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Cover: Green Bee-eater with colour pencils and watercolor wash by Elakshi Mahika Molur.

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ARTICLE

Documenting butterflies with the help of citizen science in Darjeeling-Sikkim Himalaya, India

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Abstract: The availability of information on the distribution and occurrence of different species in a landscape is crucial to developing an informed conservation and management plan, however such information in the Himalaya is often limited. Citizen science, which builds on the knowledge and interest of communities to contribute to science, can be a solution to this problem. In this study, we used butterflies as a model taxon in the Darjeeling-Sikkim Himalaya which shows how citizen science can aid in documenting biodiversity. The study employed both citizen science, and researcher-survey approaches to collect data, and the collective effort resulted in 407 species, which is the highest by any study carried out in the region. Results show that citizen science can be helpful as a supplementary tool for data collection in biodiversity documentation projects, and can aid in adding to the diversity and distribution records of species, including those that are unique, rare, seasonal, and nationally protected. Citizen science outreach was used to muster potential participants from the local community to participate in the study. Thus, it is advisable for citizen science projects to find means to recruit a larger pool of contributors, and citizen science outreach can be key to their success.

Keywords: Biodiversity documentation, community participation, data collection, outreach.

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Author Contribution AP—conceptualization, methodology analysis, organize CS events, data collection & curation, writing original draft; RG—conceptualization, methodology, organize CS events, data collection & curation, formal analysis, writing & review & editing; SD—data collection, review & editing.

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INTRODUCTION

Citizen Science (CS), which is an approach of involving the public in scientific research, has long been used to supplement collection of data required to answer research questions (Spear et al. 2017), or to document rare events in nature (Greenwood 2007). In recent years there has been an increase in the trend of using CS as a tool in research, documentation, and monitoring (Feldman et al. 2021), with a number of projects using this approach to create awareness, and as a means to engage with the local communities. This has been facilitated by the availability and development of user-friendly applications on smartphones (Land-Zandstra et al. 2016), improved internet facilities, affordable rates for internet access, and most importantly the growing popularity and the scope of CS activity (Curtis 2014). In addition, funding opportunities to implement CS related outreach activities may also have positively influenced this sharp rise (Johnson et al. 2014). The biggest advantage of using CS as a data collection tool is the assumption that vast amount of data can be collected by this approach, as the citizen scientists that this approach targets are mostly local communities who have yearlong access to areas not feasible for researchers to frequently survey or monitor due to limited time & financial constraints (Dickinson et al. 2010).

Participants of CS projects can consist of volunteers from all age-groups, different walks of life, and can be involved in a variety of roles at different stages of the study (Tulloch et al. 2013; Theobald et al. 2015). CS projects can be used in almost every field of research, ranging from marine science (van der Velde et al. 2017), to geography (Trojan et al. 2019), and from astronomy (Odenwald 2018) to biology (Greenwood 2007). This wide range of usability of CS and engagement of enthusiastic citizen scientists has enabled data collection over long periods, and covering larger gradients (Poisson et al. 2020). The use of CS in biodiversity documentation and monitoring is an example of one such long term CS engagement, and this has been dominated by projects involving a few taxa (like birds, butterflies, moths, and dragonflies), probably due to their aesthetic appeal which interests a lot of citizens to participate and contribute (Callaghan et al. 2021). However, despite the interest and the willingness of the citizens to participate and contribute in these projects, a major challenge that hinders the progress of CS projects is the difficulty to incorporate CS data into a research framework (Tulloch et al. 2013) due to the questionable issues associated with the data in terms of accuracy and precision, spatial, temporal resolution, robustness, and access (Hyder et al. 2015). Yet studies have shown that data collected through CS can be crucial for both the scientific community and decision makers (Paul et al. 2014).

Another challenge associated with CS projects is that not everyone is motivated to contribute to these CS projects due to lack of interest or material incentives (Land-Zandstra et al. 2016). The only benefit that the participants of these CS projects have is the opportunity to contribute to the world of science, public information and conservation (Silvertown 2009). Thus, CS projects that require large sample sizes must assess and understand the shared interest and unique motivations that drive their target citizen scientists to participate (Rotman et al. 2012; Wright et al. 2015), and also find means to incite motivation in them to participate (Schulwitz et al. 2021). This is where CS outreach comes into play. CS outreach brings in interested people under one platform and enables them to potentially participate in data collection (Silvertown 2009; Schulwitz et al. 2021). However, effectiveness of CS outreach needs to be tested rigorously in different fields of research, in different localities, and in studies involving different groups of participating volunteers.

As part of a project, "Key ecosystem services and biodiversity components in socio-ecological landscapes of Darjeeling-Sikkim Himalaya: deriving management & policy inputs and developing mountain biodiversity information system", an online Mountain Biodiversity Database and Information System or MBDIS (www.mbdis.in) was developed. A large part of the data in MBDIS came from CS activities implemented by the project. MBDIS was developed to be a comprehensive and interactive web-based database of biodiversity found in the Darjeeling-Sikkim Himalaya, so that students, academicians, researchers and practitioners working on biodiversity of the region could benefit from the information available here. A major component of MBDIS was to train and muster the participation of local community members to contribute photographic observations of biodiversity on already existing web-based citizen science portals. Targeted to involve local community members and nature enthusiasts from the region as citizen scientists, the project aimed at engaging them to generate new point records of biodiversity from the region, so as to create a baseline data that is accessible to anyone working on or interested to learn about the biodiversity of Darjeeling-Sikkim Himalaya.

The CS approach as a tool to collect biodiversity information is still a relatively new concept in the Himalaya, but has the potential to be an important tool in biodiversity documentation (Devictor et al. 2010), as a large swathe of land falls outside the protected area regime in human-modified and -dominated landscapes where the communities are an important source of information. The Himalaya is one of the richest places on earth in terms of species diversity, however these landscapes are still poorly explored, and are vulnerable to increasing anthropogenic pressures, land-use change, and climate change. Thus, developing informed conservation and management plans require distribution and ecological information on species (Tobler et al. 2008), which is relatively scarce in the Himalaya.

The concept of CS is gaining rapid popularity in India and it is estimated that more than 25 CS projects in ecology are operational in India (Sharma 2019). Today in India, there are numerous web-based citizen science projects where the citizens can make their amateur contributions, for example India Biodiversity Portal, eBird India, and iNaturalist, where citizens can contribute their precious observations in the form of photographs or checklists. Thus, in recent years, there have been a number of scientific publications based on use and outputs of CS from India. These publications range from assessments of some CS projects (for example, Vettakaven et al. 2016; Datta et al. 2018), trends based on CS data (for example, Arjun & Roshnath 2018; State of India's Birds 2020), to new species descriptions and discoveries (for example, Kulkarni & Joseph 2015; Jaiswara et al. 2022). Similarly, distribution and locality record available on web-based CS platforms are cited and resulted in scientific publications (for example, The Biodiversity Atlas - India projects have resulted in more than 20 publications). Thus, highlighting the potential and importance of data gathered by citizen scientists in India.

Here, we present how CS can help in biodiversity documentation by adding to the data collected by the researchers. We also explore the effectiveness of CS outreach activities in mustering the participation of local communities and nature enthusiasts in such projects. The study uses butterfly observations as a proxy for this purpose, the reasons being: (1) butterflies are one of the most popular taxa among the local communities, (2) butterflies can be easily photographed by the local communities using camera phones, and thus can be uploaded into citizen science portals, (3) butterflies are one of the most diverse taxa in the Darjeeling-Sikkim Himalaya with 691 species (Haribal 1992; Kamrakar et al. 2021). Therefore, this paper also aims to add to the limited literature on distribution, diversity, and status of butterflies in the Darjeeling-Sikkim Himalaya.

METHODS AND MATERIALS

Study area

The study was conducted in multiple sites across the Darjeeling-Sikkim Himalaya that fall outside the protected areas (Figure 1), which are characterized by traditional agricultural systems, historical tea plantations, and residential areas, interspersed by differently-managed forests. The landscape is an integral part of the Eastern Himalayan region of the Himalaya Biodiversity hotspot, and comprises the two hill districts (Darjeeling & Kalimpong) of West Bengal, and the Himalayan state of Sikkim in India. The region is also an important transboundary landscape sharing its boundary with Nepal, Bhutan, and China. The elevation here ranges 250–>5000 m, and is traversed by three important river systems, Teesta, Rangeet and Balasan.

Data collection

Data collected during the study included GPS location, date, identity of the observer, photograph of the observation, and/or the species identity of the butterfly observed. These were collected by two different approaches: CS, and researcher surveys. Overall data were collected until 15 February 2021, while researcher survey data were collected between October 2018 and September 2021. Later, for comparative analysis, CS data was filtered to match the survey-location and time period of the researcher survey data.

Citizen Science Approach

In the initial stages of the study, information on different local institutions (like village council, clubs, committees, and local NGOs) actively working in the region were collected to identify key informants and organize inception cum awareness workshops in different villages (n = 22), prior to data collection. These workshops were organized as community consultations with a purpose to discuss the key components of the study, and also to seek coordination and partnership with interested groups and local institutions (as done by Pradhan & Khaling 2023). These partners were then approached in the later part of the project to organize CS outreach events in the landscape. CS outreach activities conducted during the study (n = 15) included CS workshops (n = 9), butterfly walks (n = 4), and butterfly documentation events (n = 2), and these were carried out in multiple locations across the study area (Figure 1).

CS outreach activities were used to muster the participation of local community members during data collection. Here, CS outreach refers to the workshops,

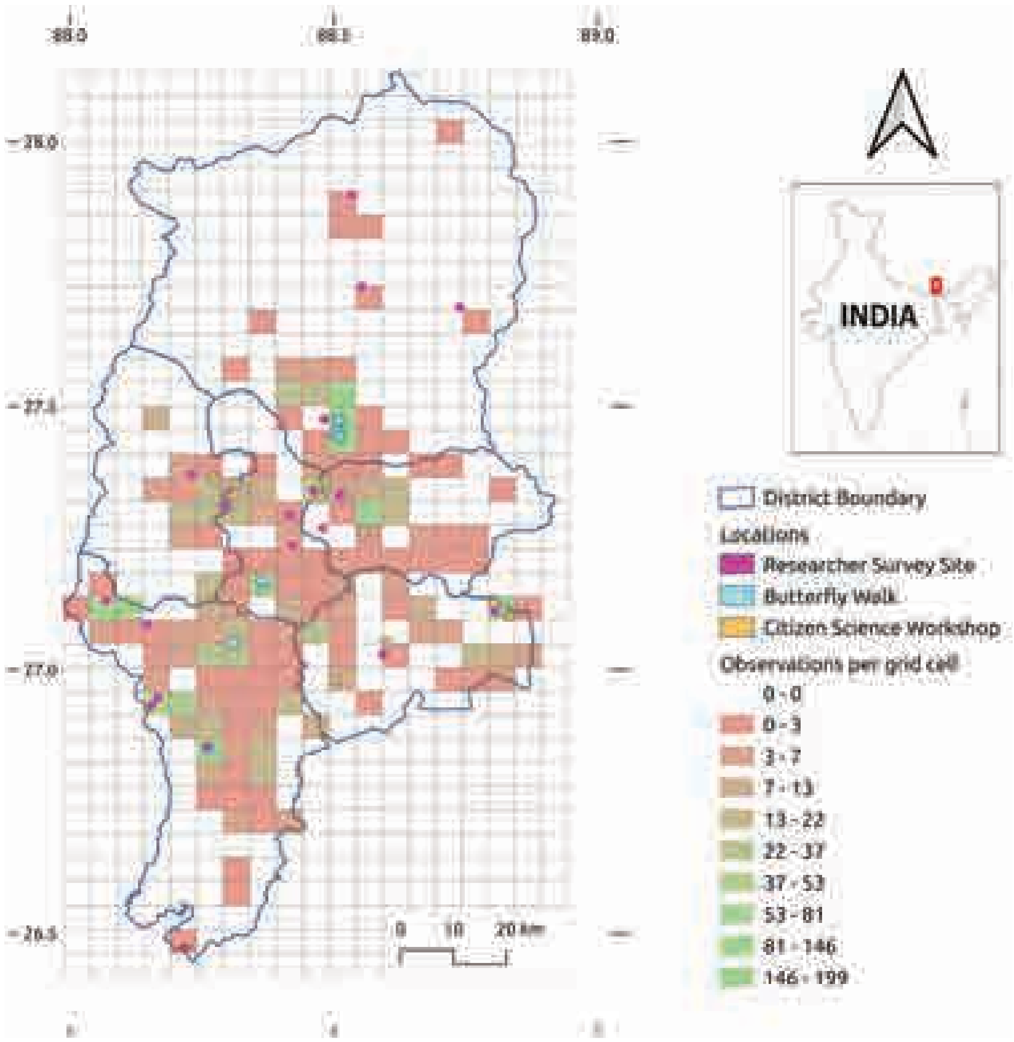

Figure 1. Map showing the location of the study area in India where research surveys, butterfly walks, and workshops were carried out. The map is divided into 5X5 km grids, and shows the number of observations made from each grid during the study period including those observations that have not been identified to the species level.

butterfly-walks, and online documentation events (discussed in following paragraphs), that were organized with an aim to reach out to interested local community members in different localities across the landscape. Data collected using the CS approach included all observations uploaded on iNaturalist (www.inaturalist.org) (identified up to species level) from within the study area. In all these activities, the local communities were neither forced, nor paid in any way to contribute to the documentation process. Hence, the participation mustered by the project was fully dependent on personal interest of the local community.

CS workshops: These were conducted in nine spatially different villages across the study area (Figure 1), targeting school students, teachers and community members, with an objective to train them on how to photograph biodiversity and contribute their observations to iNaturalist, which is an online citizen science platform. Each of these workshops had a theory session, which was followed by a hands-on session, where participants were taken for a short field visit, where they were assisted with registration, and other technicalities associated with uploading photographic observations they recorded in the field.

Butterfly walks: These were organized in four different villages across the study area (Figure 1), with an aim to muster participation of the local community members in documenting butterflies in their respective villages. During this event, participants were taken to a field location, where they were assisted by members of the research team on how to photograph butterflies, and how to upload their observations on iNaturalist. Each of these events lasted for 3–4 hours in the field.

Butterfly documentation events: These events were organized during the Big Butterfly Month (a national butterfly documentation event in India held during the month of September) of 2020 and 2021, where the local communities across the study area were supplied with written and video instructions on how to photographically document butterflies and contribute them to iNaturalist. The butterfly documentation events were carried out through online medium due to COVID-19 related lockdown and safety restrictions that were in place during this period in India. These events were carried out across the entire landscape, and information about them were spread through local contacts of the project team, and through social media.

Researcher survey approach

Two researchers of the project team conducted surveys to document butterflies in different sites across the study area (Figure 1). All species of butterflies encountered by the researchers in these locations were recorded along with their GPS coordinates. Additionally, butterflies were photographed whenever possible to aid in confirmation of species identities. Butterflies were identified using field guides (Kehimkar 2016), and web-based resources (www.ifoundbutterflies.org). To avoid confusion and double counts of the same species while data curation and analyses, taxonomic nomenclature used by iNaturalist was followed during the study.

Data Analysis

All the observations of butterflies from the Darjeeling-Sikkim Himalaya currently available on iNaturalist (accessed on 15 February 2023) were downloaded (n=5026) and those that have been identified to species level (n = 3,746) were filtered out. Since the two researchers conducting opportunistic surveys for this study are also active on this CS platform, observations added (n = 564) by them were removed from the final dataset, leaving only those records contributed by the local communities (n = 3,182). Among these, 101 were added before our project began (in October 2018), 1,291 during the project period, and 1,790 records after the project period (after September 2021)

To create the researcher survey dataset (data collected by the researchers), the researchers directly submitted their data as excel sheets on MBDIS. The dataset contained a checklist of species recorded in spatially different sites, and was also accompanied by polygons of sampling locations in each study site.

A point-in- polygon analysis was performed in QGIS to find out how many of the CS records from the study area fell within the study site polygons (with a 500 m buffer). This was used to compare the datasets created from the CS approach and researcher survey approach. 294 CS observations were determined to fall within the study site polygons.

A circular polygon of 1-km radius was prepared around the workshop and butterfly walk locations, and CS records within these polygons were taken to evaluate the extent to which local communities participated in the outreach events. Similarly, to determine the level of engagement resulting from the butterfly documentation events, observations from the study area that were added on iNaturalist during the online documentation events in September of 2020 & 2021 were tabulated.

To understand the distribution of observations across the study area, and the level of engagement of individual citizen scientists, the study area was divided into grids measuring 5x5km, and the number of observations made in each grid, as well as the number of grids covered by individual participants were enumerated.

RESULTS

CS and Researcher data

By a combined effort of CS and researcher surveys, 331 species of butterflies across six families were recorded from the socio-ecological landscapes of Darjeeling-Sikkim Himalaya (407 species, including those contributed outside the study period) (Table 1). Localities in the landscape from where these species were recorded can be seen in Figure 1.

Eighty-six species of the total recorded species of butterflies are protected in India, among which 12 species are protected under schedule I and 74 species are protected under schedule II under Wildlife Protection Act I972 (Amended through Wild Life (Protection) Amendment Act, 2022). Of the protected species, 66 species (38 within the study period) were recorded by the citizen scientists, while only 27 were recorded by the researchers.

The CS approach documented 1,717 observations resulting in 260 species belonging to six families within the study period, which increases to 4,307 observations (357 species) when we include records before and after the project period (Table 1). During the current study, the most common species observed and submitted by the citizen scientists from the study area was the Indian Tortoiseshell *Aglais caschmirensis*, which was observed 54 times by 37 participants, Popinjay *Stibochiona nicea* observed 31 times by 21 participants, Red Lacewing Butterfly *Cethosia biblis*, 28 times by 15 participants,

Table 1. Checklist of all the butterfly species recorded during the current study from Darjeeling-Sikkim Himalaya, India.

	Species	Common name	Family	WPAA (2022)	CS dataset	RS dataset
1	*Abisara chela*	Spot Judy	Riodinidae	-	#	-
2	*Abisara echerius*	Plum Judy	Riodinidae	-	*	-
3	*Abisara fylla*	Dark Judy	Riodinidae	-	*, PS, BW, WS, OE	*
4	*Abisara neophron*	Tailed Judy	Riodinidae	-	*, OE	*
5	*Abrota ganga*	Sergeant-major	Nymphalidae	-	#	-
6	*Acraea issoria*	Yellow Coster	Nymphalidae	-	*, PS, BW, WS, OE	*
7	*Acraea terpsicore*	Tawny Coster	Lycaenidae	-	#	-
8	*Acupicta delicatum*	Dark Tinsel	Lycaenidae	Schedule II	#	-
9	*Acytolepis lilacea*	Lilac Hedge Blue	Lycaenidae	Schedule II	-	*
10	*Acytolepis puspa*	Common Hedge Blue	Lycaenidae	-	*, PS, BW, WS	*
11	*Aeromachus jhora*	Grey Scrub Hopper	Hesperiidae	-	-	*
12	*Aeromachus pygmaeus*	Pygmy Scrub Hopper	Hesperiidae	-	-	*
13	*Aeromachus stigmata*	Veined Scrub Hopper	Hesperiidae	-	#	-
14	*Aglais caschmirensis*	Indian Tortoiseshell	Nymphalidae	-	*, PS, BW, WS, OE	*
15	*Aglais ladakensis*	Ladakh Tortoiseshell	Nymphalidae	-	-	*
16	*Ampittia dioscorides*	Indian Bushopper	Hesperiidae	-	#	*
17	*Ancistroides nigrita*	Chocolate Demon	Hesperiidae	-	*	-
18	*Anthene emolus*	Common Ciliate Blue	Lycaenidae	-	#	-
19	*Appias albina*	Common Albatross	Pieridae	Schedule II	-	*
20	*Appias indra*	Plain Puffin	Pieridae	Schedule II	#	-
21	*Appias lalage*	Spot Puffin	Pieridae	-	#	*
22	*Appias libythea*	Striped Albatross	Pieridae		*, BW, OE	*
23	*Appias lyncida*	Chocolate Albatross	Pieridae	Schedule II	*, PS, OE	*
24	*Appias wardii*	Lesser Albatross	Pieridae		-	*
25	*Argynnis childreni*	Large Silverstripe	Nymphalidae	-	*, PS, BW, OE	*
26	*Argynnis hyperbius*	Tropical Fritillary	Nymphalidae	-	*, PS, BW, WS, OE	*
27	*Arhopala amantes*	Large Oakblue	Lycaenidae	-	*	-
28	*Arhopala bazalus*	Powdered Oakblue	Lycaenidae	-	#	-
29	*Arhopala centaurus*	Centaur Oakblue	Lycaenidae	-	*, PS, BW, OE	-
30	*Arhopala fulla*	Spotless Oakblue	Lycaenidae	-	*	-
31	*Arhopala khamti*	Luster Oakblue	Lycaenidae	-	-	*
32	*Ariadne merione*	Common Castor	Nymphalidae	-	*, PS, BW, OE	*
33	*Arnetta atkinsoni*	Black-tufted Bob	Hesperiidae	-	-	*
34	*Artipe eryx*	Green Flash	Lycaenidae	Schedule II	#	-
35	*Athyma cama*	Orange Staff Sergeant	Nymphalidae	-	*, PS, BW, OE	*
36	*Athyma jina*	Bhutan Sergeant	Nymphalidae	Schedule II	#	*
37	*Athyma nefte*	Colour Sergeant	Nymphalidae	-	*	-
38	*Athyma opalina*	Himalayan Sergeant	Nymphalidae	-	*, PS, BW, OE	-
39	*Athyma orientalis*	Elongated Sergeant	Nymphalidae	-	*, BW, OE	-
40	*Athyma perius*	Common Sergeant	Nymphalidae	-	#	*
41	*Athyma ranga*	Himalayan Blackvein Sergeant	Nymphalidae	Schedule II	#, PS, BW	-
42	*Athyma selenophora*	Staff Sergeant	Nymphalidae	-	*, PS, BW, OE	*
43	*Athyma zeroca*	Small Staff Sergeant	Nymphalidae	-	#	-
44	*Atrophaneura varuna*	Sylhet Common Batwing	Papilionidae	-	*	-

	Species	Common name	Family	WPAA (2022)	CS dataset	RS dataset
45	*Aulocera padma*	Great Satyr	Nymphalidae	-	#	-
46	*Auzakia danava*	Commodore	Nymphalidae	Schedule II	*, PS, BW, OE	-
47	*Baoris farri*	Paintbrush Swift	Hesperiidae	-	#	-
48	*Baoris pagana*	Figure-of-eight Swift	Hesperiidae	-	*	*
49	*Bibasis amara*	Small Green Awlet	Hesperiidae	-	#	-
50	*Bibasis gomata*	Pale Green Awlet	Hesperiidae	-	*	-
51	*Bibasis harisa*	Orange Awlet	Hesperiidae	-	#, PS, BW	-
52	*Bibasis jaina*	Common Orange Awlet	Hesperiidae	-	#	-
53	*Bibasis vasutana*	Green Awlet	Hesperiidae	-	*, OE	-
54	*Borbo bevani*	Lesser Rice Swift	Hesperiidae	-	-	*
55	*Borbo cinnara*	Rice Swift	Hesperiidae	-	*, PS, WS, OE	-
56	*Byasa dasarada*	Great Windmill	Papilionidae	-	*, OE	*
57	*Byasa latreillei*	Rose Windmill	Papilionidae	-	-	*
58	*Byasa plutonius*	Pink-spotted Windmill	Papilionidae	Schedule I	#	-
59	*Byasa polyeuctes*	Common Windmill	Papilionidae	-	*, PS, OE	*
60	*Caleta elna*	Elbowed Pierrot	Lycaenidae	-	*, PS, BW, OE	*
61	*Callerebia hyagriva*	Brown Argus	Nymphalidae	Schedule II	#	-
62	*Caltoris philippina*	Philippine Swift	Hesperiidae		#	-
63	*Capila lidderdali*	Ringed Dawnfly	Hesperiidae	-	*	-
64	*Capila zennara*	Pale Striped Dawnfly	Hesperiidae	-	-	*
65	*Castalius rosimon*	Common Pierrot	Lycaenidae	-	*	*
66	*Catapaecilma major*	Common Tinsel	Lycaenidae	Schedule II	#	*
67	*Catochrysops panormus*	Silver Forget-me-not	Lycaenidae	-	-	*
68	*Catochrysops strabo*	Forget-me-not	Lycaenidae	-	#	*
69	*Catopsilia pomona*	Lemon Emigrant	Pieridae	-	*, OE	*
70	*Catopsilia pyranthe*	Mottled Emigrant	Pieridae	-	*	*
71	*Celaenorrhinus badia*	Scarce Banded Flat	Hesperiidae	-	-	*
72	*Celaenorrhinus leucocera*	Common Spotted Flat	Hesperiidae	-	*, PS, OE	*
73	*Celaenorrhinus munda*	Himalayan spotted flat	Hesperiidae	-	*	*
74	*Celaenorrhinus pulomaya*	Multi-spotted Flat	Hesperiidae	-	*	*
75	*Celaenorrhinus putra*	Restricted Spotted Flat	Hesperiidae	-	#, PS, BW	-
76	*Celastrina argiolus*	Hill Hedge Blue	Lycaenidae	-	*	*
77	*Celastrina lavendularis*	Plain Hedge Blue	Lycaenidae	-	*	-
78	*Cephrenes trichopepla*	Yellow Palm Dart	Hesperiidae	-	*	-
79	*Cepora nadina*	Lesser Gull	Pieridae	Schedule II	*, PS, OE	*
80	*Cepora nerissa*	Common Gull	Pieridae	Schedule II	*, PS	*
81	*Cethosia biblis*	Red Lacewing	Nymphalidae	Schedule II	*, PS, BW, WS, OE	*
82	*Cethosia cyane*	Leopard Lacewing	Nymphalidae	-	*, PS, BW, OE	*
83	*Charaxes arja*	Pallid Nawab	Nymphalidae	-	-	*
84	*Charaxes bernardus*	Tawny Rajah	Nymphalidae	Schedule II	#	-
85	*Charaxes dolon*	Stately Nawab	Nymphalidae	Schedule II	-	*
86	*Charaxes marmax*	Yellow Rajah	Nymphalidae	Schedule II	#	-
87	*Cheritra freja*	Common Imperial	Lycaenidae	-	*, PS, BW	-
88	*Chersonesia risa*	Common Maplet	Nymphalidae	-	*, PS, BW, OE	*
89	*Chilades lajus*	Lime Blue	Lycaenidae	-	#	-
90	*Chitoria sordida*	sordid emperor	Nymphalidae	Schedule II	#	-

	Species	Common name	Family	WPAA (2022)	CS dataset	RS dataset
91	*Choaspes benjaminii*	Indian Awlking	Hesperiidae	-	*	-
92	*Chonala masoni*	Chumbi Wall	Nymphalidae	-	-	*
93	*Cigaritis lohita*	Long-banded Silverline	Lycaenidae	Schedule II	*, OE	*
94	*Cigaritis syama*	Club Silverline	Lycaenidae	-	#	-
95	*Cirrochroa aoris*	Large Yeoman	Nymphalidae	-	*, PS, BW, WS, OE	*
96	*Cirrochroa surya*	Little Yeoman	Nymphalidae	-	*	-
97	*Cirrochroa tyche*	Common Yeoman	Nymphalidae	-	*, OE	*
98	*Coladenia agni*	Conjoin-spotted Pied Flat	Hesperiidae	-	#	-
99	*Colias croceus*	Clouded Yellow	Pieridae	-	-	*
100	*Colias fieldii*	Dark Clouded Yellow	Pieridae	-	*	-
101	*Colias stoliczkana*	Orange Clouded Yellow	Pieridae	-	*	-
102	*Ctenoptilum vasava*	Tawny Angle	Hesperiidae	-	#	-
103	*Cupido argiades*	Tailed Cupid	Lycaenidae	-	#	-
104	*Curetis acuta*	Angled Sunbeam	Lycaenidae	-	*, PS, BW, OE	-
105	*Curetis bulis*	Bright Sunbeam	Lycaenidae	-	*	*
106	*Cyrestis thyodamas*	Common Map	Nymphalidae	-	*, PS, BW, OE	*
107	*Danaus chrysippus*	Plain Tiger	Nymphalidae	-	*	*
108	*Danaus genutia*	Striped Tiger	Nymphalidae	-	*, OE	*
109	*Delias acalis*	Red Breast Jezebel	Pieridae	-	#	*
110	*Delias agostina*	Yellow Jezebel	Pieridae	-	*	*
111	*Delias belladonna*	Hill Jezebel	Pieridae	-	*, PS, BW, OE	*
112	*Delias descombesi*	Red-spot Jezebel	Pieridae	-	*, PS, WS, OE	*
113	*Delias hyparete*	Painted Jezebel	Pieridae	-	*, OE	-
114	*Delias pasithoe*	Red-based Jezebel	Pieridae	-	*, PS, BW, WS, OE	*
115	*Dercas verhuelli*	Tailed Sulphur	Pieridae	-	-	*
116	*Deudorix epijarbas*	Cornelian	Lycaenidae	Schedule I	#	-
117	*Discophora sondaica*	Common Duffer	Nymphalidae	Schedule I	*, BW	-
118	*Dodona adonira*	Striped Punch	Riodinidae	Schedule II	#	-
119	*Dodona dipoea*	Lesser Punch	Riodinidae	Schedule II	*, OE	*
120	*Dodona egeon*	Orange Punch	Riodinidae	Schedule II	*	*
121	*Dodona eugenes*	Tailed Punch	Riodinidae	-	*, PS, OE	-
122	*Dodona ouida*	Darjeeling Mixed Punch	Riodinidae	-	*	*
123	*Doleschallia bisaltide*	Autumn Leaf	Nymphalidae	Schedule II	*, PS, BW, OE	*
124	*Elymnias hypermnestra*	Common Palmfly	Nymphalidae	-	*	-
125	*Elymnias malelas*	Spotted Palmfly	Nymphalidae	Schedule II	*, PS, BW, OE	*
126	*Elymnias patna*	Blue-striped Palmfly	Nymphalidae	-	*	*
127	*Elymnias vasudeva*	Jezebel Palmfly	Nymphalidae	Schedule II	#	-
128	*Enispe euthymius*	Red Caliph	Nymphalidae	-	*	-
129	*Ethope himachala*	Dusky Diadem	Nymphalidae	-	*, OE	-
130	*Euchrysops cnejus*	Gram Blue	Lycaenidae	Schedule II	#	-
131	*Euploea algea*	Long-branded Blue Crow Butterfly	Nymphalidae	-	-	*
132	*Euploea core*	Common Crow	Nymphalidae	-	*, PS, OE	*
133	*Euploea klugii*	King Crow	Nymphalidae	-	#	*
134	*Euploea midamus*	Blue-spotted Crow	Nymphalidae	Schedule II	#	-
135	*Euploea mulciber*	Striped Blue Crow	Nymphalidae	-	*, PS, BW, WS, OE	*
136	*Euploea sylvester*	Double-branded Crow	Nymphalidae	-	-	*

