al. 2014).

#### **DISCUSSION**

During the course of this study, five distinct species were collected from three sections of the Sidi Kacem region, representing three families: Acrididae, Gryllidae, and Trigonidiidae. These insect families are widely distributed and typically abundant in nature. However, it is worth noting that the level of biodiversity within the Sidi Kacem region is relatively low for these three families. This can be attributed to the prevalence of anthropogenic pressures in the area. The expansion of agricultural areas and the intensification of management practices are significant factors that contribute to the loss of terrestrial biodiversity at both local and global scales (El Harche et al. 2022). Specifically, the application of pesticides, tillage, and the timing of harvest periods have all had a detrimental impact on the fauna's biodiversity (El Harche et al. 2023). The current state of low biodiversity within these families highlights the potential ecological consequences of human activities. As agricultural areas continue to expand and management practices become more intensive, the delicate balance of the ecosystem is disrupted, leading to a decline in biodiversity (El Harche et al. 2023). Ecosystem alterations have a profound impact on the behavior of organisms, particularly poikilotherms like grasshoppers, which heavily rely on plant matter for sustenance (Bronwyn 2013). Latchininsky et al. (2011) have demonstrated that certain grasshopper species face significant threats from anthropogenic pressures, such as overgrazing and ploughing. The limited number of orthopteran species discovered in our study sites suggests that these species have developed adaptive strategies to withstand human disturbances (Havyarimana et al. 2013).

Orthopterans are widely recognized for their herbivorous habit and are generally regarded as a dominant group of insects in terrestrial habitats. They have a voracious appetite for various types of plants, often leading to significant economic losses. The extent of crop damage is contingent upon the populations and movements of orthopterans within the fields. This poses a significant threat to food crops in Africa.

In our current investigation, the orthopterans were

found to be omnivorous or phytophagous, exclusively consuming plants and seeds. This finding underscores the importance of understanding their feeding habits and preferences. The impact of orthopteran pests on agricultural productivity cannot be overstated. Their relentless feeding habits and ability to decimate crops pose a serious challenge to farmers' livelihoods. Consequently, it is crucial to develop effective strategies for managing and mitigating the damage caused by these insects.

Gryllus sp. and Dociostaurus maroccanus pose a significant threat to agricultural crops. These voracious locusts indiscriminately target a wide range of crops, including cereals, vegetables, forage crops, oilseed crops, fruit trees, date palms, and even conifers (Latchininsky 1998). The destructive impact of D. maroccanus on crops has been documented in more than 25 countries, often necessitating military intervention for effective control (Latchininsky 1998). This species feeds on over 150 plant species from 33 different families, with 50 of them being important agricultural crops (Latchininsky & Launois Luong 1992). However, despite its potential for devastation, the detailed study of this species has been limited due to its relatively low occurrence rate.

This investigation, conducted in the Sidi Kacem region, has enabled us to compile an initial inventory of the local orthopteran biodiversity of most frequent species that exists in this area. It is important to note that this list can be expanded and updated as new species could be discovered in the future. The Orthoptera, being highly sensitive to changes in vegetation structure, seasonal temperature, and humidity, play a crucial role in ecological studies. They serve as effective indicators of environmental changes (El Harche et al. 2023)

This comprehensive list serves as a valuable reference for future studies aimed at examining the development of biodiversity and determining the real impact of landscape change & degradation on entomofauna. The article focuses mainly on the description of the specimens collected and their distribution, providing an in-depth analysis of the subject. The article presents detailed observations and measurements of each specimen, highlighting its morphology, nutrition, habitat, and life cycle. In addition, this study examines the distribution patterns of these specimens in various locations in Morocco and abroad. This meticulous examination provides valuable information on the range and abundance of different species in a given area, revealing complex relationships within ecosystems. Overall, this article is a crucial resource for scientists in this field, documenting important results on specimen collection and distribution patterns, while opening the door to further research opportunities.

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ARTICLE

# Impact of root harvest on *Decalepis hamiltonii* Wight & Arn. population across habitats in Savandurga Reserve Forest, Karnataka, India

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Abstract: Decalepis hamiltonii Wight & Arn., is a woody climber, endangered due to the destructive harvest of fragrant roots and substituted for Hemidesmus indicus (L.) R.Br. (Nannari). We assessed the density, size class, distribution across habitat types, disturbance types, and environmental variables that influence the D. hamiltonii population in the Savandurga Reserve Forest (SRF). Method: The entire forest was divided into 1 km² grids, with 10 plots of 5 x 5 m established in each of the 24 grids, totaling 240 plots. Disturbances were categorized as low, medium, and high, while plots were classified into dense, mixed, and rocky outcrop habitats. Results: D. hamiltonii density ranged from 0.1 ± 0.32 to 5.2 ± 2.66 per 25 m² across the grids with an overall mean density of 675 ± 455 stems per ha. The size class distribution showed a typical inverted "J" curve, with fewer saplings (3.01–6.0 cm class), indicating potential future population instability. Stem density was lower in dense vegetation and higher in mixed vegetation and rocky outcrops, with greater densities in areas of higher disturbance. Adult density was mainly influenced by harvesting (78% variation), saplings by NDVI (2.09%) & altitude (18.72%), and seedlings by aspect (4.44%), increasing from the south to the north. Conclusion: Strict monitoring and periodic assessment of the population are essential to protect the seedlings to the sapling stage, regulation of selective harvesting of the adults, and control of the herders feeding the leaves. Encouragement of local and large-scale cultivation to reduce pressure on the wild source and to improve livelihood. Capacity and confidence building of the community with citizen science reporting of destructive harvesting will help the forest department to save the declining population.

Keywords: Aspect, density, inverted "J" curve, MPCA, NDVI, Nannari sharbat, NTFP, root harvesting, size class, woody climber.

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#### **INTRODUCTION**

In India, over 1,178 medicinal plants are commercially demanded for raw drugs. Of these 53% of plants are destructively harvested by collecting roots, wood, bark, or the entire plant (Goraya & Ved 2017). The concern is among the non-timber forest product (NTFP), harvesting of roots, whole stems, bulbs, and bark, especially in perennials is usually lethal or fatally weakens the plant species, and further achieving sustainability becomes difficult (Davenport & Ndangalasi 2002; Ticktin 2004; Hernandez et al. 2015). Of the 242 species in high commercial demand, 72% are sourced from the wild and they lack information on the impact of harvesting on the wild population (Fuller 1991; Cunningham 1993a; Ticktin 2004; Goraya & Ved 2017). These conditions make ecological conservation even more critical when there are increased collections. Research from the perspective of assessing the threats and the impact of harvesting of climbers are less studied compared to other life forms likewise there is a dearth of information on root-harvested plants also (Ticktin 2004; Stanley et al. 2012). Most of the studies on woody climbers have focused on community level analysis, in fact, generally climbers are neglected and less included in ecological studies (Gerwing 2004; Pandi et al. 2022). Decalepis hamiltonii is a woody climber, a medicinal non-timber forest product (NTFP), destructively harvested for fragrant roots, coupled with habitat loss the population is declining (IUCN ver. 3.1, Ravikumar & Ved 2000). We focus on studying D. hamiltonii which is harvested for roots and quantify the density, size class, and distribution of the population from the perspective of harvesting impact which is fairly unique. This attempt enhances our understanding of the status and also from the literature by touching upon studies related to woody climbers as well as root harvested plants.

For sustainable management of resources Shankar et al. (1996), emphasize the need for information on extraction and productivity levels per unit area which are unknown for most of the species being harvested. Studies have demonstrated that both natural and anthropogenic disturbances are crucial for the sustainability of the population of many such species (Mandle & Ticktin 2012; Mandle et al. 2013; McKechnie & Sargent 2013). Illegal collections have driven certain species population to an endangered status (Pfab & Scholes 2004). Destructive practises of harvesting plant parts such as bark, foliage, fruit, pith, seeds, and cone have led to population decline (Ticktin 2004; Stanley et al. 2012; Krishnamurthy et al. 2013). Best lessons

have also been adopted from traditional ecological knowledge for root harvesting methods, life history & mode of reproduction to sustain the population from studies conducted on Dichelostemma capitatum, Harpagophytum procumbens, Nardostachys grandiflora and Neopicrorhiza scrophulariiflora (Anderson & Rowney 1999; Ticktin & Johns 2002; Stewart & Cole 2005; Ghimire et al. 2008; Ticktin et al. 2012). Trade and demand for roots affected the population of Rauwolfia serpenting as the gatherers were offered high prices at the retail level, which led to a sixfold increase in the harvesting rate of immature stages of the rhizomes and roots (Mishra 2001). Asparagus racemosus (Satawari), Chlorophytum borivilianum (Safed Musli), and Cyperus rotundus (Nagarmotha) are some of the well-known tubers /rhizomes facing destructive harvesting. Unsustainable harvesting practices like uprooting plants at the immature stage, not placing them back in the soil with proper coverage, or the entire primary /core root cut reduces the chances of survival. Root harvested plants remain exposed to sunlight leading to death and collection done throughout the year, roots/tubers of all age groups being harvested are highly unsustainable. Such activities reduce the quality and quantity, and pose a challenge to the survival and sustenance of the individuals (Mishra 2000; Prasad et al. 2002; Mishra & Kotwal 2003, 2007; Mishra et al. 2003).

Excessive harvesting has resulted in a typical population with many resprouts (small diameter sizes) but very few mature stems in both disturbed and severely disturbed sites (Hall & Bawa 1993; Ndangalasi et al. 2006). Over collections of fragrant medicinal roots of Mondia whitei, and other species under trade led to the extermination of the population locally in South Africa (Cunningham 1993b). Extraction likely impacts negatively on plant population leading to changes in population structure found in perennials, e.g. some trees and climbers (Ganesan & Setty 2004; Bitariho & Emmanuel 2019; Soumya et al. 2019b). Loeseneriella apocynoides is a slow-growing woody climber and its whole plant stem is harvested, so it takes over 20 years to reach a harvestable size (Muhwezi 1997). Long-term harvesting had a strong impact on reducing the root size over a period of time and noted changes in the biology of Philodendron corcovandense population (Valente & Negrelle 2011). Coscinium fenestratum is another woody climber species that is harvested for both stem and roots (Ved & Goraya 2007). The study by Thriveni et al. (2015) across disturbance levels found that none of the C. fenestratum individuals recorded a diameter of more than 3.5 cm, but the forest department records

suggested the existence of individuals that were larger than 25.0 cm diameter indicating the intense effect of harvesting which has resulted in a smaller size class representation. Likewise, McGeoch (2004) found Mondia whitei roots preferentially harvested for adults and juveniles which had led to disjunct patches of over or under abundance of size class representation with very few fruiting individuals. Since the root is an organ available in the plant from the initial growth stage found in all size classes, it is not a part like flowers/leaves/ fruits which are produced, every year to shed. Rather the role of a root is on a long term for storage, transport, anchoring, developing new shoots after disturbance and much more. Unless the marketable size is specific to only a certain size or quality, demand for the material can lead to harvest of all size classes.

Woody climbers grow well in forest edges, gaps and disturbed habitats compared to undisturbed forest which is one of their characteristic features (Schnitzer & Bongers 2002). It is observed in C. fenestratum population tolerating a wider range of light levels, and was found to have lowest survival under the lowest light treatment (Kathriarachchi et al. 2004). Similarly, McGeoch (2004), found Mondia whitei growing well in all habitats as it is a wind dispersed species and germinated very well in disturbed areas. Light levels (sunflecks) effected the local demography in root harvested ginseng Panax quinquiefolius (Wagner & McGraw 2013). Lack in information on over-harvesting and quantification of the resources leads to decline of the population and could lead to conflicts among the stakeholders (Homma 1992; Chamberlain et al. 1998; Dovie 2003).

According to the International Union Conservation of Nature (IUCN ver. 3.1), Decalepis hamiltonii Wight & Arn. (Apocynaceae) is assessed as endangered due to destructive root harvesting and habitat loss (Molur & Walker 1997; Ved et al. 2015). It is endemic to the Deccan plateau, found wild in Karnataka, Tamil Nadu, Telangana, Andhra Pradesh, and Kerala, within an altitudinal range of 1,200 m (Ravikumar & Ved 2000; Reddy & Murthy 2013). It is a woody climber found in seasonally dry forests, mixed and dry deciduous forests, and semi-evergreen forests (Muthumperumal & Parthasarathy 2010; Anburaja et al. 2012; Rao & Reddy 2022; Pandi et al. 2022). Roots of this plant are traded nationally at 100-200 MT (Goraya & Ved 2007) and traders have reported a decrease in availability through the years which is a forewarning (Goraya & Ved 2017). Roots are medicinal, aromatic and substituted for Hemidesmus indicus, and it is known as "Makaliberu" in Kannada, "Magali kizhangu" in Tamil, "Maredu kommulu" or "Nannari kommulu" in Telugu, and Swallow root in English. Collectors prefer *D. hamiltonii*'s stout and thicker tuberous roots, which yield 40–45 kg per plant, over those of *H. indicus*. To meet the demand for roots, other *Decalepis* species like *D. arayalpathra* and *D. salicifolia* are also used as substitutes. However, both species are narrow endemics with limited ecological range and small populations, making them vulnerable to extinction (Mishra et al. 2015, 2017; Rodrigues et al. 2021). Sourced largely from the wild but origin of collection sources in the wild are not known. Unfortunately, there is no large-scale cultivation to meet the demands for the future or reduce the pressure on wild resource except for few farmers.

Studies have reported that the D. hamiltonii root extracts have the potential to cure many diseases, insecticidal, antifungal, and antioxidant properties (Reddy & Murthy 2013; Sharma & Shahzad 2014; Pradeep et al. 2016; Kharat & Mokat 2020; Ahmad et al. 2022). Potentially a new ecofriendly bioinsecticide and grain protectant of natural origin for agriculture (Mohana et al. 2008; Rajshekhar et al. 2010). Phytochemical investigations find a cocktail of active principles (Srivastava & Shivanandappa 2009) and researchers consider it to be an inspiration for discovering new herbal drugs and active compounds to treat many incurable diseases in the future (Naveen & Khanum 2010). It is considered as a wild food resource in the diet of tribal communities for enhancing nutrients, therapeutic practices, and strengthening their relationship by sharing the tubers frequently among themselves (Harisha et al. 2021). Roots are of multiple uses, required for Ayurvedic formulations, pickling and beverage industries, and to prepare traditional health drinks such as Nannari Sharbat or Rayalseema sharbat (Raju & Ramana 2009). Sold in local vegetable markets for pickling in households, the recent increase in people's preference is for 'makali' root juice and tea recipes are naturally drawing crowds at local food festivals (Vedavathy 2004). Naturally, the tubers of this endangered plant are highly exploited for medicinal, culinary, and confectionary uses and applications through destructive harvesting methods. Hence, its conservation in the wild is crucial. There is a need for alternative methods to meet the growing demand and reduce the pressure on wild resources through local or mass cultivation. Recent increase in the awareness of this plant for natural flavor extracts has created huge interest and its commercial cultivation by farmers can provide scope for looking at unexplored R&D avenues of D. hamiltonii (Shankar 2022).

The scope for demand increases because these roots are 10 times stronger than a 20-fold Vanila Bourbon concentration (John et al. 2007). The plant's tuberous roots possess a characteristic vanillin-like aroma due to the compound 2-hydroxy-4-methoxybenzaldehyde (2HMB) (Nagarajan et al. 2001). Biotechnological approaches are reviewed and recommended to mass multiply, and produce this vanillin flavour in tubers naturally in large scale (Shankar 2022). Globally vanillin biosynthesis is essentially becoming a need to meet the growing demand for natural vanillin productivity in a sustainable and efficient way (Xu et al. 2024). Though, there are several methods extensively reviewed (Reddy & Murthy 2013; Shankar 2022) plant tissue culture is also one of the methods to have demonstrated the scope to increase the yield of tubers. Increasing the vanillin and vanillic acid (Matam et al. 2017; Xu et al. 2024) and field transfer in rooted plantlets have been successful (Giridhar et al. 2003, 2004, 2005). Shoot multiplication and elongation methods have produced 40–48 plants from a single explant within four months followed by transfer from green house to field which resulted in survival of approximately 80-90% of the plantlets (Giridhar et al. 2003). Further, a higher yield in tuber biomass and maintenance of relative content of flavour compounds was also observed (Kamireddy 2017; Shankar et al. 2022). These studies provide evidences that can contribute towards local and mass cultivation and reduce pressure on the wild resource.

Field studies on D. hamiltonii revealed several issues related to its population and ecology. For instance, Raju & Ramana (2009) identified bottlenecks in sexual reproduction and seedling establishment. Meanwhile, in the Bili Giri Rangan hills (BR hills), root extraction experiments showed that the population is sensitive to even small intensities of 25% harvest and recommended the need for curtailment of wild collection (Murali 2008). A population estimation study in the Seshachalam hills using Adaptive Cluster Sampling utilized decadal data from the Girijan Co-operative Corporation (GCC), and aimed to estimate productivity (Mishra & Naidu 2014). The focus was on quantification of the root yield but not population status, structure or rotation period and the study recommended a long-term monitoring to suggest sustainable harvesting practices. Apart from species specific focus there are studies specific to climbers where the presence of D. hamiltonii has been recorded from various sites (Muthumperumal & Parthasarathy 2010; Anburaja et al. 2012; Pandi et al. 2022; Rao & Reddy 2022).

Conditions for D. hamiltonii's inadequate

regeneration is reported to be the impeding factors such as the hard seed coat, and the short viability of seeds and the need for a good aerated substrate (Anandalakshmi & Prakash 2010). Factors regulating the population size of D. hamiltonii in natural areas are found to be extended flowering pattern, pollinator limitation, selfincompatibility, abortion of a considerable percentage of seedlings prior to establishment and absence of seed dormancy (Raju & Ramana 2009). The tuberous roots of D. hamiltonii are over exploited prior to reproductive maturity due to their economic and medicinal values and this is the main cause for the gradual decline of population (Anandalakshmi & Prakash 2010; Shankar 2022). As it is a tropical species, it requires higher alternate temperature of 20/30 ° C or 30 ° C rather than low temperature for germination and it was observed that among the three tests seed germination showed better results for the ones kept in between the paper than placing it on top of the paper or in the cocopeat (Navya et al. 2019). Similarly, soaking in hot water (60°C) for 24 h significantly improved the germination percentage from 83% to 98% on moist filter paper than on sand (Anandalakshmi & Prakash 2010). Storage of seeds at lower temperature of 15°C, having at least 8% moisture results in maintenance of seed viability and longevity (Navya et al. 2019). Storage of seeds at ambient temperature probably favored the increase of those enzymes, which positively affected germination.

Novelty in research information was found to exhibit significant variations observed in the quantity of phenolics, flavonoids in the tubers that were collected from different natural habitats which showed 10-16 % difference in the content of 2H4MB, Biligiri Rangaswamy Temple (BRT) being the highest, followed by Mysore, Tirumala, Kurnool, Trichy, and Palakkad (Pradeep et al. 2019). A similar study on Ichnocarpus frutescens demonstrated variations between the two phytogeographical zones and also within the same zone, as they were affected by various edaphic factors which is indicating that the micro-environment of the location plays a considerable impact on the yield of secondary metabolite contents in plants (Nirala et al. 2024). The composition of secondary metabolites is influenced by a range of biotic and abiotic factors, diverse environmental conditions that significantly impact the composition of medicinal plants (Li et al. 2020). Since roots serve as the primary channel for nutrient uptake from the external environment to the plant's internal system, edaphic conditions mainly play a pivotal role in metabolite production (Liu et al. 2022). Recent findings on D. hamiltonii study revealed that compared to the



first stage (12 months old) tuber there was a decrease in the expression of vacuolar transporters at second stage of the tubers (48 months old), indicating that the normal functioning of vacuoles in first stage transforms into a storage organ/tuber for 2HMB accumulation in the second stage (Kamireddy et al. 2021). It is indicative of the accumulation of fragrant compound in *D. hamiltonii* gradually increasing at the older stage of 48 months, than the first stage and this information is helpful in avoiding root harvesting of small size class individuals and it can also be chosen as a parameter for standardizing root harvesting. It is evident from the study that *D. hamiltonii* plants may adapt to various ecological / microclimatic conditions in the habitat and vary in accumulation of secondary metabolites.

Around 150 ha area in SRF has been designated as medicinal plant conservation area (MPCA) for in situ conservation of medicinal plants which includes D. hamiltonii population also (Somashekhar 2013). The botanical survey resulted in 355 medicinal plants including D. hamiltonii (Ved et al. 2004) and Dhatchanamoorthy et al. (2021) studied exclusively grasses. Trainings have been imparted to the community on conservation and sustainable harvesting methods of medicinal plants which includes root harvesting of D. hamiltonii also (Kinhal & Jagannatha 2006; Kinhal et al. 2006; Jagannatha & Kinhal 2008). However, attempts have not been made so far to assess the species-specific population size, density, demography, threats/disturbances for the complete landscape of SRF. Hence, addressing this primary need of assessing and quantifying the population status will be helpful in formulating management plans, assessment of Red List status, sustenance and conservation of the population in the wild.

The major objective of this study was to understand the population status of *D. hamiltonii* with key research questions such as (a) what is the density and size class distribution of the *D. hamiltonii* population in Savandurga RF?, (b) what is the differences in *D. hamiltonii* densities and regeneration across different habitats?, (c) Does the density and regeneration vary across harvesting regimes?, (d) What are the environmental variables influencing the density and demography of *D. hamiltonii*? Therefore, in the current study we aim to understand sustainable use and suggest conservation strategies to save its population.

#### **MATERIALS AND METHODS**

#### Study area

The SRF is situated approximately 70 km west of Bengaluru and 12 km from Magadi town in Karnataka, India. The largest monolith in the area, the Savandurga hill, stands at 1226 m, and is part of the Deccan plateau. The hill comprises two peaks, the black hill (Karigudda) and the white hill (Biligudda), separated by a deep valley. The scrub, dry deciduous natural forest vegetation in the area is characterized by large bald rocky surfaces. The rocks include the Closepet granite series, peninsular gneiss, granites, basic dykes, and laterites. The forest type is classified as a shrub and tree savanna type comprising the Anogeissus latifolia - Chloroxylon swietenia - Albizia amara series (Champion & Seth 1968; Pascal & Ramesh 1996). Accepted name of Anogeissus latifolia is Terminalia anogeissiana Gere & Boatwr. as per POWO (2024). The forest between 750 m and 1,200 m has a sparser distribution of trees such as Wrightia tinctoria, Wrightia arborea, Holarrhena pubescens, Polyalthia cerasoides (Accepted name-Huberantha cerasoides) Albizia amara, Terminalia anogeissiana, and Pterocarpus marsupium. However, SRF is invaded by weeds such as Lantana camara, Chromolaena odorata, and Pterolobium hexapetalum, which affect the vegetation distribution. The forest's vegetation, combined with factors such as altitude, slope, sunlight, and edaphic factors, create a habitat for species growth, leading to three main vegetation categories: dense vegetation (closed canopy, no sunlight), rocky vegetation (open canopy, extreme sunlight), and mixed vegetation (sparse vegetation with sunlight penetration) at the boundary between the two types. The dense and mixed vegetation comprises loamy soil mixed with rocks and open rocky areas that receive sunlight have patches of soil deposited on the rocky slopes.

#### **Population sampling**

To assess the population size, distribution, and habitat characteristics of *D. hamiltonii*, sampling was conducted between April end to September 2019 in the SRF. Considering the financial constraints, accessibility issues, manpower constraints, and the wind dispersal of this climber species, fixed area plots were adopted as a sampling design. First, the forest boundary was digitized using Google Earth, and the entire area was divided into 1 × 1 km grids with QGIS. Only grids with over 50% forest coverage were selected for sampling, while those located in fringe forest areas were excluded from the analysis. Consequently, the study included 24



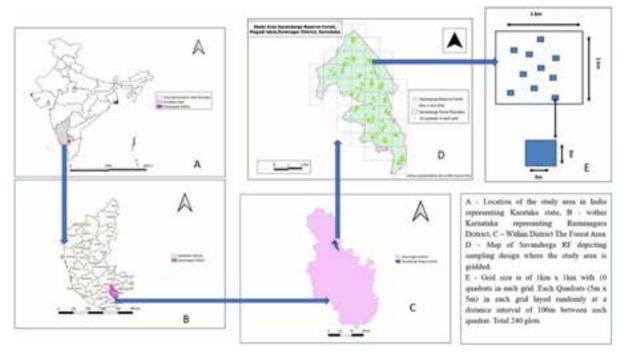


Figure 1. Study area and schematic representation of sampling quadrants of 5 × 5 m within 1 × 1 km grids (Total 24 grids).

Table 1. List of study sites selected for sampling *Decalepis hamiltonii* in Savandurga Reserve Forest.

No.	Grid	Site Code	No.	Grid	Site Code
1	610	ктк	13	G39	IXB
2	G16	ITM	14	640	DAD
3	G17	GKM	15	645	NK
4	G18	BKM	16	G46	KOH
5	G22	ITG	17	G47	DBO
6	G23	TTE	18	G53	HCG
7	624	SFH	19	G54	MST
8	625	MPCA	20	660	MR1
9	G31	AAS	21	G61	MR2
10	G32	DGK	22	G62	MR3
11	633	VBD	23	669	KPN
12	G38	VTK	24	676	KPS

grids measuring  $1 \times 1$  km, covering around 2,300 ha of forest area (Table 1). In each grid, 10 sample quadrants measuring  $5 \times 5$  m were established randomly (Figure 1). Each  $5 \times 5$  m quadrant was surveyed to identify and enumerate all stems of *D. hamiltonii*. The girth was measured at 1.3 m from the rooting point, referred to as the point of measurement (POM) (Gerwing et al. 2006). However, Gerwing et al. (2006) recommends a minimum diameter limit of 0.5 cm for density, species richness, regeneration, and succession studies to help accurate representation of stem size. Smaller diameter

classes can capture the dynamics of the community, including growth, mortality, and recruitment. If the seedlings were small and not yet established, they were measured using digital vernier callipers, otherwise, big ones were measured with a measuring tape. Each quadrant's reproductive status was recorded, and its habitat was evaluated and classified as dense, mixed, or rocky. Elevation, latitude, longitude was recorded for each plot by using the Garmin GPS (map 64) instrument.

Climber inventories generally captured evidence from a community perspective but with variations in research objectives, methods, plot sizes, and stem diameter. Studies enumerated stems ≥1 cm dbh (Proctor et al. 1983; Putz & Chai 1987; Chalmers & Turner 1994; Rice et al. 2004; Burnham 2004; Muthuramkumar et al. 2006; Reddy & Parthasarathy 2003, 2006; Campanello et al. 2007; Ding & Zang 2009) studies enumerating stems ≥1.6 cm dbh (Padaki & Parthasarathy 2000). However, it is important for identifying small-sized regeneration of climbers to know the consequences of forest dynamics (Gerwing et al. 2006), but such studies received little attention (Chazdon 2003). Some studies enumerating smaller stems to mention are ≥0.5 cm dbh studied by Dewalt et al. (2000), Yuan et al. (2009), and much lesser stem girth of ≥0.2 cm dbh by Mascaro et al. (2004), Cai et al. (2009), and Chettri et al. (2010). The above differences considered in the studies make comparison of data across the tropics difficult. In the present study,

we have considered all size classes beginning from 0.5 cm onwards to capture the population of all size classes. Though, studies by Gerwing (2004), Schnitzer et al. (2004), and Anbarashan & Parthasarathy (2013) show a positive correlation between disturbance and climber abundance, lower climber densities in more disturbed areas have been observed by Rice et al. (2004). No relationship between disturbance and climber abundance was observed by Mascaro et al. (2004). However, Hall & Bawa (1993), emphasize the importance that monitoring an annual species for which the entire individual is harvested requires a completely different sampling design from monitoring a large tree species from which only fallen fruits are harvested. Likewise, sampling designs may differ based on the life form, history, method, and plant part harvested. The sampling design chosen here is on a targeted woody climber species and the attempt here is to capture the type of disturbances, habitats, and the influencing factors that determine the population density. Our study is from the perspective of a single climber species population disturbed by root harvest, which is unique.

#### Description of stages of plants and size-class distribution

The stems were classified based on their girth size, with adults having a GBH ≥6 cm onwards, saplings having a GBH between 3 cm and 6 cm, and seedlings having a GBH >3 cm (0.5 to 3cm). The measurements for all *D. hamiltonii* stems followed the protocol of Schnitzer et al. (2006) and Gerwing et al. (2006).

**Seedlings:** Seedlings range in size from 0.5 cm to 3 cm, they stand stiff without support up to 30–40 cm in height (Image 1a). Leaves may wither due to deciduous nature, grazing, or other disturbances like small fires. 1 cm stem girth corresponds to at least 750 g of underground roots (Sathya Sangeetha: Unpublished Thesis). As stem girth increases from 2 to 3 cm, climber length increases by 1 to 1.5 m, falling on taller grasses, herbs, and nearby shrubs. Between 0.5 cm to 3 cm the un-established seedlings (height <1 m) and established seedlings (height >1 m) are included.

**Saplings:** Individuals with stem girths ranging from 3.1 to 6 cm experience growth in length but do not exhibit flowering (Image 1b). Increase in length is noted in the stem between the two nodes and are found to be greenish pink in colour. It was observed from the overall survey that the flowering and fruiting started above 5 or 6 cm onwards. At this stage, climbers establish support through multiple hosts as they grow typically, the plant twines onto from the nearby grasses, herbs, and shrubs ascending onto the nearest branch extension of the host

tree, choosing the shortest distance towards light. If no hosts are found, the stems lie on rock boulders.

Adults: Individuals with stem girths more than 6.1 cm are considered adults, as they flower and fruit (Image 1d). At this stage, the plant has 4–5 branches with flowers in terminal and axillary cymose inflorescences. Each branch has about 15–20 inflorescences and increases based on the stem size and length of the branch. After pollination, fruits develop, with each weighing around 40–50 g and measuring 20–35 g when dried. The number of branches, flowers, and fruits increases with stem size. The colour of the stem varies from greenish pink, to maroon and as they grow old the colour turns to dark brown and finally blackish grey with prominent lenticels (Image 1g & h).

Overall, the stem girth size was divided into 11 size classes with equal intervals of 3 cm ranging from 0.5 to 31 cm. The size classes are as follows: a) 0.5–3 cm; b) 3.01–6 cm; c) 6.01–9 cm; d) 9.01–12 cm; e) 12.01–15 cm; f) 15.01–18 cm; g) 18.01–21 cm; h) 21.01–24 cm; i) 24.01–27 cm; j) 27.01–30 cm; k) 30.01–33 cm (Figure 7; Image 1). The largest size class of one or two adult stems was observed in closed canopy areas, where they were found within the large trees in dense forests. These stems were identified by the presence of fruits hanging above the closed canopy. (Note–0.5 cm, 0.8 cm, 0.9 cm stems are placed within the 0.5–3 cm range).