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137	*Eurema andersoni*	One-spot Grass Yellow	Pieridae	-	*, BW, OE	-
138	*Eurema blanda*	Three-spotted Grass Yellow	Pieridae	-	*, PS, BW, WS, OE	*
139	*Eurema brigitta*	Small Grass Yellow	Pieridae	-	*, BW, OE	*
140	*Eurema hecabe*	Common Grass Yellow	Pieridae	-	*, PS, BW, OE	*
141	*Eurema laeta*	Spotless Grass Yellow	Pieridae	-	*, WS	*
142	*Eurema simulatrix*	Changeable Grass yellow	Pieridae	-	-	*
143	*Euripus nyctelius*	Courtesan	Nymphalidae	Schedule II	*, PS, BW, OE	-
144	*Euthalia aconthea*	Common Baron	Nymphalidae	-	*, PS, OE	*
145	*Euthalia alpheda*	Streaked Baron	Nymphalidae	-	*	-
146	*Euthalia durga*	Blue Duke	Nymphalidae	-	*, PS, BW, OE	*
147	*Euthalia lubentina*	Gaudy Baron	Nymphalidae	-	#	-
148	*Euthalia monina*	Powdered Baron	Nymphalidae	-	*	*
149	*Euthalia nara*	Bronze Duke	Nymphalidae	Schedule II	*, OE	*
150	*Euthalia phemius*	White-edged Blue Baron	Nymphalidae	-	*, PS, BW, OE	*
151	*Euthalia sahadeva*	Green Duke	Nymphalidae	-	*, OE	*
152	*Euthalia telchinia*	Blue Baron	Nymphalidae	Schedule I	*, PS, BW, OE	*
153	*Flos areste*	Tailless Plushblue	Lycaenidae	Schedule II	#	-
154	*Flos fulgida*	Shining Plushblue	Lycaenidae	-	*	-
155	*Gandaca harina*	Tree Yellow	Pieridae	-	*	*
156	*Gangara thyrsis*	Giant Redeye	Hesperiidae	-	*	-
157	*Gerosis phisara*	White-banded Flat	Hesperiidae	-	-	*
158	*Gerosis sinica*	White Yellow-breasted Flat	Hesperiidae	-	#	-
159	*Graphium agamemnon*	Tailed Jay	Papilionidae	-	*, PS, BW, OE	-
160	*Graphium antiphates*	Five-bar Swordtail	Papilionidae	-	*	-
161	*Graphium doson*	Common Jay	Papilionidae	-	#	-
162	*Graphium eurous*	Six-bar Swordtail	Papilionidae	-	*	-
163	*Graphium eurypylus*	Great Jay	Papilionidae	Schedule II	#	-
164	*Graphium macareus*	Lesser Zebra	Papilionidae	-	-	*
165	*Graphium sarpedon*	Common Bluebottle	Papilionidae	Schedule II	*, PS, BW, OE	*
166	*Graphium xenocles*	Great Zebra	Papilionidae	-	*, OE	-
167	*Halpe porus*	Moore's Ace	Hesperiidae	-	#	-
168	*Halpe zema*	Dark Banded Ace	Hesperiidae	-	*, PS, BW, OE	-
169	*Hasora badra*	Common Awl	Hesperiidae	-	#	-
170	*Hebomoia glaucippe*	Great Orange Tip	Pieridae	-	*, PS, OE	*
171	*Heliophorus brahma*	Golden Sapphire	Lycaenidae	-	*, PS, BW, WS, OE	*
172	*Heliophorus epicles*	Purple Sapphire	Lycaenidae	-	*, PS, BW, OE	*
173	*Heliophorus ila*	Restricted Purple Sapphire	Lycaenidae	-	*, PS, BW, OE	-
174	*Heliophorus indicus*	Dark Sapphire	Lycaenidae	-	#	*
175	*Heliophorus moorei*	Azure Sapphire	Lycaenidae	-	*, OE	*
176	*Heliophorus tamu*	Powdery Green Sapphire	Lycaenidae	-	*	*
177	*Hestina persimilis*	Siren	Nymphalidae	Schedule II	#	-
178	*Hestinalis nama*	Circe	Nymphalidae	-	*, PS, BW, OE	*
179	*Horaga onyx*	Common Onyx	Lycaenidae	Schedule II	#	-
180	*Hypolimnas bolina*	Great Eggfly	Nymphalidae	-	*, PS, BW, OE	*
181	*Hypolycaena erylus*	Common Tit	Lycaenidae	-	*	*
182	*Hypolycaena kina*	Blue Tit	Lycaenidae	-	*, PS, OE	-

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183	*Hypolycaena othona*	Orchid Tit	Lycaenidae	Schedule I	*	-
184	*Iambrix salsala*	Chestnut Bob	Hesperiidae	-	*, PS, OE	*
185	*Ideopsis vulgaris*	Glassy Blue Tiger	Nymphalidae	-	-	*
186	*Issoria gemmata*	Gem Silverspot	Nymphalidae	-	-	*
187	*Issoria issaea*	Himalayan Queen Fritillary	Nymphalidae	-	*	-
188	*Issoria lathonia*	Queen of Spain Fritillary	Nymphalidae	Schedule II	-	*
189	*Ixias marianne*	White Orange Tip	Pieridae	-	#	*
190	*Ixias pyrene*	Yellow Orange Tip	Pieridae	-	*, PS	*
191	*Jamides alecto*	Metallic Caerulean	Lycaenidae	-	*, PS, BW, OE	*
192	*Jamides bochus*	Dark Cerulean	Lycaenidae	-	*	-
193	*Jamides caerulea*	Royal Cerulean	Lycaenidae	Schedule II	-	*
194	*Jamides celeno*	Common Caerulean	Lycaenidae	-	*, WS, OE	*
195	*Jamides elpis*	Glistening Cerulean	Lycaenidae	-	-	*
196	*Jamides pura*	White Cerulean	Lycaenidae	Schedule II	-	*
197	*Junonia almana*	Peacock Pansy	Nymphalidae	-	#	*
198	*Junonia atlites*	Grey Pansy	Nymphalidae	-	#	*
199	*Junonia iphita*	Chocolate Pansy	Nymphalidae	-	*, PS, BW, WS, OE	*
200	*Junonia lemonias*	Lemon Pansy	Nymphalidae	-	*, PS, BW, OE	*
201	*Junonia orithya*	Blue Pansy	Nymphalidae	-	*	*
202	*Kallima inachus*	Orange Oakleaf	Nymphalidae	-	*, PS, BW, WS, OE	*
203	*Kaniska canace*	Blue Admiral	Nymphalidae	-	*, PS, OE	-
204	*Lampides boeticus*	Pea Blue	Lycaenidae	Schedule II	*, PS	*
205	*Lasippa tiga*	Malayan Lascar	Nymphalidae	-	*	-
206	*Lebadea martha*	Knight	Nymphalidae	-	*	-
207	*Leptosia nina*	Psyche	Pieridae	-	*, PS, BW, OE	*
208	*Leptotes plinius*	Zebra Blue	Lycaenidae	-	*, PS, BW, OE	*
209	*Lestranicus transpectus*	White-banded Hedge Blue	Lycaenidae	-	#	-
210	*Lethe chandica*	Angled Red Forester	Nymphalidae	-	*, PS, BW, OE	-
211	*Lethe confusa*	Banded Treebrown	Nymphalidae	-	*, PS, BW, WS, OE	*
212	*Lethe dakwania*	White-wedged Woodbrown	Nymphalidae	-	*	-
213	*Lethe dura*	Scarce Lilacfork	Nymphalidae	Schedule I	*, WS	*
214	*Lethe goalpara*	Large Goldenfork	Nymphalidae	-	#	-
215	*Lethe isana*	Common Forester	Nymphalidae	Schedule II	*	*
216	*Lethe kansa*	Bamboo Forester	Nymphalidae	-	*, PS, BW, OE	*
217	*Lethe latiaris*	Pale Forester	Nymphalidae	Schedule II	#	*
218	*Lethe maitrya*	Barred Woodbrown	Nymphalidae	-	*	*
219	*Lethe mekara*	Red Forester	Nymphalidae	-	#	-
220	*Lethe nicetella*	Small Woodbrown	Nymphalidae	Schedule II	*	-
221	*Lethe portlandia*	Southern Pearly-eye	Nymphalidae	-	*	-
222	*Lethe serbonis*	Brown Forester	Nymphalidae	Schedule II	*	*
223	*Lethe sidonis*	Common Woodbrown	Nymphalidae	-	*, PS, OE	*
224	*Lethe sinorix*	Tailed Red Forester	Nymphalidae	Schedule II	*	*
225	*Lethe sura*	Lilacfork	Nymphalidae	-	#	*
226	*Lethe verma*	Straight-banded Treebrown	Nymphalidae	-	*, PS, BW, WS, OE	*
227	*Libythea myrrha*	Club Beak	Nymphalidae	-	-	*
228	*Loxura atymnus*	Yamfly	Lycaenidae	-	*, OE	-

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229	*Luthrodes pandava*	Plains Cupid	Lycaenidae	-	#	-
230	*Matapa aria*	Common Redeye	Hesperiidae	-	*	*
231	*Meandrusa lachinus*	Brown Gorgon	Papilionidae	Schedule II	*, PS	-
232	*Megisba malaya*	Malayan	Lycaenidae	Schedule II	*, OE	-
233	*Melanitis leda*	Common Evening Brown	Nymphalidae	-	*, PS, BW, OE	*
234	*Melanitis phedima*	Dark Evening Brown	Nymphalidae	-	*, PS, BW, OE	*
235	*Melanitis zitenius*	Great Evening Brown	Nymphalidae	Schedule II	*, PS, BW	*
236	*Mimathyma ambica*	Indian Purple Emperor	Nymphalidae	-	#	*
237	*Mimathyma chevana*	Sergeant Emperor	Nymphalidae	Schedule II	-	*
238	*Moduza procris*	Commander	Nymphalidae	-	*, PS	-
239	*Mooreana trichoneura*	Yellow Flat	Hesperiidae	-	*, PS, BW, OE	-
240	*Mycalesis anaxias*	White-bar Bushbrown	Nymphalidae	Schedule II	*, PS, BW, OE	*
241	*Mycalesis francisca*	Lilacine Bushbrown	Nymphalidae	-	*, OE	*
242	*Mycalesis intermedia*	Intermediate Bushbrown	Nymphalidae	-	-	*
243	*Mycalesis mineus*	Dark-branded Bushbrown	Nymphalidae	-	*, PS, BW, OE	*
244	*Mycalesis perseus*	Dingy Bushbrown	Nymphalidae	-	*, PS, BW, OE	*
245	*Mycalesis visala*	Long Brand Bushbrown	Nymphalidae	-	*, OE	*
246	*Nacaduba kurava*	Transparent 6-line Blue	Lycaenidae	-	-	*
247	*Nacaduba pactolus*	Large Four Lineblue	Lycaenidae	Schedule II	-	*
248	*Neocheritra fabronia*	Pale Grand Imperial	Lycaenidae	Schedule II	#	-
249	*Neope armandii*	Yellow Labyrinth	Nymphalidae	-	#	-
250	*Neope bhadra*	Tailed Labyrinth	Nymphalidae	-	*	*
251	*Neope pulaha*	Veined Labyrinth	Nymphalidae	Schedule II	*	-
252	*Neope yama*	Dusky Labyrinth	Nymphalidae	Schedule II	#	-
253	*Neorina hilda*	Yellow Owl	Nymphalidae	Schedule II	*, PS, OE	-
254	*Neptis ananta*	Yellow Sailer	Nymphalidae	-	*, PS, OE	-
255	*Neptis clinia*	Southern Sullied Sailer	Nymphalidae	-	#	*
256	*Neptis hylas*	Common Sailer	Nymphalidae	-	*, PS, BW, OE	*
257	*Neptis mahendra*	Himalayan Sailer	Nymphalidae	-	-	*
258	*Neptis miah*	Small Yellow Sailer	Nymphalidae	-	-	*
259	*Neptis nashona*	Less Rich Sailer	Nymphalidae	Schedule II	*, PS, BW	-
260	*Neptis nata*	Sullied Brown Sailer	Nymphalidae	-	*, PS, WS	-
261	*Neptis pseudovikasi*	False Dingy Sailer	Nymphalidae	-	#	-
262	*Neptis sankara*	Broad-banded Sailer	Nymphalidae	Schedule II	-	*
263	*Neptis sappho*	Pallas' Sailer	Nymphalidae	-	*, PS, WS, OE	*
264	*Neptis soma*	Cream-spotted Sailor	Nymphalidae	Schedule II	*, PS, BW, OE	*
265	*Niphanda cymbia*	Small Pointed Pierrot	Lycaenidae	Schedule II	#	-
266	*Notocrypta curvifascia*	Restricted Demon	Hesperiidae	-	*, PS, BW, OE	*
267	*Notocrypta feisthamelii*	Spotted Demon	Hesperiidae	-	*, OE	*
268	*Notocrypta paralysos*	Common Banded Demon	Hesperiidae	-	*	-
269	*Odontoptilum angulata*	Chestnut Angle	Hesperiidae	-	*	-
270	*Oriens gola*	Common Dartlet	Hesperiidae	-	*, OE	*
271	*Oriens goloides*	Smaller Dartlet	Hesperiidae	-	#	-
272	*Orinoma damaris*	Tigerbrown	Nymphalidae	-	*, OE	*
273	*Orsotriaena medus*	Medus Brown	Nymphalidae	-	*, PS, WS, OE	*
274	*Orthomiella pontis*	Straightwing Blue	Lycaenidae	Schedule II	#, PS	-

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275	*Pachliopta aristolochiae*	Common Rose Swallowtail	Papilionidae	-	*, PS, OE	*
276	*Pachliopta hector*	Crimson Rose Swallowtail	Papilionidae	-	#	-
277	*Pantoporia hordonia*	Common Lascar	Nymphalidae	-	*, PS, OE	*
278	*Pantoporia sandaka*	Extra Lascar	Nymphalidae	-	#	-
279	*Papilio agestor*	Tawny Mime Swallowtail	Papilionidae	-	#	-
280	*Papilio alcmenor*	Redbreast Swallowtail	Papilionidae	-	*, BW, OE	-
281	*Papilio arcturus*	Blue Peacock Swallowtail	Papilionidae	-	*, PS, OE	*
282	*Papilio bianor*	Common Peacock	Papilionidae	-	*, BW, OE	*
283	*Papilio bootes*	Tailed Redbreast	Papilionidae	Schedule II	-	*
284	*Papilio castor*	Common Raven	Papilionidae	-	#	-
285	*Papilio clytia*	Common Mime Swallowtail	Papilionidae	Schedule II	*, PS, OE	-
286	*Papilio demoleus*	Lime Swallowtail	Papilionidae	-	#	-
287	*Papilio helenus*	Red Helen Swallowtail	Papilionidae	-	*, PS, BW, OE	*
288	*Papilio krishna*	Krishna peacock	Papilionidae	Schedule I	*, PS, BW, OE	*
289	*Papilio machaon*	Old World Swallowtail	Papilionidae	-	-	*
290	*Papilio memnon*	Great Mormon Swallowtail	Papilionidae	-	*, PS, BW, OE	*
291	*Papilio nephelus*	Yellow Helen	Papilionidae	-	*, PS, BW, WS, OE	*
292	*Papilio paris*	Paris Peacock Swallowtail	Papilionidae	-	*, PS, BW, OE	*
293	*Papilio polytes*	Common Mormon Swallowtail	Papilionidae	-	*, PS, BW, OE	*
294	*Papilio protenor*	Spangle Swallowtail	Papilionidae	-	*, PS, OE	*
295	*Papilio slateri*	Blue Striped Mime Swallowtail	Papilionidae	Schedule II	*	-
296	*Parantica aglea*	Glassy Tiger	Nymphalidae	-	*, PS, BW, WS, OE	*
297	*Parantica melaneus*	Chocolate Tiger	Nymphalidae	-	*, OE	*
298	*Parantica pedonga*	Pedong Tiger	Nymphalidae	-	*	-
299	*Parantica sita*	Chestnut Tiger	Nymphalidae	-	*, PS, OE	*
300	*Parasarpa dudu*	White Commodore	Nymphalidae	Schedule II	*, PS, BW, OE	-
301	*Parasarpa zayla*	Bicolor Commodore	Nymphalidae	-	*, PS, OE	*
302	*Pareronia avatar*	Pale Wanderer	Pieridae	Schedule II	#	-
303	*Parnara bada*	Oriental Straight Swift	Hesperiidae	-	*, PS, OE	-
304	*Parnassius hardwickii*	Common Blue Apollo	Papilionidae	-	*	*
305	*Pedesta masuriensis*	Mussoorie Bush Bob	Hesperiidae	-	*	-
306	*Pedesta pandita*	Brown Bush Bob	Hesperiidae	-	*	*
307	*Pelopidas agna*	Little Branded Swift	Hesperiidae	-	#	-
308	*Pelopidas assamensis*	Great Swift	Hesperiidae	-	*	-
309	*Pelopidas conjuncta*	Conjoined Swift	Hesperiidae	-	-	*
310	*Pelopidas mathias*	Small Branded Swift	Hesperiidae	-	*, BW, OE	-
311	*Petrelaea dana*	Dingy Lineblue	Lycaenidae	-	*	-
312	*Phalanta alcippe*	Small Leopard	Nymphalidae	Schedule II	*	-
313	*Phalanta phalantha*	Common Leopard	Nymphalidae	-	-	*
314	*Pieris brassicae*	Large White	Pieridae	-	*	*
315	*Pieris canidia*	Indian Cabbage White	Pieridae	-	*, PS, BW, WS, OE	*
316	*Pieris melete*	Asian Green-veined White	Pieridae	-	-	*
317	*Pieris rapae*	Cabbage White	Pieridae	-	-	*
318	*Polytremis discreta*	Himalayan Swift	Hesperiidae	Schedule IV	#	-
319	*Polytremis eltola*	Yellow-spot Swift	Hesperiidae	-	*, BW, OE	*
320	*Polyura athamas*	Common Nawab	Nymphalidae	Schedule II	*, OE	*

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321	*Polyura bharata*	Indian Nawab	Nymphalidae	-	*	-
322	*Polyura eudamippus*	Great Nawab	Nymphalidae	-	*, PS, BW	-
323	*Pontia edusa*	Eastern Bath White	Pieridae	-	*	-
324	*Poritia hewitsoni*	Common Gem	Lycaenidae	Schedule II	*, PS, BW	-
325	*Potanthus confucius*	Chinese Dart	Hesperiidae	-	*	-
326	*Potanthus omaha*	Lesser Dart	Hesperiidae	-	*, PS, BW	-
327	*Potanthus trachala*	Detached Dart	Hesperiidae	-	-	*
328	*Prioneris thestylis*	Spotted sawtooth	Pieridae	-	*, PS	*
329	*Prosotas aluta*	Banded Lineblue	Lycaenidae	Schedule II	-	*
330	*Prosotas bhutea*	Bhutya Lineblue	Lycaenidae	-	#	*
331	*Prosotas dubiosa*	Tailless Line Blue	Lycaenidae	-	*, PS, BW, WS, OE	*
332	*Prosotas nora*	Common Line Blue	Lycaenidae	-	*, PS, OE	*
333	*Prosotas pia*	Margined Lineblue	Lycaenidae	-	-	*
334	*Pseudergolis wedah*	Tabby	Nymphalidae	-	*, PS, BW, OE	*
335	*Pseudoborbo bevani*	Bevan's Swift	Hesperiidae	-	*, OE	-
336	*Pseudocoladenia dan*	Fulvous Pied Flat	Hesperiidae	-	*, PS, BW, OE	*
337	*Pseudozizeeria maha*	Himalayan Pale Grass Blue	Lycaenidae	-	*, PS, BW, OE	*
338	*Rachana jalindra*	Banded Royal	Hesperiidae	-	-	*
339	*Rapala manea*	Slate Flash	Lycaenidae	-	*, PS, OE	-
340	*Rapala nissa*	Common Flash	Lycaenidae	-	*	*
341	*Rapala pheretima*	Copper Flash	Lycaenidae	-	*, PS	-
342	*Rapala rectivitta*	Shot Flash	Lycaenidae	Schedule II	*	-
343	*Rapala tara*	Assam Flash	Lycaenidae	-	#	-
344	*Remelana jangala*	Chocolate Royal	Lycaenidae	Schedule II	#	-
345	*Rhaphicera moorei*	Small Tawny wall	Nymphalidae	-	*	-
346	*Rhaphicera satricus*	Large Tawny wall	Nymphalidae	-	#, PS	*
347	*Rohana parisatis*	Black Prince	Nymphalidae	-	*, PS, BW, OE	*
348	*Sarangesa dasahara*	Common Small Flat	Hesperiidae	-	*, PS, BW, OE	*
349	*Sephisa chandra*	Eastern Courtier	Nymphalidae	Schedule I	*, PS, BW, OE	-
350	*Seseria sambara*	Notched Seseria	Hesperiidae	-	*, OE	-
351	*Sinthusa nasaka*	Narrow Spark	Lycaenidae	Schedule II	#	-
352	*Spalgis epius*	Apefly	Lycaenidae	-	#, PS	-
353	*Spialia galba*	Indian Skipper	Hesperiidae	-	#	-
354	*Spindasis zhengweilie*	Contguous Silverline	Lycaenidae	-	-	*
355	*Stibochiona nicea*	Popinjay	Nymphalidae	-	*, PS, BW, WS, OE	*
356	*Stichophthalma camadeva*	Northern Jungle Queen	Nymphalidae	-	*	*
357	*Suastus gremius*	Indian Palm Bob	Hesperiidae	-	#	-
358	*Sumalia daraxa*	Green Commodore	Nymphalidae	-	*, PS, BW, OE	*
359	*Surendra quercetorum*	Common Acacia Blue	Lycaenidae	-	*, PS, BW, OE	*
360	*Surendra vivarna*	Acacia Blue	Lycaenidae	-	*	-
361	*Symbrenthia brabira*	Yellow Jester	Nymphalidae	-	#	-
362	*Symbrenthia hypselis*	Himalayan jester	Nymphalidae	-	*, PS, BW, WS, OE	*
363	*Symbrenthia lilaea*	Common Jester	Nymphalidae	-	*, PS, BW, WS, OE	*
364	*Symbrenthia niphanda*	Bluetail Jester	Nymphalidae	Schedule II	*, PS, WS, OE	-
365	*Symbrenthia silana*	Scarce Jester	Nymphalidae	Schedule I	*	-
366	*Tagiades gana*	Suffused Snow Flat	Hesperiidae	-	#	-

	Species	Common name	Family	WPAA (2022)	CS dataset	RS dataset
367	*Tagiades litigiosa*	Water Snow Flat	Hesperiidae	-	*, PS, OE	*
368	*Tagiades menaka*	Dark-edged Snow Flat	Hesperiidae	-	*, PS, BW, WS, OE	*
369	*Tagiades parra*	Straight Snow Flat	Hesperiidae	-	*	-
370	*Tajuria diaeus*	Straightline Royal	Lycaenidae	Schedule II	*	-
371	*Tajuria maculata*	Spotted Royal	Lycaenidae	-	*	-
372	*Talicada nyseus*	Red Pierrot	Lycaenidae	-	*	-
373	*Tanaecia julii*	Common Earl	Nymphalidae	-	*, PS, BW, WS, OE	*
374	*Tanaecia lepidea*	Grey Count	Nymphalidae	Schedule II	*, PS, WS, OE	*
375	*Taraka hamada*	Forest Pierrot	Lycaenidae	-	*, PS, WS	*
376	*Tarucus ananda*	Dark Pierrot	Lycaenidae	-	#	-
377	*Teinopalpus imperialis*	Kaiser-i-Hind	Papilionidae	Schedule II	*	-
378	*Telicota ancilla*	Dark Palm Dart	Hesperiidae	-	#	-
379	*Telicota bambusae*	Dark Palm Dart	Hesperiidae	-	*, PS, OE	*
380	*Telinga malsara*	White-line Bushbrown	Nymphalidae	-	*, PS, BW	*
381	*Thaumantis diores*	Jungle Glory	Nymphalidae	-	*, OE	-
382	*Ticherra acte*	Blue Imperial	Lycaenidae	-	*, PS, BW, OE	*
383	*Tirumala limniace*	Blue Tiger Crow	Nymphalidae	-	#	*
384	*Tirumala septentrionis*	Dark Blue Tiger	Nymphalidae	-	*, PS, BW, OE	*
385	*Troides helena*	Common Birdwing	Papilionidae	-	*, BW, OE	*
386	*Udara dilecta*	Pale Hedge Blue	Lycaenidae	-	*, PS, OE	*
387	*Udaspes folus*	Grass Demon	Hesperiidae	-	*	*
388	*Vagrans egista*	Vagrant	Nymphalidae	-	#	-
389	*Vanessa atalanta*	Red Admiral	Nymphalidae	-	-	*
390	*Vanessa cardui*	Painted Lady	Nymphalidae	-	*, PS, OE	*
391	*Vanessa indica*	Indian Red Admiral	Nymphalidae	-	*, PS, BW, WS, OE	*
392	*Vindula erota*	Cruiser	Nymphalidae	-	*, OE	*
393	*Ypthima asterope*	Common Three Rings	Nymphalidae	-	-	*
394	*Ypthima avanta*	Jewel Five-ring	Nymphalidae	-	#	-
395	*Ypthima baldus*	Common Five-ring	Nymphalidae	-	*, PS, BW, WS, OE	*
396	*Ypthima horsfieldii*	Malayan Five-ring	Nymphalidae	-	#	-
397	*Ypthima huebneri*	Common Four-ring	Nymphalidae	-	*, PS, WS, OE	-
398	*Ypthima inica*	Lesser Three-ring	Nymphalidae	-	#	-
399	*Ypthima newara*	Newar Three Ring	Nymphalidae	-	*, PS, BW, OE	*
400	*Ypthima nikaea*	MooreÃ¢s Fivering	Nymphalidae	-	*	-
401	*Ypthima parasakra*	Dubious Five-ring	Nymphalidae	-	*, PS, OE	-
402	*Ypthima sakra*	Himalayan Five-ring	Nymphalidae	-	*, PS, OE	*
403	*Zeltus amasa*	Fluffy Tit	Lycaenidae	-	*, PS, BW, OE	*
404	*Zemeros flegyas*	Punchinello	Riodinidae	-	*, PS, BW, WS, OE	*
405	*Zipaetis scylax*	Dark Catseye	Nymphalidae	-	#	-
406	*Zizeeria karsandra*	Dark Grass Blue	Lycaenidae	-	#	*
407	*Zizina otis*	Lesser Grass Blue	Lycaenidae	-	#	*

CS—Citizen Science | RS—Researcher Survey | WPAA (2022)—Wildlife (Protection) Amendment Act (2022) | -—unrecorded or unlisted | *—recorded during the project period | #—recorded outside project period | PS—recorded from a project site | BW—recorded after butterfly walks | WS—recorded after workshop | OE—recorded during the online documentation event.

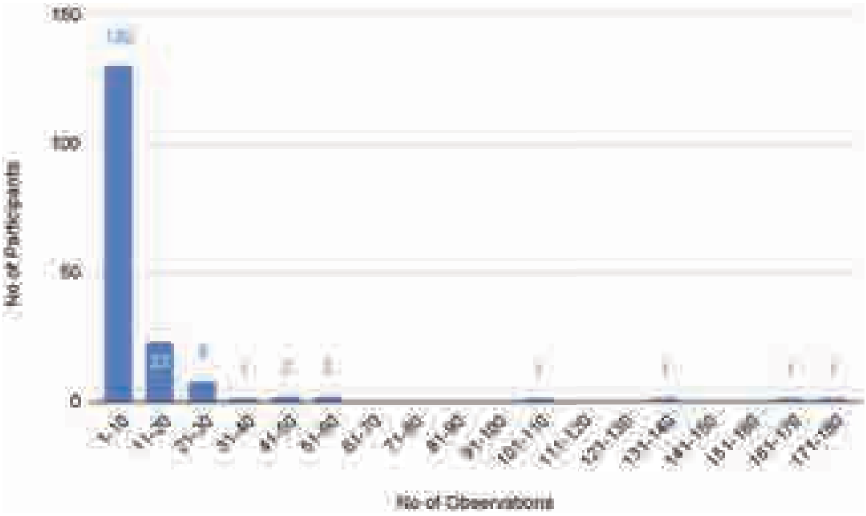

Figure 2. Bar graph showing the total no. of observations (including those that have not been identified to species level) and the corresponding no. of citizen science participants making the observations during the course of the study in Darjeeling-Sikkim Himalaya.