Associated vegetation was also recorded from each plot. Trees, shrubs, climbers, herbs, and grasses were identified in the field, and the doubtful species were compared with the authentic specimens in FRLH herbarium and also the field images. The herbarium specimen was deposited in FRLH Herbarium (Voucher specimen number: 122072, Date: 25.05.2019, Location: Savandurga MPCA). All trees measuring >10 cm DBH and the stems of shrubs (ranging from 1 to 10 cm DBH) were counted, and the species were identified. Disturbances affecting the D. hamiltonii population in the SRF were recorded through field observations and each of the 24 grids was categorized based on the number of stems harvested, uprooted or had branches chopped, as well as the occurrence of fire, grazing (Image 2 a-g), presence of weeds and distance from each site to villages and roads (Appendix 1).

#### **Habitat variables**

To investigate the potential factors that may be influencing the distribution and abundance of *D. hamiltonii*, the study selected eight habitat variables (Table 2). Normalized Difference Vegetation Index (NDVI), elevation, slope, aspect, hill shade illumination,





Image 1. Sampling *Decalepis hamiltonii* population in Savandurga Reserve Forest. a—Seedling | b—Sapling | c—Measuring the stem girth of an adult plant | d—Adult climber supported on *Euphorbia antiquorum* | e&f—Laying sample plots and assigning plot numbers | g—Large adults—woody climber | h—Adult plant grown luxuriously on rocks and surrounding grasses, herbs, and shrubs. © M. Sathya Sangeetha.

associated species, and the distance from the forest boundaries, as well as two categorical variables: habitat types and harvesting regimes. The NDVI, elevation, slope, aspect, and hill shade illumination variables were obtained from satellite imagery, namely LANDSAT-8 (Scene ID LC81440512019104LGN00; acquired in 2019; http://glovis.usgs.gov/) and elevation data from ALOS (JAXA Global ALOS portal; scene ALPSMLC30-N014E077—

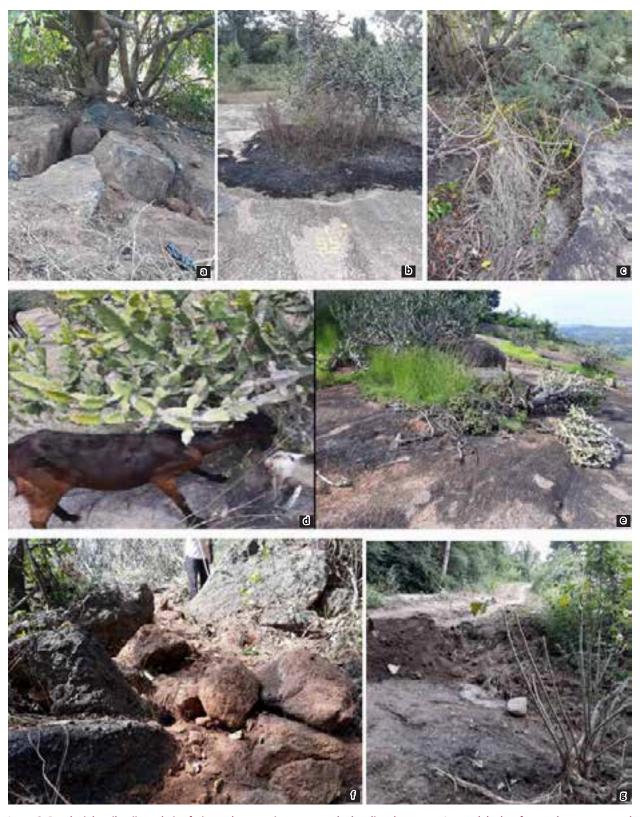


Image 2. Decalepis hamiltonii population facing anthropogenic pressure and other disturbances: a—Large adult plant from rocky area extracted for roots by breaking the rock slope into pieces | f—Large adult plant roots extracted from loamy soil by digging and twilting the rock boulders | b—Fire set only to the sample plot where D. hamiltonii sapling was killed (note the assigned plot no 55) | c—Fruiting branches pulled and chopped by herders for feeding goats | d—Goat eating the leaves of D. hamiltonii which was growing near the Euphorbia antiquorum plant | e—Supporting plant E. antiquorum branches chopped while root harvesting observed in the interior most site which is the peripheral edge of SRF | g—Small mud roads created inside the forest by clearing the vegetation. © M. Sathya Sangeetha.



Table 2. Summary of the eight environmental variables included for the regression analyses, apart from the two categorical variables (Habitat types and harvesting regimes).

Variable	Mean	Median	Range
Elevation (m)	844.7	847	754.0–960.0
Slope (Sexagecimal degree)	0.9246	10.0301	0.5072-30.1230
Eastness	-0.1384	-0.3698	-0.9997–0.9994
Northness	0.05566	0.04859	-0.99925-0.99992
Hill shade illumination	170.6	172	79.0–236.0
NDVI	0.14757	0.14377	0.07176-0.33512
Associated species	21.18	21	1.00-57.00
Distance to forest boundary (m)	563.7	530	70.0–1700.0

DSM) and Google Earth (http://earth.google.com). The NDVI values for vegetation quadrants were derived from the Landsat image with a 30 m resolution, using Band 4 (Red) and Band 5 (NIR). The NDVI was validated in the field. In the SRF, the NDVI ranged -0.1-0 for water bodies, 0.1-0.17 for bare soil and rock regions, 0.18-0.2 for sparse vegetation, and 0.2-0.4 for less dense vegetation up to the closed canopy. The topographical variables, including slope, elevation, aspect, and hill shade illumination, were extracted from the digital elevation model (DEM) raster with 30 m resolution. These variables represent the basic elements used when analyzing and visualizing ecological problems related to forest and wildlife habitat suitability site analyses (Al-Kindi et al. 2017). The aspect was transformed into a linear north-south gradient (Northness) and an eastwest gradient (Eastness) by performing cosine and sine transformations, respectively, to facilitate statistical analysis. Northness ranged from -1 (south-facing) to 1 (north-facing), while Eastness ranged from -1 (westfacing) to 1 (east-facing). The values were extracted using the ARCGIS 10.2, 3D analyst tool/raster surface option, and Q GIS 3.10.2 and ArcMap 10.2 were used for data extractions. Furthermore, surrogates of disturbance such as settlements, villages, and the distance from quadrants to villages and roads were also mapped. Distribution of plant species on a landscape depends on surface soil moisture balance, moisture holding capacity of the soil which is related to topographical variables like elevation, aspect, slope, hillside position, curvature (Franklin et al. 2000). Soil moisture is strongly correlated with the amount of radiation received on the Earth's surface and the sun's radiation is described often by aspect (Najafifar et al. 2019). Aspect plays an important role in evaporation, temperature change and solar radiation these conditions create micro niches for the plant's establishment and survival period.

#### **Environmental variables**

Atotal of 10 environmental variables were considered: NDVI, altitude, slope, northness, eastness, habitat types, harvesting intensities, hill shade illumination, associated species, and the distance from the forest boundaries (Table 2). Prior to regression analysis, collinearity among predictor variables was assessed using Pearson's crosscorrelations (Figure 2) and the variance inflation factor (VIF) (Neter et al. 1996). The VIF measures the increase in variance of an estimated regression coefficient due to collinearity. Whenever high collinearity was observed (Pearson's r  $\pm$  0.40 and VIF >10) (Neter et al. 1996), ecologically more relevant predictors were retained, while others were discarded. Therefore, the final set of explanatory variables consisted of nine variables, excluding habitat types, as they were highly correlated with the NDVI (Figure 3).

Although the quantile-quantile plots and Shapiro-Wilk test for normality indicated non-normal variance in all the response variables, the approach by Chapagain et al. (2019) was employed to address the large number of zeros in the datasets. Moreover, the best-fitting multiple regression model was determined based on the lowest value of the bias-corrected Akaike's Information Criterion for small samples (AICc; Burnham & Anderson 2002) among all potential regression models (equation X).

$$AICc = AIC + \frac{2k(k+1)}{n-k-1}$$
 equationX

where, AICc is the lowest value of the bias corrected AIC,

n is the sample size,

AIC is Akaike's Information Criterion, and is given as: AIC = 2k - 2ln(L)

where, k is the number of parameters in the statistical model.

L is the maximum value of the likelihood function for the estimated model.

The resultant best predicted variables were used to explain the variations observed in the *D. hamiltonii* distributions across SRF.

#### Data analysis

The data analysis focused on comparing the densities and relative densities of *D. hamiltonii* across three habitat types (rocky vegetation, mixed vegetation, dense



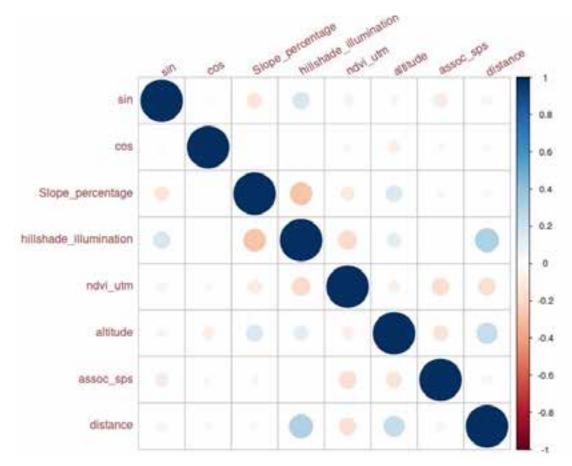


Figure 2. Correlation plots for environmental variables as part of the multidisciplinary test.

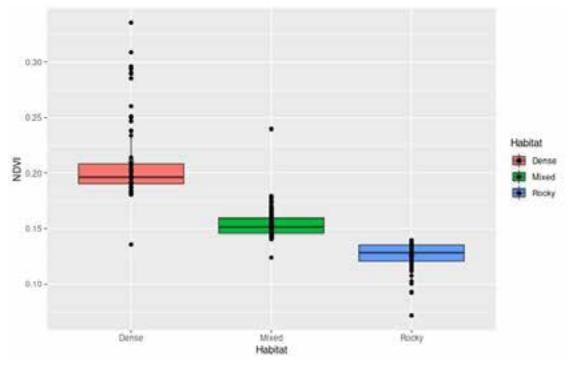


Figure 3. Box plots for comparing the mean NDVI values across the habitat classification in Savandurga Reserve Forest.



vegetation) and three different harvesting intensities (low, medium, high) to identify any variations due to these factors. Since density (D) and population size (N) are related, with  $N = D \times A$  area, we estimated the density for the sample and used it to compute the total population. We used the formula D(p) = N/A, where D(p) represents population density, N represents the number of stems (population size), and A represents the area. The quadrat and environmental data were analysed using R software version 3.6.3 (R Core Team 2020).

Next, we standardized the densities of D. hamiltonii adults, saplings, and seedlings per hectare for all sites in the SRF. We then compared the relative densities of D. hamiltonii across habitat types and harvesting intensities. We used the Wilcoxon test to calculate pairwise comparisons between group levels, with corrections for multiple testing. We also plotted population structure curves using the GBH-based size class distribution of D. hamiltonii stems. The smallest stems were 0.5 cm which was placed between 0.1 to 3 cm class. We formed 15 size classes ranging 0.1-45 cm with an equal interval of 3 cm and statistically compared the relative densities and size classes using non-parametric ANOVA (Kruskal-Wallis test). Whenever the Kruskal-Wallis test resulted in a significant difference, we performed pairwise Wilcoxon rank sum tests.

To compare the densities and relative densities, we derived four datasets from the *D. hamiltonii* database: (1) entire *D. hamiltonii* stems across SRF; (2) adults; (3) saplings; and (4) seedlings. We attempted a regression analysis to examine the impact of various environmental factors on the distribution and abundance of *D. hamiltonii* in SRF. We initially considered the eight habitat and two categorical variables as predictor variables, and after checking for collinearity among them, we used a final set of nine explanatory variables for regression, excluding habitat types due to high correlation with NDVI.

#### **RESULTS**

From the 240 sample plots, total 405 stems of the *D. hamiltonii* species were enumerated from 6000 m² (5 x 5 m = 25 m² x 240 plots = 6000 m²). Adults made up the largest portion of them (188 stems, or 46%), followed by seedlings (152 stems, or 37%), and saplings (65 stems, or 16%). Among the 24 grids density ranged from lowest  $0.1 \pm 0.32/25$  m² (Mean  $\pm$  S.D.) to the highest  $5.2 \pm 2.66/25$  m² (Mean  $\pm$  S.D.). The estimated densities of the *D. hamiltonii* per plot (25 m²) and for each grid were the highest in DBO, KOH, KPN, MPCA, and MST (Table

Table 3. Estimated densities per hectare and per sampled plot (25 m2) for *Decalepis hamiltonii*.

Grids	Density / 25 m² (Mean ± S.D)	Density / ha
AAS	1.3 ± 0.95	520
ВКМ	0.9 ± 1.20	360
DAD	2.4 ± 1.35	960
DBO	2.8 ± 2.30	1120
DGK	1.5 ± 0.71	600
GKM	1.4 ± 0.97	560
HCG	2 ± 2.98	800
ITE	0.8 ± 1.03	320
ITG	1.5 ± 1.65	600
ITM	1.6 ± 1.43	640
JKB	0.5 ± 0.85	200
кон	2.8 ± 2.39	1120
KPN	3.5 ± 4.62	1400
KPS	1.9 ± 1.97	760
ктк	1.1 ±2.13	440
MPCA	5.2 ± 2.66	2080
MR1	0.8 ± 1.75	320
MR2	0.8 ± 1.03	320
MR3	1.5 ± 1.65	600
MST	2.8 ± 2.86	1120
NK	1.9 ± 2.60	760
SFH	0.1 ± 0.32	40
VBD	0.4 ± 0.52	160
VTK	1 ± 0.67	400

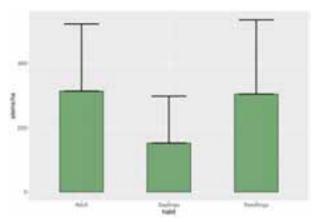


Figure 4. Distribution of the *D. hamiltonii* adult, sapling, and seedling densities per hectare across the Savandurga Reserve Forest.

3), while the grids SFH and JKB had the least densities. The density of the adults was highest (Mean  $\pm$  S.D.: 313  $\pm$  210 stems/ha, N = 24), followed by the density of the



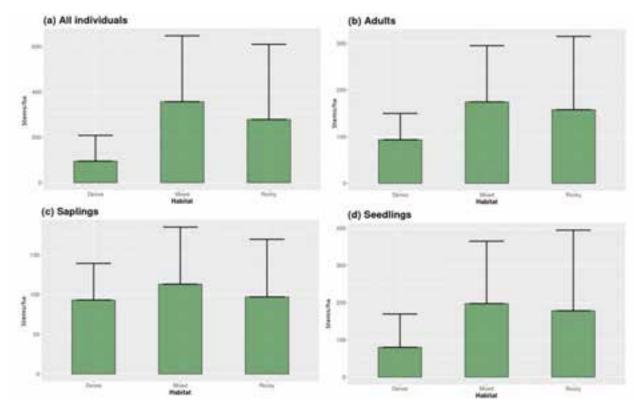


Figure 5. Comparison of the Mean  $\pm$  S.D. in the *D. hamiltonii* stems from the sites across the three habitat types (dense vegetation, mixed vegetation, rocky outcrops) for: (a)—all the stems | (b)—adults | (c)—saplings | (d)—seedlings.

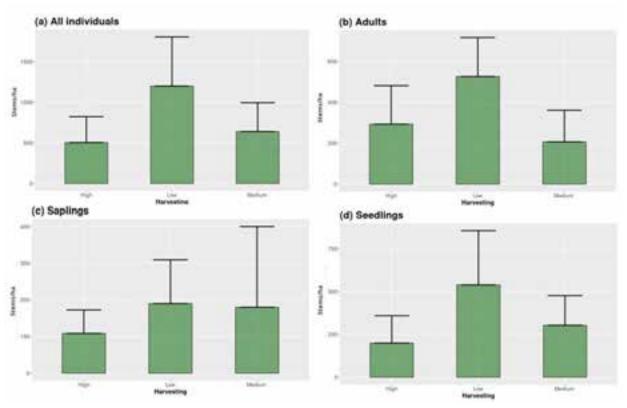


Figure 6. Comparison of the Mean ± S.D in the *D. hamiltonii* stems from the sites across the harvesting regimes (low, medium, high) for: (a)—all the stems | (b)—adults | (c)—saplings | (d)—seedlings.

6

Table 4. Nonparametric ANOVA results for the comparison of adults, saplings, and seedlings of *Decalepis hamiltonii* compared among sites under different harvesting regimes (high, medium, and low).

		Harvesting intensity				
Response	High	Medium	Low	Kruskal-Wallis	P value	
variable	Mean ± S.D. (N) stems/250 m <sup>2</sup>	Mean ± S.D. (N) stems/250 m <sup>2</sup>	Mean ± S.D. (N) stems/250 m <sup>2</sup>	ANOVA		
Adults	293 ± 188 (15)	206 ± 154 (6)	470 ± 247 (4)	χ2(2,23) = 3.291	0.19	
Saplings	108 ± 64 (7)	180 ± 220 (6)	190 ± 119 (4)	χ2(2,15) = 1.003	0.6	
Seedlings	200 ± 160 (12)	304 ± 77 (5)	540 ± 317 (4)	χ2(2,19) = 5.880	0.06	

Table 5. Multiple linear regression models based on the AIC model selection for (a) total stems, (b) adults, (c) saplings, and (d) seedlings.

Response variable	Predictors	parameter estimate	т	P- value	AIC values	
	Harvest_high	0.822	1.924	0.056		
A.II	Harvest_medium	1.264	2.803	<0.01	720.075	
All stems	Harvest_less	2.585	5.649	<0.0001	720.075	
	Associated species	0.038	2.344	<0.05		
	Harvest_high	1.529	13.686	<0.0001	225.13	
Adults	Harvest_medium	1.193	8.327	<0.0001		
	Harvest_less	1.682	13.503	<0.0001		
C. II.	NDVI	3.257	1.161	0.2503		
Saplings	Altitude	0.001	2.609	<0.05	241.227	
Seedlings	Northness	-0.655	-2.006	<0.05	384.043	

seedlings (Mean  $\pm$  S.D. :304  $\pm$  232 stems/ha; N = 20) and density of the saplings (Mean  $\pm$  S.D.: 153  $\pm$  144 stems/ha, N = 17). Overall mean density (Mean  $\pm$  S.D.) observed was 675  $\pm$  455 stems/ha in SRF.

When the total number of D. hamiltonii individuals at each site was compared, the sites that are subjected to high harvesting had, on average, the fewest individuals (less than ten), with the exception of sites AAS (13 individuals), GKM (14 individuals), KTK (11 individuals), and VTK (10 individuals). The areas that had medium levels of harvesting had a comparatively greater number of plants to begin with (10-20 individuals per site). And the locations that had the fewest plants harvested had more than 20 individuals per site; the site that was protected by the MPCA had the largest number of plants (52 individuals). When compared to the mature plants in the less harvested sites, the medium and highly harvested sites had a much lower number of individuals, with most having fewer than 10 individuals, while the less harvested sites had more than 10 individuals.

When the densities of adults, saplings, and seedlings were compared across different sites in SRF, significant differences were found (Figure 4; Kruskal-Wallis ANOVA:  $\chi^2_{(2,21)} = 14.09$ ; P <0.001). According to the results of the

pair wise comparison tests, the sapling densities were substantially lower compared to those of the adults (P <0.001), as well as those of the seedlings (P <0.05). On the other hand, there was no significant difference in the densities of the adults and the seedlings (P = 0.2032).

From the Figure 5a, the densities of the *D. hamiltonii* were significantly less in the dense vegetation (Mean±S.D.: 95 ± 113, N = 16) as compared to the mixed vegetation (Mean±S.D.: 356 ± 290, N = 24) and the rocky outcrops (Mean±S.D.: 278 ± 331, N = 22). This was confirmed using Kruskal-Wallis ANOVA ( $\chi^2_{(2,59)}$  = 13.728; P <0.01). Furthermore, the pair wise comparison tests revealed that the abundance in the dense vegetation were significantly lower compared to that in the mixed vegetation (P <0.01) and the rocky outcrops (P <0.05). However, there was no differences in the densities of the *D. hamiltonii* in the mixed vegetation and the rocky outcrops (P = 0.22).

When the adult populations were compared across the different types of habitats (Figure 5b), the densities were relatively higher in the mixed vegetation (Mean  $\pm$  S.D.: 174  $\pm$  120, N = 22) and the rocky outcrops (Mean  $\pm$  S.D.: 158  $\pm$  157, N = 18) than they were in the dense vegetation (Mean  $\pm$  S.D.: 93  $\pm$  56, N = 9). However, the

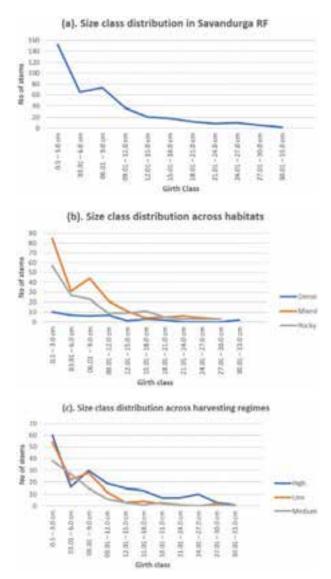


Figure 7. Comparison of the size class distributions of the  $\it D. hamiltonii$  stems for: (a)—entire SRF | (b)—across habitat types | (c)—across harvesting regimes.

statistical differences between the populations were not significant (Kruskal-Wallis ANOVA:  $\chi^2_{(2,46)} = 3.282$ ; P = 0.19). The densities of the saplings were comparable across all three habitat types (Figure 5c; dense vegetation: Mean±S.D.:  $93 \pm 46$ , N = 3; mixed vegetation: Mean ± S.D.:  $113 \pm 72$ , N = 12; and rocky outcrops: Mean±S.D.:  $96 \pm 68$ , N = 9, and the differences between the three habitat types were not statistically significant (Kruskal-Wallis ANOVA:  $\chi^2_{(2,21)} = 0.032$ ; P = 0.85). In a manner analogous to that of the saplings, the densities of the seedlings did not vary across the three different habitat types (Figure 5d; dense vegetation: Mean ± S.D.:  $80 \pm 89$ , N = 5; mixed vegetation: Mean ± S.D.:  $177 \pm 167$ , N = 17; and rocky outcrops: Mean±S.D.:  $178 \pm 216$ , N =

13, and the differences were not statistically significant (Kruskal-Wallis ANOVA:  $\chi^2_{(2,32)} = 2.544$ ; P = 0.28).

According to Figure 6a, the densities of D. hamiltonii were lower in the sites that were highly harvested (Mean±S.D.: 5483±342, N=15) and moderately harvested (Mean  $\pm$  S.D. : 640  $\pm$  554, N = 6) as compared to the sites that had less harvesting which had densities of (Mean  $\pm$  S.D. : 1280  $\pm$  355, N = 4) The Kruskal-Wallis analysis of variance ( $\chi^2_{(2,22)}$  = 8.04; P <0.05) provided evidence in support of this hypothesis. According to the results of the pair wise comparison tests, the mean densities of D. hamiltonii were substantially lower in heavily harvested sites in contrast to those in less harvested areas (P <0.01). However, there was no difference in the mean densities of D. hamiltonii between highly harvested sites and moderately harvested sites (P = 0.32), nor was there a difference between moderately harvested sites and less harvested sites (P = 0.06). When the densities of adults, saplings, and seedlings were examined across the three harvesting regimes, similar patterns were found; however, the Kruskal-Wallis ANOVA revealed that there were no statistically significant differences (Table 4).

#### Size class distributions

The size class distribution for the D. hamiltonii exhibited close to a typical inverted "J" curve (Figure 7a); however, it can be inferred that the size class distributions for the entire population of D. hamiltonii in SRF is unstable due to the lowest densities of the saplings (size class: 3.01-6.0 cm). In a similar manner, when the size class distributions were analyzed across the various habitat types (Figure 7b), it was discovered that the populations are unstable as a result of the decreased abundance of the saplings. Compared to the thick vegetation and the mixed vegetation the rocky outcrops had a greater recruitment into the populations than the dense vegetation. The Kruskal-Walli's analysis of variance revealed very significant differences between the three habitat types ( $\chi^2_{(14,42)}$  = 34.576; P <0.01). Furthermore, when the size class distributions were examined among the different harvesting regimes (Figure 7c), it was observed that the populations are unstable due to lower abundance of the saplings. However, the sites subjected to high harvesting pressure had higher recruitment into the populations than compared to that in the sites with moderate and less harvesting pressure. Statistical analysis of size classes reflected high significant differences between the three harvesting regimes (Kruskal-Wallis ANOVA:  $\chi^2_{(14,42)}$  = 36.741; P < 0.001). On the contrary to our expectations, the sites with higher harvesting pressure had a greater



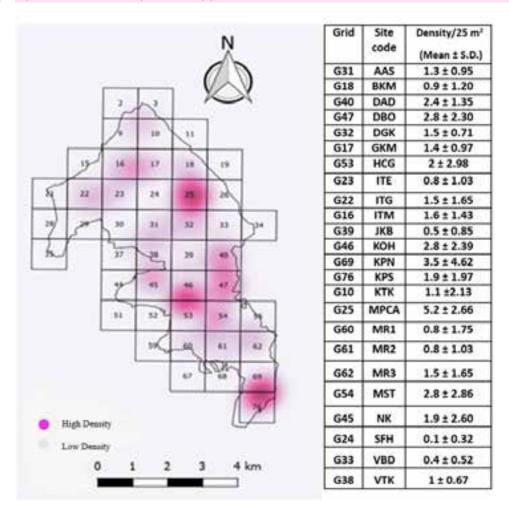


Figure 8. D. hamiltonii population density in SRF for 24 grids with IDs.

number of adults with girth ranging 6-33 cm (Figure 7c).

# Influencing variables determining the adult, sapling and seedling distribution

The regression analysis based on AIC model selection indicated that the adult D. hamiltonii distribution in the sampled sites of the SRF was determined by harvesting alone with 78% variation explained ( $F_{(3.120)} = 146.3$ ; P <0.001). Contrary to expectations, the coefficients for the adult plant abundance were more in high and less sites than in moderately harvested sites (Table 5). The sapling distributions in the SRF were mostly determined by the NDVI (2.09% variation explained), a surrogate for habitat type, along with altitude (18.72% variation explained) that explained a total variation of 56.58%. Furthermore, the NDVI and altitude positively correlated with the sapling abundance (Table 5). The seedling distributions in the SRF was determined by the transformed aspect variable-cosine or the northing. The cosine or the northing variable negatively correlated with the seedling abundance suggesting that the densities increased as one travels from north to south in SRF. However, the directional variable explained a mere 4.44% variation observed in the seedling distribution (Table 5).

#### **DISCUSSION**

This study serves as the first baseline to assess *D. hamiltonii* population density and size class across habitats and disturbance levels for the entire landscape of SRF, Karnataka, India. The focus on a specific climber species is also fairly unique since most studies of have focused on community-level analysis (Gerwing 2004). Climbers in life forms and root-harvested plants are rarely attempted for research from the perspective of harvesting and its impact on the population is less known (Ticktin 2004; Stanley 2012). Totally, 405 stems were enumerated from the sampled area of 6,000 m² and the density ranged from lowest (Mean  $\pm$  S.D.: 0.1  $\pm$  0.32/25 m²) to the highest mean density of (Mean  $\pm$  S.D.:

 $5.2 \pm 2.66/25 \text{ m}^2$ ) across 24 grids. The overall estimated mean density/ha of *D. hamiltonii* in SRF is (Mean  $\pm$  S.D.: 675  $\pm$  455 stems/ha) and details for each grid/site is provided in Table 3.

Though the adults are represented in all grids we found that in SFH except for one adult individual none of the other classes was spared as it was a highly disturbed area. Sites such as DGK have the maximum number of adult plants, while others BKM and VBD, are inaccessible peripheral portions of the MPCA having less density. Though, all grids have some kind of disturbances categorized into low, medium, and high levels based on signs of being chopped, uprooted, hanging dead stems, uncovered dug pits, and drying of collected roots, some of these peripheral grids are part of the MPCA which is interior and inaccessible but requires protection for recouping of the population naturally. Seedlings were missing in four grids, which could eventually be the result of harvesting adults, leading to a reduction in fruits or in seed quality which is not germinating well. The second reason is goat herders cutting / pulling the branches with fruits to feed the goat with leaves. This is a matter of concern as it causes great loss by wasting the reproductive effort and material of the plant. Usually, the goat feeds only on the leaves there is no threat to the survival of the plant as the roots are intact.

One would expect that areas of high adult density would also have high sapling and seedling densities since most of the adult plants' have wind-dispersed seeds which place the seeds in open rocky, and dense areas indiscriminately. Grids facing harvest compared with no harvest helps in distinguishing between the varying levels of harvesting intensities and the effects of harvesting on population dynamics (Hall & Bawa 1993). The density map depicts most of the peripheral grids being disturbed and exhibiting unequal patchy density either over-representation or under-representation of size classes and this could have been a result of harvesting largely or due to other kind of disturbances happening over a period of years now affecting the central, interior portions of the forest. Similar patches of disjuncture alternatively, representing areas where adults or seedlings have been preferentially harvested, leaving an overabundance and an under abundance of one size class was observed in Mondia whitei harvested for roots in Kakamega forest (McGeoch 2004). It is evident from the study that the highest number of 52 stems were found from the sample plots laid in the MPCA (Grid no 25), with a (Mean  $\pm$  S.D.: 5.2  $\pm$  2.66/25 m<sup>2</sup>). It is worth mentioning that this area had all size classes spread naturally which are not captured in our plots and such

population was not observed in any other grids. This is an indication of management effort and a restricted gated area or 'hands off' or 'no harvest area' where the central portion of MPCA is not disturbed, unlike the peripheral grids. The central portion of the MPCA is the only grid with a healthy population representing all size classes. Strict monitoring and regulations are required to support the natural regeneration of the population to support the in situ conservation area as well as throughout the landscape.

Size class distribution exhibits close to a typical inverted "J" curve with lower densities of the saplings (class: 3.01-6.0 cm) compared to seedlings and adults. The abundance of seedlings is an indicator of good regeneration but the low density of the saplings stage poses a future bottleneck (Bitariho & Emmanuel 2019). Reasons for the low density of the sapling class could be due to low recruitment or mortality of the individuals or both. Savandurga RF is a site where sustainable harvesting methods have been trained by the experts and implemented by the community for harvesting D. hamiltonii individuals and this could be one of the reasons contributing to the presence of a greater number of adult class (Kinhal & Jagannatha 2006; Kinhal et al. 2006). Unscientific, premature, unregulated root harvesting or uprooting of size classes like saplings do have good yield but may not be able to withstand the intensity of harvest and survive like the adults but rather lead to mortality or delay in their growth progress to reproduction (Image 1b&e). Reduction in sapling class is considered a forewarning as it indicates an unstable population generally observed in tropical forests and reasons could be many factors. One prominent factor to mention is fire which is almost an annual occurrence could also kill the seedlings and saplings (Sukumar et al. 2005). Studies by Parthasarathy (2000), Parren (2003), and Rai et al. (2016) also showed the inverse "J" pattern generally observed in climber communities. Bitariho & Emmanuel (2019) have studied harvested climber populations and found a similar inverted "J" curve pattern in five species whereas other climbers represented different patterns in population structure depending on the intensity of harvest. Similar to our findings in D. hamiltonii observations made in perennials like trees for ex Phyllanthus emblica population had higher mortality of the sapling size class, as well as Boswellia serrata (Ganesan & Setty 2004; Soumya et al. 2019a). These outcomes show similarity in the methods of representing the size class and also representing the negative consequences of the harvested perennials leading to poor representation of size classes, in a dry



tropical ecosystem. Contrary to our finding's density of saplings in C. fenestratum was positively associated with the adults and exhibited a healthy population but, during the survey they did not find size class above 3.5 cm (Thriveni et al. 2015). In case of climbers like Mondia whitei people harvest roots before fruit development and such premature harvesting disturbs the growth of the plant and because of this it is very hard to find adult fruiting plants (McGeoch 2004). As a result of intense stem harvesting in both areas of very disturbed and less disturbed sites there was a prevalence of small diameter sizes in the Loeseneriella apocynoides which is typical of a population with many resprouts but very few mature stem (Ndangalasi 2006; Hall and Bawa 1993; Bitariho et al. 2006). The studies mentioned above shows the intensity of harvest on the climber population in M. whitei (roots), L. apocynoides (stem) and C. fenestratum (stem & roots) where only small diameter classes were present but with the absence of adult size classes and very few fruiting individuals. These studies mentioned above and our results, invariable of life form indicate that a species-specific scientific approach unfolds more insights about the population and size classes. Hall & Bawa (1993) emphasize that the method of representing size class distribution is useful to identify the poorly represented size classes and it may differ based on habitats or due to various level of harvesting intensities. Considering the effect of harvesting on different life forms (herbs, shrubs, climbers, trees) the life history (annual, biennial and perennial) or plant parts being harvested (leaf, flower fruits, bark, resin and root) the negative consequence of harvesting is expressed in the process at different life stages. Invariable of the life forms, the plant parts are stored carbon resources for the plants to continue the life cycle, and removal of those parts hampers their growth process.

Harvesting history of the forest is of consideration (Ticktin 2004) because SRF is an area where sustainable harvesting methods for root harvesting of *D. hamiltonii* has been imparted to the community (Kinhal & Jagannatha 2006; Kinhal et al. 2006; Jagannatha & Kinhal 2008). These practices may ensure and support survival of the adult plants after harvest but to what extent the methods are still followed?, how frequently each stem is harvested?, how healthy and productive are the harvested plants?, do the adult plants have their roots intact or lost due to harvest?, are points necessarily to be investigated. Saplings were not found in seven grids and found less in other grids compared to adults and seedlings. This scenario is indicating that the root harvesting is not restricted to only mature adults

(>6.1 cm onwards) but being collected from smaller size classes also. This type of harvests may not allow the harvested stems to reach reproductive maturity and eventually recruit (Hall & Bawa 1993). The quantity of root available, for e.g., in a six-month-old plant, i.e., a seedling has minimum 500 g of root below ground. A sapling size class (3-6 cm stem girth) will have, not less than 2-3 kg of roots (minimum). Increased demand and pressure on the collectors, less equipped or unskilled harvesters, plants grown in favorable soil conditions which could be easily harvested are possible options for collecting roots from smaller size class. From the perspective of root harvest and its impact on the growth, survival, duration required to reproduce flower, fruits and potential seeds again are topics for further investigations. Unless monitored, managed and regulated spatially it is difficult to save premature harvest and selective harvest of adults.

Less number of stems is found in the dense vegetation, abundant in mixed vegetation and rocky outcrops. More in areas with higher disturbance which is a characteristic feature of climbers. Density of adults are determined by harvesting alone (78% variation), saplings by NDVI (2.09% variation) a surrogate for habitat type correlating along with altitude (18.72% variation). Seedlings were determined by directional variable (aspect) - 4.44%. (densities increased as one travels from south to north) adult stems had the highest density, followed by seedlings and saplings. The sites with high levels of harvesting had the fewest stems on average, while the protected site had the largest number of plants. The impact of harvesting on the population densities of D. hamiltonii was found to be significant, with heavily harvested sites having significantly lower densities compared to less harvested areas.

The densities of *D. hamiltonii* were significantly lower in areas with dense vegetation compared to areas with mixed vegetation and rocky outcrops. Interestingly the dense vegetation had less density but D. hamiltonii stems had attained higher diameter like single stems with gbh ≥28 cm to 30 cm. Reason is that some portion of forest fragments have not faced disturbance for a longer period of time and secondly in such dense vegetation these stems are not visible to the human eyes, so in such places the plants have attained higher stem diameters. Likewise, we found stems in undisturbed rocky areas also of gbh ≥20 cm single stems fallen on the rock without host. Similar results have been observed and confirmed in undisturbed, unaffected forests for long periods and also in old growth forests depicting dbh ≥30 cm in climber community studies (Dewalt et al. 2000;

Anbarashan & Parthasarthy 2013).

The destructive root harvesting of Swertia chirayita is another example of a habitat-specific species facing similar threats (Pradhan & Badola 2015). Studies have shown that the quality of the microhabitat significantly affects the availability of the Swertia chirayita population, with more stems observed in open habitats and on open grassy slopes, possibly due to lower interspecific competition for sunlight, and fewer stems observed in wetland habitats. Houttuynia cordata is restricted to specialized moist habitats in Assam (Bhattacharyya & Sarma 2010). Many medicinal plants which are root harvested are habitat-specific for example Picrorhiza kurroa (Kutki) grows only in moist rocks and steep slopes at an altitude of 3,500 m (Chandra et al. 2020). Nardostachys grandiflora (Accepted name - Nardostachys jatamansi) prefers rocky steep areas on the southeast slopes and in alpine grasslands, it is found on southwest facing slopes (Ghimire et al. 2005). In the case of D. hamiltonii, it is a wind-dispersed species that can colonize all three habitats in the SRF, but its adaptability to microsite habitat determines its survival as an adult.

Plant density, recruitment and survival rates are found to be more in less harvested areas than high harvest area with decreased densities (Ghimire et al. 2005; Bhattacharyya & Sarma 2010; Chandra et al. 2020). It was observed in *Coscinium fenestratum* population that they survive and regenerate naturally in disturbed habitats compared to undisturbed forest (Kathriarachchi 2004). The larger adults of *D. hamiltonii* were found in dense vegetation and seedlings within the canopy gaps and all classes were found in open areas, rocky outcrops and mixed vegetation in our study. Similarly, *Mondia whitei* also germinates well specifically in disturbed areas and forest gaps, also they are able to survive in all extreme conditions of Kakamega forests (McGeoch et al. 2008).

The mixed vegetation in SRF features an open canopy that allows sunlight to penetrate through exposed rocky areas and gaps in the foliage. *D. hamiltonii* is commonly found in this habitat alongside other plant species such as bamboo and *Sterculia urens*. The species also shows regeneration in the dense vegetation of the forest, particularly in canopy gaps and rock gaps where sunlight can reach. Climbing plants like *D. hamiltonii* often grow on shorter trees such as *Wrightia* spp. (in fact all size classes) and also large trees like *Albizia* spp. Additionally, *Euphorbia antiquorum* which thrives in barren rock fissures and depressions, provides a suitable habitat for *D. hamiltonii for regenerating seeds* by offering

protection from grazing and support up to a large adult stage. Rocky outcrops serve as favorable habitats for *D. hamiltonii* as they act as water filters, improving soil moisture. Moreover, the hair-like appendages connected to the seeds aid in wind dispersion, allowing the species to propagate and renew populations in rocky patches.

Apart from cattle grazing, D. hamiltonii leaves are extensively browsed by goats of course no damage to the roots. Shepherds also chop down the large climbers with flowers and fruits to feed the goats which is a loss to the population. Similar cases could be found in Hydrastis canadensis population, though overharvesting lead to severe decline, browsing of white-tailed deer also was an additional cause (Mulligan & Gorchov 2004). D. hamiltonii density is primarily impacted by destructive root harvesting practices. Flat and lowlying regions face higher levels of anthropogenic pressure compared to higher elevations, which results in a decrease in pressure as elevation increases. The reduced recruitment and density are likely due to the prevalent harvesting strategy and the chopping of the climbers that flower and fruit, used for feeding goats. This decrease in recruitment and higher mortality of seedlings and saplings will make it difficult to replace the existing adult population in the future. Although there is a higher number of adult population every other plant has been harvested in previous years, and the adults are preferred by the harvesters due to their potential for producing the highest yield.

The results of this study have clear management implications, primary focus is to mitigate destructive extraction pressure and save the remaining population in SRF by enforcing laws to control harvesting for the entire landscape. The management strategy should prioritize the protection of early life stages (seedlings to saplings stage) to avoid premature harvest and regulate selective harvesting of the adult stems. Goat herders, pulling the fruiting branches to feed the goat should be restricted. It would be worthwhile to protect the adjacent grids surrounding the MPCA also which harbor good density. The area within the MPCA needs protection to support natural regeneration and sustenance of the gene pool. Most of the peripheral grids are disturbed and needs proper planning to restore the species in open and disturbed areas provided protection is ensured (Figure 4. Density Map).

The entire landscape is worth protection as it is one of the best habitats for conserving *D. hamiltonii* population due to the natural design of the topography and supporting associated species. It provides open areas, gap vegetation and rock crevices, slopes, aspect,

hillocks which create innumerable micro niches. These micro niches ensure seedling establishment and forms vegetation patches with host species which can provide shelter from seedling to adult stage. Studies deepening the role of these variables will be useful for future studies. These micro niches make this habitat a refuge site for the population to establish, recover and sustain in spite of disturbances.

Our results underscore the necessity of long-term studies to monitor the population at different stages and develop appropriate management plans (Nakazono et al. 2004). The effect of harvesting on different size classes and their response to survival, growth, yield and reproduction necessitates investigation to tease apart the effects and enlighten future research on climbers as well as root harvesting species. All plant species are threatened by changes in temperature, rainfall, disruption of associated species, pests, pathogens, anthropogenic influence, habitat fragmentation, destructive harvesting, which could be the causes to push the populations to extinction. World scientists warning to humanity highlights the fact that medicinal plant species are often harvested unsustainably, when combined with the above pressures the stress level increases and the responses can result in decline in biomass, changes in chemical content affecting the quality and safety of medicinal plants. (Applequist et al. 2020). D. hamiltonii is holding a cocktail of medicinal properties and expected to be a potential source for more discoveries to unfold in the future, and so it is of high importance which needs to be conserved.

Harvesting *D. hamiltonii* roots can be one of the reasons which disturbs the host as well as the surrounding associated species because these activities enable gaps and create scope for growth of *Lantana camara* and *Hyptis suaveolens* (Accepted name: *Mesophaerum suaveolens*) bushes accommodating the area. The post effect of disturbance after roots of *D. hamiltonii* is harvested needs investigation.

Though rocky areas encourage regeneration, long and thick roots, in our study the big size stems were found to be growing very well in undisturbed dense forest and also in loamy soil. This information needs to be shared with the stakeholders to grow/cultivate and reduce the pressure from the wild. It is recommended that future studies focus on identifying similar suitable areas to grow, reintroduce, restore *D. hamiltonii* by using technological tools like Ecological niche modelling. Secondly, monitor the effect of harvest on different size classes.

Encouragement of cultivation locally as well as at

large-scale can be provided for the farmers through loans or under the NMPB subsidy schemes. Connecting the product to market will ensure livelihoods and reduce pressure from the wild (Homma et al. 1992). Periodic training on sustainability for harvesters, certification of the raw material and programs to monitor the population involving all the stake holders is essential.

Consequences of root harvested plants are difficult to be traced because the loss is below ground and scope of enquiry is invisible and difficult to gauge the population unless monitored periodically. The results from SRF may serve as an example to encourage more such studies across the distribution range, trigger research on root harvested climber species and compare the evidences across the sites. Such efforts would help in formulation of management plans, assessment of its Red List status and help in sustenance of the remaining endemic population.

The presence of many native species like *Wrightia tinctoria*, *Albiza amara*, *Psydrax dicoccos*, and *Euphorbia antiquorum* are potential hosts which supports the growth of *D. hamiltonii* at all stages in the forest. *Wrightia tinctoria* is present in all size classes supporting *D. hamiltonii* abundance both naturally as well as through management efforts. These plants can be used for supporting the climbers while planting or domesticating. Conservation organisations and citizen groups alike must foster a collective responsibility towards preserving this habitat and the species by engaging communities and raising awareness about it. Preserving the habitat is not just a matter of ecological conservation but also a means of securing a sustainable future for both the environment and the communities.

Confidence and capacity building among local people (Rist et al. 2016), citizen science reporting of harvesting/disturbances, will help the Forest Department to save the population. Field demonstration plots on how *D. hamiltonii* can be grown with interpretation units for surrounding villages, schools and panchayats needs to be encouraged. This effort will be useful to disseminate the value of the resource, habitat and engage community.

#### **CONCLUSION**

The high and increasing demand for roots, place undue pressures on the wild population and dynamics of *D. hamiltonii*. The management strategy should prioritize the protection of early life stages (seedlings to saplings stage) to avoid premature harvest, equally regulate selective harvesting of the adult stems and

mitigate grazing by strict monitoring and periodic assessment of the population. The impact of root harvest on the reproduction and yield needs to be investigated and monitored on a long-term basis. Intensifying the protection for the MPCA towards the peripheral grids to help natural regeneration and restoring of the population outside the MPCA is highly recommended. Encouragement of local plus large-scale cultivation is inevitable and local citizen science awareness and reporting mechanism of destructive root harvesting will help the forest department to take action and save the remaining population in the wild. It is submitted that the results of this study are based on one site (Savandurga RF) only, hence comparative studies carried out on a regional scale that include both social and ecological analyses will allow to gain better insight into the current population status of *D. hamiltonii*. More collection from the wild and missed conservation actions from the stakeholders at this juncture will lead to depletion of the population in this landscape.

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#### Appendix 1. Disturbance scoring and conservation implications of Savandurga Reserve Forest (24 sites/grids) with site description.

Grid	Site	Fire	Inva- sive plants	Collection of fruits, green, etc.	Grazing, chopping	Herbi- vory	Transport- frequency	Path- ways	People Footfalls	Adven- ture sports	Distance from villages	Drying of roots	Roots removal	Distur- bance score	Dist. code
G10	КТК	1	3	0	0	0	0	1	0	0	1	1	4	11	High
G17	GKM	1	2	0	0	0	0	1	1	0	1	1	4	11	High
G18	ВКМ	0	1	0	0	0	0	0	0	0	1	10	4	16	High
G23	ITE	0	1	1	1	1	0	1	1	0	3	0	2	11	High
G24	SFH	0	3	0	1	1	3	2	2	4	4	0	3	23	High
G31	AAS	1	2	1	3	1	3	2	2	4	4	0	3	26	High
G33	VBD	0	1	0	0	1	0	0	0	0	3	2	4	11	High
G38	VTK	0	3	1	1	1	3	2	2	4	4	0	4	25	High
G39	JKB	1	1	0	1	0	0	1	0	0	4	0	4	12	High
G60	MR1	1	3	1	1	0	1	1	0	0	4	0	4	16	High
G61	MR2	0	1	1	1	1	2	0	0	0	4	0	4	14	High
G25	MPCA	0	1	0	0	0	0	0	0	0	0	0	0	1	Less
G40	DAD	0	1	0	0	0	0	0	0	0	4	0	0	5	Less
G46	КОН	0	1	0	0	0	0	0	0	0	2	0	2	5	Less
G47	DBO	0	1	0	1	0	0	1	0	0	2	0	0	5	Less
G53	HCG	0	1	0	0	0	1	0	0	0	3	0	0	5	Less
G54	MST	0	1	0	0	0	0	0	0	0	4	0	0	5	Less
G69	KPN	0	1	0	0	0	0	0	1	0	3	0	0	5	Less
G16	ITM	1	2	0	0	0	0	1	1	0	1	0	0	6	Medium
G22	ITG	1	1	1	1	0	0	1	0	0	1	0	1	7	Medium
G32	DGK	0	1	0	0	0	0	0	0	0	4	0	2	7	Medium
G45	NK	0	1	0	0	0	0	1	0	0	4	0	4	10	Medium
G62	MR3	0	1	0	0	1	2	0	0	0	3	0	0	7	Medium
G76	KPS	0	1	0	1	0	1	0	0	0	3	0	1	7	Medium

#### Criteria for disturbance level calculated for each grid/site level based on field observation.

Site disturbance scores were obtained by assessing all 24 grids/sites which include the following:

- 1. Resource removal, roots harvested by different methods— uprooted, chopped up to the stem, no of uprooted holes vary— absent— 0, partially harvested— 1, completely harvested, uprooted chopped (>5 ind.), 5— 10 stems dug in that area— 3, more than 10 stems harvested— 4
- 2. Accessibility at a Minimum Distance of nearby villages.— 500m— 4, 1000m— 3, 1500 m- 2, 2000 m- 1.
- 3. Pathway— Road absent— 0, Mud Road— 1, Tar Road— 2.
- 4. People footfalls— Absent- 0, interior small worship places visited rarely once in a year by very few families— 1, regular visit to main temples- 2. The main temples of SRF are visited frequently weekly (rare-frequently).
- 5. Adventure habits—rock climbing, nature park, trekking. Trekking, birding, boating camps on the other side of the hill i.e., near the Manchanabele waters.
- **6. Herbivory observed** Porcupines— 1, Wild boar— 2.
- 7. Fire-- Low- level fires to clear the weeds.— observed fire-- yes-- 1, no-- 0.
- 8. Grazing and chopping of *D. hamiltonii* climbers— Absent— 0, cattle— 1, goats— 2.
- 9. Invasive species— 1— less, 2— medium, 3— high.
- 10. Drying roots— absent- 0, evidence of people staying to process the roots— 5, cooking also— 10, (high weightage has been given this point).
- 11. Collection of other NTFPs or medicinal plants— Bela (Feronia elephantum), Soppu (Greens), Genasu (Tubers), medicinal plants— yes— 1, no— 0.
- 12. Transport frequency— absent— 0, adventure cycles and two- wheelers- 4, weekend 2 wheelers, & four wheelers— 3, local autos and transport— 2, Govt vehicles— 1.

Overall Disturbance score of each site—Values ranging from 0—5 as Less Disturbed, 6—10 as Medium Disturbed, and above 10 onwards - Highly Disturbed.



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COMMUNICATION

#### Small Wild Cats Special Series

# Rare encounters: Jungle Cat *Felis chaus* Schreber, 1777 (Mammalia: Carnivora: Felidae) in the lower reaches of the Jordan River, Jordan

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**Abstract:** The Jungle Cat *Felis chaus* is classified as critically endangered in Jordan due to its limited distribution and presumed population decline. We present new evidence for its presence in the lower Jordan River region, where it was recorded opportunistically during a monitoring program focused on the Golden Jackal *Canis aureus*. Five photographs of the Jungle Cat were obtained between June 2020 and February 2022, primarily during night-time and early morning hours. In view of these findings, we recommend urgent implementation of conservation measures, including other effective area-based conservation measures, particularly in military-controlled zones with limited accessibility where confirmed sightings occurred.

**Keywords:** Bycatch, camera trap, citizen science, habitats suitability, human-induced threats, northern ghor, other effective conservation measures, private farms, riverbed, threatened species.

تم تصنيف قط الغابات في الأردن كنوع مهدد بالانقراض بشكل حرج نظراً لتوزيعه الجغرافي المحدود والانخفاض المحتمل في أعداد جموعه, نقدم في هذا البحث أدلة جديدة تثبت وجود هذا النوع في المناطق السفلية من نهر الأردن، حيث تم تصويره بشكل انتهازي خلال برنامج مراقبة لحيوانات ابن أوى. تم التقاط خمس صور لقط الغابات في ساعات الليل والصباح الباكر في الفترة الواقعة ما بين شهر حزيران للعام 2020 وشهر شباط من العام 2022. في ضوء هذه النتائج، نوصي بتطبيق تدابير عاجلة أخرى لحماية المناطق بفعالية، لا سيما تلك المناطق التي تخضع لإدارة القوات المسلحة الأردنية والتي توفر ملاذات للأنواع لمحدودية إمكانية الوصول إليها.

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Author details: MR. EHAB EID is a steering committee member in the IUCN SSC, vice chair for West Asia, and an editorial board member at IUCN, with over 22-years of experience in terrestrial and marine biodiversity conservation and protected areas management in the Middle East. MR. MOHAMMAD FARID ALAYYAN has worked with INGOs such as OXFAM and managed a family agriculture venture focused on citrus. His experience in this private enterprise at a migration stopover site has enriched his knowledge of species and conservation efforts.

Author contributions: E.E and M.F.A participated in research design and data collection. E.E. analyzed data and wrote the manuscript. E.E and M.F.A reviewed the article and gave final approval for publication.

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#### **INTRODUCTION**

Consensus across various red list assessments indicates a decline in the Jungle Cat *Felis chaus* population at national, regional, and global levels, in addition to the paucity of available information. It has been assessed as Least Concern on the IUCN Red List, and the global population is thought to be declining (Gray et al. 2016). However, regional assessments in West Asia vary. It is classified as Data Deficient in the Mediterranean Region and the Arabian Peninsula (Temple & Cuttelod 2009; Jdeidi et al. 2010; Mallon et al. 2023), but Critically Endangered in Jordan due to limited occupancy and presumed population decline (Eid et al. 2020).

The Jungle Cat is distributed across a wide range, from the Anatolian region in Turkey (Gerngross 2014; Ünal & Eryilmaz 2020) southward to the Nile River valley in Egypt (Basuony 2000). In the Levant, it occurs in southern Lebanon, Palestine and western Jordan (Qumsiyeh 1996; Amr 2000; Tohme & Tohme 2000). To the east of the Levant, its range extends to the Euphrates and Tigris Rivers in Syria and Iraq (Masseti 2009; Al-Zubaidi et al. 2017; Mallon et al. 2023), the Iranian Plateau and the Caucasus (Sanei et al. 2016; Askerov et al. 2022) to central, southern, and southeastern Asia (Gray et al. 2016).

The Jungle Cat is considered rare in Jordan, with a limited distribution mainly confined to the country's northwestern region (Eid et al. 2020). Information about it is scarce, possibly due to restricted access to the Jordan River, designated as a military zone with limited access for people (Abu Baker et al. 2003). The only known records are two deceased specimens found on 10 February 1998 in Al–Baqurah within the Yarmouk River Valley (Abu Baker et al. 2003; Eid et al. 2020). Since then, there have been no further records on its status or potential distribution in Jordan. Abu Baker et al. (2003) suggested that its range might extend to the lower regions of the Jordan River and its main tributaries.

Here we report photographic evidence of the continued presence of the Jungle Cat in Jordan obtained during a monitoring survey targeting the Golden Jackal *Canis aureus* in northwestern Jordan.

#### Study area

Our survey was conducted in the lower reaches of the Jordan River between the Sea of Galilee and the Dead Sea in Jordan (Ibrahim et al. 1976; Katz 2022). The study area encompassed a 9.9 ha private farm in Sheikh Hussein, northern Ghor (Figure 1), cultivating citrus varieties using irrigation, fertilization, and

herbicide control. The farm boundaries extend to the Jordan River, and the farm's landscape features riparian vegetation dominated by Common Reed *Phragmites communis*, Cattail *Typha domingensis*, and Athel Tree *Tamarix aphylla*. Additionally, various shrubs and herbs thrive, including Sieber's Wormwood *Artemisia sieberi*, Christ's Thorn Jujube *Ziziphus spina-christi*, Arabian Fagonia *Fagonia arabica*, and Common Mallow *Malva sylvestris*. The farm serves as a sanctuary for migratory birds such as ducks, herons, egrets and storks. Despite this, the dense reed may pose a fire hazard, prompting farmers to actively manage it through removal, controlled burning or herbicide application to maintain a sustainable farming environment.

#### **MATERIALS AND METHODS**

We deployed four Dark Ops HD MAX Browning camera traps that were set to a trigger speed of 0.6 seconds with a trigger delay of one second. The cameras were active for 24 hours at the same locations during the entire study period and were checked monthly. They were fastened to iron sticks anchored in the riverbed and positioned approximately 40–50 cm above ground in both south and north directions to avoid false records during sunrise and sunset. No bait was used to ensure neutral data collection. Their locations were determined using a Garmin eTrex 20x device set to datum WGS84.

#### **RESULTS**

Our total survey effort covered 2,548 camera trap days at four locations from 1 June 2020 to 28 February 2022. We obtained five photographs of solitary Jungle Cats at all four camera trap locations. Four records occurred during late-night or early-morning hours and one in the afternoon. In 2021, the Jungle Cat was photographed on 12 January at 1258 h, 17 January at 2133 h, 11 April at 2135 h, and 3 September at 2241 h. The only record in 2022 occurred on 30 January at 0212 h (Image 1).

During the survey, several other species were recorded, including the Golden Jackal *Canis aureus*, Egyptian Mongoose *Herpestes ichneumon*, Wild Boar *Sus scrofa*, Red Fox *Vulpes vulpes*, various rodent and numerous bird species.

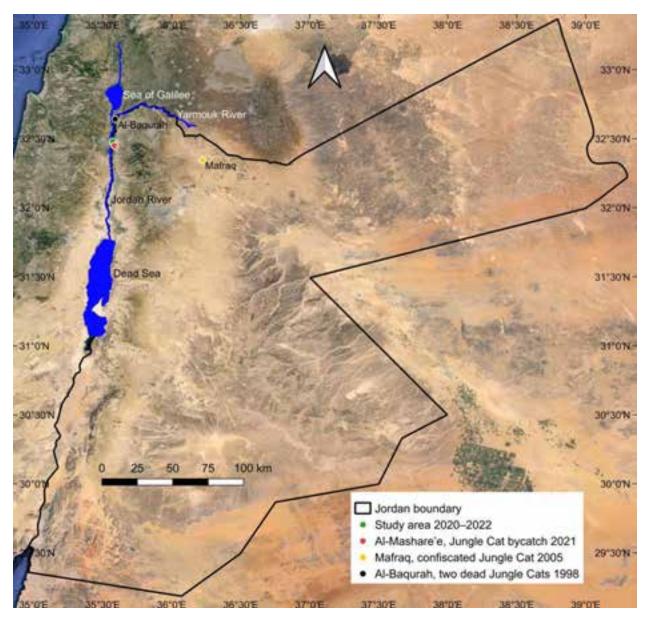


Figure 1. Map showing study area and locations where Jungle Cats were encountered between February 1998 and January 2022 in Jordan.

### **DISCUSSION**

Our survey is the first camera-trapping study in the Jordan River basin. Our results confirm the continued presence of the Jungle Cat in the lower reaches of the Jordan River nearly 22 years after two dead specimens were found near the Yarmouk River, a tributary of the Jordan River (Abu Baker et al. 2003). However, the limited number of photographs did not allow to determine the number of individuals in our study area.

We consider the agricultural setting of our study area and its surroundings to represent a suitable habitat for the Jungle Cat. Thick and dense riparian vegetation along riverbeds has been suggested to provide ideal hiding and movement spaces (Abu Baker et al. 2003; Masseti 2009; Sanei et al. 2016; Eid et al. 2020; Mishra et al. 2020; Desai et al. 2022). As documented by our survey, the area also hosts abundant rodents, which constitute the most important prey items of the Jungle Cat (Mukherjee et al. 2004; Majumder et al. 2011; Rostro-García et al. 2021).

The camera traps frequently documented four feral dogs *Canis familiaris* on the farm, which the owner kept after rescuing them. Despite more than 18 months of study duration, the Jungle Cat was recorded rather infrequently. The low detection rate can be attributed to the dense growth of reed plants, which significantly hindered visibility, affected the quality of photographs





Image 1. Jungle Cats photographed in the study area between 12 January 2021 and 30 January 2022. © Ehab Eid.

and resulted in numerous photographs of plants swaying in the wind. This situation required considerable effort to repeatedly remove reed growth. Active agricultural operations of workers, roaming feral dogs, Golden Jackals and other wildlife using the same habitat day and night added complexity to the recording environment. However, this challenging setting might also benefit the Jungle Cats, allowing them to manoeuvre quietly through dense vegetation (Abu Baker et al. 2003) and

avoid encounters with humans, feral dogs and other competitors. The low detection rate of the Jungle Cat can also be attributed to the exceedingly small population within our study area and its surroundings.

We identified several human-induced threats to the Jungle Cat population, consistent with findings by other scholars (Abu Baker et al. 2003; Eid et al. 2020, 2022). These threats include habitat alteration, fragmentation and degradation due to agricultural expansion,

the burning of wetland reeds, prey depletion from widespread pesticide use, and bycatch. An incident of bycatch involved the capture of an adult Jungle Cat in November 2021 in the Al-Mashare'e area, approximately 6 km south of our study area. The cat was caught in a net intended for chicken protection but escaped after being video-recorded. Further investigations using citizen science may reveal more instances of accidental or incidental hunting of the Jungle Cat, potentially challenging literature that suggests no hunting of this species occurs in Jordan (Abu Baker et al. 2003; Eid & Handal 2018). Elsewhere in its range, the Jungle Cat is threatened by the conversion of natural wetlands to agricultural lands, excessive destruction and burning of reeds, pollution due to extensive pesticide use, and poaching (Ogurlu et al. 2010; Gray et al. 2016; Sanei et al. 2016; Barkat et al. 2021).

Furthermore, the restricted niche where wild and domestic species coexist exacerbates the threat to the Jungle Cat. This was confirmed through species distribution models assessing suitable habitats for the Jungle Cat in Jordan, which revealed a significant decline in these habitats. Projections indicate a 33% reduction by 2050 and a staggering loss of 90% by 2070 under the Representative Concentration Pathways (RCP) 2.6 scenario. Even under the RCP 8.5 scenario, an 84% habitat loss is forecasted by 2070 (Eid et al. 2022). These alarming trends emphasize the need for conservation efforts to protect the Jungle Cat from extinction. It was not documented in Jordan's trade or folk medicine (Eid et al. 2011; Aloufi & Eid 2016). A single individual, claimed to be brought from Syria, was confiscated at a private farm in Mafraq city in eastern Jordan in 2005 (Ehab Eid, unpublished data).

The designation of Yarmouk protected area in 2010 in the Yarmouk River valley and the military control of border areas may be beneficial for the conservation of the Jungle Cat, in view of historical records of dead specimens in this area (Abu Baker et al. 2003). No sightings have been recorded within the protected area to date (Sufian Al-Yahya, pers. comm. 3 February 2024). This absence of sightings may be due to restricted site access or limited survey efforts. Therefore, targeted research and collaboration with the military are essential to determine the Jungle Cat's status.

Despite the Jungle Cat being listed in Appendix I of the Wildlife Protection of the Agricultural Law Number 13 of 2015, based on Regulation Number 43 of 2008, enforcing these legal instruments in Jordan is ineffective. Mitigating threats and preventing species extinction requires a thorough review and development of the legal

framework associated with effective implementation.