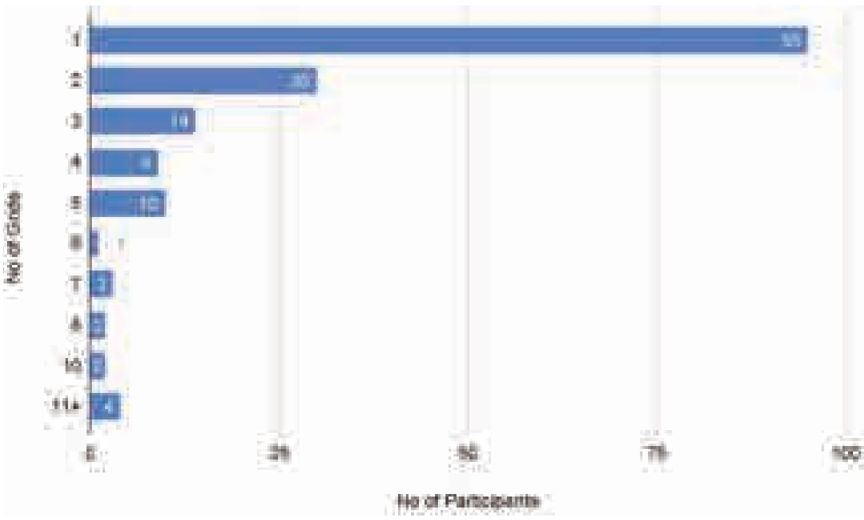

Figure 3. Bar graph showing the number of spatial locations from where the observations were made by each participant during the current study in Darjeeling-Sikkim Himalaya.

Straight-banded Treebrown *Lethe verma,* 28 times by 22 participants and Punchinello *Zemeros flegya*s, 28 times by 22 participants. Similarly, the researcher survey approach was able to document 233 of the 265 species belonging to six families across the study area, during the study period. Again, Indian Tortoiseshell was the most common species which was observed in all sites surveyed by the researchers.

Among the 331 species that were recorded during the study period, the CS dataset was found to have recorded 107 species that were unique from the researcher dataset, while 71 unique species were recorded by the researcher survey. This may be due to the limited number of sites that the researchers could survey within the study period, while CS data were collected from a larger spatial area. A point in polygon analysis was performed to compare the two datasets collected from the same study sites (with a 500 m buffer) and from within the same time period. The results showed 427 observations made by 33 CS participants, which amounted to 131 species, with 32 species unique from the researcher data.

CS outreach and participation

One-hundred-and-seventy community members participated as citizen scientists in the butterfly

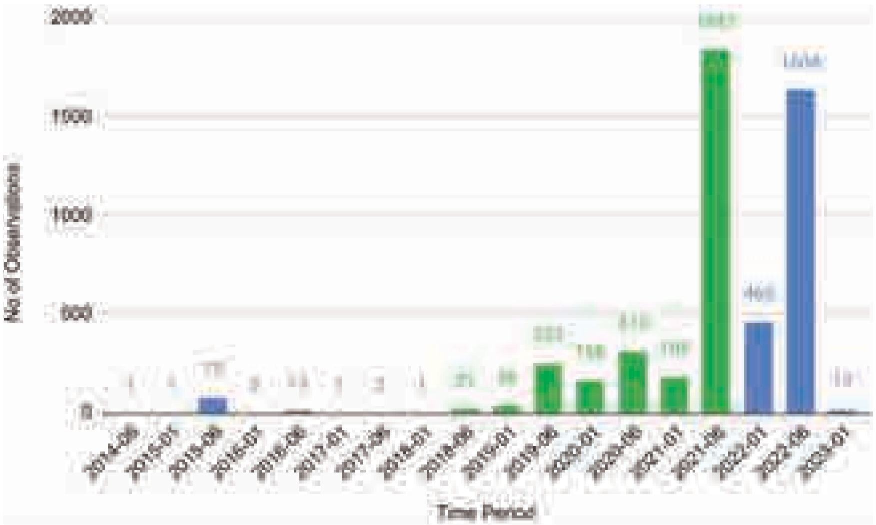

Figure 4. Bar graph showing the number of observations of butterflies contributed by citizen scientists to iNaturalist before and after the study period (blue) and during the study period (green) in Darjeeling-Sikkim Himalaya, assessed after every six months.

documentation project on iNaturalist during the course of the current study. Forty participants contributed to the database more than 10 times (Figure 2), with the highest record of 178 submissions from the same participant (out of which 120 have been identified to species level, till date). A majority of the citizen scientists in the current study, contributed their observations from a limited number of spatial locations. Yet, a few participants appeared to record and submit observations from multiple locations, with four participants submitting their observations from more than 11 spatial locations (Figure 3).

Three-hundred-and-eighty community members participated in nine CS workshops, while the four butterfly walks and two online documentation events had participation of 63 and 81 community members, respectively. The workshops and walks yielded 84 and 492 observations respectively, while 1,187 observations were made during the online documentation events.

The CS outreach during the study in Darjeeling-Sikkim Himalaya resulted in 62.26% (amounting to 181 species) of all CS observations made from the study area during the study period, with 15.11% (92 species) of these being recorded from sites after at least one CS outreach event was organized, while 47.14% of observations (175 species) were contributed during the butterfly documentation events (Table 1 & 2). Results also show that the number of observations of butterflies contributed to iNaturalist from Darjeeling-Sikkim Himalaya sharply increased during the study period, and is still increasing even after the life of the project (Figure 4). Since the end of the project, 144 users have contributed butterfly observations from the region, of which 127 users joined iNaturalist after the end of the project.

Table 2. Summary of the data contributed following citizen science outreach events in terms of observations contributed, species recorded, and participants, with respect to the overall data collected by citizen science approach during the study in Darjeeling-Sikkim Himalaya.

CS data collected	Observations	Species	Participants
Before the study period	106	67	7
During the study period	1,717	268	170
During the study period from the researcher study sites	427	131	33
From workshop locations after the event	80	50	20
From butterfly walk locations after the event	315	121	24
During online documentation events	912	236	74
After the study period	2,484	287	144

DISCUSSION

The use of citizen science approaches in biodiversity documentation is gaining pace in both rural and urban settings across the globe, with the most effective programs targeting to engage local communities (Pandya 2012). However, the reliability of the CS datasets is still a topic of discussion among the scientific community (Chatzigeorgiou et al. 2016). The current study, which incorporates both researcher and CS datasets, presents how CS approach in biodiversity documentation adds to the data collected by the researchers.

Usefulness of CS in documenting butterflies across Darjeeling-Sikkim Himalaya

The current study was conducted in one of the global biodiversity hotspots and uses one of the most diverse taxa here, the butterflies, for this purpose. Butterflies are one of the most diverse taxa in the Himalaya, and Darjeeling-Sikkim Himalaya, where the study was carried out, is a hotspot for butterfly diversity, harboring 46% of all butterflies found in India (Sharma et al. 2020). There have been numerous studies to document the diversity of butterflies in these landscapes across both protected & non-protected areas, however no single study has been able to report even close to 50% of its butterfly diversity, the main challenges being the topographical, temporal, logistical and financial constraints to carry out surveys at a larger scale. This is where CS is very useful. The current study used the traditional researcher survey approach (where the number of researchers carrying out surveys, and number of sites that could be covered by them were limited due to logistical and financial constraints), and the CS approach (where the main challenge was to reach out to, and recruit as many potential citizen scientists as possible). Thus, with a mixed approach, the study was able to document approximately 48% (331 species) of total reported butterfly diversity from the region, which is higher than that reported by any other study conducted in the Darjeeling-Sikkim Himalaya till date, with the previous highest being 43% (268 species) recorded by Sharma et al. (2020). CS alone contributed 43% of the total, while also recording 107 species that were unique from the researcher dataset. The high number of unique, rare, seasonal, and nationally protected butterflies observed by the citizen scientists in the current study, suggests that CS can be an important tool when conducting distribution studies in data deficient corners of the world, as supported by Amano et al. (2016). This is also in line with other studies that suggest CS can effectively supplement data collection in a documentation project of a large scale (Spear et al. 2017). However, the result is contrary to belief that professional surveys report more endangered species and species of special interest for research (Galvan et al. 2021), and may be due to the limited number of professionals used in the current study. The study also reiterates the fact that CS as the only data collection tool (without the use of professionals) may not be able to fully deliver the desired outcomes in a biodiversity documentation project (Pernat et al. 2021).

The use of CS data (in breeding ecology of birds, monitoring migration of birds, bird counts, etc.,) has resulted in a number of publications in recent years (Donnelly et al. 2014; Arjun & Roshnath 2018; State of India's Birds 2020), thus providing evidence on the usefulness of CS data in scientific studies. However, these publications have often been criticized by the scientific community for using CS data due to issues associated with their value and quality. Some of the major challenges of incorporating CS in large projects include lack of organized structure, haphazard coverage, repeat counts, and lack of coordination (Rahmani et al. 2003). Yet, a number of studies have advocated that these challenges can be resolved with better research design, adequate training of citizen scientists, and ground truthing (Bird et al. 2014). Thus, in light of these debates happening across the scientific community, this study adds to the limited literature that supports the theory that large-scale long-term monitoring of biodiversity can be answered through the CS approach. This is especially true when the collection of data from a large area by researchers alone, requires vast amounts of budget, time and effort (Dickinson et al. 2010). However, success of these CS-based projects will depend on the extent of volunteer engagement and training, also called CS outreach (Mason & Arathi 2019).

CS outreach and participation

The current study used outreach materials, theory sessions, field-based training, and online events, as a part of CS outreach activities to overcome the challenges of recruiting citizen scientists across a large spatial area. Here, CS outreach activities conducted prior to data collection was found to be an important step in mustering the participation of target citizen scientists, which in this study were the local community members. Similar observations were made by Feldman et al. (2018). CS outreach has been found to be effective in reaching out to, and generating interest among the potential participants, and is thus useful in mustering local participation (example van der Velde et al. 2017). Among the CS outreach activities used in the current study, butterfly walks (which involved field-based training) were found to be the most effective in mustering local participation. Similar activities have been reported to be successful by other studies (example Matteson et al. 2012). Additionally, online butterfly documentation events which were supplemented with pinpoint instructions, were found to be an effective outreach event capable of reaching out to a larger number of participants across a larger spatial area, and they hugely contributed to the final CS dataset. Online documentation events have also been found to be hugely successful in acquiring large amounts of data elsewhere (Moskowitz & Haramaty 2013), however these have been associated with the highest number of dropouts, meaning the citizen scientists who participate in these events eventually stop contributing once the event period is over (Aristeidou et al. 2021). This suggests that such events are not helpful in ensuring long term participation in science.

The outreach activities carried out during the study was able to create awareness among the local community members on the importance of biodiversity documentation, while also providing a platform for them to contribute to science. The impact made by the study, and the willingness of the participants to participate in such CS projects, can be observed from the fact that the number of observations uploaded on iNaturalist from the landscape sharply increased during the study period, and is still increasing even after the life of the project. However, despite the observable success of the CS outreach in terms of the number of observations, it was found that a large portion of data were contributed by precious few participants, while the majority contributed only a few records. This result exhibits a long tail distribution, as has been reported by other similar CS projects (Segal et al. 2015). Also, a select few participants were found to be contributing data records from multiple locations, while an average participant would only contribute data from a small area, suggesting that a participant is more interested in documenting biodiversity from locality that is easily accessible to the participant. This may also be due to the differences in levels of skill sets and motivation (West et al. 2021). These further suggests the need to reach out to a larger pool of citizen scientists from different corners of the landscape when planning a similar biodiversity documentation project in future, in order to find these precious few who can champion the documentation process, further emphasizing that reaching out to the right audience makes an immense difference to the success of a CS project.

Conservation implications

Developing informed conservation and management plans require distribution and ecological information on species (Tobler et al. 2008), which in the Himalayas are limited. The current study shows how CS can contribute to adding important locality records of rare and lesser known butterflies species, which would remain undocumented without local participation. Thus, CS which effectively accentuates the potential of local communities as knowledge partners, can be a solution to this challenge of limited information on biodiversity. However, this requires good planning, execution, and need for an efficient CS outreach program, has been suggested here. CS outreach, apart from being a means to recruit citizen scientists as data contributors, also has an immense potential in creating awareness, and can be effective in bridging the gap between humans and nature. The role of knowledge-building programs that promote CS, is important in creating positive influence on attitudes and behavior towards biodiversity has also been recently highlighted from the same landscape (Pradhan & Yonle 2022). This further adds to the importance of CS in conservation.

Study perspectives

The study presents how citizen participation in a biodiversity documentation project can aid in adding to the diversity and distribution records of different species, including those that are unique, rare, seasonal, and nationally protected. In the current study, the participation of the citizens was purely interest-based and depended on the participant's interest to learn and record biodiversity from his/her locality. Through this study, the participants gained knowledge and awareness on the local biodiversity, and were provided with a platform where he/she could contribute important biodiversity data. Some of the citizen scientists whose participation was acquired during the study period are still actively contributing to the platform, which shows that they would participate and contribute again. Thus, provided that similar future projects manage to reach out to interested sections of the community, the citizens would be willing to participate in such projects in the future.

Although the goal of the study was to muster as many CS participants as possible from the study area, the current study could only muster limited participation of local communities due to logistical, financial, and time constraints. Also, limited internet connectivity and lack of camera phones with a number of interested participants, hindered the community participation. Hence, if similar studies are carried out in future, CS outreach events that encourage the participation of local communities and help reach out to interested participants, should be organized in multiple locations, and in different seasons. These outreach activities can also be planned in such a way that different events target different potential groups, like students, teachers, farmers, nature guides, etc. This would help in maximizing the number of participants, and thus will maximize the number of observations from within the study area. Similarly, gathering basic information about a participant like, gender, age, occupation, education, etc., would give meaningful insights into the attitude, behavior, and motivation of the participating citizens.

CONCLUSION

CS can be an important tool to fill the spatial gaps in global biodiversity information, and thus can have a crucial role in the data deficient and poorly explored parts of the Himalaya, a global biodiversity hotspot. The study found that conducting CS outreach activities at the field-level prior to data collection, and online events that have the potential to reach out to a larger pool of citizen scientists is beneficial for the overall success of a CS project. The results of the current study show that the CS approach can be a useful supplemental tool in collecting distribution data, as citizen scientists (local communities in this study) have yearlong access to sampling sites. Thus, the study advises other biodiversity documentation projects in data deficient areas to try and accommodate the CS approach in data collection. Finally, MBDIS that aims to incorporate both CS and researcher data in the Darjeeling-Sikkim Himalaya can have immense potential to bring together both the scientific as well as nature enthusiasts of the region under one platform, thus creating an opportunity for the local communities to contribute and learn about the biodiversity of the region.

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COMMUNICATION

Determinants of diet selection by Blackbuck *Antilope cervicapra* at Point Calimere, southern India: quality also matters

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Abstract: Unlike the wide-ranging habitat generalists that move seasonally across heterogenous habitats to optimize the energy intake, short-ranging habitat specialists fulfil the same by restricting to single habitat. Understanding how habitat-specialists do this is an interesting question and essential for their conservation. We studied the diet composition and evaluated the covariates belonging to climate, habitat and grass dynamics to assess the determinants of seasonal diet selection by Blackbuck *Antilope cervicapra*, an antelope endemic to the Indian subcontinent, at Point Calimere Wildlife Sanctuary, southern India. Diet composition studied following feeding trail observation (n = 102322) and the influence of covariates on the top five major diet species selected seasonally was tested using Regression with Empirical Variable Selection. The results showed that overall Blackbucks consumed 30 plant species—six browse and 27 grass species. While wet season diet was less diverse (22 species) with higher dependency on principal diet *Cyperus compressus* (>40%) and *Aeluropus lagopoides* (24%), the dry season diet was more diverse (30) species, with decreased dependency on principal diet. Among 13 covariates belonging to climate, habitat, and grass dynamics tested against selection of top five major diet plants by Blackbucks, grass dynamics covariates alone entered as the predictors both in wet and dry seasons. While cover and green leaves of the grass were the most common predictors in the top-five diets selection during wet season, in dry season besides cover and green leaves, grass texture (hard and soft), also entered as the most common predictors. The entry of grass cover, a quantitative related measure, and texture and green condition of the grass, quality related measures, as the drivers indicate that diet selection by Blackbuck is not just a matter of grass quantity, but also its quality.

Keywords: Diet selection, feeding site examination, grass dynamics, grassland, native species, quality of grass, soft texture grass, Ungulate.

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Author contributions: SS—Data collection, analyses, and draft preparation. SA—Data collection, pruning, analyses, and draft preparation. NB—Conceptualizing, supervising, data analyses, and final draft preparation.

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INTRODUCTION

In natural environment, ungulates exploit the heterogeneity of resources through selective grazing, choosing a diet of better quality than the average vegetation; on offer by preferring habitats that meet their foraging requirements (Prache et al. 1998). The habitat and the physical arrangement of various factors act as key ecological attributes influencing environmental conditions (Bell et al. 2012). The diverse topography along with remarkable variation in precipitation level in tropical environment results in spaciotemporal variations in resource quality and quantity (Baskaran et al. 2018). Habitat generalists, with wide-ranging nature, in heterogenous landscape use different habitats annually by moving among habitats in relation to season and resource availability. On the other hand, the habitat specialists, with restricted movement, fulfil their requirements within a given habitat round the year (Owen-Smith 2002). Understanding how habitat specialists cope-up within a habitat round the year and the factors that influence their resource-use pattern is an interesting area of research pertaining to long-term conservation. Diet selection and forage preference play a vital role in understanding the ecology of a species, as obtaining adequate quantity and quality of food for their survival and reproduction (Weterings et al. 2018).

Foraging decision and diet selection determine both; the nutrient intake by the animals and their impact on the vegetation. Thus, they are important for animal and vegetation management (Owen-Smith 1979; Prache et al. 1998). Earlier studies have reported that diet selection by ungulates is widely determined by many factors including forage quality, e.g., fiber, protein, micronutrients, secondary compounds (Forsyth et al. 2005; Renecker & Hudson 2007), plant phenology (Bee et al. 2010; Zweifel-Schielly et al. 2012) and forage availability/ quantity (Danell & Ericson 1986), time of day (Newman et al. 1995), interspecific competition (Dailey et al. 1984). Grasses, laden with fresh young leaves, are the prime forage of grazers during monsoon. Studies report that fresh leaves with soft texture, that are essentially more palatable due to lesser fiber and cellulose content and high protein content are preferred over dry and hard-textured grass (De Jong et al. 1995; Treydte et al. 2011; Kunwar et al. 2016). On the other hand, seasonal dry out or drought conditions, even periods of low water table, turn out to be a critical period for grazing, during which the forage species transform into leafless, dried and hard textured grass. This becomes a challenging situation for grazers to meet the minimal nutritional requirements. Hence, the quality and quantity of food that is available during the dry period must be the determining factor of ungulates diet selection. Thus, the determination of grass dynamics including phenology indicating the seasonality, is suggested as the primary need (Treydte et al. 2011; Kunwar et al. 2016). Further, the environment plays a major role in forage quantity and quality, which in turn are expected to greatly influence reproduction, as the process of reproduction is energetically demanding for ungulates (Sadleir 1969; Sinclair 1977; Bronson 1989; Schmidt-Nielsen 1997; Pekins et al. 1998; Sinclair et al. 2000).

Blackbuck *Antilope cervicapra* an endemic species to Indian subcontinent; found in southern and central India, ranges in tropical and subtropical woodland, dry deciduous forests, open plain grasslands, riverbanks, semi-desert habitats, crop and pasture lands (Long 2003). The species is currently categorized under the least concerned category by the IUCN, but is listed under Schedule I species under the Indian Wildlife (Protection) Act 1972. Among the current populations in southern India, the Point Calimere Wildlife Sanctuary in Tamil Nadu harbors the largest population. Nevertheless, this population is also declining, from over 2,300 individuals in 1995 (Tamil Nadu Forest Department Census) to around 1,500 individuals in 2005 (Ali 2005) and 2010 (Jagdish 2011). A recent study using Multi Covariate Distance Sampling estimates the population at 750–900 individuals (Arandhara et al. 2020). Blackbucks are known to rely primarily on short grasses (<50 cm); with various research works reporting grasses as their major food resource under free-ranging conditions. A profound seasonality in its nutritional ecology is reported in regions where the species have access to high quantity and quality forage during the monsoon coinciding with periods of grass growth (Schaller 1967; Chattopadhyay & Bhattacharya 1986; Goyal et al. 1988; Henke et al. 1988; Pathak et al. 1992; Jhala 1997; Solanki & Naik 1998; Garg et al. 2002; Das et al. 2012; Jhala & Isvaran 2016; Baskaran et al. 2016, 2020; Frank et al. 2021). In contrast, browse species like *Prosopis juliflora* and *Acacia nilotica* can also form a significant portion of their diet (Ghosh et al. 1987; Ranjitsinh 1989; Jhala 1997). Further, studies based on gastrointestinal-digestive physiology classified the Blackbuck as intermediate feeder, which is reported to include considerable amounts of browse and other trees in its diet (Schaller 1967; Solanki & Naik 1998; Hummel et al. 2015).

The determinants of diet selection remain obscure and an understanding of whether it is quantity or quality or a combination of both influence diet selection and feeding behaviour of herbivores is crucial to manage the endemic species (Shrestha & Wegge 2006); and in devising conservation measures for their long-term survival (Belovsky 1997; Ahrestani & Sankaran 2016). Studies directed towards herbivore management suggest that; procuring reliable information on aspects of basic life history and ecology, key uncertainties rise from diet selection and nutrition (Newmaster et al. 2013). In this study, assessing the diet composition and evaluating weather, and habitat conditions, grass dynamics including phenology; we address the (i) seasonal diet selection and (ii) influence of climate, habitat and grass dynamics covariates on the individual selection of top five major/principal diet species by Blackbuck at Point Calimere, southern India. This study provides data on principal and preferred food plants and the factors influencing the principal diet species selection that are crucial for the management of Blackbuck population, the iconic species of the sanctuary concerned. We use regression with empirical variable selection (REVS) approach, which is shown to be more useful for ecological data than typical regression approaches (Goodenough 2012).

MATERIALS AND METHODS

Study area

The study was conducted between November 2017 and October 2018 at Point Calimere Wildlife Sanctuary (PCWS), (10.30N–79.85E and 10.35N–79.42E), which is home to the largest population of the Blackbuck in southern India. The area derives its name from the coast that takes 90° at 'Point Calimere', where the Bay of Bengal and Palk Strait confluence, spreading over an area of 21.5 km^2 and is situated in Tamil Nadu, southern India (Figure 1). The sanctuary was established in 1967 for the conservation of Blackbucks. The area receives 1366 mm of annual rainfall with wet season running from October to January, and dry season running from February to September. The sanctuary has diverse habitats ranging from tropical dry-evergreen to grassland with patches of open-scrub and mudflats (Ali 2005). The grasslands situated in the southern region of the sanctuary are potential habitats for Blackbuck, along with the presence of other herbivores like chital *A. axis* & Black-napped Hare *Lepus nigricollis* and the introduced horse *Equus caballus* (Ramasubramaniyan 2012). There is no large carnivore, but jackal *Canis aureus*, often prey on Blackbuck fawns (Baskaran et al. 2016), and domestic dogs occasionally prey on adults and fawns (Selvarasu

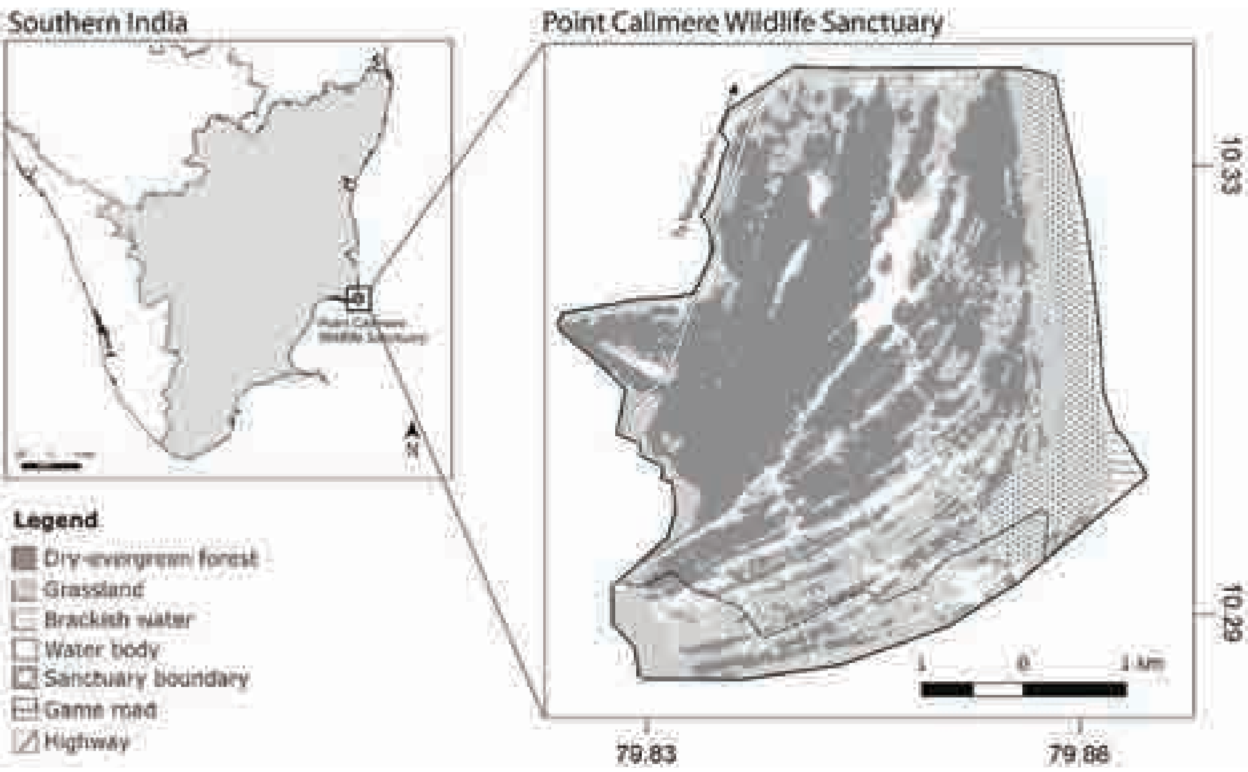

Figure 1. Point Calimere Wildlife Sanctuary, southern India.

Sathishkumar pers. obser. 05 April 2018). Its natural habitat experiences anthropogenic pressure in the form of cattle grazing (Baskaran et al. 2016) and proliferation of *Prosopis juliflora*, an alien invasive species (Ali 2005).

Feeding site examination

The diet composition or food habits of ungulates is often studied by direct observation while feeding or noting down the locations where the animals grazed/browsed. Subsequently, the site is inspected to record the species consumed (Wallamo et al. 1973). The first of these methods is called grazing minutes or seconds (Hahn 1945; Buecher 1950), and the latter is feeding site examination by direct observation (Lovaas 1958). Since the study species is primarily a grazer (Prater 1965), we adopted the method of feeding site examination. Thirty quadrats of 1m^2 each were laid at six feeding sites examined per month and the feeding sites were chosen from areas where the study species were found in their peak feeding time at 0600–1000 h and 1500–1800 h. A feeding site examination refers to the observation of study species feeding for an hour and subsequent recording of the plant species devoured in the observed area. At each feeding site, 5–7 quadrats measuring 1 m^2 were placed at uniform intervals along a feeding site as suggested elsewhere (Lovaas 1958; Baskaran 2016) and the frequency of various plant species eaten were recorded based on fresh feeding signs such as exudation of sap, crushed tissue and fresh clippings (Shrestha & Wegge 2006; Baskaran et al. 2016). Overall, 270 1-m^2 quadrats consisting of 1,02,322 fresh feeding signs were recorded, with feeding signs during the wet season accounting for marginally higher (n = 52,938 or 52%) from 121 quadrats (mean 438 feeding signs/quadrat) compared to the dry season (n = 49,384 feeding signs or 48%, from 149 quadrats - mean of 331 feeding signs/quadrat). The duration of observation during the wet season was 119 h (mean of 7.4 feeding signs/min) and 143 h during the dry season (mean of 5.8 feeding signs/min). In addition, 13 covariates that are likely to influence the diet selection belonging to climate (n = 3), habitat (n = 3) and grass dynamics (n = 7), as listed in Table 1, were assessed at the respective feeding sites, following standard procedures, as given in Table 1. All covariates pertaining to grass dynamics were obtained using quadrats of one 1 m^2 as suggested by Baskaran et al. (2010).

Statistical analysis

The compiled data were checked for homogeneity of variance and normality prior to detailed analysis. The Kolmogorov-Smirnov (KS) test on major five food plants in both the season showed that the distribution of *A. lagopoides* (KS: 0.165; *p* = >0.05), *D. aegyptium* (KS: 0.402; *p* = >0.05), *C. compressus* (KS: 0.234; *p* = >0.05), *C. barbata* (KS: 0.422; *p* = >0.05), *C. polystachyos* (KS: 0.487; *p* = >0.05), and *B. barbata* (KS: 0.483; *p* = >0.05) was neither normal, nor could be transformed to normal with four different transformations. Therefore, the difference in the selection of this species between seasons were tested using non-parametric Mann-Whitney U-test. All statistical tests were run using SPSS program (v.23). To comprehensively provide baseline data on how the five major diet species were selected in relation to each covariate, we split each covariate level into two categories as low and high and tabulated the consumption rate of five major diet species respectively. For example, in case of the covariate on ambient temperature, the replicates with temperature range ≤30^0 C were categorized as low level and those of >30^0 C level, as high level and the observed difference in consumption of major five diet between the two levels were tested using Mann-Whitney U-test. The seven covariates belonging to grass dynamics were tested with the selection of each major diet, in three different combinations, viz.: (i) effect of a grass dynamics covariate, for example grass height of a given major diet species on its own selection, similarly, (ii) the collective effect of the same covariate i.e., grass height belonging to (ii) the other four major diets species and (iii) also the rest 17 minor diet species during wet season and 25 species during dry season on the selection of a given major diet.