The Jungle Cat's presence within this limited access protected zone underscores the critical need for targeted conservation measures. The Jordanian government, particularly the Ministry of Environment and conservation organizations such as the Royal Society for the Conservation of Nature, should explore establishing Other Effective Area-Based Conservation Measures (OECMs). Farhadinia et al. (2022) recommended this approach to meet post-2020 biodiversity targets in Asia, noting that achieving the ambitious 2030 goal requires a substantial increase in protected area coverage. Currently, the riverbeds of the Yarmouk and Jordan Rivers are under army protection, presenting a valuable opportunity for species and habitat conservation and implementing OECMs, especially in view of the area's limited accessibility. Specifically, implementing OECMs through strategic partnerships with military units along the Yarmouk and Jordan rivers is essential to safeguard the Jungle Cat population and prevent its potential extinction in Jordan.

The results of this study are highly significant as they pave the way for further research into the status of the Jungle Cat across the entire Jordan River basin and its tributaries, extending northward to include the Yarmouk River. Establishing effective communication and collaboration with military units will facilitate and support the implementation of surveys. Regional cooperation with the West Bank in Palestine and southeastern Syria adjacent to the Yarmouk River valley is crucial, as it will likely yield important insights into the Jungle Cat's status and habitat use. Understanding the regional context will aid conservation efforts, particularly during species Red Listing and considering the rescue effect. These findings highlight the need for a coordinated approach to wildlife management and conservation, enhancing our understanding and enabling more effective protection measures for the Jungle Cat.

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OMMUNICATION

# Diversity of bird species in Ebpanan Marsh, Maguindanao del Norte, Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), Philippines

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Abstract: Wetland areas such as the marsh provide vital habitats for birds. However, marshes in the Philippines have been threatened by anthropogenic disturbances and may be further degraded. This study conducted a rapid bird assessment in Ebpanan Marsh located in Maguidanao del Norte within the Bangsamoro Autonomous Region in Muslim Mindanao (BARMM) on 06–12 March 2022. A total of 36 bird species belonging to 24 families were recorded during the rapid assessment. Among the bird species recorded, two species— Anas luzonica and Streptopelia dusumieri are considered as Vulnerable, while Padda oryzivora is considered 'Endangered' in the IUCN Red List of Threatened Species. Eight of the bird species recorded are endemic and are observed in the remaining freshwater swamp forest of the Ebpanan Marsh. Despite habitat degradation and anthropogenic disturbance, the Ebpanan Marsh can still accommodate threatened and endemic bird species. Increasing the number of observation sites and extending the sampling duration of bird assessment will be required in order to complete the list of birds on the Marsh and understand the spatial and temporal variation in bird populations in the area.

**Keywords:** Anthropogenic disturbance, conservation assessment, endemic birds, freshwater swamp forest, habitat degradation, rapid assessment, species richness, threatened bird species, wetland conservation.

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**Author contributions:** GRAL—paper conceptualization, data collection, writing and editing the manuscript. BATP—data collection and writing the manuscript. PJDDV—research design, paper conceptualization, data collection, writing and editing the manuscript, and corresponding journal submission.

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#### **INTRODUCTION**

A wetland is an area of land that is either covered by water or saturated with water (PAWB-DENR 2013). These areas are varied and include lakes, rivers, marshes, swamps, and reservoirs (Lopez-Calderon & Riosmena-Rodriguez 2016). The Philippines has about 216 lakes, 421 principal rivers, and 22 marshes, swamps, and reservoirs (Scott 1989). Several studies have been conducted highlighting the importance of wetland areas in the country in providing refuges for birds (van Weerd & van der Ploeg 2004; Villamor 2006; Lador & Seronay 2020). These ecosystems provide critical habitats for a wide range of bird species. However, wetland areas in the country, such as marshes, are considered to be one of the threatened ecosystems as the result of changes in land use, pollution, and climate change (Sespeñe et al. 2016). Marsh, a wetland ecosystem mostly dominated by grasses (Keddy 2010), provides a habitat for a rich diversity of flora and fauna, especially birds (Raj et al. 2010; Mohan et al. 2023).

Currently, in the Mindanao Island of the Philippines, there are around 455 species of birds documented of which 49 are globally threatened and 39 are endemic (Avibase 2023). The diversity of bird species has been noted to be a good bio-indicator of ecosystems (Datta 2011; Roshnath & Shruthi 2015), especially in wetland areas (Khadka et al. 2017; Studholme et al. 2022). However, in the Philippines' wetland areas, the diversity of birds is declining due mostly to anthropogenic pressures particularly the conversion of wetland areas for agricultural purposes (Haribon Foundation 2014).

Ebpanan marsh is a wetland area that stretches along different municipalities in Maguindanao del Norte and Maguindano del Sur provinces, and is part of the largest marsh in the country by area, the Ligawasan marsh. The Ligawasan marsh has been considered an Important Bird Area (IBA) and key biodiversity site and this warrants the need to for regular monitoring and updates of the bird composition in the area (Donald et al. 2018). This study provides a list of bird species recorded during the rapid bird assessment conducted in one of the conglomerates of the Ligawasan Marsh, the Ebpanan Marsh located within the Kabuntalan municipality, Maguindanao del Norte. Data from this study may contribute to the knowledge of the current list of birds in Ebpanan Marsh and is possibly the first published report of birds in the area. Government agencies, private stakeholders, and local communities may benefit from this study as this may serve as the basis for crafting conservation and protection policies for Ebpanan Marsh.

#### **METHODS**

#### Study area

The localized rapid bird assessment was conducted in the Ebpanan marsh (7.1451, 124.3391) that is located in Barangay Lower Taviran, Kabuntalan, Maguindanao del Norte, BARMM. The surveyed area for rapid bird assessment was approximately 64.23 hectares and is at least 5 km away from the national road. The marsh is one of three marshes, including the Ebpanan Marsh, Libungan Marsh, and Ligawasan Marsh proper, that collectively form the Ligawasan Marsh, one of the largest wetland areas in the Philippines. The southern to the western portion of the marsh is surrounded by concrete roads and residential areas, making the area easily accessible. The northern portion of the marsh is bordered by the Libungan Marsh while the lower eastern portion is bordered by the Ligawasan Marsh proper. Perennial agroforestry plants such as coconut Cocos nucifera, mango Mangifera indica and cashew Anacardium occidentale are common within the residential areas of the marsh. The study area also included marsh habitats, such as freshwater swamp forests and herbaceous swamp habitats. The presence of Terminalia catappa 'Talisay', Terminalia copelandii 'Lanipau', Antidesma ghaesembilla 'Binayuyo', and Pterocarpus indicus 'Narra' was common in the freshwater swamp forest while Water Hyacinth Eichhornia crassipes, Water Lettuce Pistia stratioides, and Duckweed Lemna pauciflora were present in the herbaceous swamp habitat. Most of the residents along the Ebpanan marsh depend heavily on its aquatic resources for food and as a source of income, evidenced by the presence of fish pens and nets. Furthermore, when the dry season arrives and floodwater recedes, certain swamp areas are utilized for cultivating watermelon Citrullus lanatus providing the residents with an alternative source of income.

#### **Bird Sampling Technique**

Localized rapid bird assessment was conducted between 06–12 March 2022 through the 'look and see', point count, and oppurtunistic listing methods in the Ebpanan marsh located in Barangay Lower Taviran, Kabuntalan municipality, Maguindanao del Norte. Field reconnaissance was conducted to explore the area and to establish the observation sites. Observation sites were established based on accessibility and safety. 10 strategic observation sites were established in the study area (5 in freshwater swamp forest, and 5 in herbaceous swamp habitat). Observation of birds was done from 0600–0900 h and 1500–1730 h by three field observers. Bird



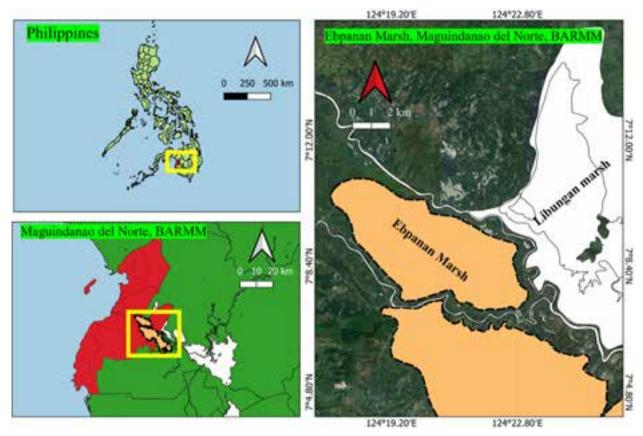


Figure 1. Map of Ebpanan Marsh, Maguindanao del Norte, Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), Philippines (QGISv.3). The map on the upper left shows the map of the Philippines. The map on the lower left is the subset of the yellow polygon in the Philippines map, which is the Ebpanan Marsh located in the province of Maguindanao del Norte in Bangsamoro Autonomous Region in Muslim Mindanao (BARMM). The map on the right is the is the subset of the yellow polygon from the map of Maguindanao del Norte, BARMM. The part of Ebpanan Marsh located in the Barangay Lower Taviran, Kabuntalan municipality (7.1451, 124.3391) was the study area.

documentation was carried out using field notebooks, binoculars, and a DSLR camera. Identification of birds and their respective spatial distribution and habitat range used different available reference materials such as 'Birds of the Philippines' (Allen 2020), eBird (2023), and Birdlife International (2023). The weather was generally sunny during the rapid bird assessment.

#### **RESULTS AND DISCUSSION**

Thirty-six species of birds belonging to 24 families were documented during the rapid bird assessment conducted in the Ebpanan Marsh (Table 1). Among the documented species, Ardeidae was the most represented family with seven species. The results of this study show a similar trend in terms of bird species richness in Agusan Marsh (Ardeidae = 13 species) in which species belonging to this family are also abundant. Bird species from this family prefer still open water habitats (Choi et al. 2007) which is common in the

marsh. Anecdotal reports mentioned that in some cases the residents of the Ebpanan Marsh hunt egrets for food. Although most of the species of the family Ardeidae are listed as of Least Concern, continuous hunting may threaten their population (Benitez-Lopez et al. 2017). On the other hand, 22% of the bird species documented in Ebpanan Marsh are classified as endemic. This number is low compared to Agusan Marsh (ASEAN Centre for Biodiversity 2023) thus increasing the number of bird surveys may significantly improve the list of birds in the marsh.

Among the 36 bird species recorded, two bird species—the Philippine duck *Anas Iuzonica* and the Philippine Collared Dove *Streptopelia dusumieri*—are considered 'Vulnerable' and *Padda oryzivora* as 'Endangered' as per the IUCN Red List of Threatened Species (Figure 2). The nearest sightings of *A. Iuzonica* from the Ebpanan Marsh are in Timaco Hill, Cotabato City (de Vera et al. 2023). Locals in the Ebpanan Marsh hunt and trap the *A. Iuzonica* along with the Common Moorhen *Gallinula chloropus* for food, thus further



Table 1. List of birds recorded at Ebpanan Marsh, Kabuntalan, Maguindanao del Norte, BARMM.

Family	Scientific name	Common name	Endemism*	Conservation status**
Accipitiridae	Elanus caerulus	Black-winged Kite	Resident	Least Concern
Accipitinade	Haliastur indus	Brahminy Kite	Resident	Least Concern
Acrocephalidae	Acrocephalus stentoreus	Clamorous Reed Warbler	Resident	Least Concern
Alcedinidae	Alcedo atthis	Common Kingfisher	Migrant	Least Concern
	Todiramphus chloris	Collared Kingfisher	Resident	Least Concern
Anatidae	Anas luzonica	Philippine Duck	Endemic	Vulnerable
	Ardea alba	Great White Egret	Resident	Least Concern
	Ardea intermedia	Intermediate Egret	Resident	Least Concern
	Ardea purpurea	Purple Heron	Resident	Least Concern
Ardeidae	Ardeola speciosa	Javan Pond Heron	Resident	Least Concern
	Bulbucus ibis	Cattle Egret	Resident	Least Concern
	Butorides striatus	Green-backed Heron	Resident	Least Concern
	Egretta garzetta	Little Egret	Resident	Least Concern
Artamidae	Artamus leucorynchus	White-breasted Woodswallow	Resident	Least Concern
Columbidae	Geopelia striata	Zebra Dove	Resident	Least Concern
	Streptopelia dusumieri	Philippine Collared Dove	Endemic	Vulnerable
- "	Centropus viridis	Philippine Coucal	Endemic	Least Concern
Cuculidae	Eudynamis scolopaceus	Asian Koel	Resident	Least Concern
Dicaeidae	Dicaeum austral	Red-keeled Flowerpecker	Endemic	Least Concern
Estrildidae	Lonchura atricapilla	Chestnut Munia	Resident	Least Concern
	Padda oryzivora	Java Sparrow	Introduced	Endangered
Laniidae	Lanius cristatus	Brown Shrike	Migrant	Least Concern
Laridae	Gelochelidon nilotica	Gull-billed Tern	Resident	Least Concern
Locustellidae	Megalurus palustris	Striated Grassbird	Resident	Least Concern
Nectariniidae	Cinnyris jugularis	Olive-backed Sunbird	Resident	Least Concern
Oriolidae	Oriolus chinensis	Black-naped Oriole	Resident	Least Concern
Passeriformes	Passer montanus	Eurasian Tree Sparrow	Resident	Least Concern
Psittacidae	Bolbopsittacus lunulatus	Guaiabero	Endemic	Least Concern
Psittaculidae	Loriculus philippensis	Philippine Hanging Parrot/ Colasisi	Endemic	Least Concern
Pycnonotidae	Hypsipetes phillipinus	Philippine Bulbul	Endemic	Least Concern
D-III-	Gallinula chloropus	Common Moorhen	Resident	Least Concern
Rallidae	Amauronis cinerea	White-browed Crake	Resident	Least Concern
Recurvirostridae	Himantopus himantopus	Black-winged Stilt	Migrant	Least Concern
Rhipiduridae	Rhipidura nigritorquis	Philippine Pied- Fantail	Endemic	Least Concern
Scolopacidae	Gallinago gallinago	Common Snipe	Migrant	Least Concern
Sturnidae	Aplonis panayensis	Asian Glossy Starling	Resident	Least Concern

<sup>\*—</sup>eBird Cornell Laboratory for Ornithology (2023) | \*\*—IUCN Red List of Threatened Species (2023).

making them vulnerable to population decline (BirdLife International 2023). The Philippine Collared Dove is classified as Vulnerable due to its rapid decrease in population in Luzon, Philippines mainly attributed to competition from the Red-collared Dove *Streptopelia* 

tranquebarica and Spotted Dove Streptopelia chinensis (BirdLife International 2023). Informing the locals of the status of *A. luzonica* and *S. dusumieri*, and establishing key protected areas, are actions that should prevent further population declines (Nori et al. 2020).

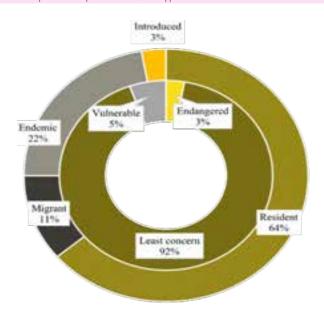


Figure 2. Endemism\* and conservation status\*\* of birds documented in Ebpanan Marsh, Maguindanao del Norte, BARMM (\*eBird 2023; \*\*International Union for Conservation of Nature Red List of Threatened Species 2022).

The introduced Java Sparrow Padda oryzivora was recorded during the rapid bird assessment in Ebpanan Marsh. A flock was recorded near the rice field within the study area. This bird species was considered to be a pest in rice crops. Eight out of the 36 recorded bird species in Ebpanan marsh were classified as endemic. Twentythree of the 36 species were classified as a resident, four bird species were migrants, and one bird species as introduced (Figure 2). All endemic birds recorded in this study were observed in freshwater swamp forests. Despite the presence of residential areas and the conversion of some areas to agricultural land, the marsh can still harbor endemic bird species. Unfortunately, during the conduct of rapid bird assessment in the area, one G. chloropus was trapped and captured, however, this was later freed by local inhabitants. They also mentioned that, based on their observations, there was a decline in the number of bird species and their frequency in the Ebpanan Marsh, and they attributed this to climate change and bird hunting. Aside from bird hunting, another threat that the birds in Ebpanan Marsh are facing is the conversion of freshwater swamp forests into agricultural land. Thus, conservation measures, such as hunting regulations and identifying protected areas within the marsh, are recommended (Dudley 2008).

#### MANAGEMENT RECOMMENDATIONS

This study recorded a total of 36 bird species from the Ebpanan Marsh. Despite the area being degraded, it still harbors important endangered, vulnerable, and endemic bird species and this underlines the importance of the marsh as an important habitat for birds. Immediate conservation measures should be developed and implemented to prevent further degradation, particularly in the remaining freshwater swamp forest, where all the endemic bird species were recorded. Raising the awareness of the locals regarding the conservation status of the documented birds in the Ebpanan Marsh is deemed necessary to conserve the presence and population of the endangered, vulnerable, and endemic bird species in the area. The data presented in this study provides an indication of the total number of bird species in the Ebpanan Marsh. Therefore, it is recommended that the number of observation sites be increased and the sampling duration extended to permit a complete list of birds to be obtained and more fully understand the spatial and temporal variation of birds in the area.

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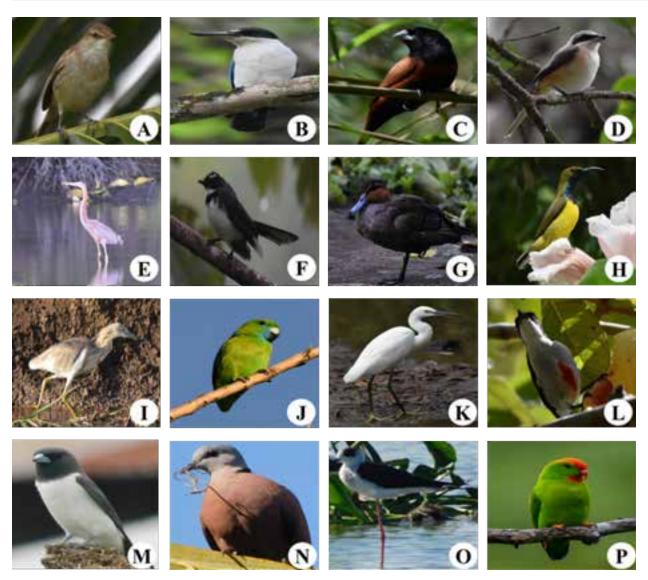


Image 1. Birds found in Ebpanan Marsh, in Ebpanan Marsh, Maguindanao del Norte, BARMM: A—Clamorous Reed Warbler Acrocephalus stentoreus | B—Collared Kingfisher Todiramphus chloris | C—Chestnut Munia Lonchura atricapilla | D—Brown Shrike Lanius cristatus | E—Purple Heron Ardea purpurea | F—Philippine Pied Fantail Rhipidura nigritorquis | G—Philippine Duck Anas Iuzonica | H—Olive-backed Sunbird Cinnyris jugularis | I—Javan Pond Heron Ardeola speciosa | J—Guaiabero Bolbopsittacus lunulatus | K—Little Egret Egretta garzetta | L—Redkeeled Flowerpecker Dicaeum australe | M—White-breasted Woodswallow Artamus leucorynchus | N—Philippine Collared-Dove Streptopelia dusumieri | O—Black-winged Stilt Himantopus himantopus | P—Philippine Hanging-Parrot/Colasisi Loriculus philippensis. © B.A. Pingoy & G.R. Limbaro.

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# Heleocoris stephanus (Heteroptera: Naucoridae: Laccocorinae), a new species of creeping water bug from Kallada River, Kerala, India

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**Abstract:** A new species of *Heleocoris*, Stål (1876) was collected from the upstream Kallada River in Kerala, southwestern India. This discovery brings the number of described species for this genus in India to twelve. This species can be diagnosed by the overall body coloration and distinct morphological & genital features. Descriptions including a habitus photograph, ecology, and diagnostic comparisons among congeners are provided.

Keywords: Coloration, congeners, genitalia, morphology, upstream, Urukunnu.

Malayalam: ഹെലിയോകോറിസ് ജനുസ്സിൽ പെട്ട ഒരു പുതിയ സ്പീഷീസിനെ ഇന്ത്യയുടെ തെക്കുപടിഞ്ഞാറൻ സംസ്ഥാനമായ കേരളത്തിലെ കല്ലട നദിയിൽ നിന്ന് കണ്ടെത്തി. ഈ കണ്ടെത്തലോടു കൂടി ഇന്ത്യയിൽ ഈ ജനുസ്സിലെ സ്പീഷീസുകളുടെ എണ്ണം പന്ത്രണ്ടായി. ശരീരത്തിലെ വ്യത്യസ്തരീതിയിലുള്ള ആകമാന നിറവും അടയാളങ്ങളും രൂപശാസ്ത്രപരവും ജനനേന്ദ്രിയ സംമ്പലമായ മറ്റു സവിശേഷതകളുമാണ് ഈ പുതിയ സ്പീഷീസിനെ മറ്റു സ്പീഷീസുകളിൽ നിന്നും വ്യത്യസ്തമാക്കുന്നത്. വിശദമായ വിവരണങ്ങളും അനുബന്ധ ചിത്രങ്ങളും താരതമ്യ പഠനങ്ങൾ ഉൾപ്പെടെയുള്ള വിവരണങ്ങളും ഈ പ്രസിദ്ധീകരണത്തിൽ നൽകിയിരിക്കാന്നാ.

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

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Heleocoris stephanus sp. nov. Benchamin et a

#### **INTRODUCTION**

The creeping water bugs are subaquatic, but some species are present in the water covering a rock surface (hygropetric). Most Indian species present adjacent to the streams. The genus Heleocoris Stål (1876) was first included in the division Laccocoraria, which later was raised to the subfamily Laccocorinae, Montandon (1897). Heleocoris contains 29 species, of which 11 are known from India (Polhemus & Polhemus 2013). The recent decade witnessed the discovery of a new species: Heleocoris mcphersoni, Sites and Vitheepradit, 2011 from Thailand. Globally, the Naucoridae consists of 401 species while the Indian sub-region consists of 20 species under seven genera (Chandra et al. 2017). There are few taxonomic records of Heleocoris in the southern part of India, and faunal surveys in waterfalls, streams of mountain ranges, and forests are still required. Heleocoris stephanus sp. nov. from Kollam District of Kerala state, is described as new to science in the present paper. This species was generally found associated with plant debris on the edge of the rivers. A diagnosis and comparison with congeners in Kerala, Tamil Nadu and other states congeners is presented..

#### MATERIALS AND METHODS

Bug samples were collected by hand-picking with either fingers or soft forceps and use of an aquatic net

to sweep through submerged vegetation and organic debris along shorelines of river Urukunnu, Kerala (Image 1). Specimens were placed into vials with 90% ethyl alcohol and brought to the laboratory of Zoology Research Centre, St. Stephen's College, Pathanapuram. Morphological terminology in the description mostly follows that of Xie & Liu (2015) and Sites & Vitheepradit (2011). All measurements are given in mm. Olympus CX33 and Weswox SZM-105 microscopes were used to obtain images and followed by its preparation with Photoshop CS5 (Adobe Systems Inc., San Jose, CA). The holotype (ZSI/WGRC/I.R.-INV.26976) was deposited in the Zoological Survey of India, Western Ghats Regional Centre (WGRC), Kozhikode, Kerala, India. Paratypes (SSCDZ/Hem01/2024 and SSCDZ/Hem02/2024) were deposited in the Department Museum, Zoology Research Centre, St. Stephen's College, Pathanapuram.

#### **RESULTS**

# Heleocoris stephanus sp. nov. (Image 2)

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Material examined: Holotype (ZSI/WGRC/I.R.-INV.26976): 07 October 2023, macropterous male, India: Urukunnu station, Kollam District, Kerala, 8.5905 °N & 77.0124 °E, 45 m, upstream Kallada River, coll. Dani & Arya, Paratypes (SSCDZ/Hem01/2024 and SSCDZ/



Image 1. Urukunnu Station in the upstream of Kallada River, Kollam District, Kerala, India, the type locality of Heleocoris stephanus sp. nov.

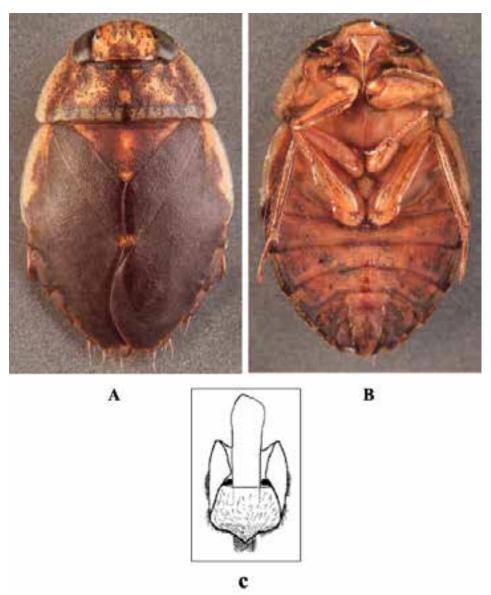


Image 2. Diagnostic features of *Heleocoris stephanus* sp. nov. (holotype): A—dorsal habitus | B—ventral habitus | C—phallotheca. © Dani Benchamin.

Hem02/2024): same data as primary type.

**Description:** Macropterous male. Holotype, length 9.78; width 6.20. Paratypes (n = 4), length 9.44–9.88 (mean = 9.69); maximum width 6.09–6.29 (mean = 6.17). General shape is ovate, slightly flattened; widest across embolia. Description of *Heleocoris stephanus* sp. nov. and other congeners are presented in Table 1.

Coloration: Overall body color is anteriorly yellowish with black mottling, scutellum dark brown with irregular yellow apex, yellowish, and thick median longitudinal patch. Head with black spotting, generally over the entire yellowish dorsal surface; patterns coalescent at the posterior margin, reducing anteriorly as black spots adjoining anterior margin, eyes blackish with

pale yellowish anterolateral margin, rostrum last two segments dark brownish. Pronotum with black markings scattered over the entire dorsal surface; laterally reduced to a few black spots; black coalescent patches in the mid-anterior region; a yellowish transverse band with irregular black blotches at the posterior margin. Hemelytra black. Forelegs and hindlegs are dorsally brownish and ventrally pastel yellowish. Connexiva yellowish-brown antero-posterior, showing checkered appearance (Image 2B). Ventrally yellowish cephalothorax, brownish abdomen, and pygophore (Image 2B).

**Head:** Densely punctured with black. Single sclerite at posterior corner of eye, directed ventrad, anterior

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end simple. Dome-shaped, blackish spot on the base of the head in between the eyes. Compound eyes blackish, convergent anteriorly, convex on lateral posterior margin. Broad semi-circular labrum, rostrum short with three visible segments, first segment partially covered with labrum, each following segment progressively narrower, reaching to near anterior prothoracic coxae. Antennae, thickened and hairy: not extending to lateral margin of head.

**Thorax:** Pronotum broad and maximum width at rounded posterolateral corners; tuft of setae in anterior end, transverse sulcus band ending at anterior end of clavus with ventral tuft of setae; ventro-lateral margins with blackish punctations; tuft of elongate sensory setae on anterior surface behind eye. Scutellum is finely granulose, tumescent, and elevated above level of wings; sinuated lateral margins. Well-developed hindwings. Hemelytron blackish-brown; largely yellowish embolium with black spots.

Legs: Foreleg with large femur, strongly widened antero-basally, lateral margins with thick bunch of setae; tibia narrow, with bunch of setae at lateral margins; tarsus bristled and two-segmented with two razor shaped claws. Hindleg with wide femur have small bulbous projections at its posterior end, tibia narrow with a profusion of swimming hairs; tarsus with small dorsal bristles and ventral elongated tuft of setae.

**Abdomen:** Abdomen ventrally covered with appressed hair; large blackish spots; ventrally with heavy marginal spines on laterotergites III–VI and elongate light-coloured hair.

**Genitalia:** Pygophore brownish and hairy, posterior margin acuminated with dense brush; parameres reduced, blackish, and symmetrical; phallosoma elongate, linear, widest & rounded triangular at apex (Image 2C).

Female: Unknown

**Diagnosis:** Heleocoris stephanus sp. nov. is similar to the congener H. vicinus (Montandon, 1910), by size, and overall coloration. However, H. stephanus is notably different from H. vicinus by the finely granulose, dark brown scutellum with irregular yellow apex and thick median longitudinal yellowish patch. H. majusculus (Montandon, 1908) and H. rotundatus (Montandon, 1908) are other congeners reported from the neighbouring state of Tamil Nadu. The former drastically differs from H. stephanus by its entirely black scutellum and overall body size, while the latter differs from H. stephanus by its black coloured body beneath with greyish pilose and body size. H. breviceps (Montandon, 1897) is another congener reported from the Indian

states such as Andhra Pradesh, Himachal Pradesh, Maharashtra, and Tamil Nadu. This congener differs from *H. stephanus* by largely blackish abdomen beneath and brownish scutellum. As the name implies, H. elongatus (Montandon, 1897) differs from H. stephanus by its body shape. Additionally, H. elongatus can be distinguished from H. stephanus by its entirely yellowish body beneath. H. elongatus was reported from the Indian states Bihar, Madhya Pradesh, Maharashtra, and West Bengal. H. acutus (Spinola, 1837) was reported only from Maharashtra and it can be distinguished from H. stephanus by its distinct acute posterior angles of pronotum. H. bengalensis (Montandon, 1910) was reported from the central states such as Madhya Pradesh, Uttar Pradesh, and West Bengal. H. stephanus can be distinguished from this congener by the distinct characteristics of genitalia such as brownish & hairy pygophore, posterior margin acuminated with dense brush, and blackish & reduced parameres. H. bergrothi (Montandon, 1897), reported from the southern Indian states of Karnataka, Kerala, and Tamil Nadu is similar to *H. stephanus* in a few characteristics such as body size, shape, and v-shaped discal spot in scutellum. H. stephanus differs from H. bergrothi by its distinct colouration of scutellum, such as, dark brown with irregular yellow apex, thick & yellowish median longitudinal patch, and distinct black mottling of head & pronotum. Lastly, the closest congener H. indicus (Montandon, 1897) shares a few similar features with H. stephanus like flavescent with brown punctures on head and pronotum, overall coloration, and v-shaped discal spot in scutellum. H. indicus has a number of contrasting characteristics such as entirely yellowish underbody, pronotum with blackish transverse colouration on the posterior margin, while the H. stephanus has a ventrally yellowish cephalothorax, brownish abdomen and pygophore, pronotum with irregular black blotches on the posterior transverse margin, embolium of the hemelytra yellowish with distinct blackish colouration, and abdomen with appressed hairs and large blackish spots, heavy marginal spines on laterotergites III-VI, and elongate light-colored hair.

**Etymology:** This specific epithet "stephanus" refers to the Institution where this significant research was conducted at the Zoology Research Centre, St. Stephen's College, Pathanapuram. St. Stephen's College was founded in 1964 after the name of St. Stephen, one of the first seven ordained Deacons and the first Martyr of Christianity.