Influence of covariate on principal diet selection (Multivariate analysis - REVS)

To identify the covariates influencing the selection of individual principal diet species by the Blackbuck, regression with empirical variable selection (hereafter REVS) was employed using LEAPS package in R Library, in R Software Version 3.3.3 (R Core Team 2019; Ihaka & Gentleman 1993). This method employs all subsets in regression to quantify empirical support for every covariate. To quantify and assign empirical support to the simultaneous effects of each covariate belonging to climate, habitat and grass dynamics, the REVS analysis branch and bound all subsets regression technique. Further, the REVS analysis can handle collinearity (Goodenough et al. 2012), therefore we did not test our data for collinearity. These criteria allowed the REVS approach to be the better approach than multiple stepwise regression. Initially, we incorporate the data

Table 1. Details of covariates belonging to environmental, habitat, and grass dynamics assessed to identify their influence on diet selection by Blackbuck at Point Calimere.

	Covariate	Description
Climate		
1	Ambient temperature (°C)	Measured using a generic digital thermometer-cum-hygrometer device (model: HT01) at each observation at the feeding site.
2	Humidity (Relative %)	As described above.
3	Weather	Recorded visually as cloudy or sunny weather at the start of each feeding site examination.
Habitat (m)		
4	Distance to water	Measured as the distance from a given quadrate to the water source using a rangefinder or obtained from land-use land-cover map.
5	Distance to shade	Measured as the distance from a given quadrat to the nearest canopy cover area using a rangefinder.
6	Distance to road	Measured as the distance from a given quadrat location to the nearest road or obtained from land-use land-cover map.
Grass dynamics (%)		
7	Grass height (cm)	Grass height was measured using a measuring scale, from the ground level to the highest leaf blade bend, at five points (one each at four corners and one at the center) of the quadrate.
8	Grass cover	Assessed visually assuming 100% for the entire quadrat and estimating the proportion of area within a quadrat covered by each grass.
9	Soft texture	Examined crushing the leaves by hands, if leaf's structure could be squashed into a ball- proportion of such leaves for a given grass species in quadrat was rated in % rating.
10	Hard texture	Examined crushing the leaves by hands, if leaf's structure could not be squashed into a ball- proportion of such leaves for a given grass in quadrat was rated in % rating.
11	Green leaves	Assessed visually quantifying the proportion of leaves in a given species with green grass, assuming 100% for all the leaves of the same species.
12	Dry leaves	Assessed visually quantifying the proportion of leaves in a given species with dry grass, assuming 100% for all the leaves of the same species.
13	Reproductive phase	Evaluated visually quantifying the proportion of a given grass with flowers fruits and seed in % rating.

into R Program following the code of Goodenough et al. (2012) and obtained the best regression model ranked by AIC and subject it to interpretation and incorporate into the results (Goodenough et al. 2012). The seven grass dynamics covariates were fed into REVS in respect to (i) a given major diet species, (ii) other four major diet species (arriving a mean from the four species) and (iii) the minor species (arriving a mean from them); in 21 columns (7 covariates x 3 different set of species, as listed above = 21). Therefore, the effect of a set of given covariates (for example grass height) belonging to a given major diet, the rest four major diet species and all other 17 minor diet species during wet season or 25 minor diet species during dry season, was tested against the selection of given major diet. In addition, six covariates belonging to climate and habitat were also included into the REVS equation. The analysis was carried out for each major five diet species season-wise separately.

RESULTS

Diet selection

During the two years of study, Blackbucks consumed 30 plant species, which include six browse and 24 grass species (Supplementary Table 1). However, the number of species and their proportion in the diet varied seasonally (Table 2). For example, during wet season Blackbucks were dependent on just 22 food plant species, with *C. compressus* (>40%) and *A. legopoides* (<25%) contributing more than two thirds of the wet season diet. Contrarily, during the dry season, their dependency on individual species decreased, more specifically on *C. compressus* (11%), with the exception of *A. legopoides* (29%) but relied on more varieties (n = 30). Two major species, viz., *A. legopoides* (>35%) and *C. compressus* (>20%) formed more than two-fourth of the available fodder species of Blackbuck in the environment during the wet season. However, the Blackbucks relied on later species double the quantity that of former species. During dry season, the later species availability reduced considerably (<10%) and similarly the *C. polystachyos*, resulting a marginal increase in the availability of *A. legopoides* (>40%; Figure 2).

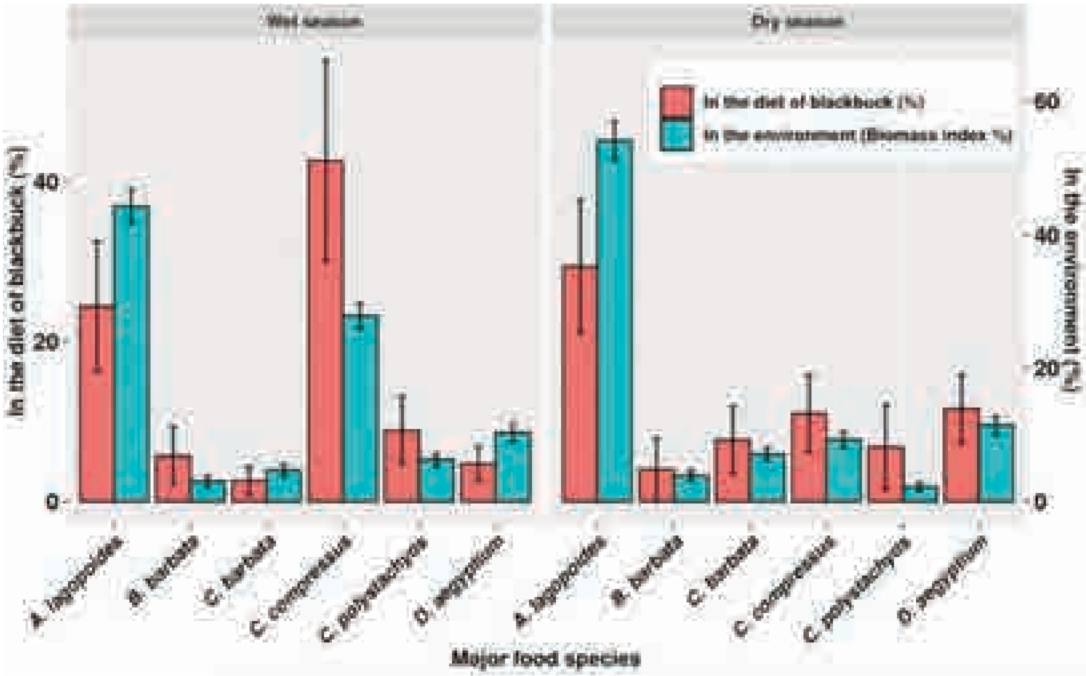

Figure 2. Seasonal selection of five major diet species by Blackbucks and their availability in environment at Point Calimere Wildlife Sanctuary, southern India.

Table 2. Seasonal variation in diet selection by Blackbuck at Point Calimere.

Diet species	Composition (%) Mean ± SE		Mann-Whitney U	p
	Wet season (n = 52938)	Dry season (n = 49,384)		
Aeluropus lagopoides	24.4 ± 8.12	29.4 ± 8.28	8699.5	0.617
Bulbostylis barbata	5.7 ± 3.63	4.0 ± 3.93	8629.0	0.306
Chloris barbata	2.6 ± 1.75	7.7 ± 4.18	7376.0	0.001
Cyperus compressus	42.6 ± 12.55	11.0 ± 4.79	4324.0	0.000
Cyperus polystachyos	8.9 ± 4.20	6.8 ± 5.26	8107.0	0.024
Dactyloctenium aegyptium	4.7 ± 2.15	11.6 ± 4.26	7106.5	0.000
Other species	11.13 ± 7.12	29.51 ± 9.05	6416.5	0.000

Influence of covariates on diet selection

REVS analysis on the influence of 13 covariates belonging to climate, habitat and grass dynamics factors on the selection of the five major diet species during wet season showed that in all the five major diet species selection, only grass dynamics factors entered as the key predictors. Further among the grass dynamics covariates, cover of the same species in all the five major species, and green leaves in four out of five major species and soft-textured grass of minor diet species in three out of five species appeared as the predictors during wet season. Overall, during wet season, 26 covariates entered as the significant predictors, explaining a mean variation of 65% for the top five species selection, minimum with three covariates explaining 44% of the variations in *D. aegyptium* selection and maximum seven covariates explaining 86% of the variations in *C. compressus* (Table 3).

During dry season, like the wet season, though grass dynamics covariates alone entered as the key predictors, the grass cover and green leaves were influencing in the selection of all the five major diet species, the hard texture of the other four major species and 21 minor diet species influenced significantly in four out of five species. Note that the same covariate (hard-texture) influenced only in one species during wet season. Unlike the wet season, during dry season more covariates (33) influenced a higher % of the selection (mean 75%) of five major diet species, a minimum with five covariates explaining 59% of the variations in *A. lagopoides* selection and a maximum 89% of the variations in *C. polystachyos,* but by five covariates (Table 4).

DISCUSSION

Our study based on a large sample size (1,02,322) and duration (2 years) produces a comprehensive data on dietary composition including its seasonality and

Table 3. Regression with empirical variable selection (REVS) to assess the effect of climatic, habitat and grass dynamics covariates on the selection of principal diet by Blackbuck during wet season at Point Calimere Wildlife Sanctuary.

Dependent factors	Predictor (covariate)	Coefficient ± SE	t	Pr (>|t|)	f	p	AIC	Adjusted R^2
Aeluropus lagopoides	(Intercept)	-204.7 ± 83.85	-2.44	0.015	28.4	0.000	1693	0.61
	Grass cover of *A. lagopoides*	2.6 ± 0.31	8.36	0.000				
	Grass height of *A. lagopoides*	0.3 ± 0.07	4.68	0.000				
	Dry leaves of 17 minor species	0.5 ± 0.29	1.63	0.000				
	Soft texture of *A. lagopoides*	1.5 ± 0.68	2.17	0.013				
	Green leaves of 17 minor species	-0.2 ± 0.08	-2.52	0.000				
	Grass cover of rest top four major species	-0.6 ± 0.22	-2.49	0.013				
Bulbostylis barbata	(Intercept)	17.9 ± 43.87	0.40	0.683	94.3	0.000	1513	0.82
	Grass cover of *B. barbata*	2.9 ± 0.27	-7.16	0.000				
	Hard texture of rest top major species	4.7 ± 1.12	4.15	0.000				
	Green leaves of *B. barbata*	3.8 ± 0.73	5.17	0.000				
	Hard texture of 17 minor species	1.6 ± 0.32	5.19	0.000				
	Grass height of rest top four major species	-0.4 ± 0.17	2.30	0.022				
	Soft texture of 17 minor species	-0.4 ± 0.18	2.06	0.040				
Cyperus compressus	(Intercept)	2.2 ± 9.01	0.25	0.802	129	0.000	1667	0.86
	Grass cover of *C. compressus*	1.9 ± 0.66	2.93	0.003				
	Green leaves of *C. compressus*	9.7 ± 4.88	-1.99	0.048				
	Soft texture of *C. compressus*	11.6 ± 4.81	2.41	0.017				
	Soft texture of 17 minor species	-0.6 ± 0.19	-3.56	0.000				
	Green leaves of 17 minor species	-0.8 ± 0.26	2.97	0.003				
	Dry leaves of 17 minor species	1.8 ± 0.7	2.36	0.019				
	Dry leaves of rest top four major species	11.9 ± 5.46	-2.19	0.029				
Cyperus polystachyos	(Intercept)	-2.6 ± 3.47	-0.73	0.462	107	0.000	1532	0.54
	Grass cover of *C. polystachyos*	2.4 ± 0.56	4.23	0.000				
	Green leaves of *C. polystachyos*	0.6 ± 0.17	3.4	0.000				
	Grass cover of rest top four major species	-0.9 ± 0.31	2.89	0.004				
	Green leaves of rest top four major species	-0.5 ± 0.22	2.38	0.018				
Dactyloctenium aegyptium	(Intercept)	0.8 ± 6.70	0.11	0.09	11.8	0.000	1584	0.44
	Grass cover of *D. aegyptium*	0.5 ± 0.30	1.65	0.011				
	Green leaves of *D. aegyptium*	0.6 ± 0.14	4.13	0.000				
	Soft texture of 17 minor species	-0.2 ± 0.13	2.08	0.039				

associated covariates influence on the selection of major diet species by Blackbuck at Point Calimere, southern India. Overall Blackbucks diet consists of 30 plants, with richness of diet species being more during dry (n = 30) compared to wet season (n = 22). The diet species richness recorded in the present study is double that of Baskaran et al. (2016) (n = 14), which was restricted to only seven months (January -June and December). During the wet season, both grass availability and quality (crude protein and digestibility) are generally higher and thus ungulates find more nutritive and palatable grasses everywhere. In contrast, during dry season owing to unfavorable conditions particularly with severe dryness, both above-ground productivity or biomass and palatability of grass drop (Murray 1995; Jhala, 1997; Pradhan et al. 2008; Jhala & Iswaran 2016), leading to herbivores dependence on a wide spectrum of plants unlike wet season. These findings go in support of earlier studies on other herbivores in India (Four-horned Antelope: Kunwar et al. 2016; Asian Elephant: Baskaran et al. 2010). The inadequate quantity and quality during dry season, especially the principal diet,

Table 4. Regression with empirical variable selection (REVS) to assess the effect of climatic, habitat and grass dynamics covariates on the selection of principal diet by Blackbuck during dry season at Point Calimere Wildlife Sanctuary.

Dependent factors	Predictor (covariate)	Coefficient ± SE	t	Pr (>|t|)	f	p	AIC	Adjusted R^2
Aeluropus lagopoides	(Intercept)	-87.0 ± 24.01	-3.63	0.000	22.9	0.000	1868	0.59
	Grass cover of *A. lagopoides*	1.1 ± 0.16	7.23	0.000				
	Soft texture of rest top four major species	-1.9 ± 0.26	7.42	0.000				
	Grass cover of rest top four major species	-0.5 ± 0.19	3.08	0.000				
	Green leaves of *A. lagopoides*	0.7 ± 0.29	2.38	0.020				
	Dry leaves of 25 minor species	0.5 ± 0.15	3.26	0.000				
Chloris barbata	(Intercept)	-38.1 ± 16.80	-2.26	0.020	29.3	0.000	1594	0.64
	Grass cover of *C. barbata*	2.4 ± 0.32	7.54	0.000				
	Soft texture of *C. barbata*	1.4 ± 0.37	3.83	0.000				
	Green leaves of *C. barbata*	1.3 ± 0.30	4.40	0.000				
	Soft texture of rest top four major species	-1.4 ± 0.32	-4.55	0.000				
	Hard texture of 25 minor species	1.5 ± 0.37	-4.10	0.000				
	Grass cover of 25 minor species	-0.3 ± 0.13	2.97	0.000				
	Grass cover of rest top four major species	-0.4 ± 0.02	2.10	0.040				
Cyperus compressus	(Intercept)	4.2 ± 4.93	0.85	0.390	59.6	0.000	1522	0.82
	Grass cover of *C. compressus*	1.6 ± 0.31	5.04	0.000				
	Soft texture of *C. compressus*	2.6 ± 0.37	6.82	0.000				
	Green leaves of *C. compressus*	0.7 ± 0.14	5.23	0.000				
	Green leaves of rest top four major species	-2.1 ± 0.35	-6.03	0.000				
	Dry leaves of rest top four major species	0.3 ± 0.14	2.14	0.030				
	Grass cover of rest top four major species	-0.8 ± 0.23	-3.47	0.000				
	Hard texture of 25 minor species	0.2 ± 0.09	-2.19	0.030				
	Hard texture of rest top four major species	0.2 ± 0.09	2.11	0.040				
	Green leaves of 25 minor species	-0.3 ± 0.09	-3.05	0.000				
Cyperus polystachyos	(Intercept)	-1.4 ± 1.64	-0.86	0.390	216	0.000	1369	0.89
	Grass cover of *C. polystachyos*	60.3 ± 8.27	-7.29	0.000				
	Green leaves of rest top four major species	-1.2 ± 0.13	-8.96	0.000				
	Green leaves of *C. polystachyos*	32.8 ± 4.39	7.48	0.000				
	Green leaves of 25 minor species	-63.5 ± 8.49	-7.48	0.000				
	Hard texture of 25 minor species	93.7 ± 12.67	7.40	0.000				
Dactyloctenium aegyptium	(Intercept)	32.1 ± 20.0	1.60	0.110	65.8	0.000	1505	0.79
	Grass cover of *D. aegyptium*	1.4 ± 0.16	9.08	0.000				
	Hard texture of 25 minor species	0.1 ± 0.05	-2.52	0.010				
	Hard texture of rest top four major species	0.3 ± 0.10	3.39	0.000				
	Grass cover of rest top four major species	-0.4 ± 0.20	-2.02	0.040				
	Green leaves of *D. aegyptium*	0.2 ± 0.12	2.27	0.020				
	Dry leaves of 25 minor species	0.7 ± 0.16	4.79	0.000				
	Soft texture of rest top four major species	-1.1 ± 0.16	2.63	0.010				

resulting a lower contribution of individual diet species to the diet, perhaps force herbivore to rely on a more diverse spectrum of food plants.

The higher consumption of *C. compressus* during wet season, despite higher availability of *A. lagopoides* in the environment indicate that Blackbucks are selective feeder. Further, the *C. compressus* is found mostly in high moisture area and its leaves are fleshy and succulent than *A. lagopoides.* Studies on nutrient composition of grass species show that *C. compressus* constitutes more moisture content (83%) and less crude protein (7.8%) than *A. lagopoides* (moisture 60% and crude protein 9.07%) (Mohsenzadeh et al. 2006; Moinuddin et al. 2012 ; Nurjanah et al. 2016). The fleshy and succulent quality leaves of *C. compressus* perhaps increases the digestibility, palatability and would also meets the water requirements. In addition, it could also be related to the level of secondary component found in the diet species, as herbivores are known to avoid plants with higher secondary metabolites (Owen-Smith 2002; Weterings et al. 2018).

Influence of covariates on principal diet section

The present study with empirical data on 13 covariates belonging to climate (n = 3), habitat (n = 3) and grass dynamics (n = 7) tested against the selection five major diet species as the dependent factor using REVS. The results revealed that grass cover and green leaves of grass are the most appeared significant predictors in the selection all five major food plants both during wet and dry season. This followed by soft-texture of grass in four out five major species both during wet and dry seasons, dry leaves in two out of five species during wet and three out of five during dry season and hard texture of grass in one out of five during wet season and four out of five during dry season and grass height in two out of five species during wet season. Among the six significant predictors, the grass cover and height covariates are associated to quantity and the rest four predictors viz. green leaves, dry leaves, soft-texture and hard-texture of grass are the covariates associate with digestibility and palatability, which indicate quality.

Soft-textured green grass owing to lower fiber and cellulose content and higher protein content is more appetizing and easily digestible than dried and hard-textured grass, which is higher in fiber, cellulose, and low in protein (Sukumar 1989; Sivaganesan 1991; Jhala 1997; Klaus-Hügi et al. 1999; Jhala & Iswaran 2016). Therefore, green leaves and soft-texture act as indicators of higher palatability and nutrient level compared to dry-leaves and hard-texture. Thus, the higher level of former two covariates (i.e., green leaves and soft-texture) in each major diet positively influenced its selection, while their higher level in other four major diets or minor species (17 and 20 during wet and dry season, respectively), negatively influenced the selection of a given major diet. Further, plant species during reproductive phase contains more secondary metabolites (Hartmann 2004) and high fiber, and cellulose and low protein content (Sukumar 1989; Jhala 1997). Thus, the negative influence of hard-textured dry grass could be to avoid secondary metabolite and higher fiber, and cellulose, and low protein as reported elsewhere in Blackbuck (Jhala 1997; Jhala & Iswaran 2016) on other antelope (Bongo *Tragelaphus eurycerus* Klaus-Hügi et al. 1999), ungulates (Owen-Smith 2002), and Asian Elephants (Sukumar 1989; Sivaganesan 1991). Our findings go in support of earlier studies on Blackbuck that reports that Blackbucks in Velavadar National Park, northern India, depended on high quantity and quality (crude protein 11%) food during monsoon and early winter (7%) coinciding with period of grass growth. But after seeding, the grasses lose nutritive quality rapidly during late winter and in summer seasons, when Blackbucks experience nutritional bottlenecks as their diet become less digestible and with low protein content (Jhala & Iswaran 2016). The selection of soft-textured green grass by Blackbuck reported in this study is a quantitative assessment. Similar observations were also made on other antelopes (Four-horned Antelope: Kunwar et al. 2016, Oli et al. 2018; Thomson's Gazelle: Talbot &Talbot 1962; Sable Antelope: Le Roux 2010, Duncan 1975), ungulates (Lowland Tapir: Prado 2013; Sheep & Goat: Bartolome et al. 1995; Impala & Blue wildebeest: Treydte et al. 2011), and Roe deer (De Jong et al. 1995). Further, the grass height although entered as one of the predictors, its influence only during the wet season, where grass growth is not limited, and only in two out of the five major diet species, which indicate the species can withstand or dependence on shortgrass. This finding goes in support of the earlier findings that Blackbuck is a selective feeder and adapted to feed on shortgrass (Prater 1965), which is predominantly available in open habitats (Baskaran et al. 2020).

Further, as reported by Jhala & Iswaran (2016), during summer though the protein content of the Blackbucks' diet drops significantly (>4%), well below the maintenance requirement for ruminants, which is between 5.5–9 % (Robbins 1983), with negative protein balance (as they lose more protein via feces than they can obtain from the forage during summer) and a significant drop in dry mater digestibility (from a high of 76.5% during the monsoon to a low of 32% during summers), their ability to catabolize proteins with reduced forage-intake and movement during summer ensure them to survive on seasonally low-quality diets and live as a primary grazer. Such adaptation could be a trade-off strategy perhaps Blackbuck uses to fulfill it requirements within a single habitat, mostly of open shortgrass land, unlike wide-ranging species that overcome by moving to other optimal habitats. Overall, as covariates associated to both quantity and quality entered as the predictors of the principal diet selection, the study points out in addition to quantity, quality also matters in the selection of major diet species by Blackbuck.

Conclusion and management recommendation

Overall, the study quantitatively assessing the covariates belonging to climate, habitat and grass dynamics and comparing them with the seasonal diet composition of Blackbuck demonstrated that the principal diet selection is determined not only by just the quantity, but also quality in the form of soft-texture green grass due to higher palatability, digestibility and nutrients. The findings indicate the selective feeding on palatable short-grass by Blackbucks. Thus, the need for maintaining the grasslands habitats to support a viable population of Blackbuck and wild ungulates. The Blackbuck being the flagship species of the sanctuary, managing grassland habitat free of invasive species like feral-horse, an effective competitor of Blackbuck (Baskaran et al. 2016, 2020; Arandhara et al. 2020) and *Prosopis juliflora*, an alien invasive affecting grassland habitat (Baskaran et al. 2020; Arandhara et al. 2021), would benefit the conservation of Blackbuck population. Further, we suggest, the need for future focus on the influence of nutritional composition in diet species selection by Blackbuck.

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Supplementary Table 1. Season-wise percentage contribution of various food plants to the diet of Blackbuck and in the environment at Point Calimere Wildlife Sanctuary.

	Food plant species (Grass/Browse)	Wet season		Dry season	
		% cover ± SE in environment	% consumption ± SE in the diet of Blackbuck	% cover ± SE in environment	% consumption ± SE in the diet of Blackbuck
1	*Acacia nilotica* (B)	0.0 ± 0.00	0.3 ± 1.19	0.6 ± 0.32	0.1 ± 0.01
2	*Aeluropus lagopoides* (G)	36.9 ± 2.15	**24.4 ± 8.12** *Second*	45.1 ± 2.42	**29.4 ± 8.28** *First*
3	*Aristida adscensionis* (G)	0.8 ± 0.66	0.0 ± 0.00	1.7 ± 0.74	0.5 ± 0.16
4	*Brachiaria ramosa* (G)	2.5 ± 0.67	0.0 ± 0.00	0.2 ± 0.13	0.1 ± 0.04
5	*Bulbostylis barbata* (G)	2.5 ± 0.67	**5.7 ± 3.63** *Fourth*	3.2 ± 0.62	4.0 ± 3.93
6	*Canthium parviflorum* (B)	0.3 ± 0.17	0.4 ± 1.19	0.4 ± 0.20	0.2 ± 0.40
7	*Cenchrus ciliaris* (G)	0.3 ± 0.17	1.6 ± 1.68	0.6 ± 0.25	1.4 ± 2.51
8	*Chloris barbata* (G)	3.9 ± 0.78	2.6 ± 1.75	5.9 ± 0.82	**7.7 ± 4.18** *Fourth*
9	*Chrysopogon aciculatus* (G)	2.0 ± 0.32	0.6 ± 2.01	0.5 ± 0.22	0.5 ± 1.15
10	*Commelina benghalensis* (B)	0.2 ± 0.13	1.2 ± 2.20	0.1 ± 0.12	0.6 ± 1.89
11	*Cyanotis axillaris* (B)	0.6 ± 0.20	0.0 ± 0.00	0.6 ± 0.16	1.2 ± 1.48
12	*Cynodon dactylon* (G)	0.0 ± 0.00	0.0 ± 0.00	0.2 ± 0.11	0.2 ± 0.33
13	*Cyperus compressus* (G)	23.2 ± 1.60	**42.6 ± 12.55** *First*	7.7 ± 1.03	**11.0 ± 4.79** *Third*
14	*Cyperus polystachyos* (G)	5.2 ± 0.80	**8.9 ± 4.20** *Third*	1.8 ± 0.50	**6.8 ± 5.26** *Fifth*
15	*Cyrtococum trigonum* (G)	0.0 ± 0.00	3.3 ± 3.11	0.3 ± 0.15	0.4 ± 0.73
16	*Dactyloctenium aegyptium* (G)	8.6 ± 1.19	**4.7 ± 2.15** *Fifth*	9.5 ± 1.13	**11.6 ± 4.26** *Second*
17	*Desmodium triflorum* (B)	6.3 ± 0.52	0.3 ± 0.67	4.7 ± 0.70	5.2 ± 3.75
18	*Dichanthium annulatum* (G)	0.2 ± 0.15	0.0 ± 0.16	1.5 ± 0.44	1.7 ± 2.19
19	*Digitaria longiflora* (G)	0.1 ± 0.07	0.2 ± 0.58	0.6 ± 0.18	0.5 ± 0.60
20	*Eriochloa procera* (G)	0.1 ± 0.05	1.2 ± 1.27	0.7 ± 0.22	0.7 ± 0.76
21	*Fimbristylis cymosa* (G)	5.7 ± 0.60	0.1 ± 0.32	8.1 ± 1.02	5.8 ± 3.33
22	*Fimbristylis ovata* (G)	0.3 ± 0.20	0.0 ± 0.00	0.4 ± 0.20	1.8 ± 2.78
23	*Hemarthria compressa* (G)	0.0 ± 0.00	0.0 ± 0.00	0.1 ± 0.04	0.0 ± 0.00
24	*Heteropogon contortus* (G)	0.0 ± 0.00	0.8 ± 1.64	0.2 ± 0.08	0.0 ± 0.12
25	*Kyllinga nemoralis* (G)	1.2 ± 0.35	0.2 ± 0.56	3.6 ± 0.62	6.2 ± 3.67
26	*Paspalum paspaloides* (G)	0.0 ± 0.03	0.8 ± 1.41	0.1 ± 0.05	0.3 ± 0.62
27	*Pedalium murex* (B)	0.0 ± 0.04	0.8 ± 1.42	0.1 ± 0.06	0.2 ± 0.63
28	*Perotis indica* (G)	1.4 ± 0.28	0.0 ± 0.00	2.9 ± 0.35	1.6 ± 1.58
29	*Trachys muricata* (G)	0.0 ± 0.00	0.2 ± 0.58	0.1 ± 0.06	0.1 ± 0.30
30	*Urochloa maxima* (G)	0.2 ± 0.14	0.0 ± 0.00	0.2 ± 0.08	0.4 ± 0.74

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COMMUNICATION

An update on the conservation status of Tibetan Argali *Ovis ammon hodgsoni* (Mammalia: Bovidae) in India

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Abstract: Mountain ungulates are important for alpine ecosystem ecology, yet are understudied, particularly in Asia. Tibetan Argali *Ovis ammon hodgsoni* occurs across Tibet, with Trans-Himalayan India forming the edge of its distribution. We studied their conservation status in India. We compiled published data and secondary information about the occurrence of argali. We then focused on Ladakh, the remaining stronghold of argali in India. Based on literature from Ladakh and after consulting key-informants, we delimited two major populations of argali and estimate population density and demography using the double-observer method. We found 27 studies on argali in India. Studies covered four major themes: records (n = 12), conservation (n = 7), ecology (n = 7), and evolution (n = 1), with studies increasing after 2000. Estimated argali density in Tsaba was 0.34 argali km^{-2} (0.32–0.40) and in Chushul-Mirpal Tso was 0.15 argali km^{-2} (0.12–0.30). Both populations had comparable demography including age-sex ratios. We need to urgently consider argali as a priority species for conservation in India particularly as threats—including transboundary concerns, lack of coordinated conservation across the international border, anthropogenic disturbances, competition & disturbance from livestock grazing, and habitat loss—are a reality. Towards that, we delimited knowledge gaps and set robust population baselines for the two important argali populations in India. As the Tibetan Argali here co-occur with people, it will be crucial to ensure conservation is done in partnership with local communities.