Heleocoris stephanus sp. nov.

Table 1. Comparison among the congeners in the genus *Heleocoris* reported from Kerala, Tamil Nadu, and central states of India.

Taxon	Size	Shape	Head	Pronotum	Scutellum	<b>Hemelytro</b> n	Abdomen
H. bergrothi	Length 9.7; width 6.8 mm	Oval	Palely yellow with indistinct brown spots	Colouration same as head, the lateral margins arcuate, the lateral posterior angles acute, the surface densely and finely punctate	Black with yellow apex, very finely granulose, with a yellow v-shaped discal spot	Blackish-brown with largely yellowish embolium	Connexivum, abdomen, and legs entirely pale yellowish
H. vicinus	Length 9–10	Oval	Twice as broad at base between eyes, rounded in front, ochraceous with black speckles	Ochraceous with black speckles, the lateral margins pale ochraceous, distinctly subampliate, the posterior margin a little paler than the disk	Black with ochraceous apex	Black, lateral margin of corium pale ochraceous with two blackish macular suffusions	Connexivum pale ochraceous with the posterior margins black; abdomen and legs pale ochraceous, left sixth ventral laterotergite is completely flat
H. breviceps	Length 9.5; width 6.5 mm	Oval	Ochraceous; intraocular area marked on each side by two small foveations	Ochraceous	Blackish-brown with the disk narrowly yellow	Hemelytra brownish; embolium ochraceous	Abdomen beneath largely blackish with yellowish apex; legs entirely yellow
H. indicus	Length 8; width 5 mm	Oval	Brown punctures more or less dense forming small ill- defined shadings	Colouration same as head	Dark brown with a median longitudinal line.	Embolium yellowish with a medial brown spot on its inner margin and another spot at its apex	Connexivum yellow, very narrowly darkened on the outer edge of the posterior angles of each segment; underside of body entirely yellowish
H. majusculus	Length 13; width 8.8 mm	Oval	Surface superficially punctate, two impressions, one before the other on each side near the eyes	Very transverse, yellowish, finely granulose, punctured on all the surface, with the anterior margin obtusely sinuate on each side behind the eyes	Entirely black; claval commissure nearly as long as the scutellum	Embolium very much dilated behind	Connexivum yellow, with the posterior angles of the segments a little acuminate; underside of body pitchy brown
H. rotundatus	Length 12; width 9.4 mm	Oval	Thick, finely granulose, brownish-ochraceous, centrally blackish	Brownish- ochraceous much suffused with blackish except on the lateral margin areas which are distinctly rounded	Black with apex ochraceous, strongly and mostly transversely wrinkled; clavalsuture longer than scutellum and apically spotted with ochraceous	Black, thick, finely punctate, lateral margins of embolium ochraceous.	Connexivum ochraceous; under side of the body blackish; legs ochraceous; abdomen beneath with a central longitudinal series of black spots, the lateral margins ochraceous.
H. acutus		Oval		Shape is not perceptibly "flange" with no marginal furrow, the posterior angles acute			
H. elongatus	Length 8; width 4.5 mm	Oval	Clear yellow tint densely punctured with black	Colouration same as head; lateral margins very slightly arcuated nearly straight, the lateral posterior angles rounded, not prominent behind, the posterior edge straight	Brownish; claval suture a little longer than half the length of the scutellum	Embolium little enlarged and largely yellowish and along the outer margin, the yellow margin bisinuated at its inner edge by two brown spots	Connexivum yellow, with a straight transverse brown fascia on the posterior margins of the segments; underside of the body entirely yellow

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Taxon	Size	Shape	Head	Pronotum	Scutellum	Hemelytron	Abdomen
H. bengalensis	Length 8 mm	Oval	Posterior margin with brown punctures coalescent and dark punctuation and associated maculation denser	Dark punctuation and associated maculation denser; posterior margin with longitudinal marks		Large yellow patch on embolium.	Abdomen laterally slightly surpassing hemelytra in males; ventral laterotergites II–VII each light anteriorly, dark posteriorly
H. stephanus sp. nov.	Length 9.78; width 6.20 mm	Oval	Densely punctured with black. Single sclerite at posterior corner of eye, directed ventrad, anterior end simple. Goblet shaped black colouration in the dorsal surface	Finely granulose, broad and maximum width at rounded posterolateral corners; tuft of setae in anterior end, transverse sulcus band ending at anterior end of clavus with ventral tuft of setae	Finely granulose, tumescent and elevated above level of wings, sinuated lateral margins.	Blackish-brown, largely yellowish embolium with distinct black colouration	Abdomen ventrally covered with appressed hairs; heavy marginal spines on laterotergites III–VI and brush of elongate light- coloured hairs; legs brownish with setae and bristles.

#### **DISCUSSION**

This species was found in the upstream of Kallada River, Kerala, India. The type locality is located in the Western Ghats of the southwestern region of the country. The sampling area of the present investigation was characterized by partially decomposed plant parts. Studies on saucer bugs are scanty in our country, and the sampling area is an undisturbed and unexplored area of Kallada River. There were no detailed recent taxonomical reports on creeping water bugs from the freshwater ecosystems of Kerala. As part of our macroinvertebrate sampling, we explored several ignored parts of the upstream forested area of Kallada River with a rich diversity of creeping water bugs. No additional Heleocoris species were collected. Males of this species are most similar to H. bergrothi and H. indicus based on some external features like coloration pattern, though, these species are clearly distinct from H. stephanus in several aspects. Previously, 11 species were reported in India. The present discovery brings the total number of Heleocoris species in India to 12.

#### **CONCLUSION**

Heleocoris stephanus sp. nov. shows close morphological similarity with *H. bergrothi,* and *H. indicus*; The latter species are differentiated by distinctive morphological and genital features.

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## Incidence and risk factors associated with parasitic infections in captive wild mammals and birds in Indian zoos

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Abstract: Present study was conducted to record the seasonal incidence and worm burden (eggs per gram of faeces) of helminthic infections and to evaluate the efficacy of deworming protocols followed for control of parasitic infections in captive animals (including birds) at the zoo. Freshly voided faecal samples were collected during winter, monsoon and summer from 150 captive animals including wild mammals (n = 95) and birds (n = 55) between 1–15 years of age kept at Rajiv Gandhi Zoo and Wildlife Research Centre Karaj, Pune (Zoo-I) and Nisargakavi Bahinabai Chaudhary Zoo, Pimpri Chinwad (Zoo-II) in Maharashtra, India. Samples were processed and examined by standard sedimentation and floatation methods to assess the prevalence of helminth infections. Faecal samples of positive animals were collected pre and post-treatment, and the efficacy of the drugs used was evaluated based on faecal egg count reduction test (FECRT). The overall seasonal prevalence of gastro-intestinal parasitic infection in mammals varied among seasons with the highest prevalence (29.50 %) in monsoon followed by winter (26.30 %) and lowest in summer (8.40 %), while the same was found non-significant in birds. Of 19 bird species screened, 25 % of peafowl were positive for Ascardia spp., 25 % of crested eagles for Capillaria spp., and 50% of brown fish owls for Strongyloides spp. Among mammals, 75 % of Black Buck, 50 % of Leopards and 25 % of Giant Malabar Squirrels were positive for Strongyloides spp., while all four Bonnet Macaques were positive for Balantidium coli. The range of eggs per gram (EPG) of faeces recorded was 50-300 in mammals and 100-350 in birds. At Zoo I (Rajiv Gandhi Zoo and Wildlife Research Centre Karaj, Pune), there was 85.89 and 77.36 per cent reduction in egg counts after treatment with fenbendazole @5 mg/kg in herbivores and birds, respectively. While in carnivoresthe reduction was 69.93 % after treatment with a drug combination@10 mg/kg (Praziquantel 50 mg + Pyrantelembonate 144 mg + fenbantel 150 mg). In Zoo II (Nisargakavi Bahinabai Chaudhary Zoo, Pimpri Chinwad) the reduction in EPG was 72.35 % in carnivores with drug combination @10 mg/kg (Praziquantel 50mg + pyrantelembonate 144 mg + fenbantel 150 mg) and 68.98% in birds with albendazole @10 mg/kg.

**Keywords:** FECRT, helminthic infections, prevalence, preventive management, worm.

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#### **INTRODUCTION**

India is the World's 8th most bio-diverse region with a 0.46 BioD score on the diversity index, including 1,02,718 species of fauna (ZSI 2021). The nation is unique in having immense natural beauty, rich, and diverse wildlife comprising mixed species of different types of animals. Many countries including India in different parts of the world have adopted the strategy to protect wild animals via the use of parks and the construction of zoological gardens (Parasani et al. 2001). The zoological gardens display wild animals for aesthetic, recreational, educational, and conservation purposes (Varadharajan & Pythal 1999). Wild mammals and birds act as reservoirs and amplifiers of emerging human and domestic livestock pathogens (including parasites) of public health significance which has gained considerable attention in recent years (Moudgil et al. 2015).

The mortality in animals in captivity has been reported to be at a higher rate due to a variety of factors, including various bacterial, viral, fungal, and parasitic infections (Rao & Acharjyo 1984). Knowledge on parasitic diseases of wildlife is still in infancy in India, and data is on the baseline to understand the epidemiology of parasitic diseases in wild fauna kept in Indian zoos (Singh et al. 2009). Only a few researchers have carried out basic work on the prevalence of parasites in captive wild animals in India (Singh et al. 2006; Gupta et al. 2011; Jaiswal et al. 2014; Mir et al. 2016) except for recent comprehensive studies on animals (Moudgil et al. 2020a,b) from Punjab state.

The parasitic control and prevention programs for wildlifemainly depend on different factors like financial resources, public health structures, reduction of parasitic load, action on the animal reservoirs & vectors, improved diagnostic tools, environmental & ecological changes, human behaviours, education of the people that are involved in the wildlife, and domestic animal chain (Chomel 2008).

Though we can achieve better animal health in zoological gardens by quarantining newly inducted animals, improving hygiene practices and enforcing policy for not allowing visitors to feed animals (Singh et al. 2006), however, the most important part is the implementation of strategic prevention and control programs for prevalent parasitic species in wild animals based on parasitological analysis. The present study was planned to record the seasonal incidence of helminthic infections, assess the worm burden based on estimation of the eggs per gram (EPG) of faeces, and evaluate the efficacy of regular scheduled anthelmintic treatment

administered in zoo mammals and birds.

#### **MATERIALS AND METHODS**

#### Area of the study

The study was conducted in two zoos located at Pune, Maharashtra, India (18.5204 °N & 73.8567 °E), the first zoo (Zoo I) was Rajiv Gandhi Zoological Park and Wildlife Research Center (RGZP) andthe second zoo (Zoo II), Nisargakavi Bahinabai Chaudhary Zoo, Pimpri Chinchwad. Freshly voided faecal samples of 150 wild animals including Indian Peafowl (6), Pariah Kite (6), Brown Fish Owl (1), Shikra (2), Flying Fox (1), Eagle Owl (1), Brown Owl (1), Long-billed Vulture (2), Black Kite (2), Ring-neck Dove (1), Great Horn Owl (1), Painted Stork (1), Crested Eagle (2), LaggarFalcon (2), Amazon Parrot (4), African Grey Parrot (4), Plum-headed Paraket (1), Spot-billed Duck (4), Rose Ring Parakeet (1), Budgerigar (2), Cockatiel (2), Tiger (7), Leopard (6), Jungle Cat (16), Indian Wolf (1), Lion (2), Hyena (2), Jackal (5), Sloth Bear (2), Black Buck (4), Chinkara (2), Four-horned Antelope (5), Gaur (4), Indian Elephant (2), Barking Deer (2), Sambar (3), Spotted Deer (5), Blue Bull (4), Malabar Giant Squirrel (4), Bonnet Macaque (4), Rhesus Macaque (4), Trinket Snake (2), Bamboo Pit Viper (5), Reticulated Python (1), King Cobra (3), Common Krait (1), Banded Racer (1), Earth Boa (4), Indian Rat Snake (1), and Star Tortoise (1) were collected and examined.

A total of 450 faecal samples were collected during three different seasons, 150 each time (110 samples from Zoo I and 40 samples from Zoo II) throughout 2021. Along with the faecal sample, individual data regarding age, sex, and captivity were recorded separately. The data regarding the drug used for deworming, dose and period of deworming was also noted separately. Fresh faecal samples were collected randomly and pooled together from enclosures premises.

#### **Coprological evaluations**

The faecal samples were subjected to detailed parasitological analysis to confirm parasitic eggs /oocysts by direct smear examination. These samples were also subjected to standard sedimentation and floatation techniques (Soulsby 1982). Positive samples were further analysed quantitatively to indirectly calculate the parasitic load by eggs per gram of faeces (Gupta & Singla 2012).

#### **Treatment given**

The treatment at Zoo I was carried with fenbendazole

for herbivores @5 mg/kg body weight (BW) for two days (Table 7). The carnivores and reptiles were treated with a combination of praziquantel 50 mg, pyrantel-embonate 144 mg and fenbantel 150 mg @10 mg/kg BW for three days. Whereas, for the birds fenbendazole was given orally @5 mg/kg body weight (with restricted feed) once and repeated after 14 days during the study period.

The deworming zoo II in birds was carried out with albendazole@ 10 mg/kg as a single dose orally. The carnivores were treated with a combination of praziquantel 50 mg, pyrantel-embonate 144 mg and febantel 150 mg) @ 10 mg/kg BW for three days (Table 8).

The faecal samples of positive animals were collected before treatment and  $14^{\text{th}}$  day post-treatment. The efficacies of the above drugs were assessed based onthe faecal egg count reduction test (FECRT).

(Pre-treatment mean EPG -Post-treatment mean EPG)

Percent efficacy (FECRT) = 
$$\frac{1}{100}$$
 x 100

Pre-treatment mean EPG

#### Statistical analysis

The data was processed in Microsoft Excel, and descriptive analysis was done using SPSS statistic software for Windows, Version 20 developed by IBM Company, USA. Parametric and non-parametric statistical tests including the Chi-square test, student t-test and faecal egg count reduction test was used to interpret the final results.

#### **RESULTS AND DISCUSSION**

#### Overall seasonal prevalence

The seasonal prevalence of gastro-intestinal parasitic infection in mammals varied significantly (P<0.01) with a higher prevalence (29.50%) in monsoon followed by winter (26.30%) and summer (8.40%). At the same time, the same was found non-significant in birds

with a prevalence rate of 21.80, 20.00, and 18.80% in monsoon, winter, and summer, respectively (Table 1). During monsoon season, high humidity and suitable environmental temperatures can prolong the survival of infective parasitic stages (Singh et al. 2009) resulting in higher prevalence rate. Mammals had a higher overall prevalence (21.50%, CI: 16.8-26.6) than birds (18.80%, CI: 13.10-25.60). The findings are similar to Moudgil et al. (2020a), who reported a 25.5% prevalence of gastrointestinal parasitism in zoo animals. In contrast, Muraleedharan et al. (1990) and Singh et al. (2006) recorded a higher prevalence of gastrointestinal parasites. Similarly, in other previous studies, higher prevalence had been recorded from different states of India, i.e., Bihar (51.90%; Modi et al.1997a), Andhra Pradesh (46.59%; Kumar & Rao 2003), and Punjab (32.98%; Moudgil et al. 2020b). Comparatively lower prevalence in the present study could be associated with the adoption of better management practices including balanced feeding, regular deworming, regular screening and treatment of positive animals and daily cage and premises cleaning.

#### Prevalence based on sex and location

Sex and location (Table 2) based variation in prevalence rate was found non-significant during all three seasons. However, Kumar & Rao (2003) found a higher prevalence of parasitic infection in males than females kept in the different zoological gardens of Andra Pradesh (India) and Nekede Owerri (Southeast Nigeria).

#### Species based seasonal prevalence

All the omnivores were positive for gastrointestinal parasites during the winter (Table 3) followed by 28.60 % prevalence in herbivores, 16.70 % in reptiles, and 5.04 % (2/37) in carnivores. The observations are similar to Thawait et al. (2014). The prevalence was significantly higher (p<0.01) in omnivores during the winter and monsoon season. The prevalence during summer

Table 1. Overall seasonal prevalence of gastrointestinal parasitic infections in mammals and birds.

	Season	Examined (mammals + birds)	Positive mammals	Prevalence (%)	Positive birds	Prevalence (%)
1	Winter	150 (95+55)	25	26.30 (CI 17.8-26.30)	11	20.00 (CI 10.40-33.0)
2	Summer	150 (95+55)	8	8.40 (CI 3.70-15.90)	8	14.50 (CI 6.50–26.70)
3	Monsoon	150 (95+55)	28	29.50 (CI 20.60-39.90)	12	21.80 (CI 11.80-35.00)
	Overall	450 (285+165)	61	21.50 (CI 16.80–26.60)	31	18.80 (CI 13.10-25.60)
	χ2			20.86**	6** 1.033 <sup>NS</sup>	

CI—95% Confidence Interval | \*—significant at P<0.05 | \*\*—significant at P<0.01 | NS—non-significance.

among different species was found lower than the other seasons, contrary to the studies from southeastern Nigeria that reported higher infections in the summer season in wild cats (Okoye et al. 2014). The hot and dry climate in the study region and clean surroundings on the premises might have attributed to the low parasitic infections during the summer season.

In the monsoon season, 10.80% of carnivores were found positive for gastrointestinal parasites. Balantidium colicysts were most commonly found in lions (Image 5) and strongyle eggs and Strongyloides larvae in Hyena (Image 6). Among herbivorous animals, gaurs and blackbucks (Image 3 & 4) were found positive for Strongyloides species, whereas chinkara and nilgai were positive for *Trichuris* eggs (Image 1) and *B. coli* (Image 2), respectively. Similar findings were previously recorded by Cook et al. (1979). Among omnivores, B.coli cysts were seen in Bonnet macaque (Image 8) and Strongyloides species larvae in Malabar Giant Squirrel (Image 7). Prevalence was found higher in monkeys as they were kept in cages, which could have led to excessive stress, further leading to a 100% infection rate of gastrointestinal parasites. Thawait et al. (2014) also recorded a similar observation where the prevalence of different gastrointestinal parasites was found higher in monkeys (60%), followed by herbivores (45.6%) and carnivores (45.2%). In python, Strongyloides species eggs (Image 13) were abundant, whereas B. coli cysts were seen in Star back

Table 2. Seasonal prevalence of gastrointestinal parasites based on sex and location in different animals.

Maniahla.	Se	ex	Location		
Variables	Female	Male	Zoo I	Zoo II	
Winter	8/29 (27.00)	4/25 (16.00)	24/85(28.20)	1/10(10.00)	
χ2	8.3	8 <sup>NS</sup>	3.74 <sup>NS</sup>		
Summer	0/29(0)	4/25(16.0)	7/85(8.20)	1/10(10.00)	
χ2	4.6	2 <sup>NS</sup>	0.48 <sup>NS</sup>		
Monsoon	9/29(31.00)	9/25(36.00)	26/85(30.60)	2/10(20.00)	
χ2 value	1.05 <sup>NS</sup>		0.48 <sup>NS</sup>		

 $<sup>^{\</sup>rm NS}-$ non-significance | Figures in parentheses indicate percentages | Zoo I—Rajiv Gandhi Zoological Park and Wildlife Research Center | Zoo II—NisargakaviBahinabaiChaudhary Zoo.

tortoise (Image 14). Similar observations were reported in Kerala by Akhila et al. (2018), i.e., overall *Strongyloides* species larvae were most prominently found accounting for 25.7% of all infections, followed by *Capillaria* species (22.8%) and strongyles (20.00%) in captive snakes.

#### Seasonal prevalence in birds

In the monsoon season, adult birds showed a significant higher prevalence of gastrointestinal parasites compared to young birds. (Table 4). Similarly, a highly significant difference was observed in males during the monsoon season. The presence of *Ascaridia galli* was

Table 3. Seasonal prevalence of gastrointestinal parasites found in different species of animals.

Variables	Carnivores	Herbivores	Omnivores	Reptiles	χ2 Value
Winter	2/37(5.40)	8/28(28.60)	12/12(100)	3/18(16.70)	25.232**
Summer	2/37(5.40)	2/28(7.10)	2/12(16.70)	2/18(11.20)	1.72 <sup>NS</sup>
Monsoon	4/37(10.80)	8/28(28.60)	12/12(100)	4/18(22.20)	35.380**

<sup>\*—</sup>Significant at P<0.05 | \*\*—significant at P<0.01 | ns—non-significance. Figures in parenthesis indicates percentages

Table 4. Age, sex, location wise seasonal prevalence of gastrointestinal parasites in birds.

Variables /season	A	ge	Se	ex	Location		
	Adult	Young	Female	Male	Zoo I	Zoo II	
Winter	11/40 (27.50)	1/15 (6.70)	4/18 (22.20)	2/14 (14.30)	8/25 (32.0)	4/30 (13.0)	
χ2 value	2.7	2.77 <sup>NS</sup>		0 .71 <sup>NS</sup>		2.78 NS	
Summer	9/40 (22.50)	2/15 (13.30)	2/18 (11.10)	4/14 (26.60)	7/25 (28)	4/30 (13.0)	
χ2 value	0.5	7 <sup>NS</sup>	1.57 NS		1.83 NS		
Monsoon	6/40 (15.00)	2/15 (13.30)	3/18 (16.70)	3/14 (21.40)	3/25 (12.0)	5/30 (16.70)	
χ2 value	17.39**		46.54**		47.43**		

<sup>\*—</sup>significant at P<0.05 | \*\*—significant at P<0.01 | NS—non-significance. Figures in parenthesis indicates percentages.

commonly observed in peafowl (Image 9) and African Grey Parrots (Image 11), followed by *Capillaria* in Longbilled Vultures (Image 10) and Great Horn Owls (Image 12). Such observation is more or less similar to the findings of Parsani et al. (2007) in birds in Ahmedabad. Sahoo et al. (2010) reported a prevalence of 29.5% from Orissa at Nandankanan Zoo in wild birds. The birds in the zoo are often subjected to the stress of caged captivity, overcrowding and environmental conditions favorable for the development of parasites. As a result, the birds in captivity generally harbor more parasitic infections than their freely living counterparts.

#### Captivity based prevalence

Captivity based prevalence found significantly higher in herds (groups), i.e., 33.30% (95% CI = 25.60-41.80), followed by birds 18.80% (95% CI = 13.10-25.60) and individually enclosed animals 9.72% (95% CI = 5.40-15.80) (Table 5). At the beginning of the study, the birds showed the highest prevalence in monsoon, i.e., 32% (95% CI = 14.90-53.50), followed by winter with 28% (95% CI = 12.10-49.40) and summer with 12% (95% CI = 2.50–31.20) at Zoo I. The parasitic gastrointestinal infections in the mammals kept in herds (groups) were highest (45.70%) in the monsoons (95% CI = 30.90–61.00) followed by winter (45.60%) (95% CI = 30.90-61.00) and summer (8.7%) (95% CI = 2.40-61). Initially, in Zoo II, the captivity-based prevalence in birds and herds during the season of monsoon was 13.3% (95% CI = 3.80-30.70) and 100% (95% CI = 2.5–100), respectively. That could be because of overcrowding and competition for food and water, causing stress and lowered immunity, making them more vulnerable to parasitic illnesses (Dhoot et al. 2002; Singh et al.2009). The individual enclosed animals had a significantly lower prevalence than herd animals and birds, as they might get special care, management and appropriate anthelmintic treatment throughout the year (Table 5). Similar observations have been reported by Moudgil et al. (2020a) from Punjab from different zoos.

#### Seasonal EPG recorded in both mammals and birds

The mean EPG of mammals of both zoos during the pre-monsoon season was the highest (183.9±16.00). The EPG recorded in the positive herbivores was moderate (50–100) while it was more in carnivores (100–300). The mean EPG observed in the pre-winter and pre-summer seasons was (156±13) and post-winter andpost-summer was(20± 6.40) (Table 6). Similar results were shown by Modi et al. (1997b) from Bihar; Kumar & Rao (2003) from Andhra Pradesh and Moudgil et al. (2014) from Punjab, where the monsoon season has the highest prevalence,

Table 5.Prevalence of gastrointestinal parasites based on captivity.

Overall/ Captivity	Frequency of positive samples	Prevalence (%)	χ2
Birds	31/165	18.80 (CI 13.10-25.60)	
Individual enclosed	14/144	9.70 (CI 5.40–15.80)	24.858**
Groups (herds)	47/141	33.30 (CI 25.60-41.80)	

Cl—95% Confidence Interval | \*—significant at P<0.05 | \*\*—significant at P<0.01 |  $^{\rm NS}$ —non-significance.

followed by winter while the summers had the lowest prevalence. During the study period, the intensity of parasitic infection was also recorded in captive birds at both zoos. The mean EPG recorded in birds was highest in the winter season (265±18.30), followed by monsoon (200±26.10) and summer (181.2±32.60).

#### **Drug-wise efficacy study**

The treatment was carried out for positive animals and birds in the present investigation as per Table 7 and 8. The faecal samples were collected two times (pre and post treatment) for the study from different animals and birds. At Zoo I (Rajiv Gandhi Zoo and Wildlife Research Centre Karaj, Pune), there was 85.89% and 77.36% reduction in eggs counts after treatment with fenbendazole @5mg/kg in herbivores and birds, respectively. In carnivores, the reduction was 69.93% after treatment with drug combination @10 mg/kg (Praziquantel 50mg + Pyrantelembonate 144mg + fenbantel 150 mg) (Table 7). In Zoo II (Nisargakavi Bahinabai Chaudhary Zoo, Pimpri Chinwad) the reduction in EPG was 72.35% in carnivores with drug combination@10mg/kg (Praziquantel 50mg + pyrantelembonate 144mg + fenbantel 150mg) and 68.98% in birds with albendazole @10mg/kg.

The drugs used were able to eliminate the development stages of the parasites in herbivores, carnivores as well as birds. Cent-percent efficacy was observed against *Trichuris* species and strongyle parasites for herbivores and carnivores, as eggs of these two species of parasites were not detected in the faeces post-treatment. Similarly, in birds, both albendazole and fenbendazole were able to eliminate *Ascaridia* sp., parasites, as no eggs of *Ascaridia* species were seen in the faeces post-treatment.

In the Nisargakavi Bahinabai Chaudhary Zoo, the faecal egg count reduction in mammals was 66.60–100 %. The average reduction in carnivores was 72.35%. The fecal egg count reduction in EPG birds was 76.20–56.30 % with an average of 68.98%. A highly significant reduction in the faecal egg count when compared to pre and post-



Table 6. Seasonal EPG recorded in both mammals and birds.

EPG	Mammals (Range)	Mean ± SE	Birds(Range)	Mean± SE
Pre-winter	50-300	156 ±13.00	150-350	265±18.30
Post-winter	0–100	20 ± 6.40	0–150	60±16.30
Pre-summer	50–300	156±13.00	100–300	181.2±32.60
Post-summer	0–100	20±6.40	0–150	56.2±17.50
Pre-monsoon	50-300	183.9±16.00	100-300	200±26.10
Post-monsoon	0–150	55.30±11.30	0–150	83.3±16.60

Table 7. Species-wise drug and dosing used for deworming at Zoo I.

	Species	drug used (contents)	Dosing	Pre- treatment EPG	Post-treatment EPG	Percentage reduction in EPG
1	Herbivores	5% suspension containing fenbendazole	@ 5mg/kg body weight once a day for 2 days.	156 ±13.00	20 ± 6.40	85.89%
2	Carnivores	Praziquantel 50mg + Pyrantelembonate 144mg +fenbantel 150 mg	@10mg/kg body weight for 3 days.	183.9±16.00	55.30±11.30	69.93%
3	Birds	5% suspension containing fenbendazole	@5mg/kg body weight for 1 day	265±18.30	60±16.30	77.36%

Table 8. Species-wise drug and dosing used for deworming at Zoo II.

	Species	Content of the drug used for deworming	Dosing	Pre- treatment EPG	Post-treatment EPG	percentage reduction in EPG
2	Carnivores	Praziquantel 50mg + pyrantelembonate 144mg + fenbantel 150 mg	@10mg/kg body weight for 3 days.	200±26.10	55.30±11.30	72.35%
3	Birds	Albendazole	@ 10mg/kg. body weight for 1 day	181.2±32.60	56.2±17.50	68.98%

treatment egg counts in both zoos may be due to proper dosing of drugs in animals according to body weight, good hygienic management practices, and individual care of wild animals and birds. It has been observed that confinement of wild animals in the zoo makes them prone to different parasitic infections despite providing adequate attention to feed, water, and maintenance of hygiene in captivity (Barmon et al. 2014). Despite regular deworming practices, the prevalence of parasitic infections varying from 25–95 % has been reported in zoo birds at various locations in India (Parasani et al. 2007). Besides proper dosing with specific and recommended drugs, in animals according to their body weight, good hygienic management practices and individual care of wild animals and birds in captivity is required for the management of these infections.

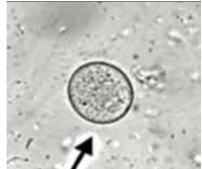
#### **SUMMARY AND CONCLUSION**

Climatic conditions during different seasons and captivity play a major role in the prevalence of parasitic infections in zoo animals. Captivity enhances the repeated exposure of the animals to the environment contaminated by the infective stages of the parasites. Furthermore, such environmental conditions can be favourable to the parasites' developmental stages being propagated as a result and the enclosure being contaminated. It is possible to infer from the study's findings that appropriate control methods, such as periodical examination, following the collection of faecal samples, and successful treatment is administered with anthelmintic. It may be suggested that cleaning the premises and proper disposal of excreta and refusals may minimize/avoid the associated losses.

Eggs of parasites observed in herbivores:



Image 1. Trichuris sp. (40X) in Chinkara.



Eggs of parasites observed in carnivores:

Image 5. Balantidium coli (40X) in African Lion.





Eggs of parasites observed in omnivores:

Image 8. Balantidum coli (40X) Bonnet Macaque.

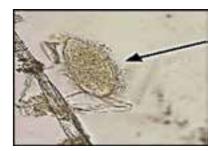


Image 2. Balantidium coli (40X) in Nilgai.

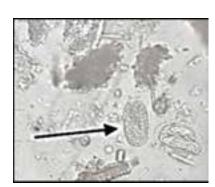


Image 3. Strongyloides (40X) in Gaur.



Image 4. Strongyloides (40X) in Black Buck.

Image 6. Strongyloides (40X) in Spotted Hyena.

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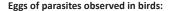




Image 9. Ascaridiagalli (40X) in Indian Peafowl.



Image 10. *Capillaria* spp.(40X) in Long-billed Indian Vulture.



Image 11. Ascaridiagalli (40X) in African Grey Parrot.

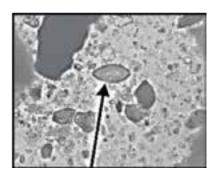


Image 12. *Capillaria* spp. (40X) in Great Horn Owl.

#### Eggs of parasites in reptiles:



Image 13. Strongyloides (40X) in Reticulated Python.