Keywords: Changthang, double observer survey, Mountain ungulate, occurrence, population, Tibet, Trans-Himalayan India.

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Author contributions: MK, RD, SL and KS did the field surveys. KRS conceived the study. MK analyzed the data and wrote the first draft with help from KRS. All author commented and revised subsequent versions. All authors agreed upon the final version.

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INTRODUCTION

Wild ungulates are key determinants of large carnivore populations whilst also playing an important role in ecosystem function including affecting vegetation composition and nutrient cycling (Karanth et al. 2004; Bagchi & Ritchie 2010; Suryawanshi et al. 2017). Ungulates typically from the sub-family Caprinae are adapted to live in the high mountains and are referred to as mountain ungulates. The mountain ecosystems of southern and central Asia are home to a diverse community of mountain ungulates (Schaller 1977). Because of the remoteness of their landscape and associated logistical challenges, studying mountain ungulates in Asia has seen considerable difficulty (Singh & Milner-Gulland 2011). This has hindered the understanding of their conservation status and limited conservation efforts in the region.

The argali is the world's largest wild sheep and occurs across large tracts of mountainous central and southern Asia. Various subspecies of argali are found across 11 central and southern Asian countries and is classified as 'Near Threatened' by the IUCN Red List (Reading et al. 2020) with their populations occurring in small and fragmented populations across their distribution range (Ekernas et al. 2016). Globally, argali populations are seeing a decreasing population trend, with a continuing decline in the population of mature individuals (Reading et al. 2020).

The Tibetan Argali *Ovis ammon hodgsoni* is found in parts of the Tibetan Plateau and its marginal mountains (Shackleton 1997). While this is a seemingly large area, its population is highly fragmented throughout this vast range (Fox et al. 1991; Schaller 1998; Namgail et al. 2009). Alongside, there is a great deal of uncertainty and varying reliability around numbers of Tibetan Argali (Reading et al. 2020). During an extensive survey of the Tibetan Plateau, argali were the least encountered wild ungulate (Schaller 1998). In India, the status of the Tibetan Argali is precarious. They are known to occur in the trans-Himalayan region of Sikkim and Ladakh, with sporadic sightings in Himachal Pradesh and Uttarakhand. Chanchani et al. (2010) estimated c. 177 individuals from Sikkim. Estimates from Ladakh have ranged from c. 200 individuals (Fox et al. 1999) to c. 360 individuals (Namgail et al. 2009). With c. 10,988 km^2 of potential Tibetan Argali habitat, Ladakh perhaps is the last remaining stronghold of argali in India (Chundawat & Qureshi 1999). Nonetheless, more recent surveys have found argali to be absent from large tracts of their potential habitat (Bhatnagar & Wangchuk 2001; Namgail et al. 2009).

Hunting for trophy and meat have been noted to contribute to a drastic decline of Tibetan Argali in Ladakh through the latter part of the 20th century (Ward 1924; Fox et al. 1991a; Namgail et al. 2004). Surveys by Namgail et al. (2009) suggested there was no substantial change in argali population in Ladakh since early 1980s, even though hunting of argali has been banned since then. The Tibetan Argali in fact are fully protected (Schedule 1) species under the Indian Wildlife Protection Act of 1972 and are also listed on Appendix 1 of CITES. Recovery of Tibetan Argali population in Ladakh might be hindered by exploitative and interference competition by livestock that is found throughout their presumed range, although more in-depth research is needed to determine this (Namgail et al. 2007).

Given this background, we compiled published data and gathered secondary information about the occurrence and abundance of argali in India. We then focused our work in Ladakh, as it is arguably the last remaining stronghold of Tibetan Argali in India (Namgail et al. 2007). Based on available literature from Ladakh and after consulting key-informants, we delimited two major populations of Tibetan Argali to estimate population density and population parameters using double observer surveys (Forsyth & Hickling 1997; Suryawanshi et al. 2012). The major goal of this work was to provide an update on the status of argali in India in order to inform regional and global assessment of conservation status of argali.

MATERIALS AND METHODS

Literature review

We conducted a scoping review of scientific literature on argali in India. Using structured search terms (India OR Ladakh OR Sikkim OR Himachal Pradesh OR Uttarakhand OR Himalaya*) AND (Argali OR "Ovis ammon") for abstracts, title or keywords. This was done to capture literature focused on argali in India. Searches were conducted in English only and were done in Web of Science (all findings) and Google Scholar (first 10 pages). Beyond this, additional searches were conducted by consulting the reference list of the literate from the structured search in November 2011. This also allowed us to capture potential multi-species study (e.g., Chanchani et al. 2010) that included argali. We used the flowchart provided in Haddaway et al. (2017) to organize our search. This included reading title and abstract of each publication, and if they didn't directly pertain to argali research in India, then they were excluded from the review. Duplicates were removed as well.

Post this first round of screening, all the applicable publications were downloaded and read in full. Information including location of the study, year, publication type, and main theme of publication (see Table 1) were recorded. The literature review was conducted to construct a knowledge of base available for argali in India and identify key gaps.

Table 1. Major themes of publication.

Theme	Definition
Records	Only presence of argali from locations is reported
Ecology	Studies that investigate how argali interact with their surroundings
Conservation	Studies that explicitly investigate conservation threats to argali and their potential solutions. These studies can have policy relevance
Evolution/ Phylogenetic	Studies that relate to the evolutionary development and diversification of argali
Other	None of the above theme are directly applicable.

Field surveys, study area and data collection

In India, Tibetan Argali are known to occur primarily across the Changthang region of Ladakh (Namgail et al. 2009; Reading et al. 2020). This falls within the Leh district of the Union Territory of Ladakh. This area is an arid and cold high elevation desert with low rainfall and low primarily productivity (Chundawat & Rawat 1994). The vegetation of Ladakh is classified as ‘Dry Alpine Scrub’ (Champion & Seth 1968). Temperature can vary from summer highs of 30^0C to below -35^0C in the winters. This cold desert is characterized by dry plateau of rolling hills. Average elevation in Changthang is 4,500 m, ranging between 4,000–6,500. Apart from the Tibetan Argali, Changthang is home to sizeable population of other ungulates including Blue Sheep *Pseudois nayaur* and Tibetan Wild Ass *Equus kiang*. Beyond these, small groups of Tibetan Antelopes *Pantholops hodgsonii*, Tibetan Gazelle *Procapra picticaudata*, and Wild Yak *Bos grunniens* are also found in remote regions of Changthang. Main predators in this region are Tibetan Wolves *Canis lupus chanco*, Snow Leopard *Panthera uncia*, and Eurasian Lynx *Lynx lynx*.

These wildlife populations are spread across the landscape and not confined to protected areas. Albeit with low densities, human populations live in this low-productivity, highly seasonal landscapes. They have evolved a distinct lifestyle and culture, and have traditionally been pastoralists and agro-pastoralists. One of the mainstay of the local culture and economy is rearing of *Changra* goats that yield ‘pashmina’, i.e., cashmere (Singh et al. 2009).

We compiled published data and gather secondary information about the occurrence and abundance of argali across Ladakh (assisted by the literature review). To do the latter, we visited local herders in the Tsokar basin, Sumdoo TR, Korzok, Nyoma, Gya-Miru, Kharnak, Chumur, Hanle, Chushul, Tsaga, Man-Merak, Tangtse, Kuyul-Demchok, Tukla, and Himya and asked if they had seen Tibetan argali (locally known as ‘Nyan’ for males and ‘Nyanmo’ for females) in their vicinity in the past two years. The population in Hemis National Park is well documented in different studies and reports and hence we did not do secondary surveys in this region. A total of 30 key-informants were engaged (i.e., two key-informants per village). Alongside, we spoke to five knowledgeable wildlife protection department officials to gain further information. Given that argali are known to be migratory, information on potential migratory routes was noted in an attempt to delimit potentially separate populations.

Upon finishing the secondary surveys, the team identified two relatively large populations of Tibetan argali in Tsaba valley (within the Gya-Miru region) and around Mirpal Tso (near Chushul village). In March 2020, we used the mark-recapture theory based double-observer method (Forsyth & Hickling 1997; Suryawanshi et al. 2012) to survey both these areas. Individually identifying ungulates is challenging given their similarities in appearance across age and sexes. Nonetheless, ungulate groups can be identified due to peculiarities such as their size, age-sex composition and location; albeit temporarily. During the surveys the units being “marked” and “recaptured” are ungulate groups. This is done by two teams surveying for and enumerating animals either simultaneously or sequentially in the same area. They do so while strictly ensuring they don’t influence each other on the animal detection. This method has been used to conduct reliable population estimation for several mountain ungulate species, including argali across central and southern Asia (Tumursukh et al. 2015; Chetri et al. 2017; Suryawanshi et al. 2020; Khanyari et al. 2021).

Both the survey area, Tsaba valley and Chushul-Mirpal Tso were further divided into smaller blocks that could be visually covered by a team of observers on a survey occasion. The terrain and logistics determined the shape and size of these survey blocks. Each block was surveyed keeping three assumptions in mind: 1) each block had entire visual coverage, 2) areas within blocks were surveyed independently by two teams who were separated by time (15 minutes), 3) ungulate groups could be individually identified based on the age-sex composition of the herd, location and any other noticeable peculiarities. The data collected included group size and group detection/non-detection by each observer team. The 'mt' model with a uniform prior was fitted using the function BBRecap to estimate number of argali groups (Ĝ) in each site. "mt" is the standard temporal effect with no behavioral effect. Owing to the fact that these were first attempted double observer surveys in the sites, we used uninformed uniform priors. We carried out 10,000 mcmc iterations with 1,000 burn in.

In Tsaba valley, we covered 6 blocks along 62 km survey trails (31 km for each observer) while in Chushul-Mirpal Tso we six blocks along 51 km survey trails (25.5 km for each observer). In both sites, each team used a pair of binoculars to scan and classify ungulate groups. Topographic maps of the areas and local knowledge of herders and wildlife protection department officials was used to determine survey trails. To account for the effect of activity patterns (if any) on sightings of the study species, we started all surveys just post sunrise (Fattorini et al. 2019). Each team had one or two trained persons. Same number of observers per team per surveys was kept to standardize effort. Overall, six observers were involved in the surveys.

Data analysis

For the literature review and the secondary surveys in Ladakh, we used descriptive statistics to display the data. For the population estimation, we used the Bayesian framework in "BBRecapture" package to estimate total number of argali groups (Fegatelli & Tardella 2013; R Development Core Team Version 3.3.4 2020). Number of groups was the unit of analysis as recommended by Suryawanshi et al. (2012). A group was coded '11' if both teams observed it, '10' if only the first team observed it and '01' if only the second team observed it.

The detection probability for observer teams one and two was interpreted from the estimated detection probability by model 'mt' for occasion one and two. The total population (Nest) for each landscape was estimated as a product of the estimated number of groups (Ĝ) and the estimated mean group size (µ). In order to estimate the confidence intervals of the population using both the mean group size and estimated number of groups, we generated a distribution of estimated group size by bootstrapping 10,000 times with replacement. A distribution of estimated population (Nest) was generated by multiplying 10,000 random draws of estimated number of groups (Ĝ), weighted by the posterior probability and draws of mean group size (µ). The estimated population (Nest) was the median of the resultant distribution while the 2.5 and 97.5 percentiles were used as the confidence intervals.

Density was obtained by dividing the estimated abundance by the total area sampled, which was obtained by summing areas of all the surveyed blocks. We demarcated and obtained areas of blocks on Google Earth Pro post the survey. These included areas that were visible from the trails.

Additionally, we conducted 10,000 bootstraps to assess the 95% confidence intervals of the proportion of individuals of different age-sex classes (adult male, adult female, and young) using herd as the sampling unit. The median values were used as the estimates, while the 0.025 and 0.975 quartiles were used as 95% confidence intervals.

RESULTS

Literature review

We found 27 studies on Tibetan Argali in India. These included 19 peer-reviewed scientific papers, seven reports and one book chapter. Majority of the studies were conducted in Ladakh (n = 19), followed by Sikkim (n = 4), Himachal Pradesh (n = 2) and Uttarakhand (n = 1). Overall studies covered four major themes: records (n =

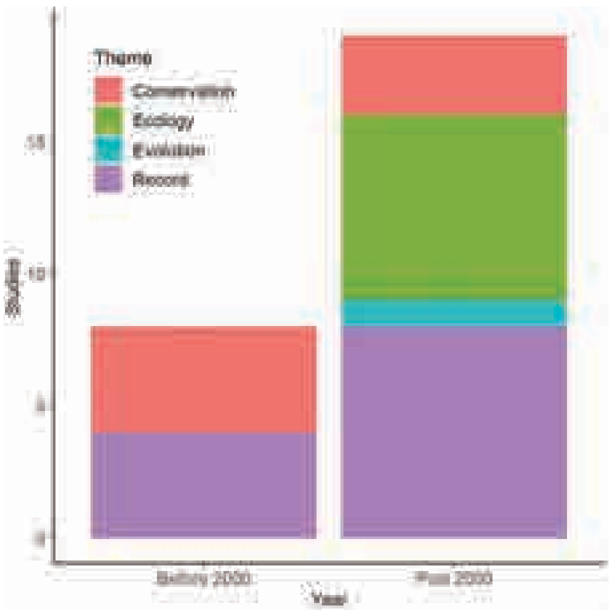

Figure 1. Stacked bar graph displaying split of research themes in publications before and post 2000.

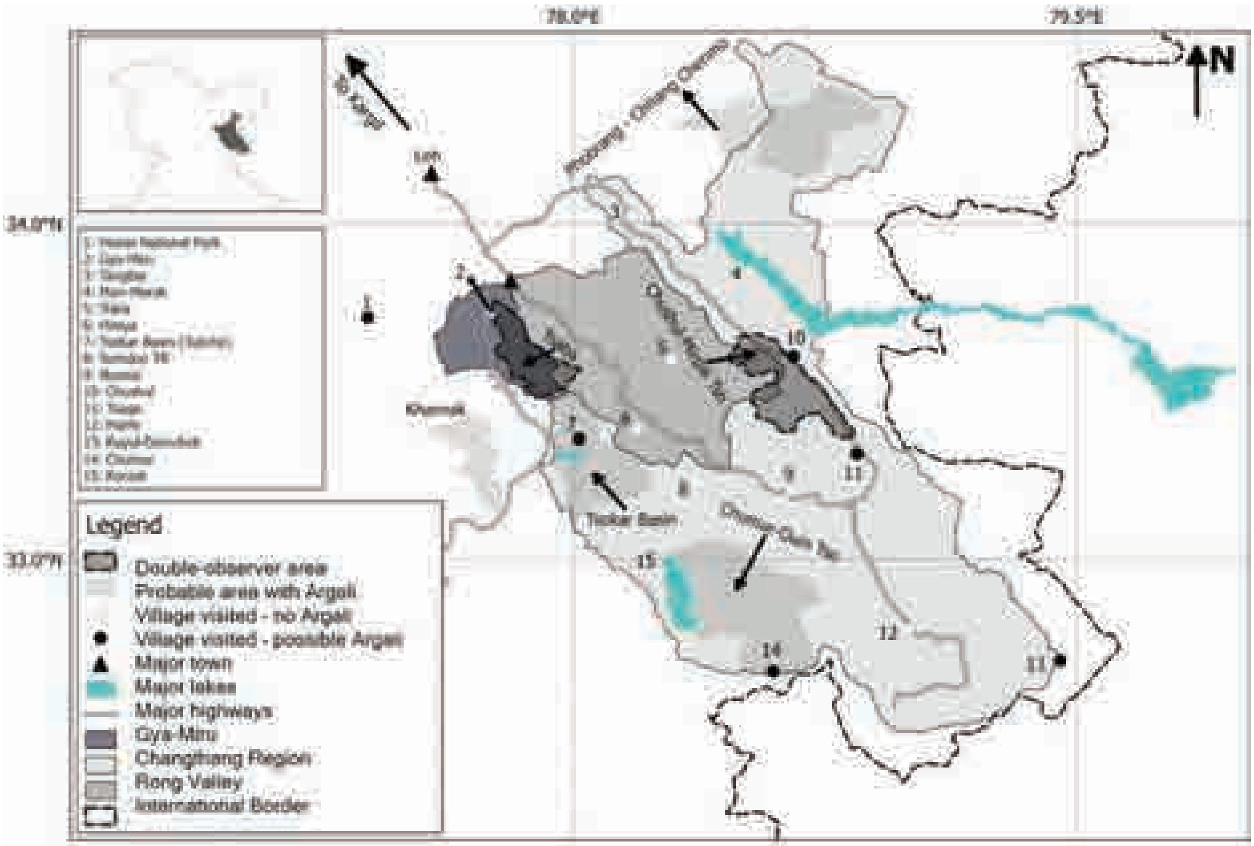

Figure 2. Map displaying the current distribution of Tibetan Argali within Ladakh. Probable areas were delimited by key-informants.

12), conservation (n = 7), ecology (n = 7), and evolution (n = 1). Interestingly, not only have the number of studies increased since 2000 (n = 19 compared to n = 8 before 2000), but researchers are also studying newer themes beyond just presenting records of Argali (Figure 1). Nonetheless, records have remained the major theme of study through time.

Tibetan Argali in Ladakh

Records of Tibetan argali in Ladakh based on compiling published data and secondary information are listed in Table 2 and displayed in figure 2. In Tsaba valley, the double-observer survey abundance of argali was 104 (98–123), whilst in Chushul-Mirpal Tso was 76 (57–148). The estimated density of argali in Tsaba was 0.34 argali km^{-2} (0.32–0.40) and in Chushul-Mirpal Tso was 0.15 argali km^{-2} (0.12–0.30) (Table 3 and Figure 3a). Table 3 summarises different parameters such as detection probabilities, estimated number of groups, mean group size, and age-sex ratios of each of the populations and species. The age-sex class proportion were comparable between Tsaba and Chushul-Mirpal Tso (Figure 3b).

DISCUSSION

India forms the edge of the distribution for the Tibetan Argali (Image 1) and often it is such at range extremities that local population decline, leading to local extinctions as populations become fragmented and disconnected (Boakes et al. 2017). Added to this is the fact that Tibetan argali is known to be found in fragmented and disconnected groups across its range in India (Namgail et al. 2009; Chanchani et al. 2010). This is similar to many other ungulate species from the Indian Himalaya like the Kashmir Red Deer (Hangul) *Cervus elaphus hangul* (Ahmed et al. 2009). Our study shows that limited research has been conducted on Tibetan Argali in India (n = 27 studies). This is perhaps similar for other sympatric Caprinae species in the region such as Ladakh Urial, *Ovis orientalis vignei* (e.g., Khara et al. 2020), highlighting the need for focussed research on mountain ungulates across the country and the larger central and southern Asian Mountain ecosystem. The total number of studies might be an underestimate considering our search didn't extend beyond the English language, and likely missed studies in grey literature that are often not easily

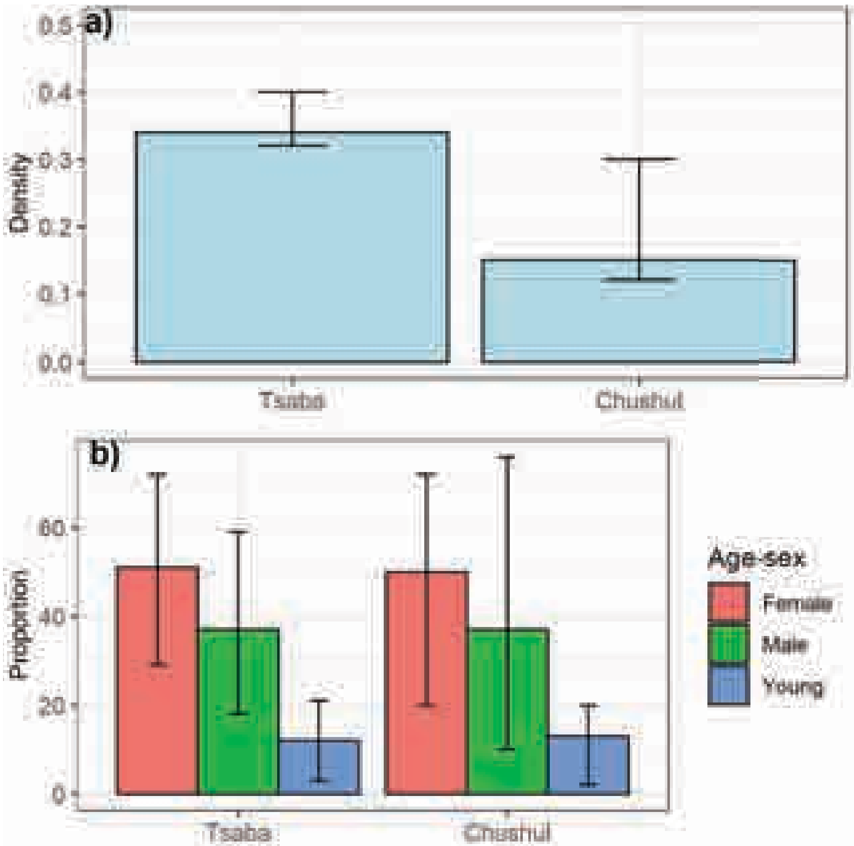

Figure 3. Panel graph showing a) density of argali and b) age-sex classes across Tsaba and Chushul. The estimated density of argali in Tsaba was 0.34 argali km^{-2} (0.32–0.40) and in Chushul-Mirpal Tso was 0.15 argali km^{-2} (0.12–0.30). The bars represent 95% confidence intervals.

Table 2. Records of Tibetan Argali in Ladakh.

Location	Occurrence and abundance*	Source of information	Notes
Hemis National Park	c. 20 argali	Namgail 2001	Interviews with wildlife protection department suggested this population persists and is likely to be resident. 3 adult males, 11 adult females, 1 yearling and 5 lambs
Gya-Miru region (Tsaba valley) and neighboring Tsokar basin	127 argali	Namgail et al. 2009	Survey area was 472 km^{-2} (0.3 argali km^{-2}) According to local knowledge this population of argali spend winter/rutting in the Tsaba valley catchment (within Gya-Miru proposed Wildlife sanctuary) and migrate to the adjacent Tsokar Basin in the summer.
Kuyul-Demchok-Skagjung region	c. 30 argali near Demchok and Skagjung	Namgail et al. 2009, anecdotal observation of our field team and interviews with local herders.	Believed to be move between India and China. Anecdotal observation by our field team in November 2020 near Demchok (4 argali).
Phobrang- Chhang Chenmo	c. 100 argali	Namgail et al. 2009; Shawl et al. 2011 and our interviews	Interviews suggested argali are found in and around the Chhang Chhenmo valley throughout the year.
Quin Tso-Chumur	c. 10–15 argali	Namgail et al. 2009 and anecdotal sighting	At least 2 adult males, 3 adult females, 3 yearlings, 2 kids sighted in November 2020 Interviews with local herders confirmed atleast 30 Argalis in the area exhibiting movement between India and China.
Tsaga la region	Present	Interviews with local herders and confirmed by wildlife protection department.	Numbers are unknown but believed to be less than 20
Kharnak	c.75 argali	Interviews with local herders and anecdotal sights	2 groups of Argali were spotted by the field team. One of the groups had 29 argali (primarily consisting of females and yearlings) and 19 argali (primarily consisting of adult males). Herders in the valley confirmed that around 75 argali might inhabit this area and exhibit movement within the valley throughout the year.
Chushul – Mirpal Tso region	c. 70–100 argali	Interviews with local herders and confirmed by wildlife protection department and wildlife researchers	Local herders suggested that argali here move between Mirpal Tso region and Upper Yaya tso region depending on the season.

accessible through online web searches (Haddaway et al. 2017). Old hunting records (dating back to early 20th century) (e.g., Ward 1924) and government archives are potential repertoire of information on argali in India, albeit with varying difficulty in accessing its content. Nonetheless, it is encouraging to see increased research outputs post 2000, particularly covering various research themes. For effective conservation of argali to occur in India, it is critical to build a research base of robust and integrated information than helps conservationists not only understand the species better but also delimit conservation priorities and test their effectiveness (e.g., Williams et al. 2020).

Furthermore, our study provides an update on the status and distribution, along with providing robust population estimates and population parameters for two important populations of Tibetan Argali in Ladakh. While an in-depth and updated threat assessment is needed to understand contemporary threats to argali, our literature review highlighted majors conservation concerns to be: transboundary concerns (lack of coordinated conservation across the international border), and anthropogenic disturbances (competition and disturbance from livestock grazing and habitat loss) as the main threats to this region's argali populations. Based on our surveys and the secondary information collected, we estimate that the Tibetan argali population in Ladakh to be slightly higher than 300–360 as reported by Namgail et al. (2009). The bulk of the argali population seems to be present in the Tsaba-Tsokar, Chushul-Mirpal Tso, Phobrang-Chhang Chenmo, and Kharnak populations, although more research is needed to understand potential seasonal movement of argali to better delimit geographically separate populations. While there are signs of some increase in numbers (e.g., Namgail et al. 2009 report around 10–20 argali from Kharnak while our surveys suggest c. 75 argalis), there seems to be no substantial change in the population of argali in Ladakh since the late 1980s.

Nevertheless, several challenges remain while studying and attempting to conserve argali populations in Ladakh an India at large. For instance, due to their proximity to the Indo-Sino border, areas supporting population like the Phobrang-Chhang Chenmmo, Quin Tso-Chumur, and Chushul-Mirpal Tso often have restricted access. This is also true for the Tso Lhamo population in Sikkim (Chanchani et al. 2010). Volatility along the borders renders these population particularly vulnerable from a conservation point of view (Mendiratta et al. 2021). Additionally, due to its vast territory, the areas home to the Kharnak population proves to be a logistic challenge to survey; especially as large area remain inaccessible for nearly six months of including and either side of winter. As most of the migrating population of argali share pastures seasonally with various transhumance pastoral communities that have their social, cultural and political differences (Singh et al. 2013), conducting meaningful stakeholder meetings to then have effective on-ground action remains a huge challenge (Allen & Singh 2016).

Even though there has been a ban on hunting of Argali since the 1980s, Ladakh has seen substantial levels of socio-economic changes primarily due to expansion of defense, tourism and development infrastructure (Bhatnagar et al. 2006; Dollfus 2012). There is a possibility that these activities are limiting argali population recovery by negatively influencing habitat use. Alongside, the role of exploitative and interference competition from livestock grazing and collateral activities in hindering recovery of argali

Table 3. Information about Tibetan Argali populations in Tsaba Valley and Mirpal Tso-Chushul landscapes, Ladakh, India.

	Tsaba Valley	Chushul-Mirpal Tso
Area (km^2)	306	497
Minimum count (Obs 1 & 2 combined)	98	57
Estimated Population (95% CI)	104 (98–123)	76 (57–148)
Density	0.34 (0.32–0.40)	0.15 (0.12–0.30)
P1	0.74	0.60
P2	0.79	0.53
Obs 1 Total	89	47
Obs 1 group	13	7
Obs 2 Total	89	32
Obs 2 group	14	6
Common Groups	11	4
Total Groups	17	9
Range of Group Sizes	3–15	5–12
Mean Group Size	6.1	6.3
Prop Male	0.37 (0.18–0.59)	0.37 (0.10–0.76)
Prop Female	0.51 (0.29–0.72)	0.50 (0.20–0.72)
Prop Young	0.12 (0.03–0.21)	0.13 (0.02–0.20)
M:F	0.72	0.74
Y:F	0.24	0.26

Note table 2: detection probabilities (P1 = first observer; P2 = second observer), individual ungulates seen by each observer (Obs 1 total and Obs 2 total), Individual number of groups seen by each observer (Obs 1 groups and Obs 2 groups), number of groups seen by both, i.e. recaptures (Common groups), mean group size, proportion of male, female, and young with 95% confidence intervals. M:F and Y:F displays the male to female ratio and the young to female ratio respectively using the estimated proportional values for each age-sex class.

Image 1. Tibetan Argali *Ovis ammon hodgsoni*. © Rigzen Dorjay.

populations needs more research (Namgail et al. 2007; Butt & Turner 2012).

Therein is an urgent need to consider Tibetan argali as a priority species for conservation in India. As a first step towards that, we set robust population baselines for two remaining strongholds of argali populations in Tsaba and Chushul-Mirpal Tso. While we intended to do so, robust on ground surveys were not possible in Kharnak and Phobrang-Chhang Chenmo due to logistical and COVID-19 related constraints. Robust population estimates over time help in determining population trends (Mihoub et al. 2017). Conservation status assessment of any species requires rigorous monitoring of their abundances (Lindenmayer et al. 2013). An initial population reference can aid in framing conservation objectives by helping assess feasibility, concentrate effort, and define time period within which progress can be evaluated (Bull et al. 2014).

Our estimated argali densities for Tsaba and Chushul-Mirpal Tso are lower than many other sites across argali's central Asia range known to harbor good argali population (e.g., Wingard et al. 2011; Khanyari et al. 2021), while being comparable to estimates presented by Tumursukh et al. (2015) from the Tost mountains, in Mongolia. A factor driving lower densities of argali from our sites might be the clustering of groups within the study sites, driven primarily by forage availability, competition with sympatric livestock and wild ungulates and species natural history. This merits further research.

Additionally, both our argali populations were female-biased, like most mountain ungulate populations (Berger & Gompper 1999). Not only are males disproportionately predated upon, but in polygynous mating species like argali, males expend higher costs than females during rut which can lead to reduced mating male survival. This can be exacerbated in resource limited systems (Berger & Gompper 1999; Toigo & Gaillard 2003). Alongside, factors such as selective hunting of prime-aged males for trophy hunting or through poaching can further aggravate the female-bias. We also find low young to female ratio for both sites (Table 3). Ekernas et al. (2016) suggest that argali populations with young to female ratios <0.5 are potentially declining. Our surveys were conducted in March. To better understand the dynamics of this population, it would be important to conduct the surveys in summer, soon after the birth season.

Finally, it would be useful to update the conservation status of argali, not only throughout their range, but particularly Tibetan Argali in neighboring regions such as China and Nepal to get a an overall status in the region. Kusi et al. (2019) compiled historical data and their own observation data to discuss the present distribution of Tibetan Argali in Nepal. They also recommend management of livestock numbers, promotion of traditional grazing practices and raising conservation awareness as long-term conservation strategies for the species. The Tibetan plateau houses the largest known Tibetan Argali population, numbering into a few thousands. However they have seen recent declines primarily due to habitat loss and hunting (Harris 2010). Schaller (1998) provides a good overview of historic records, and although some early explorers seemed to find them common, most reported them as rare. Contemporary Chinese policy aims to conserve argali using two main strategies—nature reserves and international hunting areas (Harris 2010). Given the proximity of the India, Nepalese and Chinese population to their respective country borders, it is important to consider a transboundary strategy to conserve them.

CONCLUSION

Across India and larger central and southern Asian mountain ecosystem, it is important that more research is conducted on various conservation aspects concerning the Tibetan Argali and other Caprinae species. Specifically, in Ladakh, we recommend that continual monitoring of the Tsaba and Chushul-Mirpal Tso populations are done. Alongside continually monitoring these populations, in-depth threat assessment and stakeholder engagement, especially with the agro-pastoral communities that share their pastures with argali, is needed to delimit both contextually-appropriate and effective conservation interventions for argali in India.

Research ethics/best practice statement

Research conducted complied with the laws and regulation of India (Union Territory of Ladakh) where the study was performed. The required permission was obtained from the Ladakh Wildlife Protection Department. For the primary survey, none of the procedures performed involved any animal handling and were in accordance with the 1964 Helsinki declaration and its later amendments. For the secondary surveys, were made sure to obtain oral consent for all respondents – written consent was not possible as many respondents were not literate. We ensured all information provided by informants was anonymized and interviews were not conducted if the respondent did not feel comfortable. Interviews with individuals were only conducted after taking permission from village elders and the village headman. Ethics clearance was obtained from the research ethics committee from the University of Bristol.

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COMMUNICATION

An annotated checklist of the avifauna of Karangadu mangrove forest, Ramanathapuram, Tamil Nadu, with notes on the site's importance for waterbird conservation

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Abstract: Avifaunal inventories are crucial to the formulation of conservation and management strategies for habitats and species. An annotated checklist of the birds of the Karangadu eco-tourism area located in the Palk Bay in Ramanathapuram district of Tamil Nadu, was prepared. We listed a total of 107 species belonging to 18 orders and 40 families. Orders Charadriiformes, Suliformes, and Pelecaniformes dominated the habitat. Among the families, Scolopacidae (10 species) was dominant, followed by Ardeidae (9), and Laridae (8). In addition, the study also documented three globally 'Near Threatened' species: Painted Stork *Mycteria leucocephala*, Black-tailed Godwit *Limosa limosa*, and Black-headed Ibis *Threskiornis melanocephalus.* The observed frequency of the species was: 57% (61 spp.) common, 32.7% (35 spp.) uncommon, and 10.3% (11 spp.) rare. Categorization based on the residential status of birds revealed that 31% (33 spp.) were winter visitors, and one was a passage migrant (Rosy Starling *Pastor roseus*). These baseline data highlight the importance of Karangadu as an important site on the southeastern coast of India for migratory shorebird conservation priorities.

Keywords: Central Asian Flyway, Gulf of Mannar, mangroves, Palk Bay, shorebirds, winter visitors.

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INTRODUCTION

India is biodiversity rich, with several distinct biogeographical zones and habitats housing 12.5% of world avian diversity (Praveen et al. 2016; Praveen & Jayapal 2022). Birds are indicators of ecosystem health (Bilgrami 1995; Piersma & Lindstrom 2004), and data on their occurrence is important for ecological assessments and management initiatives (Kati & Sekercioglu 2006). The significance of a specific landscape for avifaunal conservation can be recognized by assessing the structure of local bird communities (Kattan & Franco 2004). Information on avifauna is vital for an ecosystem conservation effort, as well as to understand the implications of habitat degradation/loss and climate change (Daniels et al. 1991; Peterson et al. 2000; Llanos et al. 2011).

In Tamil Nadu, several studies have been carried out on the bird diversity of wetlands including Kaliveli (Pieter 1987), Karaivetti (Gokula 2010), Pallikaranai (Raj et al. 2010), Point Calimere (Sugathan 1982), Singanallur Lake (Reginald et al. 2007), and Vaduvoor (Gokula & Raj 2011). In the Ramanathapuram District of Tamil Nadu and adjoining areas of the Gulf of Mannar & Palk Bay regions, previous studies have been carried out from Rameswaram Island (Biddulph 1938) and Mandapam & its neighbouring islands (Balachandran 1990). Byju et al. (2023) described the avifaunal distribution on the 21 islands located in the Gulf of Mannar Biosphere Reserve.

In 2022, the Government of India announced the increase of Ramsar sites to 75, which included two wetlands (GOI 2022) from the Ramanathapuram District, prioritizing this area for bird and habitat conservation. Bird survey data provide useful insights for basic and applied ecology, as well as for identifying priority conservation areas (Daniels et al. 1991; Peterson et al. 2000). Some of the recently published records from this district include sighting of Artic Skua *Stercorarius parasiticus* (Byju & Raveendran 2022a), and the first Asian record of Light-mantled Albatross *Phoebetria palpebrata* (Byju & Raveendran 2022b). Previous findings highlight the importance of this area, and prompted us to undertake a baseline survey based on a need for monitoring in new areas in the rapidly changing landscape. This study of Karangadu on the Palk Bay adjoining the Gulf of Mannar region could act as a base for further research into avian systematics, taxonomy, distribution, assessment, and management. This study also remarks on the regularity of observations, the relevance of birds, especially long-distance migratory shorebirds, and the conservation significance of this mangrove habitat.

METHODS

Study Area

Karangadu mangrove forest (9.6479^{0}N & 78.9569^{0}E) is located on the southeastern coast of India, adjoining the Palk Bay in the Ramanathapuram District of Tamil Nadu, India (Figure 1). It is an eco-tourism area run by the forest department with the support of the Eco Development Committee (EDC), involving the local fishermen community providing boating and bird-watching facilities for the public. The predominant vegetation of Karangadu is mangroves, which consists of *Avicennia marina* and *Rhizophora mucronata*. Many of unused areas were planted with mangroves in the last decade, converting the area to a mangrove forest from the estuary to the east coast road. This aids in supporting wading birds. Crabs, prawns, and other invertebrates inhabit the mangroves, regularly attracting a number of birds. Fishing activity and fish landing centres attract fish-eating birds. Small water bodies left over by unused salt pans as well as artificial pools with water released from prawn culture areas aid bird populations. The main habitat types observed in the study area include: Open water habitat and Mangroves (WL = Wetland); Trees (Tr) Palm and Tamarind trees; Shrub habitat (OS = Open Scrub type). The district receives rain from both the south-west and north-east monsoons. The district experiences a tropical climate. The months of May and June are generally hot and dry (Balachandran 1990).

This study on the avifauna of the Karangadu eco-tourism area was carried out between January 2017 and March 2018. A total of 12 field visits (one per month) were conducted to observe the diversity of birds. Field surveys were conducted in the morning (0700–1000 h) and evening (1600–1900 h), depending on the season when birds were most active. Opportunistic sightings were also made to compile the checklist of the birds of the region during the years 2019 to 2021, from September to March. Direct count for individual species and block count methods were employed for flocks for data collection (Howes & Bakewell 1989; Bibby et al. 2000). In the study area, waterbirds were counted at three scanning points (Figure 1), selected on the basis of preliminary surveys done in January 2016. Additional observations recorded while moving from one scanning point to another were treated as incidental records. Birds were observed using Nikon binoculars (10x50) and photographed with Canon 100–400 mm tele-lens, and were identified with the help of a field guide (Grimmett et al. 2011).

The residential status of birds was assessed as

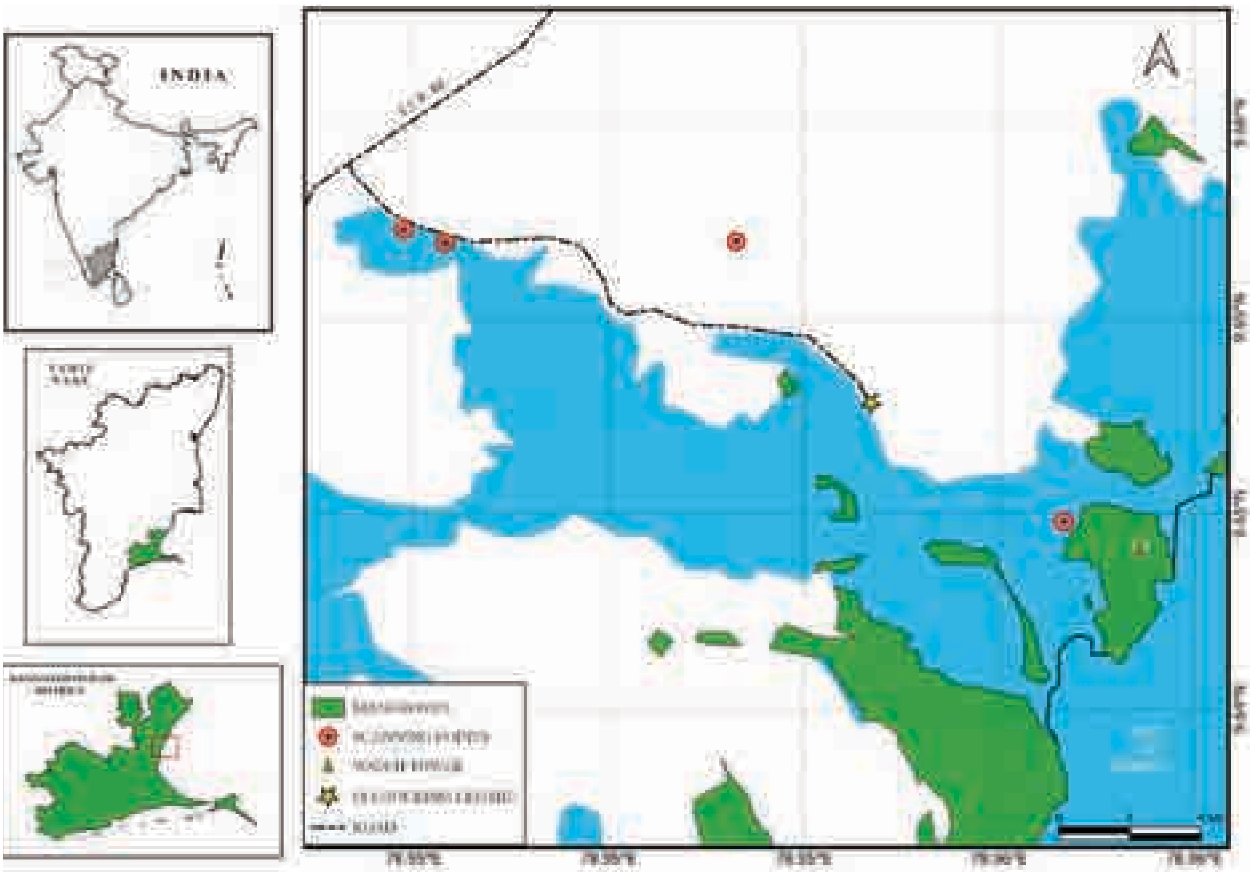

Figure 1. A map of the Karangadu mangrove ecotourism area with bird scanning points.

Resident (R), Passage Migrant (PM), and Winter Visitor (WV) depending on their timing and duration of occurrence (Grimmett et al. 2011). The International Union for the Conservation of Nature (IUCN 2022) status was additionally used to compare the local status with the global status. During the surveys, other information–like the role of EDC or threats to birds were noted. The data recorded in each survey was analyzed for relative abundance based on frequency of bird sightings, categorized as: Common (C), encountered on >60% of visits; Uncommon (UC), encountered 21–60% of visits; Rare (R), encountered on less than 20% of visits (McKinnon & Philips 1993).

RESULTS AND DISCUSSION

Avian community structure

A checklist of the Karangadu eco-tourism area in Ramanathapuram, Tamil Nadu, produced in the study includes a total of 107 avian species representing 40 families belonging to 18 orders. Passeriformes, with 17 families and 31 species, was dominant. But waterbirds (n = 45) belonging to 11 families were abundant in numbers.

Our observations revealed that the families Scolopacidae (10 species) and Ardeidae (nine species) are followed by Laridae (eight species), Accipitridae (seven species), Charadriidae, and Cuculidae (six species each), Columbidae, Threskiornithidae, and Cisticolidae (four species each), and Alcedinidae, Corvidae, Sturnidae, and Alaudidae (three species each). Phasianidae, Meropidae, Strigidae, Phalacrocoracidae, Ciconiidae, Laniidae, Hirundinidae, Muscicapidae, Nectariniidae and Motacillidae (two species each); Podicipedidae, Upupidae, Coraciidae, Apodidae, Rallidae, Recurvirostridae, Falconidae, Phoenicopteridae, Oriolidae, Dicruridae, Monarchidae, Pycnonotidae, Timaliidae, Acrocephalidae, Estrildidae, and Passeridae (one species each) represented the major bird groups of the area. An annotated checklist of birds of the Karangadu mangrove area representing the orders and families is given in Table 1.

Among the total birds documented from the area, water birds were in the majority during all the surveys. We observed that several species of gulls and terns

Image 1. A flock of Marsh Sandpiper *Tringa stagnatilis* in the mangroves of Karangadu.

used the open regions inside the mangroves (Image 1) near the watchtower mainly for roosting. Waterbird abundance and diversity were influenced by factors in wetlands such as wetland area, depth of water and its quality, trophic level, and ideal roosting and breeding locations for birds (Wiens 1989; Mukherjee et al. 2002; Ma et al. 2010). Among the waterbirds, the order Charadriiformes dominated the study area, followed by the Pelecaniformes. Since most of the areas under study were covered with mangroves in the wetland area (Image 2), waterbirds represented the majority in numbers. As a result, we have focused on waterbirds (shorebirds, large wading birds, gulls, and terns) with special focus on shorebirds, both long-distance migrants and resident birds.

We recorded 18 shorebird species, of which 15 were Winter Visitors (WV), including the 'Near Threatened' Black-tailed Godwit *Limosa limosa*. Two species, namely Black-winged Stilt *Himantopus himantopus* and the Red-wattled Lapwing *Vanellus indicus* were Resident. One species, the Kentish Plover, could either be a Resident/Winter Visitor (R/WV), as we have recorded it during the non-breeding season. The most dominant species among the observed shorebirds were Common Sandpiper *Actitis hypoleucos* (peak count at one time: 620 in January), followed by Little Stint *Calidris minuta* (peak count at one time: 245 in January). Another 19 species of waterbirds, including herons, egrets, and ibises, were also recorded from this site, including the 'Near Threatened' Painted Stork *Mycteria leucocephala* and Black-Headed Ibis *Threskiornis melanocephalus*. The most dominant group of birds were the egrets: Great Egret *Ardea alba*, Intermediate Egret *Ardea intermedia*, and Little Egret *Egretta garzetta* (peak counts between 100 and 150 at one time, throughout the year). Moreover, this area also serves as an important foraging place for Greater Flamingo *Phoenicopterus roseus* (peak count at one time was 150 in February).

Six species of terns and two species of gulls were also recorded from the eco-tourism area. This was used as a roosting site by two gull species, namely Brown-headed Gull *Chroicocephalus brunnicephalus* and Black-headed Gull *Chroicocephalus ridibundus*, as well as two tern species, Lesser Crested Tern *Thalasseus bengalensis* and Greater Crested Tern *Thalasseus bergii*. Brown-headed gulls were the most dominant gull species (peak count at one time: 225 in February), and the Lesser Crested Tern represents the most dominant tern species (peak count at one time: 325 in February) among the terns. This observation goes in parallel with the reporting from

Table 1. An annotated checklist of the avifauna recorded from the Karangadu ecotourism area.

Scientific name	Common name	Migration status	IUCN Red List status	Frequency of observation	Habitat type
Order: Galliformes Family: Phasianidae					
Pavo cristatus	Indian Peafowl	R	LC	C	OS
Francolinus pondicerianus	Grey Francolin	R	LC	C	GL/OS
Order: Podicipediformes Family: Podicipedidae					
Tachybaptus ruficollis	Little Grebe	R	LC	C	WL
Order: Bucerotiformes Family: Upupidae					
Upupa epops	Common Hoopoe	R	LC	UC	GL
Order: Coraciiformes Family: Coraciidae					
Coracias benghalensis	Indian Roller	R	LC	C	OS
Family: Alcedinidae					
Ceryle rudis	Pied Kingfisher	R	LC	UC	WL
Halcyon smyrnensis	White-throated Kingfisher	R	LC	C	WL
Alcedo atthis	Common Kingfisher	R	LC	C	WL
Family: Meropidae					
Merops orientalis	Green Bee-eater	R	LC	C	OS
Merops philippinus	Blue-tailed Bee- eater	WV	LC	C	OS
Order: Cuculiformes Family: Cuculidae					
Centropus sinensis	Greater Coucal	R	LC	C	OS
Eudynamys scolopaceus	Asian Koel	R	LC	C	OS
Phaenicophaeus viridirostris	Blue-faced Malkoha	R	LC	C	OS
Hierrococcyx varius	Common Hawk Cuckoo	R	LC	UC	OS
Cuculus canorus	Common Cuckoo	WV	LC	UC	OS
Clamator jacobinus	Pied Cuckoo	R	LC	UC	OS
Order: Psittaciformes Family: Psittacidae					
Psittacula krameri	Rose-ringed Parakeet	R	LC	C	Tr
Order: Strigiformes Family: Strigidae					
Asio flammeus	Short-eared Owl	WV	LC	R	GL/OS
Athene brama	Spotted Owlet	R	LC	UC	OS/Tr
Order: Columbiformes Family: Columbidae					
Columba livia	Rock Pigeon	R	LC	C	OS/GL
Streptopelia decaocto	Eurasian Collared-Dove	R	LC	C	OS/GL
Spilopelia senegalensis	Laughing Dove	R	LC	C	OS/GL
Spilopelia chinensis	Spotted Dove	R	LC	C	OS/GL
Order: Apodiformes Family: Apodidae					
Cypsiurus balasiensis	Asian Palm-swift	R	LC	C	Tr
Order: Gruiformes Family: Rallidae					
Amaurornis phoenicurus	White-breasted Waterhen	R	LC	C	WL
Order:Charadriiformes Family: Scolopacidae					
Tringa glareola	Wood Sandpiper	WV	LC	UC	WL

Scientific name	Common name	Migration status	IUCN Red List status	Frequency of observation	Habitat type
Actitis hypoleucos	Common Sandpiper	WV	LC	C	WL
Calidris minuta	Little Stint	WV	LC	UC	WL
Calidris temminickii	Temminick's Stint	WV	LC	R	WL
Numenius phaeopus	Whmibrel	WV	LC	UC	WL
Limosa limosa	Black-tailed Godwit	WV	NT	R	WL
Calidris pugnax	Ruff	WV	LC	R	WL
Tringa stagnatilis	Marsh Sandpiper	WV	LC	C	WL
Tringa totanus	Common Redshank	WV	LC	C	WL
Tringa nebularia	Common Greenshank	WV	LC	C	WL
Family: Charadriidae					
Charadrius dubius	Little Ringed Plover	WV	LC	UC	WL
Pluvialis fulva	Pacific Golden Plover	WV	LC	UC	WL
Pluvialis squatorala	Black-bellied Plover	WV	LC	UC	WL
Charadrius mongolus	Lesser Sand Plover	WV	LC	UC	WL
Vanellus indicus	Red-wattled Lapwing	R	LC	C	WL
Charadrius alexandrinus	Kentish Plover	WV/R	LC	C	WL
Family: Recurvirostridae					
Himantopus himantopus	Black-winged Stilt	R	LC	C	WL
Family: Laridae					
Chlidonias hybrida	Whiskered Tern	WV	LC	R	WL
Hydroprogne caspia	Caspian Tern	WV	LC	UC	WL
Gelochelidon nilotica	Gull-billed Tern	WV	LC	UC	WL
Thalasseus bengalensis	Lesser Crested Tern	WV	LC	C	WL
Thalasseus bergii	Greater Crested Tern	WV	LC	C	WL
Sternula albifrons	Little Tern	WV	LC	R	WL
Chroicocephalus ridibundus	Black-headed Gull	WV	LC	C	WL
Chroicocephalus brunnicephalus	Brown-headed Gull	WV	LC	C	WL
Order: Falconiformes Family: Falconidae					
Falco tinnunculus	Common Kestrel	WV	LC	R	OS/GL
Order: Accipitriformes Family: Accipitridae					
Milvus migrans	Black Kite	R	LC	UC	OS
Elanus caeruleus	Black-winged Kite	R	LC	C	OS
Hieraaetus pennatus	Booted Eagle	WV	LC	R	OS/Tr
Accipiter badius	Shikra	R	LC	UC	GL/Tr/OS
Pernis ptilorhynchus	Oriental Honey Buzzard	R	LC	R	Tr
Haliastur indus	Brahminy Kite	R	LC	C	WL/GL
Pandion haliaetus	Osprey	WV	LC	R	WL
Order: Phoenicopteriformes Family: Phoenicopteridae					
Phoenicopterus roseus	Greater Flamingo	R	LC	UC	WL
Order: Suliformes Family: Phalacrocoracidae					
Microcarbo niger	Little Cormorant	R	LC	C	WL
Phalacrocorax fuscicollis	Indian Cormorant	R	LC	C	WL

Scientific name	Common name	Migration status	IUCN Red List status	Frequency of observation	Habitat type
Order: Pelicaniformes Family: Ardeidae					
Ardea cinerea	Grey Heron	R	LC	UC	WL
Ardea purpurea	Purple Heron	R	LC	UC	WL
Egretta garzetta	Little Egret	R	LC	C	WL
Bubulcus ibis	Cattle Egret	R	LC	C	WL
Ardea alba	Great Egret	R	LC	C	WL
Ardea intermedia	Intermediate Egret	R	LC	C	WL
Ardeola grayii	Indian Pond Heron	R	LC	C	WL
Nycticorax nycticorax	Black-crowned Night Heron	R	LC	C	WL
Butorides striata	Striated Heron	R	LC	UC	WL
Family: Threskiornithidae					
Threskiornis melanocephalus	Black-headed Ibis	R	NT	C	WL
Plegadis falcinellus	Glossy Ibis	R	LC	C	WL
Pseudibis papillosa	Red-naped Ibis	R	LC	C	WL
Platalea leucorodia	Eurasian Spoonbill	R	LC	C	WL
Order: Ciconiiformes Family: Ciconiidae					
Anastomus oscitans	Asian Openbill	R	LC	C	WL
Mycteria leucocephala	Painted Stork	R	NT	C	WL
Order: Passeriformes Family: Oriolidae					
Oriolus kundoo	Indian Golden Oriole	WV	LC	UC	OS
Family: Laniidae					
Lanius vittatus	Bay-backed Shrike	R	LC	C	OS
Lanius cristatus	Brown Shrike	WV	LC	UC	OS
Family: Dicruridae					
Dicrurus macrocercus	Black Drongo	R	LC	C	GL/OS
Family: Monarchidae					
Terpsiphone paradisi	Indian Paradise Flycatcher	R	LC	UC	OS/GL
Family: Corvidae					
Dendrocitta vagabunda	Rufous Treepie	R	LC	UC	OS
Corvus macrorhynchos	Large-billed Crow	R	LC	C	OS/GL/WL
Corvus splendens	House Crow	R	LC	C	OS/WL/GL
Family: Sturnidae					
Acridotheres tristis	Common Myna	R	LC	C	OS/GL
Pastor roseus	Rosy Starling	PM	LC	UC	OS/GL
Sturnia pagodarum	Brahminy Starling	R	LC	UC	OS/GL
Family: Hirundinidae					
Cecropis daurica	Red-rumped Swallow	R	LC	UC	WL
Hirundo rustica	Barn Swallow	WV	LC	UC	WL
Family: Pycnonotidae					
Pycnonotus cafer	Red-vented Bulbul	R	LC	C	OS/GL
Family: Timaliidae					
Turdoides affinis	Yellow-billed Babbler	R	LC	C	OS

Scientific name	Common name	Migration status	IUCN Red List status	Frequency of observation	Habitat type
Family: Cisticolidae					
Prinia socialis	Ashy Prinia	R	LC	C	OS/GL
Prinia inornata	Plain Prinia	R	LC	C	OS/GL
Orthotomus sutorius	Common Tailorbird	R	LC	C	OS/GL
Cisticola juncidis	Zitting Cisticola	R	LC	UC	OS/GL
Family: Acrocephalidae					
Acrocephalus dumetorum	Blyth's Reed Warbler	WV	LC	R	OS
Family: Alaudidae					
Eremopterix griseus	Ashy-crowned Sparrow Lark	R	LC	UC	OS/GL
Galerida cristata	Jerdon's Bushlark	R	LC	UC	OS/GL
Alauda gulgula	Oriental Skylark	R	LC	UC	OS/GL
Family: Muscicapidae					
Copsychus fulicatus	Indian Robin	R	LC	C	OS
Copsychus saularis	Oriental Magpie Robin	R	LC	C	OS
Family: Nectariniidae					
Cinnyris asiaticus	Purple-rumped Sunbird	R	LC	C	OS/GL
Cinnyris asiaticus	Purple Sunbird	R	LC	C	OS/GL
Family: Estrildidae					
Lonchura punctulata	Scaly-breasted Munia	R	LC	UC	OS
Family: Passeridae					
Passer domesticus	House Sparrow	R	LC	C	GL
Family: Motacillidae					
Motacilla maderaspatensis	White-browed Wagtail	R	LC	C	WL
Anthus rufulus	Paddyfield Pipit	R	LC	UC	GL

LC—Least Concern | NT—Near Threatened | EN—Endangered | PM—Passage Migrant| WV—Winter Visitor | LM—Local Migrant | R—Resident | R—Rare| C—Common | UC—Uncommon| WL—Wetland| GL—Grass Land| OS—Open Scrub| Tr—Trees on the peripheries and the village area.

Image 2. A view of the water pools inside the mangrove area from the watch tower at Karangadu.

the west coast site of Kadalundi-Vallikkunu Community Reserve (Aarif et al. 2017).

In terms of distribution and migratory status, 73 species were found to be Resident, 32 Winter Visitors, one WV/R, and one Passage Migrant (PM) including the Rosy Starling *Pastor roseus* (Table 1). Based on the frequency of sightings, 61 species were Common, 35 were Uncommon, and 11 were Rare. In addition to this, habitat-wise avian richness was also recorded. The wetland had the most species presence (55 spp.), followed by open scrub (OS, 49 spp.) and grassland (GL, 29 spp.). The maximum number of different bird species occupying various habitats, shows their capacity to occupy diversified habitats. Varied feeding niches have enhanced the bird diversity of the study area. Among the land birds documented in Karangadu, eight raptor species were identified: the Black Kite *Milvus migrans*, Black-winged Kite *Elanus caeruleus*, Booted Eagle *Hieraaetus pennatus*, Brahminy Kite *Haliastur indus*, Common Kestrel *Falco tinnunculus,* Oriental Honey Buzzard *Pernis ptilorhynchus,* Osprey *Pandion haliaetus* and Shikra *Accipiter badius*. In addition to birds of prey, two nectarivorous birds, the Purple Sunbird *Cinnyris asiaticus* and the Purple-rumped Sunbird *Leptocoma zeylonica* were common in the areas adjacent to the mangroves.

Conservation Significance

Stop-over sites are critical for long-distance migrant shorebirds migrating between wintering and breeding areas (Boere et al. 2006). Karanagadu's proximity to the Important Bird Areas of Sri Lanka and the Gulf of Mannar may provide a link between the country's other major east coast shorebird sites, and other wintering sites along the Central Asian Flyway. Karangadu sandwiches the established shorebird wintering sites of Point Calimere and the Gulf of Mannar. This site serves as an ideal place of roosting for gulls, terns, long-migrant shorebirds, and Greater Flamingos, which makes this wintering site very important for further monitoring and conservation-related activities.

For effective conservation to be implemented, and for future strategies to be adopted, the role, contribution, and participation of the local community are essential (Sinthumule & Netshisaulu 2022). The forest department-initiated EDC-run eco-tourism project in Karangadu is in the right perspective because of the active involvement of the fishermen community in boating and bird watching for the public. This helps in the general upkeep of the mangrove habitat along with avian conservation, as the revenue earned from tourism is being used by the local community. The newly planted 30-acre areas of mangroves (represented by *Rhizophora apiculata*, *R. mucronata*, and *Avicennia* spp.; developed with the help of the local community in 2015), are serving as an ideal habitat for waterbirds. This might have improved the area's avian diversity and richness. Nevertheless, in the tree habitat (Tr) in the peripheries, the avian richness was quite low, as only six species were recorded (Table 1). The current observations emphasize the value of wetlands and associated areas as avian habitats, as sizable number of species was found in more than one habitat. The inclusion of local communities in conservation has helped in habitat restoration and natural resource conservation programs in recent times (Silori 2007; Nepal & Spiteri 2011; Badola et al. 2012; Scholte et al. 2016; Sinthumule 2021). Controlled seasonal tourism by boating without disturbing habitats through the water channels could boost revenue, and the money raised could be further used for conservation initiatives. Hence, we propose that to check and devise strategies and activities for conservation, Karangadu mangrove areas should be declared a bird sanctuary.