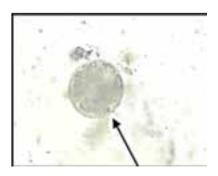


Image 14. *Balantidium coli* (40X) in Star Back Tortoise.

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OPEN



Bryophyte diversity of Berinag (Pithoragarh District) in Kumaun Himalaya, Uttarakhand, India

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Abstract: This study reports the diversity of bryophytes of Berinag (Dist. Pithoragarh), Uttarakhand (India). In the investigation, a total of 33 species were reported, out of which eight were liverworts and 25 were mosses. Majority of the taxa were terricolous and corticolous. Liverwort species belonged to six genera of five families and two orders whereas mosses belonged to 24 genera of 17 families and eight orders. Out of the 10 orders dealt in the present work, Hypnales was the largest consisting of seven families, nine genera, and nine species followed by Dicranales and Marchantiales both consisting of three families, five, and four genera, respectively. In mosses, Bryaceae was the dominant family consisting of three genera and four species followed by Calymperaceae, Leucobryaceae, Anomodontaceae, Leskeaceae, and Polytrichaceae. Out of 25 mosses, 15 were acrocarpous and 10 were pleurocarpous. Aytoniaceae was dominant among liverworts. *Marchantia, Frullania*, and *Bryum* were the dominant genera of the region.

Keywords: Acrocarpous, bryoflora, corticolous, hornworts, hypnales, liverworts, Marchantiales, mosses, pleurocarpous, Pottiaceae.

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**Author contributions:** DD collected the samples, prepared the herbarium specimens and wrote the first draft. PC conceptualized and designed the study and prepared the final draft.

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### eth

#### **INTRODUCTION**

India is one of the mega diverse countries blessed with four global diversity hotspots, home to more than 49,000 species of plants, bestowed with 18,800 species of Angiosperms, 82 species of Gymnosperms, 1,307 species of Pteridophytes, 7,434 species of Algae, 2,786 species of Bryophytes, and 2,917 species of Lichens. According to Plant Mao et al. (2020), bryophytes constitute 5.57 % of total plants of India.

State-wise distribution list of liverworts and hornworts in India (Singh et al. 2016) suggested that the state of Uttarakhand ranked second in the country holding 47 families of liverworts and hornworts. The state is home to 84 genera and 259 species and infraspecific taxa of liverworts and hornworts ranking fourth and fifth in the country, respectively, for number of genera and species. There are 339 species belonging to 129 genera of mosses in Uttarakhand (Suman et al. 2010).

Luxuriant forests, varied topography, suitable climatic conditions made Kumaun Himalaya of Uttarakhand, very rich in bryophyte diversity. Pithoragarh is the easternmost district in the Kumaun region of Uttarakhand. The study area (29.781-29.778 °N & 80.045-80.048 °E), Berinag, is a hill station and one of the six administrative subdivisions (tehsil) of Pithoragarh famous for tea estates (Figure 1). It is located at an elevation of 2,540-1,674 m (source: Google Earth). It got its name from Naga Devta temple locally called 'Bedinag' situated at the top of Berinag Hill. Limestone, sandstone, slate, gneiss, and granite rocks are very common in the region. It has a humid and cold climate. Earlier, most of the land was covered by the forest, however, due to rapid urbanization, biodiversity of this area is under threat. Today, various types of new projects like electricity production from pine needles, tourism and small-scale industries are flourishing in this area, however, threat to the biodiversity remains unaddressed. For the biodiversity conservation of the area, a complete taxonomic data of the region is essential. In the ongoing decade of ecosystem restoration (www. unep.org), diversity of the pioneers of vegetation needs to be assessed for getting the up-to-date health profile of the fragile mountain ecosystems. The present study, attempts to document the bryophytic flora of Berinag and prepare a preliminary checklist based on this.

#### **MATERIAL AND METHODS**

The present work is the outcome of several plant surveys done in the study area by the first author (DP) from September 2018 to June 2019. Systematic collection of bryoflora was done from the various localities. Plants from different terrestrial habitats were collected, air dried and were kept in paper packets (15 x 10 cm). Field data such as name, locality from where the collection was done, date of collection, habitat, substratum, collector's name, associated species were written on the paper packets followed by the identification of collected samples using the available monographs of Gangulee (1969–1980), Chopra (1975), & Aziz & Vohra (2008) for mosses and Kashyap (1929), Watson (1968), Singh & Singh (2009), & Dey & Singh (2012) for liverworts. Bryonet and bryophyte experts were also consulted to confirm the identity of some confusing taxa. Standard abbreviations of authors' names have been followed according to Brummit & Powell (1992). The collected samples have been deposited in Cryptogam section of Herbarium of G.B. Pant University of Agriculture & Technology, Pantnagar (GBPUH). Enumeration is done in accordance with the classification given by Crandall-Stotler et al. (2009) for liverworts and Goffinet et al. (2009) for mosses. Orders and families have been arranged as per adopted classifications. The genera within each family and the species within each genus have been arranged alphabetically. All species were verified against TROPICOS data base (www.tropicos. org), The Plant List 2013 (www.theplantlist.org), and Catalogue of Life (CoL 2021).

#### **RESULTS**

Increased tourism activities, unabated biological resource usage and unrestricted infrastructure development in the hills have been the major factors responsible for diminishing plant diversity of the region. Bryophytes along with pteridophytes and herbaceous angiosperms form the major ground flora of the hill forests. Besides, the miniature cryptogams also adorn the tree barks and rocky surfaces. Habitat preference of bryophytes itself signifies their role as an important indicator of ecosystem health. To recreate and restore diminishing health of fragile ecosystems of mountainous tourist spots, bryofloristic assessment and conservation is essential. Bryophytes of Berinag along with their local distribution, information about their substratum and taxonomic hierarchies of species are listed in Table 1. Out

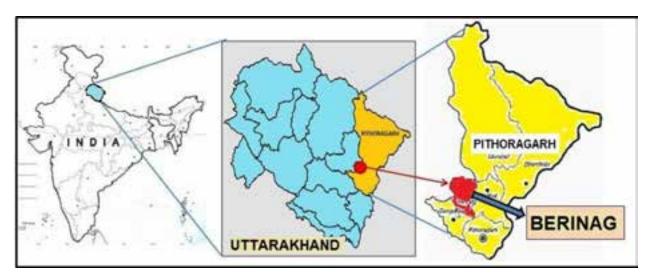


Figure 1. Map of study site in Uttarakhand, India.

of the 33 taxa reported in the region, eight taxa belonged to liverworts and remaining 25 were mosses. Hornworts were not found in the study area. This accounted for 1.18% of total bryophytes in just around 0.0002% of total geographic area of the country. Substratum details give the idea of dominance of terrestrial and corticolous forms of bryophytes in Berinag (Figure 2). Out of 25 mosses, 15 were acrocarpous mosses and 10 mosses were pleurocarpous making acrocarpous growth forms most dominant amongst mosses (Figure 3).

Hypnales and Marchantiales were the most dominant orders with representation of seven and three families respectively. Bryum, Frullania, and Marchantia were represented by two species each in the study area. A preliminary checklist enlisting 51 taxa has earlier been reported by Alam et al. (2012) from Dharchula and Munsyari tehsils of Pithoragarh wherein, Hypnales was the most dominant order represented by seven families followed by Bryales and Dicranales representing three families each among mosses. Marchantiales was the most dominant order among liverworts representing eight families. According to the moss checklist of western Himalaya (India) by Alam (2013), there are 745 species of mosses belonging to 230 genera and 55 families. Hypnales is the most diversified order of mosses followed by Pottiales and Bryales. In the present study also, Hypnales was the most dominant group of mosses followed by Dicranales.

Family wise comparison of distribution of collected genera and species in the world, in western Himalaya, in Uttarakhand, in Pithoragarh District and in the study area (Berinag) is provided in the Table 2. According to The Plant List (2013), a total of 1,822 plant genera in 177

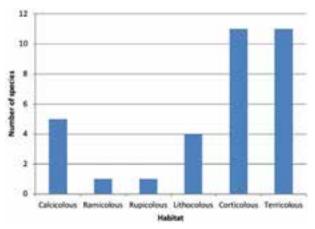


Figure 2. Distribution of mosses on the basis of their habitat.

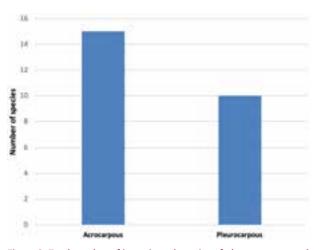


Figure 3. Total number of investigated species of pleurocarpous and acrocarpous mosses.



Table 1. Bryophytes of Berinag (Pithoragarh District), Uttarakhand.

	Family	Species	Local distribution	Substratum		
A)	LIVERWORTS			ı		
1	Aytoniaceae	Plagiochasma appendiculatum Lehm. & Lindenb.	New Bazar Berinag, 29.778°N, 80.053°E	Cemented wall		
2		Reboulia hemisphaerica (L.) Raddi	New Bazar Berinag, 29.778°N, 80.053°E	Cemented wall		
3	Dumortieraceae	Dumortiera hirsuta (Sw.) Nees	Kanera (Site rich in water) 29.672°N, 80.055°E	Stone		
4	Marchantiaceae	Marchantia papillata Raddi	Degree College Berinag, 29.775°N, 80.050°E	Cemented wall		
5	iviai criantiaceae	Marchantia polymorpha L.	Naula Berinag, 29.778°N, 80.053°E	Stone		
6	Frullaniaceae	Frullania ericoides Nees (Mont).	Kanera, 29.672°N, 80.055°E	Bark of <i>Quercus leucotricophora</i> A.Campus and <i>Mangifera indica</i> L.		
7		Frullania muscicola Steph.	Kalibinayak, 29.704°N, 80.054°E	Bark of <i>Q. leucotricophora</i> A.Campus		
8	Porellaceae	Porella caespitans (Steph.) S.Hatt.	Kanera, 29.672°N, 80.055°E	Bark of <i>Q. leucotricophora</i> A.Campus		
В)	MOSSES					
9	Bartramiaceae	Philonotis mollis (Dozy & Molk.)	Forest of Berinag, 29.780°N, 80.055°E	Soil		
10		Bryum argenteum Hedw.	Berinag Main Market, 29.778°N, 80.053° E	Humus mixed soil		
11	Bryaceae	Bryum mildeanum Jur.	Berinag forest 29.780°N, 80.055°E	Soil		
12		Ptychostomum capillare (Hedw.) Holyoak & N.Pedersen.	Berinag forest, 29.780°N, 80.055°E	Soil		
13		Rhodobryum roseum (Hedw.). Limpr.	Berinag forest, 29.780°N, 80.060°E	Soil		
14	Mniaceae	Mnium integrum Bosch & Sande Lac.	Berinag forest, 29.780° N, 80.055° E	Soil		
15	Calymperaceae	Octoblepharum albidum Hedw.	Kanera, 29.672°N, 80.055°E	Bark of <i>Q. leucotricophora</i> A.Campus		
16	Calymperaceae	Syrrhopodon gardneri (Hook.) Schwagr	Kalibinayak, 29.704°N, 80.055°E	Bark of <i>Q. leucotricophora</i> A.Campus and <i>Q. glauca</i> Thunb.		
17	Ditrichaceae	Ditrichum heteromallum (Hedw.)	Forest of Berinag, 29.780° N, 80.060° E	Rock		
18	Leucobryaceae	Brothera leana (Sull.) Müll.	Kalibinayak, 29.704° N, 80.054°E	Bark of <i>Q. leucotricophora</i> A.Campus		
19	Leucobi yaceae	Thysanomitrion involutum (Mull.Hal.) P.de la Varde	Degree College Berinag, 29.775°N, 80.050°E	Stone		
20	Funariaceae	Funaria hygrometrica Hedw.	Forest of Berinag, 29.780°N, 80.055°E	Soil		
21		Anomodon minor (Hedw.) Lindb.	Kanera, 29.672°N, 80.055°E	Bark of <i>Toona ciliata</i> M.Roem.		
22	Anomodontaceae	Herpetineuron toccoae (Sull. & Lesq.) Cardot.	Dhanouli, 29.761°N, 80.053°E	Bark of <i>Q. leucotricophora</i> A.Campus and <i>Q. glauca</i> Thunb.		
23	Brachytheciaceae	Oxyrrhynchium vagans (A.Jaeger) Ignatov & Huttunen.	Kanera, 29.672°N, 80.055°E	Soil		
24	Entodontaceae	Entodon concinnus °	Kanera, 29.672°N, 80.055°E	Cemented wall		
25	Leskeaceae	Haplocladium schimperi Ther.	Kanera, 29.672°N, 80.055°E	Bark of <i>T. ciliata</i> M.Roem. and <i>M. indica</i> L.		
26		Rozea pterogonioides (Harv.) A. Jaeger	Kanera, 29.672°N, 80.055°E	Soil		
27	Meteoriaceae	Trachypodopsis serrulata (P. Beauv.) M. Fleisch.	Naula site Berinag, 29.778°N, 80.053°E	Cemented wall		
28	Neckeraceae	Neckeropsis exserta (Hook. ex Schwagr.) Broth.	Kanera, 29.672°N, 80.055°E	Wood of <i>M. indica</i> L		
29	Thuidiaceae	Thuidium tamariscellum (Mull. Hal.) Bosch & Sande Lac.	Dhanouli, 29.761°N, 80.053°E	Stone		
30	Orthotrichaceae	Macromitrium moorcroftii (Hook. & Grev.) Schwagr.	Kalibinayak,29.704°N, 80.054°E	Branch of <i>Q. leucotricophora</i> A.Campus		
31		Atrichum obtusulum (Mull. Hal.) A.Jaeger.	Berinag forest, 29.780°N, 80.055°E	Soil		
32	Polytrichaceae	Pogonatum aloides (Hedw.) P.Beauv.	Berinag forest,29.780°N, 80.055°E	Soil		
33	Pottiaceae	Hyophila involuta (Hedw.) P.Beauv.	Kanera, 29.672°N, 80.055°E	Soil		

Table 2. Family wise comparison of distribution of genera and species in the world, in India, in western Himalaya, in Uttarakhand and in the study area.

	Number of genera and species													
	In the World In India			In western In Uttarakhand		In Pithoragarh			In Basinasa					
Family	(The Plant List 2013)		(Dandotiya et al. 2011)		Himalaya (Alam 2013)		(Suman et al. 2010)		(Sahu & Asthana 2015)		(Alam et al. 2012)		In Berinag (Dhami 2019)	
	G	S	G	s	G	s	G	s	G	s	G	s	G	s
Aytoniaceae	10	115	6	30	-	-	7	22	-	-	3	6	2	2
Dumortieraceae	-	-	1	1	-	-	-	-	-	-	1	1	1	1
Marchantiaceae	7	50	3	24	-	-	3	5	-	-	1	3	1	2
Frullaniaceae	-	-	1	42	-	-	1	7	-	-	-	-	1	2
Porellaceae	5	134	1	24	-	-	1	16	-	-	1	1	1	1
Bartramiaceae	14	686	8	48	7	28	4	12	1	2	1	1	1	1
Bryaceae	43	2108	09	103	6	45	6	32	5	9	4	5	3	4
Mniaceae	13	222	7	64	7	38	3	20	2	3	1	1	1	1
Ditrichaceae	34	347	9	30	4	12	4	9	1	1	-	-	1	1
Calymperaceae	19	417	7	35	2	4	3	4	-	-	-	-	2	2
Leucobryaceae	-	-	8	69	2	5	5	9	-	-	-	-	2	2
Pottiaceae	138	3223	38	207	32	116	17	60	6	8	3	4	1	1
Funariaceae	24	452	4	47	3	15	3	11	2	2	2	2	1	1
Anomodontaceae	-	-	5	17	-	-	3	8	-	-	1	1	2	2
Brachytheciaceae	62	1117	9	66	-	-	7	19	2	3	1	1	1	1
Entodontaceae	13	314	3	19	3	3	5	15	1	3	1	2	1	1
Leskeaceae	22	383	10	35	12	30	5	17	1	1	-	-	2	2
Meteoriaceae	30	650	21	80	11	21	4	5	4	5	3	4	1	1
Neckeraceae	37	827	19	93	8	21	6	12	1	1	-	-	1	1
Thuidiaceae	33	504	3	27	5	27	1	4	5	6	1	-	-	1
Orthotrichaceae	44	1265	13	64	1	18	2	4	1	1	1	1	1	1
Polytrichaceae	31	536	5	55	5	24	4	18	2	3	2	2	2	2

<sup>-—</sup>indicates that respective species is not included in the source | G—number of genera | S—number of species.

families of bryophytes are present in the world out of which maximum number of genera and species belong to family Pottiaceae (138 genera, 3,223 species) followed by Bryaceae (43 genera, 2,108 species). Dandotiya et al. (2011) reported total 1,786 species of mosses in 355 genera and 675 species of liverworts in 121 genera in India, out of which maximum number of taxa belonged to family Pottiaceae (38 genera, 207 species) followed by Lejeuneaceae and Notothyladaceae. Sahu & Asthana (2015) reported 72 moss taxa belonging to 24 families from Pithoragarh and adjoining areas with Bryaceaee being the most diverse family represented by nine genera. The present bryofloristic study of Berinag also reports maximum number of species belonging to Bryaceae but with a lesser representation, i.e., three genera and four species only (Tables 1 & 2). According to Alam (2013), maximum diversity of mosses in western

Himalaya (India) is represented by family Pottiaceae which included 116 species belonging to 32 genera. Unlike the general trend in western Himalaya or India or Uttarakhand, in the present study, Bryaceae is having maximum diversity. This may be due to dominance of mixed pine-oak forests in the region. Bryaceae members prefer shade and lower temperature while Pottiaceae is more common in comparatively drier and open areas. Habitat preference of the bryophytic vegetation suggests the dominance of both terricolous and corticolous forms represented by 11 species each. Habitat wise distribution of bryophytic vegetation of district Nainital and selected mineralized localities of Almora and Pithoragarh districts of Kumaun Himalaya have earlier been reported by Tewari and Pant (1994).

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SHORT COMMUNICATION





# The opportunistic feeding behaviour of *Schistura notostigma* (Teleostei: Nemacheilidae) in tropical mountain streams in Sri Lanka

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**Abstract:** *Schistura notostigma* is a bottom-dwelling freshwater fish endemic to Sri Lanka. In this study, we document an opportunistic feeding behavior exhibited by this loach species in close association with *Garra ceylonensis* and *Plesiopuntius bimaculatus* in the streams of high-elevation headwater regions of the Knuckles Mountain Range and Piduruthalagala Mountain Range in Sri Lanka.

**Keywords:** Feeding ecology, behavioural ecology, coexistence, commensalism

Schistura notostigma (Bleeker, 1863) is a small, bottom-dwelling freshwater fish endemic to Sri Lanka. It features an elongated, moderately cylindrical body that is ventrally flattened and slender, and possesses three pairs of small barbels, which characteristics are shared by many benthic freshwater fishes (Pethiyagoda 1991; Sundarabarathy et al. 2001; Herath 2009; Shirantha 2021). In this study, we document instances of opportunistic feeding behavior exhibited by this loach. These behaviors were observed frequently in the close association with two species of the family Cyprinidae,

Garra ceylonensis (Bleeker, 1863) and Plesiopuntius bimaculatus (Bleeker, 1863), in streams situated in high elevation headwater regions of the Knuckles Mountain Range (KMR) and Piduruthalagala Mountain Range (PMR).

Schistura notostigma (Image 1A) is one of the few freshwater fishes found at elevations exceeding 1,000 m in Sri Lanka, along with Garra ceylonensis (Image 01B), Plesiopuntius bimaculatus (Image 01C) and Devario spp. (Pethiyagoda & Sudasinghe 2021). Although S. notostigma can sometimes be found at elevations above 1,300 m, the other species mentioned generally inhabit elevations up to 1,000 m, albeit with rare exceptions (Pethiyagoda & Sudasinghe 2021; Sudasinghe et al. 2021, 2023). Despite our observations indicating that S. notostigma could occasionally be the sole freshwater fish in high-elevation habitats, however, it exhibits a notable inclination to associate closely with G. ceylonensis when present in the ichthyo-community as associates.

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Image 1. Three benthic co-existing species from tropical mountain streams of Sri Lanka: A—Banded Mountain Loach Schistura notostigma | B—Ceylon Stonesucker Garra ceylonensis | C—Red-side Barb Plesiopuntius bimaculatus. © Madura De Silva & Wildlife Conservation Society, Galle (WCSG).

### **MATERIALS AND METHODS**

The present study was conducted as part of an ongoing research project on Sri Lankan freshwater fishes. The study was carried out in Mandaramnuwara, situated on the eastern slopes of PMR at an elevation of 1,000 m. PMR study site is a circular area with a 5-km radius centered on coordinates 7.0638°N, 80.7652°E, situated in the Belihul Oya (river) headwaters, near Kalu Palama Ella, and in Medadumbara, on the southern flank of KMR at 590 m. KMR study site is a circular area of 5-km radius, centered at 7.3475°N, 80.8202°E in the Heel Oya and Galmal Oya headwater regions (Figure 1).

Five points were arbitrarily selected for observation from each site within the specified range. These observations spanned a 12-month period from November 2022 to October 2023. Observations were made monthly (five days per month from 0700–1800 h) using remote underwater video footage (GoPro Hero 9 action camera) and snorkelling. Fish identification was performed in the field using the latest field guides (De Silva et al. 2015; Shirantha 2021) and later confirmed by reference to recent publications (Sudasinghe 2017, 2018). During the species identification process, fishes were captured using a scoop-net and immediately released to their

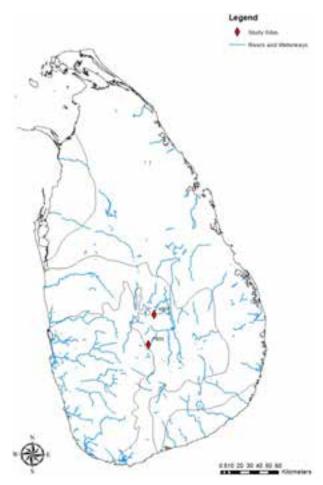


Figure 1. The locations of the study sites in Sri Lanka. KMR—Knuckles Mountain Range | PMR—Piduruthalagala Mountain Range.

original habitats.

### **OBSERVATIONS AND DISCUSSION**

Garra ceylonensis and Schistura notostigma (at an elevation range of 590–1,350 m in KMR and PMR) exhibit a close association and a commensalism relationship as follows: G. ceylonensis was observed grazing on algae on the rocky substrate, using its sucker mouth (Sudasinghe et al. 2021), causing small invertebrates and other food particles to be detached. The opportunistic species S. notostigma then exploits this disturbance by browsing on the dislodged food particles (see Image 2A,B).

Analysis of diets, notwithstanding different degrees of selectivity for various food categories, showed both species to be omnivorous (confirmed through gut content analysis in prior studies and references in: Moyle & Senanayake 1984; Wikramanayake & Moyle 1989; Sundarabarathy et al. 2001, 2005; Shirantha 2004; Pethiyagoda & Sudasinghe 2021). However, *S. notostigma* was found to primarily feed on a variety

of small invertebrates, especially caddisfly larvae (Trichoptera: Hydropsychidae and Rhyacophilidae) and small mayfly larvae (Ephemeroptera: Leptophlebeidae) (Moyle & Senanayake 1984). Furthermore, Moyle & Senanayake (1984) suggest carnivory as the dominant mode of feeding for this loach, highlighting the short intestine (less than 1 × body length) and the observed willingness of S. notostigma to attack small benthic prey. On the other hand, G. ceylonensis is mentioned as a benthic algae feeder in Sudasinghe (2021) by referring to Lujan & Conway (2015) and Kottelat (2020). Moreover, Moyle & Senanayake (1984) also showed G. ceylonensis to be primarily a detritus and diatom feeder, based on a gut content analysis in Costa & Fernando (1967). Furthermore, studies conducted by Wikramanayake & Moyle (1989) and Moyle & Senanayake (1984) in rainforest streams and lowland wet zone streams show that there is no statistically significant dietary overlap among G. ceylonensis and S. notostigma. Therefore, based on the diet and behaviour, we hypothesize this association as a commensal relationship, in which S. notostigma benefits substantially more, rather than a mutualism in which both species benefit.

A similar set of observations were also recorded with schools of *Plesiopuntius bimaculatus* in the KMR during the study period (recorded in five occasions specifically during March 2023 and April 2023), which also confirmed the opportunistic feeding behaviour of *S. notostigma*. Here, *S. notostigma* followed the schools of foraging *Pl. bimaculatus*, feeding on food particles sinking to the substrate.

In this case of *S. notostigma*, given the absence of any recorded negative interactions up to date, whether of a predatory or competitive nature between these species (*G. ceylonensis* and *S. notostigma* or *Pl. bimaculatus* and *S. notostigma*), we suggest that opportunistic feeding behaviours and close associations may enable these species to access food sources more easily and with less competition. This becomes especially pertinent in highelevation tropical mountain regions, where co-evolved fishes and other vertebrate species are recognized to have a high degree of adaptation to their environments, as described in Abell et al. (2008).

In addition to *S. notostigma*, similar opportunistic feeding behaviours have been observed in other benthic loach species, including *Misgurnus anguillicaudatus* (Cantor, 1842) as documented by VanHaitsma (2020) and McNair et al. (2023), *Misgurnus fossilis* (Linnaeus, 1758) as reported by Pyrzanowski et al. (2019), and *Barbatula barbatula* (Linnaeus, 1758) as noted by Andrei et al. (2015) and Worischka et al. (2015). Furthermore,

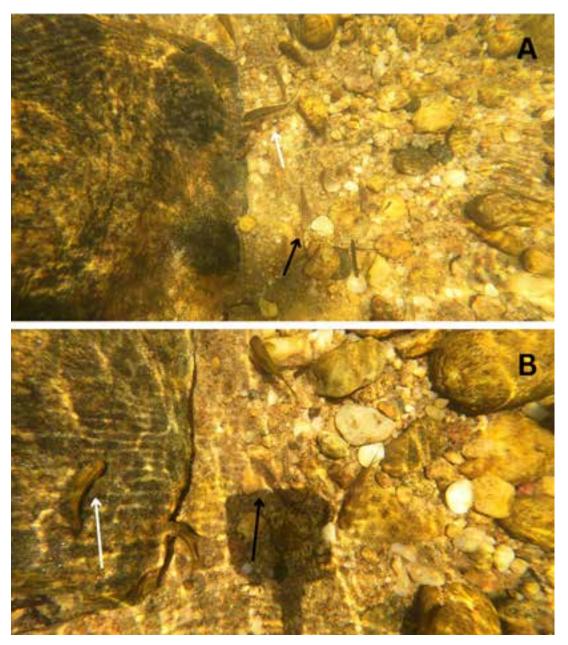


Image 2A,B. Close association of *Garra ceylonensis* (GC) and *Schistura notostigma* (SN) recorded frequently in Heel Oya, southern flank of Knuckles Mountain Range, Sri Lanka. GC pointed by the white arrowheads and SN by the black arrowheads. © Janamina Bandara.

Rice et al. (2019) too, make reference to several unique benthic feeding behaviours of fishes.

Pethiyagoda & Sudasinghe (2021) also make reference to a similar set of ecological interactions among Sri Lankan freshwater fishes, originally documented by Schut et al. (1984). These interactions involve *Rohanella titteya* (Deraniyagala, 1929) and *Pl. bimaculatus*, as well as juvenile *Dawkinsia fillementosa* (Valenciennes, 1844) and adult *Pethia cumingii* (Günther, 1868). These associations can be succinctly summarized as optimizations of dietary habits and the avoidance of

shared predators.

Apart from *G. ceylonensis*, *S. notostigma*, and *Pl. bimaculatus*, only *Devario malabaricus* (Jerdon, 1849) and *Poecilia reticulata* (Peters, 1859) were found inhabiting the high-elevation regions in KMR (600 m) (Pethiyagoda & Sudasinghe 2021; Bandara & Gunawardena 2023a,b). No other freshwater fishes were recorded in headwaters of the PMR study site, except for *G. ceylonensis* and *S. notostigma*. Generally, it shows a lower fish diversity in high-elevation tropical freshwater fish assemblages recorded above 1,000 m elevation, when compared to

lowland assemblages in the island's south west wet zone (Sumith et al. 2011; Pethiyagoda & Sudasinghe 2021; Sudasinghe et al. 2023). In this case we believe that the opportunistic feeding of *S. notostigma* reported here could be a common behaviour with the co-occurring benthic species *G. ceylonensis* and *Pl. bimaculatus*.

#### CONCLUSION

In conclusion, frequent occurrences of the aforementioned ecological associations and interactions provide insight into the feeding behaviours of this benthic loach species. Moreover, these records also offer vital information that can be used to understand the ecological structure of tropical freshwater fish assemblages in high-elevation mountain streams (1,000 m) in Sri Lanka.

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SHORT COMMUNICATION

### First record of *Pieris napi* L. (Lepidoptera: Pieridae) from Kashmir Valley, India

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Abstract: Pieris napi, a well-studied butterfly species distributed throughout the world, has remained undiscovered from Kashmir Himalaya to date. The present study reports the Pieris napi for the first time from Kashmir. This paper describes the diagnostic features and distribution patterns of the butterfly species in this region. It also includes specimen photographs and a distribution map of the species.

Keywords: Dachigam National Park, distribution, general hosts, Geranium sp., green-veined white butterfly, new record, Pir Panjal range, preservation, Rubus sp., Stellaria media, Zabarwan Range.

There are 34 different Pieris species reported in the world (Kirti et al. 2020). Pieris napi is found in the Himalayan region, including the northeastern states of India such as Arunachal Pradesh, Manipur, Assam, Himachal Pradesh, Meghalaya, Mizoram, Nagaland, and Uttarakhand, as well as Bhutan, Myanmar, China, Japan, North America, and Japan (Geiger & Shapiro 1992; Shaoji 2009; Gogoi 2013; Lodh & Agarwal 2015; Tadokoro 2015; Tadokoro 2017). The geographic distribution of the Pieris napi comprises the cool-temperate to cold wooded biomes of the Northern Hemisphere (Geiger & Shapiro 1992). In Russia, Pieris napi is present in the Caucasus; middle Siberia, and southwestern Siberia. It is a polymorphic species represented by a complex set of forms (Nuzhnova & Vasilevskaya 2013). The permanence

of habitat and the oviposition on different hosts of the P. napi were studied by Ohsaki & Sato (1999). However, some researchers considered them as individual species, while others do not discriminate between various Pieris species such as P. bryoniae, P. pseudorapae, P. euorientis, P. persis, P. narina, P. bowdeni, P. dulcinea, P. ocshenheimeri, and P. tomariana, instead, they view the P. napi complex as a superspecies in a broad sense. The P. napi has been considered as a separate species by many authors and has provided distinguishing characteristic features (Richards 1940; Ohsaki & Sato 1999; Rayor et al. 2007; Bibi et al. 2022). Richards (1940) studied in detail the structural differences in eggs, pupae, larvae, and the hosts of Pieris rapae and Pieris napi. The Pieris napi, a multivoltine butterfly is widely spread in Europe at elevations below 200 m. Females of Pieris napi produce few large eggs, tend to be sedentary, and usually select those plants whose locations are long-lasting (Ohsaki & Sato 1990). As per Ohsaki (1979) Pieris napi lays eggs only on Arabis plants in the shade in the Kyoto area, Western Japan.