CONCLUSION

Aside from the established shorebird monitoring sites, regular long-term monitoring, and assessment of the Karangadu eco-tourism area, an important wintering site on the east coast, should be carried out in the future to establish the importance of this area on the flyway. Furthermore, because the community members are engaged in ecotourism activities other than fishing and crab harvesting, they may be effectively educated on the significance and necessity of preserving and sustaining a balanced environment. This study has provided preliminary information on selected shorebirds as well as other waterfowl from the Karangadu eco-tourism area, which will be beneficial for future research in this area as well as demonstrating the importance of designating this as a protected area of conservation importance.

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COMMUNICATION

Habitats and nesting habits of Streaked Weaver *Ploceus manyar* in select wetlands in the northern districts of Tamil Nadu, India

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Abstract: The habitats and nesting habits of Streaked Weaver *Ploceus manyar* was studied in the Narrow Leaf Cattail *Typha angustifolia* (Typhaceae) wetlands in Ranipet, Kancheepuram, and Viluppuram districts of northern Tamil Nadu. A total of 349 nests, in various developmental stages, in 24 colonies and of 536 adult individuals were enumerated. Of these 349 nests, 43 nests were in wad stage, 28 in ring stage, 123 in helmet stage, 55 egg-chamber-closed stage, and 100 complete nests including two abnormal nests were recorded. Streaked Weaver used fibres of *T. angustifolia* for building nests. Females were also observed to be engaged in nest construction, while males placed blobs of cow dung on the inner or both walls of helmet stage nests before pairing with females; 88.7% helmet stage nests had deposition of cow dung on their inner walls and the remaining 11.3% nests had no such deposits. In 59% nests the entrance tubes were found facing east. They showed communal foraging and the flock size ranged from 50–80 birds. No antagonistic interactions were observed between Streaked Weavers and other species over sharing of common perching sites and foraging grounds. Harvesting of nest-supporting reeds and sightings of avian predators, such as House Crow *Corvus splendens*, Large-billed Crow *Corvus macrorhynchos*, and Rufous Treepie *Dendocitta vagabunda* near nest colonies may pose threats to Streaked Weavers.

Keywords: Abnormal nests, communal foraging, deposition of cow dung, nest colonies, nest orientation, *Typha angustifolia*.

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INTRODUCTION

Ploceidae is a family of Passerine birds, many of which are called weavers. The family is believed to have originated in the mid-Miocene (De Silva et al. 2017). The group comprises a total of 116 species in 15–17 genera (Clements et al. 2022) and is well known for their intriguing and dramatic variation in behaviour, nest structure, and plumage coloration (Collias & Collias 1964). The Afro-Asian region harbours 64 species of weavers (Dickinson & Christidis 2014). Four species alone occur in India: Baya Weaver *Ploceus philippinus*, Black-breasted Weaver *P. benghalensis*, Streaked Weaver *P. manyar*, and Finn's Weaver *P. megarhynchus* (Ali & Ripley 1987). Though the Streaked Weaver occurs widely in India, its nesting colonies are scattered across the peninsular region (Rasmussen & Anderton 2005). Detailed accounts of its breeding biology were studied in Kumaon Terai (Ambedkar 1972), Wazirabad, Delhi (Prakash et al. 2020), and Veedur wetlands, Tamil Nadu (Pandian 2022). The other published works on the Streaked Weaver are either anecdotal accounts of its natural history, e.g., roosting behaviour (Dhindsa & Toor 1981), crop damage (Dhindsa 1982), causes of nest destruction (Sharma 1997), and on nesting in *Ipomoea* reed beds (Panwar et al. 2020); or records in regional checklists, e.g., Dharmapuri (Vasanth 1990), Coringa Wildlife Sanctuary (Srinivasulu et al. 2000), Pallikaranai (Raj et al. 2010), Mayiladuthurai (Ali et al. 2011), Kalpakkam (Hussain et al. 2011), Tiruchirappalli (Siva & Neelanarayanan 2017), and Chengalpet (Nagarajan 2021).

The present study documents the various habitats and nesting habits of the Streaked Weaver in the *T. angustifolia* reeds in northern Tamil Nadu. The aim of this study was to investigate the habitats, nesting behaviours, and probable threats to their populations.

MATERIALS AND METHODS

Study area

The present study was carried out in the selected reeds covered wetlands providing definite habitats to Streaked Weavers in Ranipet, Kancheepuran, and Viluppuram districts of northeastern Tamil Nadu (Figure 1). These three districts cover an area of c. 7600 km^2 with a human population of c. 44,00,000. Monsoon-dependent Palar, Kosasthalaiyar, Ponnaiyar, Thenpennai, Varaha, and Thondi rivers pass through these districts. The reeds at the study sites comprise Narrow Leaf Cattail *Typha angustifolia*, spread over c. 4 ha. Apart from that *Chrysopogon zizanioides* and *Phragmites* spp. occur abundantly in the study sites. Agriculture is the primary occupation in the district. The major crops of the area are Rice *Oryza sativa*, Jowar *Sorghum bicolor*, Pearl Millet *Pennisetum glaucum*, Finger Millet *Eleusine coracana*, Sugarcane *Saccharum officinarum*, and pulses such as Black Gram *Vigna mungo*, Green Gram *Vigna radiata*, & Pigeon Pea *Cajanus cajan*. Monoculture of *Casuarina equisetifolia* and cultivation of vegetables & flowers are also common. Temperatures range from 20–36 ^{o}C. The average annual rainfall is c. 1,000 mm.

METHODS

Data collection

A survey was conducted in the c. 4 ha reed beds situated along the arable lands in three districts. The selected wetlands in three districts were identified that had a definite history of the existence of *P. manyar* populations and their nests in the previous years. These sites were surveyed between 0600 h and 1800 h during the breeding season from 01 February–15 July 2022. The following variables were quantified: area of nesting colonies, depth of water (measured using a bamboo pole), size of nest colonies, number of nests in each colony, the distance between adjacent nest colonies, number of nests in various developmental stages, the orientation of nests, and distance between the reeds & foraging sites, i.e., paddy fields. The communal foraging behaviours, interactions with other species, and threats to their populations were observed uninterruptedly from a reasonable distance (c. 20 m) through binoculars, without causing disturbance to the birds. The number of birds was enumerated by following the total count method (Bibby et al. 2000) and analysing photographs taken when the birds were foraging and perching on nearby plants. Bird census was conducted over four monthly sessions during the second week of every month, i.e., March–June 2022. The total number of birds is expressed as the highest count of the session's total count. Photographic documentation of various developmental stages of nests and deposition of cow dung on the wall of helmet stage nests was done. The choices of grains/seeds foraged by the Streaked Weavers were observed and identified plant species foraged by these birds. The damaged nests, size of harvested reed areas by humans, and other plants that caused disturbance to nest colonies were quantified. The sighting of avian predators in the vicinity of nest colonies

Figure 1. Study area map: a—India map indicating Tamil Nadu (Black colour) | b—Tamil Nadu map indicating study districts | c —Location of study sites showing *Typha angustifolia* reeds with nest colonies.

were observed and recorded.

Data analysis

After the breeding season was over and the nests were completely abandoned by the birds, the number of leaves attached to the roofs of nests, the length, and the diameter of entrance tubes were measured in 15 nests. The number of nests per colony, the distance between the nest colonies, and the nearest roads, buildings & electric cables were measured. Based on these data, bar charts were made. Karl Pearson's correlation coefficient (*r*) test was used to assess the significant relationship between the height of reeds above water level & the total number of nests and between the depth of water & the number of nests. No live nest, adult bird, egg or chick was handled during the study. Locations of the nest colonies in the reeds were determined using a Garmin Etrex 20x GPS device. Photographs and videography were made using a Nikon P1000 digital camera. The collected data were tabulated, analysed, and shown as a graphical representation.

RESULTS

A total of 349 nests, including some in various developmental stages in 24 nest colonies, and 536 individuals of Streaked Weaver were enumerated. Colony size ranged 4–43 nests. Of the 349 nests, 12% nests (n = 43) were in wad stage, 8% in ring stage (n =

Table 1. Details of the number of birds, nesting habits, various stages of nests, and orientation of nests of Streaked Weaver during peak breeding period (2nd week of July 2022).

	Name of village	GPS coordinates	Area size (ha)	Total number of birds	Total number of nests	Stages of nests					Total number of nests studied for orientation	Orientation of nests			
						Wad stage	Ring stage	Helmet stage	Egg-chamber closed stage	Complete nests		East	West	North	South
1	Minnal	13.082°N–79.552°E	0.20	48	30	1	0	11	1	17	18	11	2	1	4
2	Kodaikal mottur	13.060°N–79.403°E	0.28	36	19	2	0	7	0	10	10	8	2	0	0
3	Anvarthikanpet	13.044°N–79.568°E	0.36	46	25	4	0	5	4	12	16	12	3	1	0
4	Melkalathur	13.040°N–79.567°E	0.20	30	15	3	0	4	4	4	8	5	0	3	0
5	Periyakarumpur	12.911°N–79.681°E	0.16	8	4	0	0	2	2	0	2	2	0	0	0
6	Urani	12.151°N–79.907°E	0.12	68	43	4	6	19	6	8	14	1	0	13	0
7	Kazhuveli	12.152°N–79.898°E	0.20	26	22	2	4	8	4	4	8	6	2	0	0
8	Marakkanam	12.158°N–79.916°E	0.80	26	21	4	3	4	3	7	10	4	2	4	0
9	Nemili	12.064°N–79.648°E	0.24	49	37	5	0	17	5	10	15	9	2	4	0
10	Veedur	12.069°N–79.586°E	1.42	199	133	18	15	46	26	28	54	33	5	13	3
		Total	**3.98ha**	**536**	**349**	**43**	**28**	**123**	**55**	**100**	**155**	**91**	**18**	**39**	**7**

28), 35% in helmet stage (n = 123), 16% in egg-chamber closed stage (n = 55), and 29% complete nests (n = 100). The size of reeds containing nest colonies varied from a minimum of 0.04 ha to a maximum of 0.24 ha. The minimum distance between the adjacent nest colonies was 30 m and the maximum was 70 m. The depth of water in the wetland also varied. It was shallowest at 0.3 m, and deepest at 1.6 m. Maximum nests (n = 43) was observed on reeds standing in water containing 30 cm depth, followed by 41 nests on reeds having 120 cm depth, and 33 nests on reeds with 100 cm depth. The height of the reeds above the water level also varied: minimum of 1.4 m and maximum of 3 m. Maximum nests (n = 271; 77.65%) were enumerated on reeds having height ranges of 1.6 m–2 m (Table 1).

Nest construction and materials

The study revealed that Streaked Weaver used fibers of *T. angustifolia* for the construction of nests in all three districts. Both sexes contributed to nest construction. Males built nests up to helmet stages and then, after pairing with females, both sexes collected fibres and continued the building. No other plant materials were used by the birds for the construction of nests.

Selection of nesting sites and stages of nest construction

Individuals of male Streaked Weaver with breeding plumages first appeared on reeds in first week of February 2022. The males visited various leaves, perching during the day on particular leaves of the *T. angustifolia*, 1–2 m above the water except for the duration of forage, but they did not engage in nest construction. From the second week of February onwards, males brought fibres from adjacent *T. angustifolia* and started building wad stage nests (by tying knots) and later the wad stage nests were developed into various stages of nests. After selecting a nesting site, a male Streaked Weaver bends and lowers the distal ends of two *T. angustifolia* leaves, holding them together with its toes, and plaits a knot around the leaves with its beak. Once this initial knot is tied, the male easily pulls down adjacent leaf tips, one by one and in a similar manner, and makes the basal knot stronger, creating a wad. The number of leaves attached to each wad nest varies from nest to nest. Males brought fibres continuously and made the wad stronger, like an amorphous roof/ceiling-like structure into which they enmeshed many green leaves. Out of 349 nests observed, only 29 % nests (n = 100) were developed into complete nests including two abnormal nests (bistoried 1+1 type) at the end of the breeding period (Figure 2) (Table 1). Only females were observed feeding their chicks. On three occasions, males were carrying the food in their beaks to the nesting colony but it was not possible to observe whether they entered the nests with food or not.

The length of the entrance tube varies from nest to nest and the measurement of entrance tubes of 15 abandoned nests revealed variations of 9–20 cm in length and 4–5 cm in diameter. The base of the nests was attached with 30–55 leaves. Apart from leaves, the birds also enmeshed peduncles with the base of nests (n = 22). The nests did not swing freely, because they were attached to many leaves of the *T. angustifolia*. A nest rarely swung along with the nest-supporting plants, during a gust, remaining suspended, and static most of the time. The distal ends of entrance tubes were 0.8—1.5 m above the water's surface (Figure 3). Pearson's correlation coefficient test reveals that there exists no significant correlation (r = -0.0946) between the height of reeds above water level & the number of nests and similarly no correlation (r = -02475) exists between depth of water under the nest colonies & several nests.

Nest Colonies

The size of reed beds containing nest colonies varied 0.04–0.24 ha. The number of nests per colony also varied 4–43. The distance between adjacent nests ranged 1–4 m. Out of 349 nests enumerated, maximum nests were observed in the nest ranges 21–30 nests, followed by 41–43, and 1–10 nests (Table 2; Image 1i).

Preference of Streaked Weaver in building nests on reeds occurring close to human activities

The study also tested the relationship between the proximity of roads, buildings, electric cables, and the selection of nest-supporting plants by individuals of Streaked Weaver. Three percent of nests (n = 9) occurred within a 25 m radius from the nearest roads, 33% of nests (n = 116) within a 26–50 m radius, and the remaining 65% of nests (n = 224) occurred above a 51 m radius from roads. Of the verified details, 32% of the nests (n = 111) occurred within a 200 m radius of buildings such as human dwellings, cattle sheds, & motor pump sets in crop fields, and the remaining 68% of nests (n = 238) occurred above 200 m radius from buildings; 38% of nests (n = 132) were found within 50 m of electric cables and the remaining 62% of nests (n = 217) occurred above 50 m from the nearest power cables (Figure 3–5).

Orientation of nests

Examination of the orientation of 155 nests (egg-chamber closed stage-55 & complete nests-100) on the nest-supporting plants revealed that 59% of nests (n = 91) were oriented east facing the rising sun, while 12% of nests (n = 18) were oriented the west, 25% nests towards (n = 39) north and 5% nests (n = 7) were found facing south. The study reveals that entrance tubes of maximum nests were found facing the east (Figure 6).

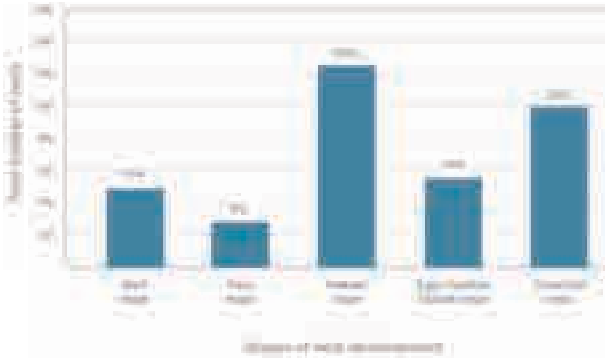

Figure 2. Bar-diagram indicating various developmental stages of nest construction.

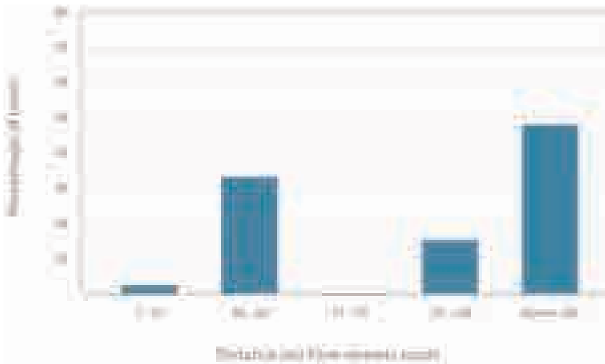

Figure 3. Relationship between a selection of nest-supporting plants and nearest roads.

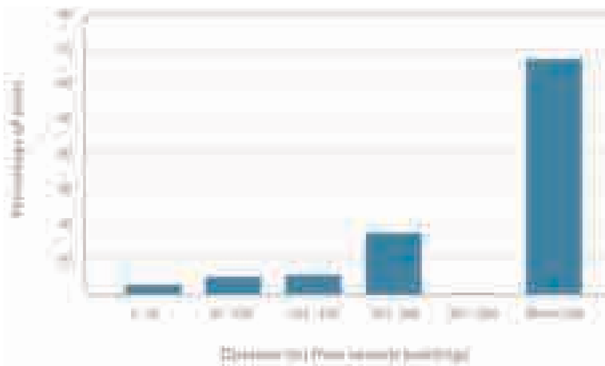

Figure 4. Relationship between a selection of nest-supporting plants and nearest buildings.

Deposition of cow dung

The males have the habit of smudging cow dung on the inner walls of helmet stage nests before pairing with a female. Out of 80 helmet stage nests that were

Table 2. Frequency distribution of nest colony sizes and number of nests in each range.

	Nest ranges (number of nests per colony)	Number of nest colonies	Percentage of number of nest colonies	Total number of nests enumerated	Percentage of number of nests in each range
1	1–10	12	50	77	22.06
2	11–20	5	21	66	18.91
3	21–30	4	17	89	25.5
4	31–40	1	4	33	9.46
5	41–50	2	8	84	24.07
	Total	24	100	349	100

studied, 88.7% helmet stage nests (n = 71) had a thick layer of dung on their inner walls (Image 2a), whereas the remaining 11.3% nests (n = 9) had no deposits of cow dung. Abnormal deposits of dung were found in seven nests, where dung had been plastered on both, inner and outer surfaces. Males had deposited cow dung abnormally like horn-shaped structures on the outer walls of these nests (Image 2b). No females were observed in the vicinity of nest colonies or seen carrying cow dung to the nests.

Communal foraging

The study revealed that all the nest colonies were surrounded by paddy crops (*Oryza sativa*: Poaceae) that occurred 25–800 m distance from the nest colonies. The Streaked Weaver always moved in flocks and the flock varied in size from 50–80 birds. The flocks contained a few individuals of other species, such as Baya Weaver *P. philippinus*, Black-breasted Weaver *P. benghalensis*, Tricolored Munia *Lonchura malacca*, White-rumped Munia *L. striata*, and Indian Silverbill *Euodice malabarica*. They strictly followed communal foraging. They forage on paddy when it is at the milky and unripe seed stage. They also perched on the spikes of *T. angustifolia*, *Setaria pallide-fusca*, and *Paspalidium geminatum* and consumed their unripe seeds. The individuals of Streaked Weaver coexisted with the aforesaid other bird species without any competition over sharing of common perching sites or food grains while foraging.

Threats

The study reveals that out of c. 4 ha of reeds in the study sites, c. 700 m^2 reeds were harvested by local villagers during the current breeding period for making mats and ropes. It was observed that 21 nests of various developmental stages (wad stage 7, ring stage 11, and helmet stage 1) were abandoned by the birds as the nearby reeds were harvested resulting in the exposure of nests to the outside. The birds might have abandoned those nests due to apprehension of movements

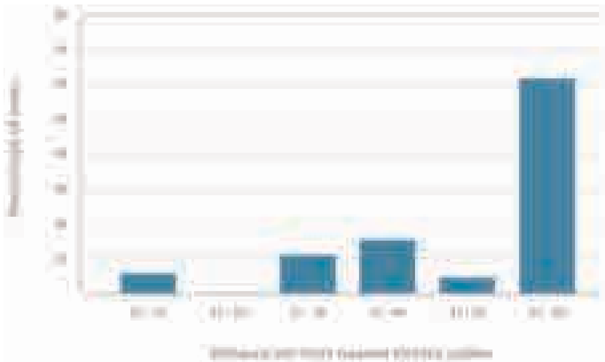

Figure 5. Relationship between a selection of nest-supporting plants and nearest electric cables.

Table 3. Details of predators observed near nest colonies of Streaked Weaver.

	Name of predator	Binomials	No. of sightings
1	House Crow	*Corvus splendens*	34
2	Large-billed Crow	*Corvus macrorhynchos*	16
3	Rufous Treepie	*Dendrocitta vagabunda*	09
	Total		59

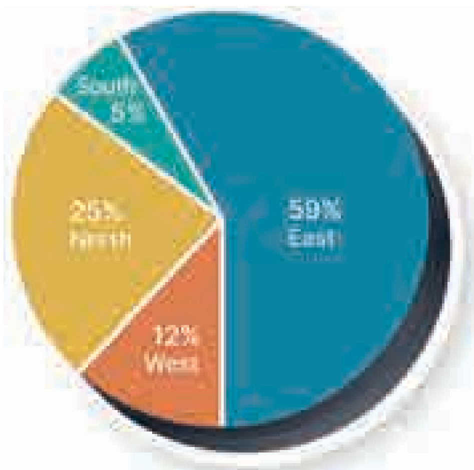

Figure 6. A pie diagram showing the orientation of nests of Streaked Weaver in the study area.

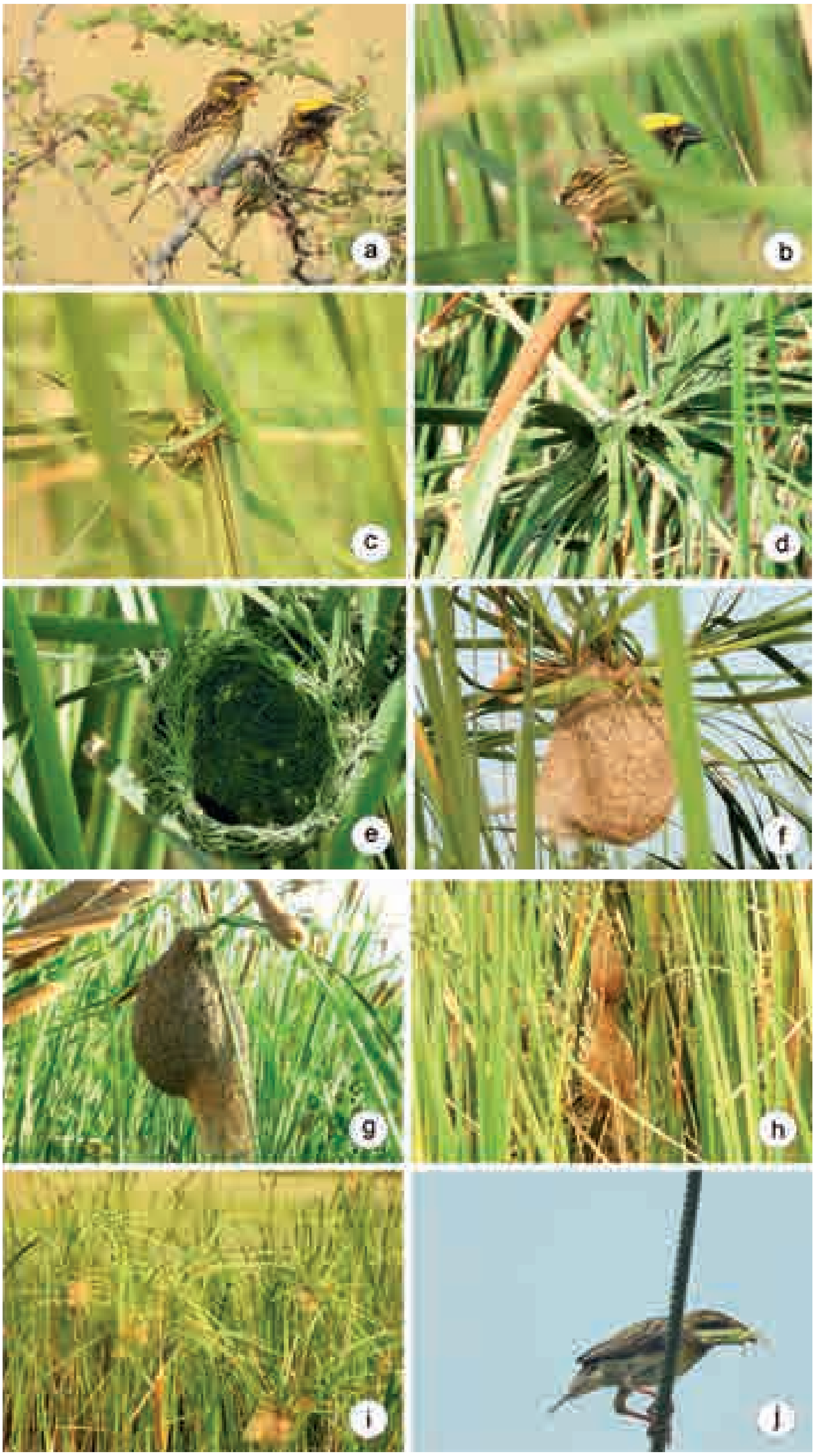

Image 1. Pictures indicating various stages of nest construction: a—A pair with breeding plumages perching on a shrub | b—Male carrying fibre for nest construction | c—Wad stage nest | d) Ring stage nest | e—Helmet stage nest | f—Egg-chamber closed stage nest | g—Complete nest with entrance tube | h—Double chambered abnormal nest | i—View of nest colony on reeds | j—Female carrying prey to chicks. © M. Pandian.

Image 2 . Pictures showing deposits of cow dung: a—Helmet stage nest contains a deposit of dung on the inner wall | b—Abnormal deposition of dung on the outer wall of the helmet stage. © M. Pandian.

of human beings, cattle, and exposure of nests to outside/predators. Birds had abandoned 12 nests (wad 8 and helmets 6) which were found enmeshed in the angiosperm climber *Oxystelma esculentum* and angiosperm parasite *Cassytha filiformis* which thickly covered and caused fallen down of *T. angustifolia* reeds. One complete nest was found torn probably due to the predators. Predatory birds, such as House Crow *Corvus splendens*, Large-billed Crow *Corvus macrorhynchos*, and Rufous Treepie *Dendrocitta vagabunda* were sighted (n = 59) in the vicinity of nesting colonies during the study period (Image 3; Table 3).

DISCUSSION

Many authors had previously studied the breeding periods of the Streaked Weaver. The breeding period was completed by the end of May in Dharmapuri District (Vasanth 1990), between February and June in Viluppuram, Tamil Nadu (Pandian 2022), and between May and July 2012 in Wazirabad (Prakash et al. 2020). But in the present study, breeding occurred between February and July as stated by Prakash et al. (2020) and Pandian (2022). Streaked Weavers used fibres from *Phragmites*, *Typha*, and *Vetiveria* in Wazirabad (Prakash et al. 2020). In the present study, the birds used fibres from leaves of *T. angustifolia* only, and not of *Phragmites* spp. or *Vetiveria* spp., as stated by Prakash et al. (2020). Though *Chrysopogon* (=*Vetiveria*) *zizanioides* and *Phragmites* spp. occur abundantly in the study sites, the birds did not pluck fibres from these plants, and the reasons for not selecting these plant species are not known and hence, it requires further studies. They attached many leaves of *T. angustifolia* with an initial knot (wad stage) and formed a strong roof/ceiling of the nests (Ambedkar 1972; Prakash et al. 2020; Pandian 2022). The present study revealed that a minimum of 30 and a maximum of 55 leaves including peduncle of the inflorescence of *T. angustifolia* were found attached to the base of the nest, corroborating the findings of Ambedkar (1972), and Prakash et al. (2020), and Pandian (2022). In Gadharpur, Uttarakhand, the number of nests per colony varied from 12–20 nests, and the largest colony contained 60 nests (Ambedkar 1972). But Prakash et al. (2020) recorded only a maximum of 11 nests per colony in Wazirabad. The present study revealed that the nest colonies contained 4–43 nests, partly corroborating the observations of Ambedkar (1972). The study revealed that maximum nest colonies occurred close to roads, buildings, and overhead power cables.

Usually, females of *Ploceus* spp. do not participate in nest construction activities (Crook 1963; Quader 2006). Ambedkar (1972) reported that the male Streaked Weaver alone build nests in the Kumaon Terai. But Prakash et al. (2020) later observed that females also participated in the nest constructions in Wazirabad. Similarly, I have also recorded that after pairing, females were also involved in nest construction.

The majority of the nests of Baya Weavers are usually oriented towards the east direction (Ambedkar 1964; Davis 1971; Quader 2006). About 87% of nests in Chorao Island in Goa (Borges et al. 2002) and 88% of nests in Vellore district, Tamil Nadu were oriented towards the east (Pandian 2021). The present study revealed that 59% of the nests of Streaked Weaver also face toward the east as in the case of Baya Weavers. The probable

Image 3. Various threats to nests of Streaked Weaver: a—Partly harvested vegetation of *T. angustifolia* | b—A torn and abandoned nest | c—Stunted *T. angustifolia* vegetation covered by an angiosperm parasite *Cassytha filiformis* | d—*T. angustifolia* vegetation covered by climber *Oxystelma esculentum*. © M. Pandian.

reasons for birds constructing many nests with entrance tubes orienting towards the east would be to protect their nests from south-west monsoon winds.

The mixed communal roosting and foraging, consisting of different species, serves as a centre for the exchange of information regarding the location of food sources and receives warning about the approach of predators (Zahavi 1971; Gadgil 1972; Ward & Zahavi 1973; Gadgil & Ali 1976). In the present study, flocks containing individuals of Streaked Weaver, Baya Weaver, Black-breasted Weaver, Tricolored Munia, White-rumped Munia, and Indian Silverbill moved collectively for foraging without any interspecific competition over sharing of food.

Abnormal nesting behaviours occur in other species of the genus *Ploceus*, such as *P. philippinus* (Sharma 1995; Pandian 2021), *P. benghalensis* (Mishra 2004), *P. manyar* (Delacour 1947; Pandian 2022), *P. ocularis* (Maclean 1985), *P. velatus,* and *P. cucullatus* (Collias & Collias 1962; Crook 1963). In the present study, only two abnormal complete nests (1+1 type) were recorded. This corroborates the findings of (Delacour 1947), Collias & Collias (1962), Crook (1963), Maclean (1985), Sharma (1995), Mishra (2004), and Pandian (2021, 2022).