Since only five Pieris species have been reported in the Union Territory of Jammu and Kashmir-Pieris brassicae, Pieris canidia, Pieris rapae, Pieris ajaka, and Pieris deota—this survey focused on the Kashmir Valley.

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It also included high-altitude forest areas like the Pir Panjal Range, Dachigam National Park, and various vegetable-growing regions of Kashmir.

### **MATERIALS AND METHODS**

**Study area:** The present study was conducted in high-altitude forest areas such as the Pir Panjal Range, Dachigam National Park, and various vegetable-growing regions of Kashmir, UT Jammu & Kashmir. The Dachigam National Park is a part of the Zabarwan range of the western Himalaya located at 34.1547°N & 74.9155°E and altitude 1634.36 m (Image 1).

**Survey and collection:** The current study highlighted all *Pieris* species of different regions of Kashmir from 2020 to 2021. Random surveys were conducted fortnightly in different months of the year depending on the prevailing weather conditions and butterfly activity. The survey was done twice a month and conducted near water sources, damp patches in the forest areas, open sunny areas, and blossoming flowers. Adult butterflies were collected with the help of an insect collecting net.

After collection, the butterflies were kept in jars and killed with ethyl-acetate. Thereafter, these specimens were then shifted in the relaxing chamber with wet sand for at least 24 hours and were properly labelled bearing (i) sample number, (ii) date of collection, (iii) name of the place, and (iv) name of the collector. The collected specimens were stretched on an insect stretching board by passing an entomological pin of size 4 through the thorax. The wings were spread in such a manner that the lower margin of the fore-wing was at a right angle to the body and the antenna in front of the head.

**Preservation:** After proper spreading, the specimens were left for about 2 to 4 days at room temperature inside the Entomology Research Laboratory, Department of Zoology, and were then shifted to wooden insect-storing boxes (Image 4). Each specimen was labelled bearing the same information as was written on the relaxing chamber previously. In order to protect the collected specimens from pests and fungus, cotton balls dipped in ethyl acetate vapours or benzene-dipped papers were periodically inserted in these boxes. The storage



Image 1. A—Map of India in the world map | B—Sampling sites in different districts of Kashmir Valley (prepared with ArcGIS, version, 10.8.1) | C—Study site map- Dachigam National Park, Srinagar (source: Wikipedia).





Image 2. Pieris napi spotted in Dachigam National Park, resting on: 1—Flowers of Stellaria media | 2—Leaves of Geranium sp. | 3 — Leaves of Rubus sp. | 4—Eggs on the leaves of Cardamine flexuosa.

boxes were kept in clean and shadow places, away from direct sunlight as per the technique adopted by Borror et al. (1976), Kunte et al. (2020), and Wynter-Blyth (1957). The specimens were identified later by using the identification keys of Evans (1932).

### **RESULTS AND DISCUSSION**

**Material examined:** 40°, 10°, Dachigam National Park along Dagwan River, 20.iv.2022, 34.154°N 74.915°E, 1634.36 m, insect collection nets, coll. Firdousa Rasool.

Diagnosis: The upper side of both Male and female is white coloured, veins conspicuously green or black, the base of the forewing is dusted with black scales, the apex and terminal border is black down to vein 2, and a black spot is present in the outer half of interspace 1. Hindwing with a black sub-costal spot. Under side veins are margined with black, the apex of the forewing and the whole surface of the hindwing are tinged with yellow, base of the costa of the hindwing is bright yellow. The female is much darker than the male, all the markings are broader. The upper side of the body is black with whitish hairs (Image 3).

Pieris napi was observed to fly inside Dachigam National Park and mostly rested on the flowers of Stellaria media, leaves of Geranium sp., and the leaves of Rubus sp. (Image 1, 2). A total of 5 specimens were collected from the same site with insect collection nets. Out of 30 different sampling sites, P. napi was spotted and trapped only in Dachigam National Park as it typically occurs in moist habitats, favours shaded or partly shaded woodland edges in a cool, moist environment as also suggested by (Howe & Bauer 1975). The present study revealed that P. napi exhibited the narrowest range of distribution being confined to only Dachigam National Park; outside the Dachigam National Park, no specimen

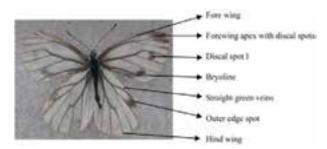


Image 3. *Pieris napi* of Kashmir, captured at Dachigam National Park, Srinagar, J & K, India



Image 4. Preservation of *Pieris napi* in Entomological Research Laboratory, Department of Zoology, University of Kashmir.

was collected. The P. napi and its narrowest natural distribution within forest edge habitat have also been proved in an experiment conducted by Ohsaki & Sato (1999) in the northern city of Kyoto, Japan. P. napi eggs and larvae were found on the plants of the Brassicaceae family like Cardamine flexuosa, acting as the host of the P. napi in the Dachigam National Park (Image 2(4)), and the same results were obtained by (Chew & Watt 2006; Friberg & Wiklund 2019). Pieris napi was very difficult to trap as it was flying high and fast. The species was captured at an elevation of 1676 m (5,500 ft) above sea level. The same results were documented by Shreeve (1981) as Pieris napi can fly high and cover large distances. Pieris napi trapped in Kashmir is not too much white but has long parallel green veins and broad discal spots that may be due to the variation in morphology due to the effect of latitude and altitude. According to Espeland et al. (2007) and Valimaki & Kaitala (2007), the morphology and life history of P. napi vary with latitude. P. napi is predominantly white at low elevations and low latitudes in Scandinavia; however, at higher elevations

and latitudes, it is darker and more melanized and is

frequently known as Pieris bryoniae (Ochsenheimer

1808; Kirby 1896) in central Europe and Pieris napi

adalwinda (Fruhstorfer, 1909) in Scandinavia (Porter

et al. 1997). Richards (1940) found that the proboscis

sheath of p. napi projects only a very short distance in

pupae, with its eggs and larvae being found on cabbage

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very rarely.

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SHORT COMMUNICATION

### Reassessment of Strobilanthes recurva (Acanthaceae), an endangered plant from Manipur, India

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Abstract: The presence and status of Strobilanthes recurva C.B.Clarke (Acanthaceae) in Manipur, northeastern India is re-assessed with a brief description and illustration. Photographs and their conservation status are also provided. The values of Extent of Occurrence (EOO) and Area of Occupancy (AOO) calculated as 16,313.797 km2 (VU), and 20.000 km2 (EN) respectively predicted S. recurva as Vulnerable and Endangered. Therefore, we proposed to treat this threatened species, S. recurva as Endangered (EN) until further information becomes available.

Keywords: Biodiversity, description, eastern himalaya, exploration, flora, northeastern India, rare, red list, taxonomy, threatened.

The genus Strobilanthes Blume of the family Acanthaceae consists of around 463 species (POWO 2024). It is distributed mostly in Asia, extending to Japan and Korea in the north, Afghanistan and Pakistan in the west, and northern Australia in the south. It is most diverse in the Indian subcontinent, southern China, and mainland southeastern Asia, but many species have also been reported to occur on the islands of southeastern Asia (Wood & Scotland 2021). Most species under the genus Strobilanthes are recorded as rare and localized in their distribution (Wood & Scotland 2009). Species

belonging to the genus Strobilanthes of peninsular India, the Himalaya, and hills of northeastern India are recorded to be varied and have no connection (Wood 1994). About 65 species were reported from peninsular India, and approximately 85 species were reported from hills of northeastern India (Wood & Scotland 2021). The Strobilanthes of the whole Himalayan region were discussed by Wood (1994), however, southwestern China, upper Burma, and the hills on the Indo-Burmese border were excluded from the study. It is reported that there is no comprehensive study on the Strobilanthes species of the hills on the Indo-Burmese border, which include the Indian states of Meghalaya, Nagaland, and Manipur.

Manipur is one of the states of the northeastern region of India. It lies between 23.83'-25.68'N and 94.31'-94.78'E and falls under the Indo-Burma Biodiversity Hotspot (https://www.cepf.net/our-work/ biodiversity-hotspots/indo-burma). In the Flora of Manipur, no species of Strobilanthes was previously recorded (Singh et al. 2000). However, a total of six species-Strobilanthes affinis Terao ex J.R.I.Wood &

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Competing interests: The authors declare no competing interests.





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J.R.Bennett, S. asymmetrica J.R.I.Wood & J.R.Benn., S. auriculata Nees, S. clarkei J.R.I.Wood, S. cusia J.R.I.Wood & J.R.Benn., and S. torrentium Benoist—were recorded by Ningombam (2012), of which S. auriculata is reported to have an eight-year plietesial cycle in Manipur (Devi et al. 2021). In addition, S. frondosa J.R.I.Wood was also reported to be rediscovered in Manipur (Wood et al. 2003). Among the other Strobilanthes species collected in the 19<sup>th</sup> century from Manipur, a newspaper article reported photographic evidence for the presence of S. recurva at Sadu chiru water fall Kangpokpi District, Manipur (https://easternmirrornagaland.com/ coneflower-photographed-in-wild-after-73-years-inmanipur/). Strobilanthes recurva, a rare species, was also collected from Mt. Tiyi in Wokha, Nagaland, India by N. Odyuo and D. Roy. Herbaria specimens are present at Botanical Survey of India, Eastern Regional Center in Shillong bearing numbers 096495 and 096496. However, these vouchers lack information on the altitude, habitat, and geographical coordinates (latitude and longitude). John R.I. Wood, an expert on the genus Strobilanthes, confirmed another collection of S. recurva from Chittagong, Bangladesh (Collector: Mr. Mohammed Uddin). No record on the description and illustration of these new collections of S. recurva are available on public domain.

A plant specimen of *S. recurva* was collected by the present author(s) from Mao Songsong, Senapati District, Manipur, India in February 2022 and the species was authenticated by J.R.I. Wood. Plants were growing at an altitude of 1,835 m on a shaded streamside at the hill slope of an open forest. Although not plentifully distributed, the collection site recorded 20–30 mature individuals. Voucher specimens were deposited in the Institute of Bioresources and Sustainable Development (IBSD), Imphal with accession number IBSD/M-279. In this study, the presence of *Strobilanthes recurva* C.B.Clarke (Acanthaceae) in Manipur, northeastern India is re-assessed with a brief description and illustration. Photographs and their conservation status are also provided below.

### **RESULTS AND DISCUSSION**

### Strobilanthes recurva C.B.Clarke

J. Linn. Soc., Bot. xxv. (1889: 53).

Type: INDIA, Naga Hills, Kohima, alt 4,500 ft (1,360 m), Clarke C.B. 41379, 30.x.1885, (Holotype K000883138!).

Description: Isophyllous to weakly anisophyllous undershrub, 0.6–0.8 m height. Stem quadrangular, swollen at the base of the node. Leaves petiolate, ovate, pubescent on both sides, 7.8–9.9 × 3.0–4.5cm, margin

crenate, apex broadly acuminate, base attenuate. Inflorescence of terminal and axillary bracteates spikes, 3.5–3.7 × 1.1–1.3 cm. Bracts imbricate, broadly ovate, persistent, green, glandular-pilose, 1.3–1.7 × 0.7–1.1 cm. Bracteoles 2, linear, obtuse, 7–8 mm. Calyx 5-parted, glandular hairs present, linear, obtuse, lobes unequal in length, 0.8 – 0.9cm. Corolla 5-lobed, curved, funnel-shaped. Corolla tube short, white, 1.5–2 × 0.2 cm, gradually widened at the mouth., purple. Stamens 4, fertile, didynamous, longer filament 2.2 cm, shorter filaments 0.3–0.4 cm; anthers included, 0.2–0.3 cm long. Style pilose, curved, 2.1 cm. Ovary 0.9 cm, hirsute at the apex. Capsule oblong, hairy at the apex 1.0–1.2 cm. 4-seeded; seeds lenticular, villous, 0.2–0.3 cm (Image 1).

**Specimen examined:** INDIA, Manipur, Senapati, Mao Songsong, 1,835 m, 09.ii.2022, RJ Devi & B.Thongam, M-279 (IBSD) (Image 2).

### Other specimens of S. recurva recorded so far.

India: hills of Kohima, alt 4,500 ft (1,360 m), C.B.Clarke 41379, 30.x.1885 (Holotype K000883138!) (http://specimens.kew.org/herbarium/K000883138); India: Manipur, 14.i.1882, Watt G. 5156, E00133469 (https://data.rbge.org.uk/herb/E00133469); India: Sirhoi, alt 6,500 ft (1,970 m), 15.iv.1948, F. Kingdon-Ward 17292 (NY2652219!) (http://sweetgum.nybg.org/science/vh/specimen-details/?irn=3451210); India: Mt. Tiyi, Wokha, Nagaland, N. Odyuo and D. Roy, 17.iv.2016, 096495! and 096496; Chittagong, Bangladesh (photographs by Mr Mohammed Uddin).

### **Conservation Status**

No earlier evaluation was made to assess the conservation status of this species. Since its type specimen collection was made in the year 1885, this species is presently known from only four locations of northeastern India, viz.: Kohima & Wokha of Nagaland State; Ukhrul & Senapati District of Manipur State; and another one from Chittagong, Bangladesh. Records available so far may indicate the confinement of this species in the higher altitudinal hills of the Indo-Burma border. Geographical coordinates are not available for the locations from where Strobilanthes species are collected so far except for the present study. Therefore, the standard Geospatial Conservation Assessment Tool (Bachman et al. 2011) was used for automatic assessment of IUCN Red Listing. The extent of occurrence (EOO) and area of occupancy (AOO) were calculated as 16,313.797 km<sup>2</sup>, and 20,000 km<sup>2</sup>, respectively.



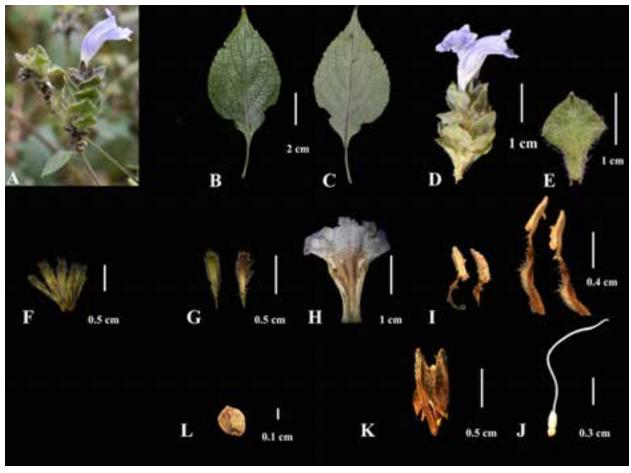


Image 1. Strobilanthes recurva: A—A twig with inflorescences | B—leaf adaxial | C—leaf (abaxial) | D—inflorescence | E—bract | F—calyx split open | G-bracteoles | H-corolla (split opened from curvy side) | I-Stamens | J-carpel | K-capsule | L-seed | © M. Aldrin & R.J. Devi.

### Keys to species of Strobilanthes recorded from Manipur, India

1a.	Inflorescence short terminal spike; Leaves petiolate; Bracts obovate – subspathulate; Bracteoles oblanceolate subspathulate; Corolla lobe pale sulphur or white
1b.	Inflorescence axillary or terminal spike; Leaves subsessile; Bracts obovate or elliptic obovate; Bracteoles oblanceolate or subacute;  Corolla lobe white or purple
2a. 2b.	Inflorescence small axillary & terminal panicle; Bracts obovate; calyx lobe equal; Corolla lobe glossy violet, curved
3a.	Anisophyllous, subsessile; Inflorescence short, nodding subcapitate spike on simple or sparsely branched axillary branchlets; Bracts elliptic-obovate with scattered gland dots; Corolla straight, lobe pale blue colour
3b.	Isophyllous to weakly anisophyllous, subsessile or petiolate; Inflorescence terminal or axillary spike; Bracts leaflike imbricate or obovate or oblanceolate; Corolla straight to slightly bent or highly curved, lobe blue colour
4a.	Leaves oblong, ovate, elliptic, glabrous or pilose; Cystolith absent
4b.	Leaves ovate to elliptic, pubescent; Cystolith present
5a.	Leaves sessile, lanceolate, oblong, cordate, both surface pilose; Bracts imbricate broadly ovate to obovate spathulate; Corolla funnel shape, lobe pale purple
5b.	Leaves petiolate, elliptic, ovate, glabrous or pubescent; Bracts leaflike imbricate broadly ovate or oblanceolate to obovate; Corolla straight to slightly bent or highly curved, lobe blue colour
6a.	Leaves both surface sparsely stringose, densely covered with subulatecystoliths; Bracts imbricate broadly obovate, rhomboidal or elliptic; Corolla purplish-blue to violet, curved
6b.	Leaves adaxially sparsely pubescent, numerous cystolith; Bracts obovate to subrhombic, distinctly angled; corolla blue to violet, curved
7a.	Isophyllous to weakly anisophyllous; Leave glabrous; Bracts oblanceolate to obovate; Corolla straight to slightly bent, lobe blue  S. cusia
7b.	Isophyllous; Leaves pubescent on both sides; Bracts broadly ovate; Corolla curved, funnel shaped, lobe purple S. recurva.





Image 2. Image of the herbarium specimen of Strobilanthes recurva deposited at IBSD (IBSD/M-279).

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SHORT COMMUNICATION

### New distribution record of Slender Wild Basil Clinopodium gracile (Benth.) **Kuntze (Lamiaceae: Nepetoideae: Mentheae)** for the flora of Himachal Pradesh, India

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Abstract: Clinopodium gracile (Benth.) Kuntze has been recorded for the first in the Chamba district of Himachal Pradesh. The study provides descriptions, illustrations, and photographs to aid in the identification of the species. The new distribution record has been entrenched upon investigation based on observation and verification obtained from herbarium, literature, and plant web databases.

**Keywords:** Calamint, distribution, flora, identification, keys, native.

The mint family Lamiaceae Martinov (also known as Labiatae Juss.) has profound distribution, confining 232 accepted genera. According to Angiosperm Phylogeny Group (APG IV), the genus 'Clinopodium' is bound within the sub-family Nepetoideae (Dumortier) Luerssen and tribe Mentheae Dumortier (Stevens 2001 onwards). Clinopodium L. comprises about 20 species in Asia and Europe (Li & Hedge 1994). In accord with Plants of World Online (POWO 2024), Clinopodium has nativity in tropical and sub-tropical regions with 186 species. The genus is commonly known as Calamint (Weakley 2010) due to the inclusion of Calamintha Mill. members. Doroszenko (1985) served Clinopodium as a section of Calamintha. However, the latter remains as a synonym for the former genus due to the priority of Clinopodium (1753) over Calamintha (1754). According to the database of POWO, Clinopodium is native to India and portrayed by seven species: Clinopodium capitellatum (Benth.) Kuntze, C. gracile (Benth.) Kuntze, C. hydaspidis (Falc. ex Benth.) Kuntze, C. javanicum (Blume) I.M.Turner, C. piperitum (D.Don) Murata, C. umbrosum (M.Bieb.) K.Koch., and C. vulgare L. The species 'Clinopodium gracile' is native to Asia (Weakley 2010). It can be allocated from China, Indonesia, Japan, Jawa, Korea, Laos, Malaysia, Myanmar, Taiwan, Thailand, Vietnam, and India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim, & Tripura). It commonly flourishes near river banks, wild/semi-wild areas, and alongside forest margin sites (Li & Hedge 1994). In accord with Cantino & Wagstaff (1998), the species is associated with a morphologically diverse and taxonomically complex group called the 'clinopodioid complex'. The species retain a 2n = 18 chromosomes number and distinct in possessing one vascular bundle in its petiole (Hsieh & Huang 1998).

Clinopodium gracile is known as slender wild Basil (Zomlefer et al. 2011). According to Mao & Dash (2020), Clinopodium is represented by three species in Himachal Pradesh: Clinopodium capitellatum, C. umbrosum, and C. vulgare. However, in accord with POWO (2024), three additional species C. hydaspidis, C. javanicum and C. piperitum can also be found. The presently

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examined `Clinopodium gracile' is the seventh addition of Clinopodium species to the flora of Himachal Pradesh.

### **MATERIALS AND METHODS**

The first author collected the plant specimen from Gajnoi region during a field trip in Chamba district, Himachal Pradesh. Field photographs, and GPS location were recorded and flowering plant parts were collected for further investigations. The morphological attributes, including stem, leaves, inflorescence, bract, flowers, calyx, corolla, androecium, and gynoecium have been studied. The vegetative and reproductive features have been examined and the species was identified as Clinopodium gracile (Benth.) Kuntze. Further, the species identity was confirmed based on microfilms of herbarium records as furnished in specimen examined section. The voucher specimen of the presently examined species has been deposited in GUBH. The map showing the precise location of Clinopodium gracile in the Chamba district of Himachal Pradesh has been laid out (Figure 1).

Also, a photoplate (Image 1) and an illustration (Figure 2) displaying the vegetative and reproductive parts have been presented.

### **Taxonomic treatment**

Clinopodium gracile (Benth.) Kuntze Revis. Gen. Pl. 2:514.1891. —Clinopodium confine (Hance) Kuntze Gen. Pl. 2:515.1891; Hara In: Jour. Jap. Bot. 101:14.1935. — Clinopodium gracile (Benth.) Matsum Index. Pl. Jap. 2:538.1912 (isonym). —Calamintha gracilis Benth. In: DC. Prodr. 12:232.1848. —Calamintha moluccana Miq. Fl. Ned. Ind. 2:968.1859. —Calamintha confinis Hance In: Journ. Bot. 6:331.1868. —Calamintha radicans Vaniot Bull. Acad. Geogr. Bot. 14:182.1904. —Calamintha argyi H.Lév. In: Repert. Spec. Nov. Regni Veg. 8:423.1910. —Satureja gracilis (Benth.) Nakai In: J. Coll. Sci. Univ. Tokyo. 31:149.1911; Bailey In: Gent. Herb. 1:43.1920. — Satureja confinis (Hance) Kudô Mem. Fac. Sci. Taihoku Imp. Univ. 2:100.1929.

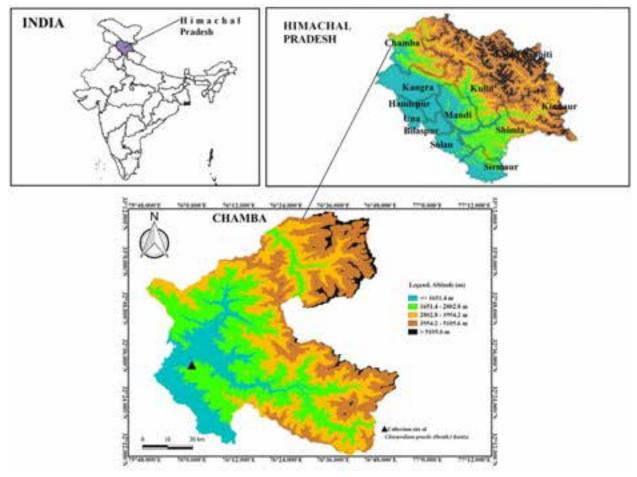


Figure 1. Map showing geographical location of *Clinopodium gracile* (Benth.) Kuntze in Chamba District of Himachal Pradesh (Map prepared through QGIS software).

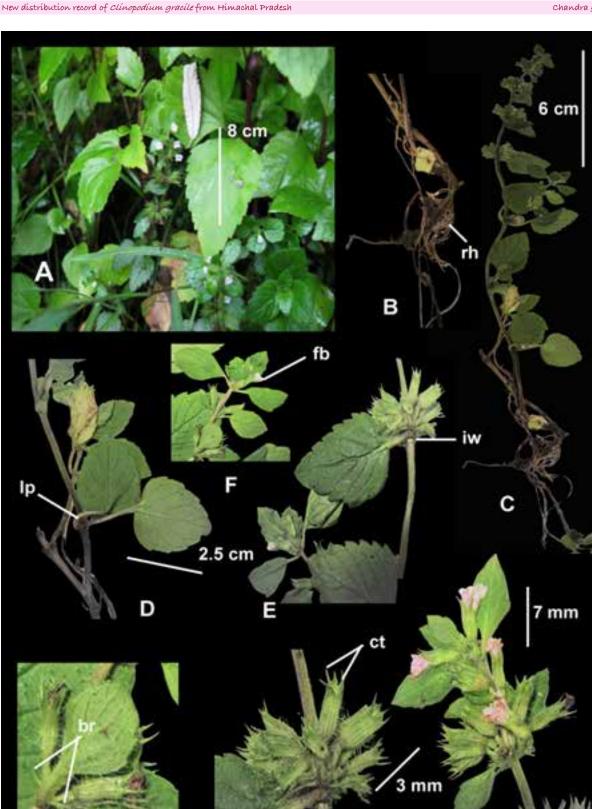


Image 1. Clinopodium gracile (Benth.) Kuntze: A—Habit of the plant | B—Stoloniferous root showing root hair (rh) | C—Whole plant revealing its vegetative and reproductive parts | D—Abaxial surface of leaf with leaf petiole (Ip) | E—Adaxial surface of leaf and inflorescence whorl (iw) | F—Young shoot displaying floral bud (fb) | G—Base of pedicel manifesting bract (br) | H—Calyx portraying its tooth (ct) | I—Verticillaster inflorescence exhibiting flower arrangement. © Rimjhim Chandra.



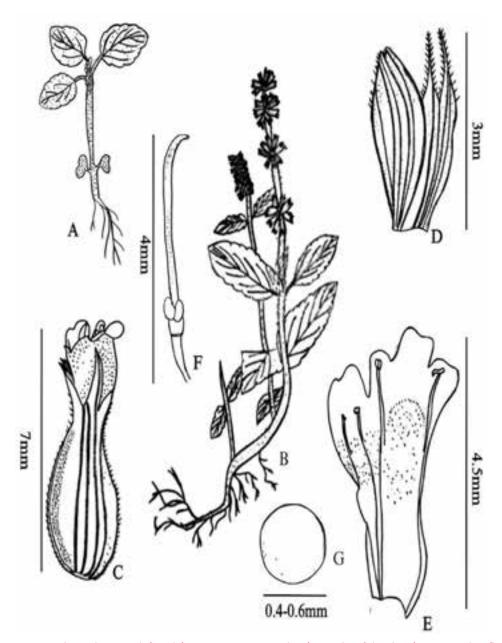


Figure 2. Clinopodium gracile (Benth.) Kuntze. A—Young sapling | B—Habit of the plant | C—A complete flower | D—Dissected view of the calyx | E—Corolla showing epipetalous stamens | F—Gynoecium | G—Nutlet. © Rimjhim Chandra.

### Description

Small perennial herb, slender, stoloniferous; Stem quadrangular, numerous, tufted, erect or procumbent at the base, ascending, 8–30 cm, retrorse, pubescent; Leaves opposite, basal leaves circular-ovate, 1.2–1.5 × 1–1.1 cm, base rounded, apex obtuse, margin remotely crenate, lower and mid stem leaves ovate, 1.5–2.5 × 1–1.5 cm, papery, sub-glabrous, abaxially sparsely hispid on veins, apex obtuse, base rounded to cuneate, margin remotely dentate or crenate-serrate, petioles 0.3–1 cm; Inflorescence verticillaster, few flowered (5–10), lax or

dense, crowded in short terminal raceme, floral leaves ovate-lanceolate,  $0.5\text{--}1 \times 0.3\text{--}0.8$  cm, margin serrate, acute; Bract linear, acute, puberulous, much shorter than pedicel; Flower rose-pink,  $5\text{--}7 \times 3\text{--}4$  mm, pedicel 1–3 mm; Calyx tubular, base rounded, ca. 3–5 mm and declinate in fruit, puberulent or subglabrous, minutely hispid on veins, throat sparsely fine pilose, teeth ciliate, lower two subulate, upper three triangular, reflexed in fruit; Corolla ca. 4.5 mm, puberulent; nutlets ovoid, smooth 0.4--0.5 mm (Image 1 and Figure 2).

Common name: Calamint, Savory, Slender Wild Basil,

### Key to four species of Clinopodium L.

- 1. Calyx with glandular hairs
  - 2. Calyx teeth ciliate; leaves ovate; verticillate flowers crowded nearby; corolla rose-pink; nutlets ovoid; stoloniferous .............

- 2. Calyx teeth long; leaves sub-obtuse; verticillate flowers distantly cyme; corolla purplish; nutlets ellipsoid; woody rootstock ......
- 1. Calyx with eglandular hairs
  - 3. Calyx with bristly hairs; leaves sharply toothed margin; many (30-40) flowered whorls; lower corolla with notched centre lobe
  - 3. Calyx with wooly hairs, leaves toothless or shallowly toothed; few (10-20) flowered whorls; lower corolla with smooth centre

### Tower Flower.

Flowering: The flowering has been observed in July. Habitat: Stream sides, open grasslands, forest margin, and thickets.

### Specimen examined

China: Si-chu-shan, C. Sampson, i.1968, coll. No. 13045, K000193480! Indonesia: Bakh; Brink, 27.vii.1920, coll. No. 4839, K000193477! Japan: Pref. Chiba, Nokogiriyama, Yoshihiro Asai, 3.v.1959, coll. No. 8518, NY 02706540! USA: Florida, J.R. Burkhalter, 30.iv.1980, coll. No. 6908, 149957! Louisiana, S.R. Hill, 20.iv.1998, coll. No. 29989, 278039! South Carolina, K.A. Bradley, 22.vi.2016, altitude 165 m, coll. No. 4693, 295537! Florida, Tallahassee, Loran C. Anderson, 25.v.2017, 30.44718 N 84.25225 W, coll. No. 30676, NY 04416374! Louisiana, Iberia Parish, Roland M. Harper, 16.vii.1934, NY 03030120! India: Himachal Pradesh, Jot Chamba, Gajnoi, Rimjhim Chandra, 27.vii.2023, 32.4868 N 76.0593 E, altitude 2,880 m, coll. No. 103.

### **Taxonomic note**

The specific epithet 'gracile' (Latin–gracilis) suggests the slender and delicate habit of the species. Calamintha gracilis Benth. is the basionym for Clinopodium gracile (Benth.) Kuntze. Bentham (1848) showed uncertainty about the species kinship to the genus 'Clinopodium'. He further considered Clinopodium gracile features somewhere between Clinopodium debilis (C. debile) and Clinopodium umbrosa (C. umbrosum). (1965) mentioned four varieties of Clinopodium gracile viz. C. gracile var. latifolium (H.Hara) Ohwi, C. gracile var. minimum (H.Hara) Ohwi, C. gracile var. multicaule (Maxim.) Ohwi, C. gracile var. sachalinense (F. Schmidt) Ohwi. However, these remain as synonyms of *C.* latifolium, C. multicaule var. yakusimense, C. multicaule, C. micranthum var. sachalinense respectively. No infra-specific ranks are available for Clinopodium gracile (POWO 2024).

### **DISCUSSION**

Chen et al. (2013) reported 34 essential oil compounds in Clinopodium gracile with higher content of sesquiterpenoids followed by monoterpenoids. Thus, the species occurring in the Himachal Pradesh region can also act as a potent therapeutic plant. The species can be efficacious in floristic, biodiversity, and conservation studies. The present examined species is similar in morphological description provided by Weakley (2010).

#### **CONCLUSION**

The perennial herb Clinopodium gracile, was collected from natural habitat of Gajnoi, Chamba district of Himachal Pradesh. The location site was specified with 12 numbers of C. gracile plants, growing in deteriorated broad-leaved oak and pine forest. Few other species flourishing along with C. gracile were Ageratum conyzoides L., grasses being Cynodon sp., Stachys sp., ferns such as Diplazium sp., and Matteuccia sp. The species might be a prospective for multitude of medicative activities. The findings can further assist in exploring the extended distribution of C. gracile in the western Himalayan belt.

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### Small Wild Cats Special Series

### Rusty-spotted Cat *Prionailurus rubiginosus* (I. Geoffroy Saint-Hilaire, 1831) (Mammalia: Carnivora: Felidae) in the semi-natural subterranean habitat in Karnataka, India

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On 29 March 2023, we conducted a rapid survey in three abandoned gold mines in Kappatagudda Wildlife Sanctuary in Gadag, Karnataka, India (Image 1) to explore the biological diversity in these artificial subterranean habitats. The habitat around these mines is characterised by dry grasslands interspersed with thorn scrub forests (Image 2). Due to the presence of groundwater, our survey of the mines was limited to the upper strata. While surveying one of the mines in Jalligeri village of Shirahatti taluka in Gadag district, we observed pug marks of a small mammal. Approximately 85 m from the tunnel's entrance, we found the carcass of a male Rustyspotted Cat Prionailurus rubiginosus (Image 3).

The carcass was taken to Gadag Zoo for further examination (Image 4). The cat measured 68.58 cm in total length, 35.56 cm in body length, with a 10.16 cm head, 22.86 cm long tail, 22.86 cm in height, and chest girth of 35.56 cm, indicating that it was an adult. The gut contained a partly digested unidentified bat species

(Microchiroptera) (Image 5). The dissection examination of the carcass revealed exophthalmia of eyeballs, gelatinisation of subcutaneous fat, paleness and friable nature of kidneys, half-digested food, severe congestion in the right lobe of the lung, passed off rigour mortis, and initiation of the secondary flaccidity. We therefore estimated that the time of death was within 48-72 hours prior to the collection (Sastry 2020). The cause of death could not be determined.

Rusty-spotted Cat predation on bats is evident from at least one instance in Gujarat (Devkar et al. 2016). Other studies on Rusty-spotted Cat diet indicate that rodents form its primary prey (Mukherjee et al. 2016; Bora et al. 2020; Chaudhary et al. 2022; Mukherjee et al. 2024). This individual possibly visited this cave for hunting, as caves have a rich presence of bats and rodents (Busch et al. 2000). The Rusty-spotted Cat is claimed to also use caves and crevices in other parts of India (Patel 2011; Vyas & Upadhyay 2014).

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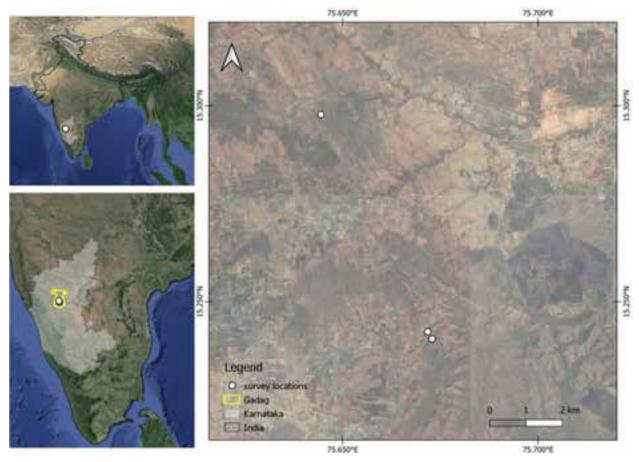


Image 1. Map showing the location of the surveyed human-made subterranean spaces in Kappatagudda Wildlife Sanctuary, Karnataka, India.



Image 2. The habitat around the abandoned gold mine. © Shirish Manchi.

Between 1900 and 1922, horizontal tunnels were dug in Indian forests for gold mining, but they have been abandoned since mining ceased in the early 1990s. The

mines have varying lengths and structures with several subterranean water patches. Due to infiltration from rainwater, these tunnels are inaccessible for most part





Image 3. The carcass of the Rusty-spotted Cat found in the abandoned gold mine, Kappatagudda Wildlife Sanctuary. © Shirish Manchi.

of the year. Horizontal branching tunnels could only be reached via wet ground and shallow water ditches. The oligotrophic subterranean habitat harboured several amphibians, sustained mainly by organic matter from the bats inhabiting the roof and dropping their guano.

Our observations suggest that the subterranean habitats are possible additional important areas of use for the Rusty-spotted Cat, especially given the availability of prey such as bats. The Rusty-spotted Cat possibly contributes to the food chain of this semi-natural subterranean habitat, both as a top predator and as an additional energy source to this oligotrophic ecosystem. Further investigation on this aspect is necessary to uncover the extent of its contribution to this ecosystem.

Ecologically, subterranean habitats are still poorly understood (Wynne et al. 2021). They contain unique and specialised biozones of high scientific interest and conservation potential (Stone et al. 2005). Abandoned



Image 4. Measuring the morphometric characteristics of the dead Rusty-spotted Cat found in the abandoned gold mine. © Shirish Manchi.



Image 5. A bat (Microchiroptera) was recovered from the gut of the carcass of the Rusty-spotted Cat. © Shirish Manchi.

mines are semi-natural habitats and complex ecosystems (Lenart et al. 2022).

The presence of a Rusty-spotted Cat, a Near Threatened (Mukherjee et al. 2016) and Schedule-I species (Ministry of Law and Justice 2022), in a human-made subterranean habitat demonstrates the high conservation value of this habitat and calls for additional research to explore underground ecosystems in the cat's range. We urge local, state and national governments and other stakeholders to safeguard and oversee both human-made and natural underground habitats in order to maintain and preserve these under-researched crucial habitats for a multitude of identified and unidentified species that rely on them.



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### First record of Scaly-breasted Munia Lonchura punctulata (Linnaeus, 1758) (Aves: Passeriformes: Estrildidae) from Kashmir, India

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Scaly-breasted Munia Lonchura punctulata is a small-sized bird from the family Estrildidae, commonly known as waxbills, grass finches, or the munia family. Scaly-breasted Munia is also sometimes referred to as Spotted Munia. This bird is indigenous to Asia with a broad geographic range, including India, China, Bangladesh, Afghanistan, Bhutan, Cambodia, Indonesia, Nepal, Singapore, Sri Lanka, Thailand, and Vietnam (Arigela et al. 2020; Bird Life International 2023). It has been introduced to Australia, Hawaii, Puerto Rico, Florida, Japan, and California. In Oahou and Hawaii this introduction has led to the establishment of wild populations that compete with other munia species, such as Tricolored Munias Lonchura malacca, which were also introduced at the same time (Moulton et al. 1992; Payne 2020). Scaly-breasted Munias live in wet and high grasslands, often with other munias. They eat grass seeds, berries, algae, and insects (Payne 2020). They roost in bushes, trees, and cycas plants. In southern India and Indonesia, they are considered crop pests.

Current literature reports Scaly-breasted Munia in Jammu province of the Union Territory of Jammu & Kashmir, India which is ecologically different from

Kashmir (Grimmet et al. 2011; Suhail et al. 2020). Here we present Scaly-breasted Munia from Kashmir province with photographic evidence (Image 1-4). During our field surveys, Scaly-breasted Munia, was observed at three locations, viz., Kaman post Uri, Salamabad Uri, and Ladoora area in Baramulla District along the river Jhelum (Image 5). Initially the bird was sighted at Kaman post (34.100°N & 74.022°E) on 2 September 2022, at 1022 h perching on the transmission wires. During a second visit on 17 June 2023 in the Salamabad area (34.095°N & 74.022°E), a flock of Scaly-breasted Munias was sighted at 1430 h near the hydropower project dam. The third sighting took place at the Ladoora (34.252°N & 74.401°E) area of Baramulla on 21 July 2023 at 1500 h. The bird was observed near a footbridge, perching on an electric wire, and feeding on grass. The first author is investigating riverine bird diversity, conducting comprehensive bird surveys in the area to identify, and document bird species. As munias are popular cage birds, the birds seen in Kashmir could be escapees. As munias have been successfully introduced in many countries, they are hardy enough to establish wild populations by the escaped birds from the cages.

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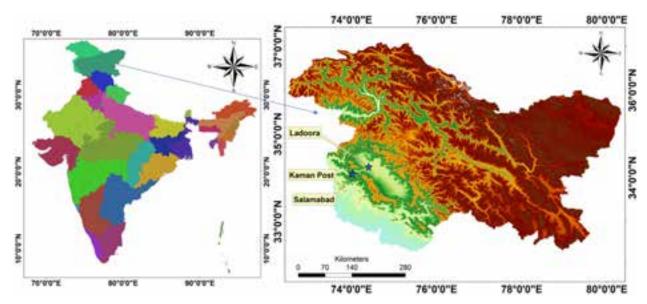


Image 1–2. Scaly-breasted Munia Lonchura punctulata at Kaman Post and Salamabad Uri, Baramulla, Kashmir. © Shazia Shafayat.





Image 3–4. Scaly-breasted Munia Lonchura punctulata at Ladoora, Baramulla, Kashmir. © Shazia Shafayat.



 $Image \ 5. \ Map \ showing \ sighting \ locations \ of \ Scaly-breasted \ Munia \ in \ Kashmir, \ India.$ 

Many times, people become tired of their pet birds and release them. Most do not survive, but many species (e.g., munias, parakeets, mynas) survive and establish wild populations.

The bird is characterized by its rich reddish-brown plumage, notably darker on the chin, and throat (Image 1–4). The breast and belly display a striking contrast, appearing white with black feather edges that create a scaled pattern which distinguishes the bird from other munias. The mantle, back, and wings exhibit a slightly paler and duller hue, adorned with subtle barring, and occasionally more prominent pale shaft streaks. The bird's eyes have a dark brown to brownish-red iris, the bill is blackish, and the lower mandible may exhibit a paler bluish-grey shade. The rump showcases distinctive dark brown and whitish or yellowish bars, while the upper tail coverts feature a subdued golden-yellow to orange-brown coloration. The species was identified using Grimmett et al. (2011).

The bird is a new member to the avifaunal diversity of Kashmir, India. There is limited scientific investigation

into the avian diversity inhabiting the northern Kashmir, which needs scientific attention in the form of intensive surveys.

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### First record of *Proszynskia diatreta* (Simon, 1902) (Araneae: Salticidae) from Gujarat, India

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The genus Proszynskia Kanesharatnam & Benjamin, 2019 was established with Proszynskia diatreta (Simon, 1902) as its type species (Kanesharatnam & Benjamin 2019; World Spider Catalog 2024). P. diatreta is presently known from India and Sri Lanka (Kanesharatnam & Benjamin 2019). In India, the species has been documented only from two localities in Tamil Nadu (Caleb 2016). In this study, we report the discovery of P. diatreta in Gujarat, making it the northernmost distributional record of this species.

The specimen was hand-collected and studied under a Leica M205 A stereomicroscope and microphotographic images were taken by a Leica DFC2900 digital camera attached to the stereomicroscope and enabled with the software package Leica Application Suite (LAS), version 4.5.0. Left palp was studied and photographed by placing in a cavity block filled with ethanol. The species was identified based on Kanesharatnam & Benjamin (2019). The examined specimen has been deposited in the reference collection of the Web of Nature (WON) Research Foundation, Gujarat, India (curator: Dhruv A. Prajapati).

Family Salticidae Blackwall, 1841 Genus Proszynskia Kanesharatnam & Benjamin, 2019 Type species: Viciria diatreta Simon, 1902

Proszynskia diatreta (Simon, 1902) (Images 1–3)

Viciria diatreta Simon, 1902: 366

Viciria diatreta Prószyński, 1984: 433, figs 42-43 Viciria diatreta Caleb & Mathai, 2014: 65, figs 38-46 Phintella diatreta Caleb, 2016: 274

Proszynskia diatreta Kanesharatnam & Benjamin, 2019: 54, figs 23E-H, 24A-E, 25A-E, 26A-D

Material examined: WON102495A, 3.viii.2016, 1 male, India, Gujarat, Ahwa village in Dang district (20.7488 °N, 73.6916 °E), 461 m, leg. D. Prajapati.

Diagnosis: The species can be easily identified by the short, straight and robust embolus directed at 12 o'clock position ventrally (Image 2); tegulum with significant prolateral proximal lobe (Image 2); retrolateral tibial apophysis bent at the tip and directed at 10 o'clock position (Image 3).

Distribution: India and Sri Lanka (Simon 1902; Kanesharatnam & Benjamin 2019) (Figure 1).

Distribution in India: Tamil Nadu (Simon 1902) and Gujarat.

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Image 1–3. Male *Proszynskia diatreta* (Simon, 1902) from Gujarat, India: 1—habitus, dorsal view | 2—left pedipalp, ventral view | 3—same, retrolateral view. Scale bars: 2 mm (1), 0.5 mm (2–3). © WON Research Foundation.

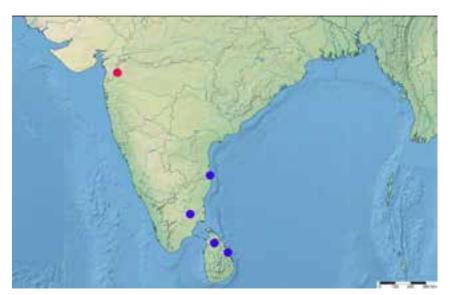


Figure 1. Distribution of Proszynskia diatreta (Simon, 1902). (pink circle—new record; blue circles—previous records)

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# Medicago monantha (Fabaceae) and Euphorbia jodhpurensis (Euphorbiaceae) as new additions to the flora of Maharashtra State, India

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Abbreviations: BLAT—Blatter Herbarium, St. Xavier's College (Autonomous), Mumbai, Maharashtra 400001 | BSJO—Botanical Survey of India, Arid Zone Circle, Jodhpur, Rajasthan, India | CAL—Botanical Survey of India, Central National Herbarium, Howrah, West Bengal, India | LE—The Komarov Botanical Institute of RAS, Saint Petersburg, Russia.

During the recent (2019–2022) botanical survey of Daund Tehsil in Pune District of Maharashtra State, two plant specimens belonging to the genera Medicago L. and Euphorbia L. were collected. On the thorough scrutiny of the literature (Bentham 1839; Trautvetter 1840; Baker 1879; Hooker 1890; Blatter & Hallberg 1920; Ali 1977; Purohit et al. 2019), they have been identified as Medicago monantha (C.A. Mey.) Trautv. (1840, Fabaceae) and Euphorbia jodhpurensis Blatt. & Hallb. (1920, Euphorbiaceae), which were hitherto not reported from the Maharashtra State. The herbarium specimens (Image 1B & 1C) of the plant species reported herein are deposited at the Blatter Herbarium (BLAT), St. Xavier's College (Autonomous), Mumbai. The detailed descriptions, distribution, and photographs of both species are provided herein for their easy identification.

## Systematic treatment Fabaceae

Medicago monantha (C.A. Mey.) Trautv., Index Seminum [Kiev] 1840: vi, adnot. No. 4. 1840; Mishra et al., Indian J. Plant Sci. 4(3): 70. 2015; Mishra et al., Int. J. Herb. Med. 3(4): 11. 2015; Mishra et al., Indian J. Plant Sci. 4 (4): 92. 2015; Pandey et al., J. Med. Plants Stud. 5(5): 53. 2017; Kaur et al., J. Threat. Taxa 9(8): 10556. 2017; Sanjappa & Ambrish in Dar & Khuroo, Biodivers. Himal.: Jammu & Kashmir State: 640. 2020; Sharma, Int. J. Innov. Res. Sci. Eng. 11(5): 6098. 2022; Sharma, Int. J. Innov. Res. Sci. Eng. 11(7): 9623. 2022; Choudhary et al., Int. Res. J. Modern. Eng. Technol. Sci. 5(8): 565-569. 2023. Trigonella monantha C.A. Mey., Verz. Pfl. Casp. Meer.: 137. 1831. Trigonella polyceratia auct. non. L. (1753): Baker in Hook. f., Fl. Brit. India 2: 87. 1879; Duthie, Fl. Gangetic Plain 1(1): 209. 1903; Bamber, Pl. Punjab.: 554. 1916; Babu, Herb. Fl. Dehradun: 168. 1977; Sharma & Dhakre, Fl. Agra Distr.: 96. 1995. Trigonella incisa Royle ex Benth., Ill. Bot. Himal. Mts.: 197. 1839; Aitchison, Cat. Pl. Punjab and Sindh: 38. 1869; Maheshwari, Fl. Delhi: 115. 1963; Nair, Rec. Bot. Surv. India 12(1): 101. 1978; Sanjappa, Legumes of India: 265. 1992; Bhellum

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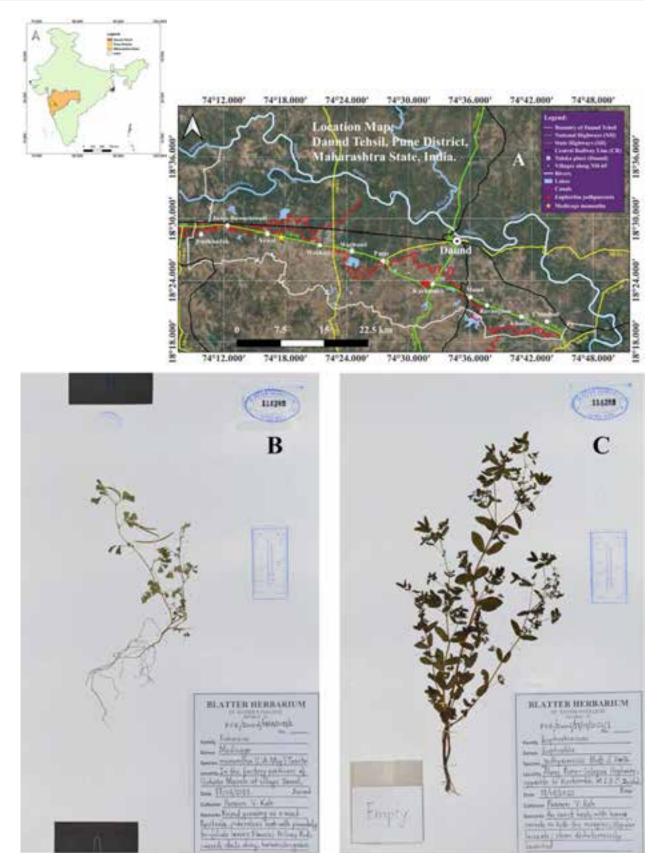


Image 1. A—Location Map | Herbarium specimens deposited at BLAT: B—Medicago monantha (C.A. Mey.) Trautv. | C—Euphorbia jodhpurensis Blatt. & Hallb. © The Director, Blatter Herbarium, St. Xavier's College (Autonomous), Mumbai.

& Magotra, J. Plant Biol. Res. 1(2): 7. 2012. *T. monantha* ssp. *incisa* (Benth.) Ali in Nasir & Ali, Fl. W. Pakistan, Papilionaceae 100: 294. 1977; Singh in Shetty & Singh, Fl. Rajasthan 1: 269. 1987. (Image 2H–J).

Annual, silvery-puberulous herb with prostrate to ascending slender stem. Leaves pinnately 3-foliolate, strongly nerved; petioles 1-1.5 cm long; leaflets 5-10 × 3–7 mm, obovate-oblong, retuse at apex, cuneate at base, toothed mainly in upper part, sub-glabrous above, sparsely silvery-puberulous beneath; lateral nerves 5-8, conspicuous; petiolules of lateral leaflets are very short, those of terminal leaflets 1-1.5 cm long. Stipules are linear-subulate, puberulous, 3-6mm long, single-nerved. Inflorescence axillary, sessile or pedunculate, 1-3-flowered in axillary sub-umbellate clusters; peduncles 0.5–1.5 cm long, puberulous; bracts linear-subulate, puberulous, 1-2 mm long; pedicels less than 1mm long. Calyx campanulate, linear-lanceolate, very acute, 3-5 mm long, puberulous outside; teeth 5, sub-equal. Corolla yellow; standard 5–7  $\times$  2–2.5 mm, obovate, narrow towards base, retuse; wings 3.5-4 × 1 mm, oblong-obovate, obtuse, claw 2.0-2.3 mm long, upper auricle 0.5-1 mm long; keels 3.5-4 x 1 mm, oblong-obovate, slightly curved towards apex, rounded, claw 2 mm long, flat, upper auricle minute. Stamens glabrous; vexillary filaments 2.3-3 mm long, flat; staminal sheath 2.5-4 mm long; free filaments 0.5-0.7 mm long, sub-equal; anthers minute. Ovary 2.7-3.3 × 0.5 mm, narrowly oblong, ovules many, very minute hairs present along dorsal side when young, densely sericeous at maturity; style 0.5 mm long, subulate, flat, curved, glabrous, persistent; stigma capitate, glabrous. Pods greyish-green, 2.7-5.5 × 0.1-0.2 cm, linearcylindrical, somewhat compressed, straight or slightly falcate, apiculate, reticulately veined, 12-15-seeded; seeds brownish-green.

Flowering & Fruiting: March-May.

Habitat & associates: In the garden premises of Pravin Masalewale (Suhana Masala) factory unit located along the Pune–Solapur Highway Road, found growing as a weed together with *Medicago polymorpha L., Solanum nigrum L., Dactyloctenium aegyptium* (L.) Willd., *Eragrostis tenella* (L.) P. Beauv. ex Roem. & Schult. and *Setaria verticillata* (L.) P. Beauv.

Distribution in India: So far has been reported from Delhi, Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab, Rajasthan, Uttarakhand, and Uttar Pradesh. The present collection from Yawat village, Daund tehsil, Pune district, Maharashtra State, India (Image 1A), forms its first report for the State.

Specimens examined: Maharashtra, Suhana Masale

Factory Premises, Yawat, Daund tehsil, Pune district, 18.469 °N, & 74.294 °E, 17.iii.2019, coll. P. V. Kale, P.V.K./Daund/17/03/2019/2 (BLAT Accession number 114280).

### Euphorbiaceae

Euphorbia jodhpurensis Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 26(4): 971. 1920; Bhandari, Fl. Ind. Desert: 342. 1978; Parmar in Shetty & Singh, Fl. Rajasthan 2: 781. 1991; Binojkumar & Balakrishnan in Balakrishnan et al., Fl. of India 23: 291. 2012; Purohit et al., Euphorbia World 15(3): 18–22. 2019; Kotiya et al., Fl. Rajasthan: 441. 2020. Euphorbia clarkeana var. erecta Hook.f., Fl. Brit. India 5: 254. 1890. Chamaesyce jodhpurensis (Blatt. & Hallb.) Raju & Rao, Phytologia 40(5): 392. 1978. (Image 2A–G).

Annual, about 15-60 cm high, glabrous, filiform; stem straw-coloured, erect, simple, or branched from base. Leaves opposite,  $12-15 \times 3-4.5$  mm, obliquely linear-oblong, base almost auricled on one side, margins usually spinulose-serrate, apex obtuse or sub-acute, coriaceous, often crowded at end of branchlets; petioles very short. Stipules lacerate. Cyathia solitary, axillary, less than 1 mm long, campanulate, glabrous; involucral lobes 5, lanceolate, triangular, toothed; glands 4, minute, rounded with pinkish-white orbicular appendages. Male flowers 3-4, pedicels ca 0.5 mm long; anthers subglobose, transversely dehiscing. Female flower solitary, pedicels ca 1 mm long, glabrous; ovary sub-globose, ca 1 mm across; style 3, ca 1.2 mm long, free from base, each bifurcated up to base; stigma capitate. Capsules yellowish-green with a reddish tinge, sub-globose 1.5-1.7 mm in diam., glabrous, obtusely keeled, cocci somewhat cornet; seeds rose-brownish, 1-2 mm long, 4-angled, attenuated and obtuse at apex, distinctly transversely rugose, delicately foveolate.

Flowering & Fruiting: September-December.

Habitat & associates: In sandy soils near the Kurkumbh M.I.D.C. along the Pune–Solapur Highway Road, found growing together with *Euphorbia hirta* L., *Tridax procumbens* L., and *Alternanthera pulchella* Kunth.

Distribution in India: The present collection of specimens of *E. jodhpurensis* collected from Kurkumbh village, Daund Tehsil, Pune District, Maharashtra State, India (Image 1A) forms its extended distribution to Maharashtra State from Rajasthan.

Specimens examined: Maharashtra, Along Pune–Solapur Highway opposite to Kurkumbh M.I.D.C, Kurkumbh, Daund Tehsil, Pune District, 18.395 °N & 74.525 °E, 17. ix.2022, coll. P. V. Kale, P.V.K./Daund/17/09/2022/1 (BLAT Accession number 114283).



Image 2. Euphorbia jodhpurensis (A–G): A—Habit | B—Lacerate stipules | C—Leaves showing margins spinulose-serrate | D—Inflorescence | E—Male flowers | F—Cyathium showing male & female flowers | G—Seeds. Medicago monantha (H–J): H—Habit | I—Single flower | J—Pods.

IUCN Red List status: Purohit & Kulloli (2022) listed *Euphorbia jodhpurensis* as 'Endangered' under criteria B2ab(iii), D.

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BOOK REVIEW

## All eyes on the island A book review of The Great Nicobar Betrayal

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I tried to stifle a yawn as my monotonic Physics teacher read out the formula of displacement from her notes.

### Thrust force = $p \times v \times g$

"Where  $\rho$  is the density of the liquid, V is the volume of liquid displaced and g is the acceleration due to gravity." I wrote it down in my notebook and tried assigning a mnemonic to commit it to memory.

As the seasons changed and my interest in environmental matters grew stronger, I learnt a new formula for displacement that involved different terms.

Displacement was now the sum total of a Transshipment port, an International Airport, a Power plant, and a Township; all coming together as the Great Nicobar mega project.

This is one formula that needs to be committed to public memory.

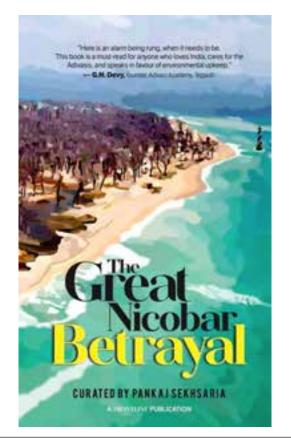
On Google Maps, one has to zoom in at least thrice for a clear view of the Andaman and Nicobar Islands. Tucked away in a remote corner of our planet, a few regions of the Indian archipelago are still home to pristine natural ecosystems due to the sea barrier and island conditions that limit mass entry and proliferation. The biodiversity that these islands hold is like no other. Its flora and fauna have deeply complex relationships with the island tribes and is one of the rarest places on Earth where there is an instinctive respect and understanding between humans, wildlife, and forests.

However, a series of decisions taken in mainland India threaten to throw this sacred connection off the course. The proposed site of the mega project in Great Nicobar sits on a zone that is prone to earthquakes, tsunamis and worst of all, apathy.

For those keen on understanding the effects of capitalism on natural ecosystems, **The Great Nicobar Betrayal** is a great place to start. It is a timely book of curated articles on the repercussions of the Great

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Nicobar mega project, weaved together masterfully by Pankaj Sekhsaria. The book explores multiple viewpoints of the project's impact from researchers, journalists, ecologists, and scientists. The story comes together like pieces of a jigsaw puzzle to give the reader a full view of the havoc that is about to be inflicted on the island.

The Government of India through NITI Aayog had proposed the mega project in 2021 in a scale that appears humungous for the mainland itself. The project envisions port connectivity, infrastructural development, and employment opportunities for the inhabitants of the Great Nicobar Island, the last land mass in the Andaman and Nicobar archipelago. The project is priced at INR 72,000 crores with grand plans that would require around 130 sq.km of primary forests to be cleared.

It seems like the apocalypse is not a single event; it is a string of careless verdicts like these. Pankaj Sekhsaria introduces the islands, the project and throws light on the absurdities of the environmental assessments, convenient legal loopholes and criminal leniencies in forest and land clearances procured.

Most earthquakes originate in the margins of Great Nicobar. 'Following building codes is one thing but going ahead and building on a fault line is reckless', the authors, Janki Andharia, V Ramesh and Ravinder Dhiman point out.

To the sane, this is a risky proposition and the fault lines are not just wobbly tectonic plates.

A planned influx of around 3,50,000 people to an island that strains to meet demands of 8,000 people is sure to drive local tribals to extinction. Marooned on an island of worry, the Particularly Vulnerable Tribal Group – the Shompens and other tribes like the Nicobarese are left to fend for themselves. Manish Chandi shares snippets of his interactions with tribal leaders and families that have been displaced post the tsunami. Some tribal members lament that they have been lied to about the scope of the project and the damage from it is expected to be worse.

The articles in the book have been arranged in a such a way that they help the reader understand the linkages between them. For example, the irrationality of compensatory afforestation in Haryana is mentioned in the earlier chapters by Sekhsaria with some authors like Ishika Ramakrishna driving the point home through further explanation. The outlined areas for forest diversion to the mainland include habitats of the Nicobar Long-Tailed Macaque and many other endemic species like the Nicobar Treeshrew. The forests themselves date back to the Pleistocene period and reducing them to a safari park in mainland India would be deplorable.

The frustrations of the authors are evident in the words that are peppered throughout their articles. Words like absurdity, monumental folly, flawed judgment, economic peril, misadventure, lethal, disastrous, marginalised, misunderstood and their variations feature across the book.

I tried to pick holes in the presentation of the book however this subject aligned with my confirmation bias on the predictability of decisions taken by people who wield power. Once this bias was recognised and kept aside, I felt that more stories on displaced tribal communities could have made the book more impactful to the uninitiated and moved them closer to the epicentre of the issue since displacement is fairly relatable. The concerns on ecological damage could have followed once a strong case on the irreversible loss of ancestral land was set. Each chapter could have also had their associated image for quicker visualization instead of placing them all towards the end of the book.

The format in which the book is presented with various angles to the issue seems like an ideal blueprint for understanding the total effects of infrastructural projects, mostly built with taxpayer money. **The Great Nicobar Betrayal** should find a good audience amongst those holding the slightest interest in environmentalism and one can positively hope that the book will bring the required focus to the issue of displacement of Great Nicobar's natural ecosystems and their custodians – the Shompens and Nicobarese.

Lastly, the timing of the book release is commendable. It works like an out-of-breath town crier arriving in the crowded places of our minds and city spaces to inform us of the happenings in our beloved archipelago.



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