The habits of smudging mud/dung on the inner walls of helmet stage nests are prevalent among Streaked Weaver, Baya Weaver, and Black-breasted Weaver when the nest reaches the helmet stage (Crook 1963; Ambedkar 1969; Borkar & Komarpant 2003). Individuals of male Streaked Weaver placed mud and cow dung on the inner wall of helmet stage nests in Wazirabad (Prakash et al. 2020) and cow dung in Viluppuram, Tamil Nadu (Pandian 2022). Similarly, in the present study, I have recorded the males smudging cow dung on the inner wall when the nest construction reached the helmet stage, and before pairing with a female. An abnormal deposition of dung on the outer wall was also recorded. The exact reasons for plastering of dung in helmet stage nests require further studies.

Incident of Rufous Treepie damaging nests of Baya Weaver was recorded in Arakkonam Taluk, Tamil Nadu (Pandian 2021). In reed beds, many nests are destroyed by grazing cattle and people harvesting reeds (https://weavers.adu.org.za; Pandian 2022). The harvesting of *T. angustifolia* vegetation in c. 700 sq. m and the subsequent abandoning of 21 nests at various developmental stages by birds in the present study sites deprives their potential breeding ground, corroborating the views of Pandian (2021, 2022). House Crows, Large-billed Crows, and Shikras are the major nest predators of Streaked Weaver (Oschadleus 2021; Pandian 2022). In the present survey House Crows, Large-billed Crows, and Rufous Treepie were observed in the vicinity of nest colonies corroborating the observations of Oschadleus (2021) and Pandian (2022). The impact of these avian predators in large geographical areas requires further studies.

CONCLUSION

The present study sites containing c. 4 ha of *T.angustifolia* reeds harbour 536 individuals of Streaked Weaver and 349 nests in various stages of development. Birds built nests using fibres of *T. angustifolia* as nest materials and followed a seemingly strict mixed communal foraging. Females also participated in nest constructions. The entrance tubes of maximum nests were found oriented towards the east. Males had the habit of plastering cow dung on the inner, or both walls, of helmet stage nests. Harvesting of nest-supporting reeds would cause not only habitat loss to this species but also the abandoning of nests due to their exposure. Avian predators such as House Crow, Large-billed Crow, and Rufous Treepie were sighted in the vicinity of nesting colonies. Despite rapid urbanization, industrialization, population increase, habitat destruction, and decreasing areas of cultivation of grains, considerable populations of the Streaked Weaver exist in the agrarian landscape of the three studied districts. A special management plan could be devised for the area, considering the anthropogenic and natural stresses that the habitat is currently subjected to.

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COMMUNICATION

Genetic evidence on the occurrence of *Channa harcourtbutleri* (Annandale, 1918) in Eastern Ghats, India: first report from mainland India

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Abstract: *Channa harcourtbutleri* (Annandale) was described from Inle Lake (Southern Shan State) in Myanmar, and is currently considered as a valid species in the *Channa gachua* species-group. Notwithstanding several detailed studies on *Channa* from India in the recent, none has mentioned the occurrence of *C. harcourtbutleri* in the Indian mainland. In continuation to the faunal diversity exploration in Eastern Ghats, India, a few specimens in the *C. gachua* species-group were collected from the river Sabri sub-basin of the river Godavari basin in the East Godavari District of Andhra Pradesh which was identified as *C. harcourtbutleri* through DNA barcoding. This is a first report on occurrence of the species in the wild in the Eastern Ghats, India.

Keywords: Channidae, DNA barcoding, Godavari basin, Inle Lake, phylogeny, taxonomy.

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

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INTRODUCTION

Snakehead fishes belong to the family Channidae, are most popular in ornamental fish trade. The species in the genus *Channa* Scopoli, 1777 are currently distributed in southern, eastern, and southeastern Asia while their allied species in the genus *Parachanna* Teugels & Daget, 1984 are endemic in Africa. Snakehead fishes are broadly categorized into two major groups based on the presence of gular scales. The first group having gular scales comprises of all the African species of the genus *Parachanna* and four Asian species of the genus *Channa* while the second group lacking gular scales comprises of all the rest of the channid species (Musikasinthorn & Taki 2001; Zhang et al. 2002). Further, the *Channa gachua* species group (sensu Britz 2008) was characterized by a varying number of dark and light semi-circular bands on the pectoral-fin, which has been subsequently followed by other ichthyologists (Britz et al. 2019; Praveenraj et al. 2019). The presence of gular scales is regarded as a plesiomorphic state within the Channidae (Li et al. 2006). As mentioned in Li et al. (2006), the African taxa of *Parachanna* differ from the Asian taxa of *Channa* by the absence of supporting 'lamellas' or process of the first epibranchial and hyomandibular in the suprabranchial organs (Senna 1924; Bonou & Teugels 1985). Further, the study has shown that the species lacking pelvic fins clade with the species having the pelvic fins, and has explained that the loss of the pelvic fins occurred at least three times independently during the evolution of the taxa in Channidae (Bonou & Teugels 1985). The gular region with scales or without scale is one of the key characters in channid taxonomy (Talwar & Jhingran 1991; Li et al. 2006), and the shape of isthmus was also shown to be a key feature in differentiating *marulius* and *gachua* species-groups (Vishwanath & Geetakumari 2009). Among the currently reported 24 channid species in India, 18 species (15 with pelvic fins and 3 without pelvic fins) are included in the *C. gachua* species-group (sensu Britz 2008). Britz et al. (2019) mentioned that snakehead fishes have a centre of diversity in the eastern part of the Himalaya Biodiversity Hotspot (Conte-Grand et al. 2017; Rüber et al. 2019). In the recent two decades, quite a good number of new species, mostly in the *C. gachua* species-group, have been described. However, a few of the recent descriptions have been retained in synonymy by Britz et al. (2019).

Channa harcourtbutleri (Annandale) was described from Inle Lake (southern Shan State) in Myanmar (Annandale, 1918), but it was placed in synonymy with *C. gachua* (Hamilton) by Hora & Mukerji (1934). However, since the latter species is younger than the former, this synonymy at its first instance appears incorrect. Ng et al. (1999) resurrected the species and discussed the differences between them. The taxonomy of *C. gachua* has been a complex problem (Ng et al. 1999), but the recent phylogenetic study suggested two distinct lineages within the *C. gachua* species-complex (Conte-Grand et al. 2017). The true *C. gachua* as referred in Conte-Grand et al. (2017) is restricted to the area west of the Indo-Burman ranges (i.e., Rakhine Yoma and Chin Hills) and covers Sri Lanka, India, Nepal, Bangladesh, and the Rakhine area of Myanmar. The taxon previously recorded as *C. gachua* from Sri Lanka has been revalidated as *C. kelaartii* (Gunther), and has its population also distributed in southern peninsular India (Sudasinghe et al. 2020). The eastern lineage of the *C. gachua* species-complex, nominally referred to as *C. limbata*, is distributed to the east of the Indo-Burman ranges from Myanmar reaching east to Vietnam and southern China and south to Indonesia and Malaysia (Conte-Grand et al. 2017). Notwithstanding several detailed studies on channid taxa from India recently, none has mentioned the occurrence of *C. harcourtbutleri* in mainland India (Conte-grand et al. 2017; Britz et al. 2019; Sudasinghe et al. 2020).

In continuation to the faunal diversity exploration in Eastern Ghats, India, several specimens of the genus *Channa* were collected from various localities. Among the examined specimens, a few specimens collected from the river Sabri sub-basin of the river Godavari basin in the East Godavari District of Andhra Pradesh, were morphologically identified as similar to *C. harcourtbutleri* and were confirmed through DNA barcoding. *C. harcourtbutleri* is a Burmese species, hitherto not recorded from mainland India.

MATERIALS AND METHODS

The study incorporates several specimens of the genus *Channa* from various localities within India. However, this study is specifically aimed to resolve the identity of the specimens in the *Channa gachua* species-group collected from the northern Eastern Ghats, within a range of around 30 km to the north-east of Papikonda National Park.

DNA isolation, PCR and DNA sequence

DNA isolation followed basic methods after partial modification (Sambrook & Russell 2001; Laskar et al. 2018). Partial segment of the mitochondrial cytochrome oxidase C subunit I (COI) gene was amplified using the primer pairs FishF1-FishR1 (Ward et al. 2005). A total of 28 COI sequences for nine channid species from India were generated in this study. All the examined specimens were registered in the National Zoological Collections of Zoological Survey of India (Freshwater Biology Regional Centre), Hyderabad, and the COI sequences generated in this study were submitted to NCBI GenBank and BOLD. The accession numbers are given in the material examined section as well as in the phylogenetic tree and are marked by orange stars. We also retrieved COI sequences from NCBI, and BOLD. Representative sequences for 22 clearly defined channid taxa from India as referred in Conte-Grand et al. (2017) were retrieved from databases. Further, the sequences of a few recently described species whose accessions are referred by their original authors were retrieved from NCBI. The dataset of 157 COI sequences included an outgroup *Parachanna obscura* (MK074551). Genetic divergence analyses and the neighbor-joining phylogenetic tree visualization were performed in MEGA7.0 (Kumar et al. 2016). Bayesian inferences were drawn in Mr. Bayes (Ronquist & Huelsenbeck 2003) and the tree topology was developed in iTOL (Letunic & Bork 2007). We used the Kimura 2-parameter model, mostly applied in DNA barcoding studies (www.bold.org), to calculate the mean genetic distance between the groups. The study is limited by the lack of COI sequence of a few of the recent species, like, *C. brahmacharyi*, *C. pomanensis*. Further, we limit to discuss only the conspecific status of the COI sequences generated in the study, *C. harcourtbutleri* in particular.

Materials examined

1. *C. gachua* (Hamilton, 1822): FBRC/ZSI/F1979, 1, Himayat Sagar, Telangana, 17.35N & 78.42E, GenBank accession: KM272635; FBRC/ZSI/F/3317, 1, Small stream at Basavagu village, Andhra Pradesh, 17.70N 81.02E, GenBank accession: MT118102; FBRC/ZSI/F/3450, 6, Stream at Mothugudem, Andhra Pradesh, 17.80N & 81.64E, GenBank accessions: MW002473, MW002474, MW002475; FBRC/ZSI/F/2662, 1, Manjeera Dam, Telangana, 17.692N & 78.171E, GenBank accession: MH795975; FBRC/ZSI/F/3628, 1, Gubbagurthi near Wyara Lake, Telangana, 17.27N & 80.37E, GenBank accession: MW002494.

2. *C. harcourtbutleri* (Annandale, 1918): FBRC/ZSI/F/3393, 3, Papikonda National Park at G. M. Valasa Road, Andhra Pradesh, 17.59N & 81.68E, GenBank accession: MW002468; FBRC/ZSI/F/3615, 4, 62.0-83.0 mm SL, Stream at Mothugudem-Donkarayi Road, E. Godavari, Andhra Pradesh, 17.84N & 81.67E, GenBank accession: MW002479; FBRC/ZSI/F/3630, 2, 72.0–78.0 mm SL, River Pamuleru at Egavalasa village, Andhra Pradesh, 17.7N & 81.78E, GenBank accession: MW002470 (Image 1).

3. *C. kelaartii* Gunther,1868: FBRC/ZSI/F/3124, 13, Nilavoor Lake, Tamil Nadu, 12.56N & 78.64E, GenBank accessions: MT720842, MT720843, MT720844, MT720845, MT720846, MT720847; FBRC/ZSI/UN9604/DNA582, 1, Small pond at Valvanthinadu village, Namakkal District, Tamil Nadu, 11.280N & 78.364E, GenBank accession: MN685707.

4. *C. punctata* (Bloch, 1973): FBRC/ZSI/F/2405, 1, Kaddem Dam, Telangana, 9.026N & 76.385E, GenBank accession: MF601323; FBRC/ZSI/F/2717, 1, Maharashtra, 20.450N & 74.403E, GenBank accession: MH795988; FBRC/ZSI/F/3627, 1, Gubbagurthi near Wyara Lake, Telangana, 17.27N & 80.37E, GenBank accession: MW002493; FBRC/ZSI/F/3421, 1, Small stream near Basavagu Village, Andhra Pradesh, 17.70N & 81.02E, GenBank accession: MT654658.

5. *C. striata* (Bloch,1793): FBRC/ZSI/DNA357, 1, Namsai, Assam, 27.57N & 95.39E, GenBank accession: MK681748.

6. *C. marulius* (Hamilton, 1822): FBRC/ZSI/F2337, 1, Singur Dam, Telangana, 17.802N & 77.892E, GenBank accession: KY694512; FBRC/ZSI/F/2715, 1, Maharashtra, 20.450N & 74.403E, GenBank accession: MH795986;

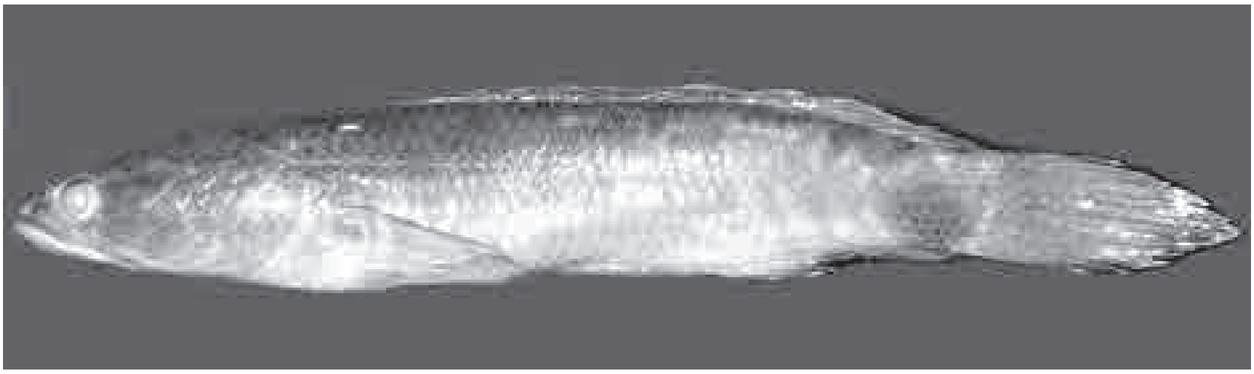

Image 1. *Channa harcourtbutleri* collected from River Pamuleru, Andhra Pradesh (preserved specimen, FBRC/ZSI/F/3630). © Boni Amin Laskar.

Table 1. Estimated genetic divergence (% K2P) between the studied groups in the genus *Channa*. The analysis reveals a low K2P genetic divergence among the specimens of *C. royi* Andaman Islands, *C. harcourtbutleri* Myanmar and *C. harcourtbutleri* India, suggesting their conspecific status.

Grouped taxa	Within-group (K2P %)	Between-group (K2P %)																							
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1. *C. harcourtbutleri* Myanmar	0.013																								
2. *C. harcourtbutleri* India	0.002	2.3																							
3. *C. royi* Andaman Islands	0.003	2.3	1.4																						
4. *C. gachua* Godavari	0.002	17.3	17.4	16.7																					
5. *C. gachua* Topotypic	0.006	19.3	19.5	19.3	3.2																				
6. *C. andrao*	0.000	16.0	14.7	15.7	18.6	19.5																			
7. *C. aurantimaculata*	0.001	12.7	13.4	13.1	15.4	16.9	15.7																		
8. *C. aurantipectoralis*	0.001	16.1	16.0	16.9	17.2	16.9	18.3	14.9																	
9. *C. barca*	0.000	16.9	16.3	17.5	15.2	16.5	15.4	8.8	15.4																
10. *C. bipuli*	n/c	17.7	17.8	18.8	15.3	18.2	16.1	12.3	18.2	12.2															
11. *C. bleheri*	0.001	17.4	16.8	16.8	15.4	16.9	16.7	14.8	17.0	14.0	12.4														
12. *C. brunnea*	0.000	16.1	16.1	16.4	16.4	17.2	17.2	14.5	17.5	14.9	12.3	9.7													
13. *C. diplogramma*	0.004	22.8	23.3	24.1	22.7	22.0	22.3	22.0	23.0	23.1	25.8	23.9	25.0												
14. *C. kelaartii*	0.007	16.3	15.5	15.8	10.5	9.9	19.6	16.3	16.4	17.7	16.5	16.2	16.5	22.1											
15. *C. lipor*	n/c	15.1	15.6	15.6	16.0	14.4	17.2	14.5	11.9	15.7	21.4	16.7	17.7	22.6	15.5										
16. *C. marulius*	0.004	24.6	24.3	24.7	21.0	24.7	19.5	21.1	21.1	20.3	18.6	18.4	21.7	19.1	21.2	23.3									
17. *C. melanostigma*	0.008	14.5	15.5	15.4	16.3	17.8	14.6	6.3	18.8	10.3	11.7	14.7	13.4	23.6	15.5	15.7	21.4								
18. *C. pardalis*	0.002	16.2	16.5	17.2	14.8	16.2	14.7	11.2	16.4	10.9	5.8	12.0	12.2	23.1	13.8	17.2	18.2	12.9							
19. *C. pseudomarulius*	0.000	23.3	22.2	23.1	22.8	25.0	18.7	19.8	20.5	19.8	19.2	19.2	23.1	16.9	20.7	24.2	4.3	21.0	18.0						
20. *C. punctata*	0.015	21.4	20.6	19.9	19.3	19.9	19.1	19.0	20.1	20.0	17.6	18.2	20.3	19.6	17.9	21.5	16.1	16.8	19.5	15.4					
21. *C. quinquefasciata*	0.008	14.9	14.3	15.3	16.8	17.2	15.9	12.6	16.0	13.3	15.0	15.7	14.4	22.2	15.7	15.4	19.2	12.3	13.7	19.1	19.3				
22. *C. rara*	0.002	16.1	16.6	15.7	9.4	10.8	19.2	16.3	15.6	18.0	18.8	17.3	18.3	22.1	8.8	14.5	25.4	17.1	15.2	25.0	19.7	18.4			
23. *C. stewartii*	0.005	17.1	16.6	17.7	17.9	19.0	16.6	12.6	15.8	13.8	15.8	16.4	12.8	21.6	15.7	16.3	20.7	12.3	12.2	19.3	19.1	8.3	19.1		
24. *C. stiktos*	0.013	23.6	22.3	22.2	21.3	21.2	22.8	21.3	20.2	19.6	18.7	17.7	19.9	24.2	20.1	20.7	21.4	20.6	18.6	22.5	23.0	21.3	22.1	18.4	
25. *C. striata*	0.001	21.1	19.8	18.4	22.0	23.2	19.3	21.0	24.0	21.2	17.0	22.2	23.8	20.3	20.6	23.0	18.1	18.7	19.4	17.5	16.7	21.7	22.1	22.0	23.7

FBRC/ZSI/DNA267, 1, Tungabhadra River, Andhra Pradesh, 16.169N & 77.934E, GenBank accession: MK336898.

7. *C. bleheri* Vierke, 1991: FBRC ZSI DNA354, 1, Tinsukia, Assam, 27.57N & 95.39E, GenBank accession: MK632315.

8. *C. aurantimaculata* Musikasinthorn, 2000: FBRC/ZSI/DNA359, 1, Tinsukia, Assam, 27.57N & 95.39E, GenBank accession: MK632318.

9. *C. stewartii* (Playfair, 1867): FBRC/ZSI/DNA356, 1, Tinsukia, Assam, 27.57N & 95.39E, GenBank accession: MK632316.

RESULTS

The phylogenetic tree shows distinctive cladding of the Asian channid taxa with reference to the African *Parachanna* used herein to root as out-group. The generated sequences of the *C. gachua* species-group from the Yelagiri Hills in Tamil Nadu form a distinct clade that comprises of a few database sequences that were referred in a recent study as *C. kelaartii*. Thus, this study contributes further specimens of *C. kelaartii* from the southern Eastern Ghats. The sequences of the *C. gachua* like specimens collected from near to the type locality of *Ophicephalus marginatus* and various other localities in the Godavari River basin are nested as a subclade to the topotypic *C. gachua*. Our generated sequences of other congeners like *C. marulius*, *C. punctata*, *C. stewartii*, *C. bleheri*, *C. aurantimaculata*, and *C. striata* show distinct cladding and each comprises of conspecific database sequences wherever available. The sequence of *C. pomanensis* (referred in Praveenraj et al. 2019) was found to be cohesively claded with a sequence of *C. melanostigma*. Hence, in the absence of further specimens for both the taxa, the identity of the two sequences is not confirmed and remained beyond the limit of this study. Our sequences of *C. stewartii* are nested in the 65th clade of *C. stewartii* of Conte-Grand et al. (2017) (BIN:AAF3764) while status of the taxonomic assignment of the 64th clade of *C. stewartii* of Conte-Grand et al. (2017) (BIN:AAF3772) is also beyond the limit of this study. However, the generated sequences of the specimens in *C. gachua* species-group from near the Papikonda National Park, East Godavari District, in the Eastern Ghats, are cohesively claded with the two database sequences from southern India of Conte-Grand et al. (2017) (MF462283 BOLD:ADL6569, MF462269) and formed a sub-clade to the species *C. harcourtbutleri* from Myanmar (BIN:AAC3926) suggesting their close genetic similarity with the latter, hence referred hereafter as *C.*

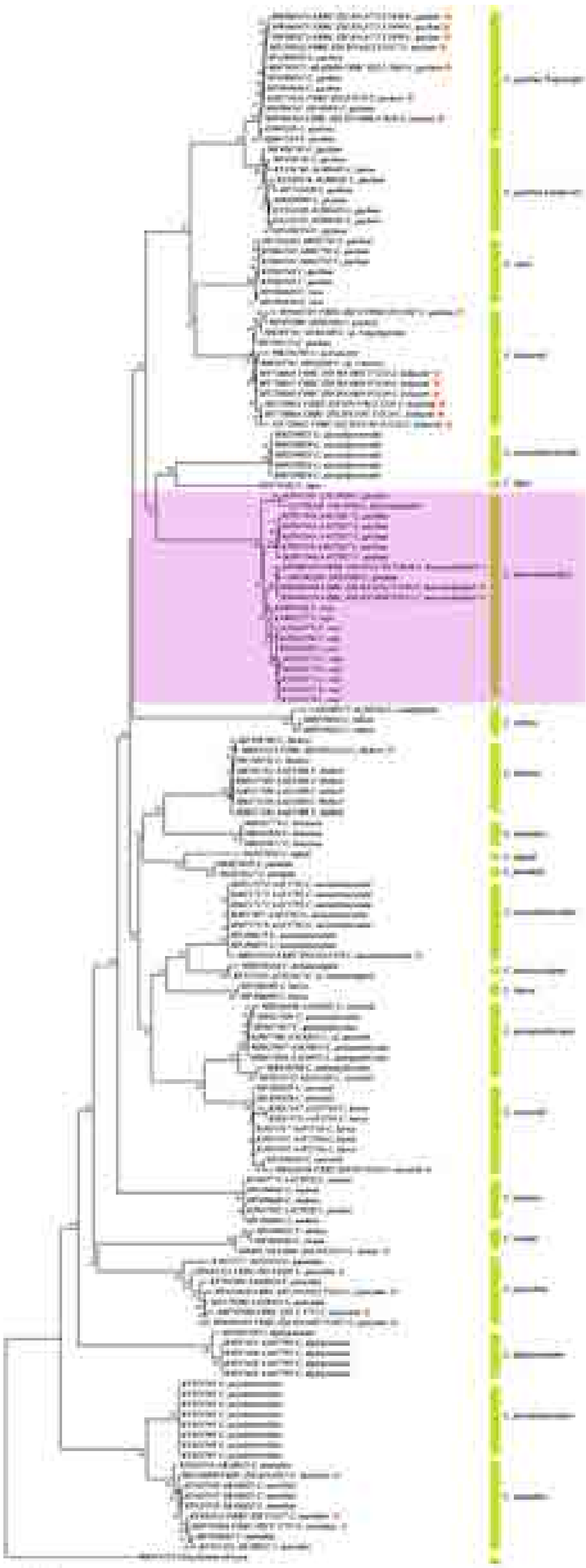

Figure 1. Neighbour-Joining phylogeny based on K2P divergence on partial mtCOI gene of the Channid taxa In India. The cohesive cladding (pink shape in figure) of the sequences of *C. harcourtbutleri* Myanmar, *C. royi* Andaman Islands, and *C. harcourtbutleri* India suggest their conspecific status due to low genetic divergence.

harcourtbutleri India.

The present dataset is comprised of representative sequences from the clearly defined taxa. However, in order to estimate the range of intra-species genetic divergence, the sequences are grouped based on the clustering inferred from neighbor-joining phylogeny (Figure 1). Sequences in the cluster with topotypic *C. gachua* (referred in Britz et al. 2019) are named as *C. gachua* Topotypic. The sequences of *C. gachua* like specimens from near to the type locality of *O. marginatus* are named as *C. gachua* Godavari. Similarly, the sequences of *C. royi* from Andaman Islands (Praveenraj et al. 2019) are tentatively named as *C. royi*, sequences in the cluster with *C. harcourtbutleri* BIN:AAC3926 from Myanmar (Conte-Grand et al. 2017) are named as *C. harcourtbutleri* Myanmar, and the sequences of *C. gachua* species-group from near the Papikonda National Park, East Godavari District, along with other database sequences in the same cluster are named as *C. harcourtbutleri* India. The overall genetic divergence between groups in the dataset is lying in the range from 1.4–25.8 % (Table 1). The divergence matrix revealed that the sequences of *C. gachua* Godavari are genetically diverged by 3.2% K2P distance from *C. gachua* Topotypic, and maintained 9.4–22.8 % K2P distance from all the congeners. *C. pseudomarulius* is diverged by 4.3% K2P from *C. marulius*, *C. pardalis* is diverged by 5.8% from *C. bipuli*, *C. melanostigma* by 6.3% from *C. aurantimaculata*, and so on. Similarly, the divergence matrix revealed that the sequences of *C. harcourtbutleri* India are genetically diverged by 1.4% K2P distance from *C. harcourtbutleri* Myanmar (BIN: AAC3926), 2.3% K2P distance from *C. royi* Andaman Islands, and maintained 13.4–24.3 % K2P distance from all the congeners.

DISCUSSION

Conte-Grand et al. (2017) recovered a total number of 90 BINs in their dataset having a total number of 38 valid species at time, and inferred higher species diversity in snakeheads. However, they neither included any COI sequence data of *C. limbata* in the phylogenetic analysis nor assigned any BIN for the species. As of now, a search for BINs with the name '*Channa*' in BOLD yielded a record of 93 BINs. Conte-Grand et al. (2017) had an extensive dataset covering various geographical areas and populations. In fact, the representative sequences for almost all the species described or validated after 2017 were present either as an unnamed clade (potential new BIN in Conte-Grand et al. 2017) or has been assigned with BOLD BIN.

The taxonomic history of the snakehead fishes finds two descriptions, *O. marginatus* and *O. coramota*, with their type locality in Vizagapattam (=Visakhapatnam, Andhra Pradesh), part of the Eastern Ghats region. However, both these species have been synonymized with *C. gachua* (Roberts 1993; Ng et al. 1999; Courtenay & Williams 2004; Kottelat 2013). In fact, Britz et al. (2019) examined specimens of topotypic *O. marginatus*, and found a very similar colour pattern as well as a very little genetic difference with topotypic *C. gachua* (2.4% uncorrected p-distance). Britz et al. (2019) pointed out several flaws in the description of *C. shingon* by Endruweit (2017) and provided various valid reasons to consider *C. shingon* as a junior synonym of *C. harcourtbutleri*. Britz et al. (2019) was also not convinced enough by the morphological descriptions to consider *C. royi* as a distinct species from *C. harcourtbutleri*, and a very low genetic distance of 2.4–2.8 % uncorrected p-distance was stated to be in the range of intra-species variation, and therefore considered Andaman *C. royi* as a junior synonym of *C. harcourtbutleri*. However, the distribution limit of *C. harcourtbutleri* and *C. limbata* is not yet clear. Conte-grand et al. (2017) mentioned an unexpected placement of two specimens from southern peninsular India in the middle of the eastern lineage of the *C. gachua* species-complex. The same statement was repeated in Ruber et al. (2019). Conte-grand et al. (2017) showed that the two specimens from southern peninsular India (one from Chunchi falls, Cauvery River, 12.351N & 77.443E; and the other from Kali River, 15.381N & 74.403E) were included in a Putative BIN:ACM5826 new that claded away from the BIN of *C. harcourtbutleri* (AAC3926).

Following the previous studies, it may be figured out that the nominal species in the genus *Channa* with fewer or no morphological differences can have intra-species genetic divergence as high as 2.2–2.4 %, and the nominal taxa falling within such range of genetic divergence could be considered as a single species. Therefore, a clear understanding of the range of intra-species genetic divergence would be helpful in taxonomic assignment of the channid taxa. Based on the analysis of COI barcode sequences, we confirm that the specimens of *C. gachua* species-group from East Godavari District, Eastern Ghats, India, along with the sequences from southern India, are actually a single species which may be named as *C. harcourtbutleri* because of low genetic divergence with the conspecific sequences from Myanmar. Thus, this study claims the presence of *C. harcourtbutleri* in the wild in Eastern Ghats region, in mainland India. Nonetheless, *C. harcourtbutleri* has already been recorded to be distributed

Image 2. *Channa harcourtbutleri*, image taken in the field immediately after collection. © Boni Amin Laskar.

in Andaman Islands through the synonymization of *C. royi* with *C. harcourtbutleri*. Hence, this study reports for the first time the occurrence of *C. harcourtbutleri* in mainland India (Image 2). Unexpectedly, no specimens from northeastern India are available to place in the clade of *C. harcourtbutleri*. In this background, the distribution of *C. harcourtbutleri* is appearing disjunct.

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COMMUNICATION

Redefining *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933 from two freshwater fishes of Channidae family of Hooghly District, West Bengal, India

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Abstract: The genus *Pallisentis* has been mostly found among the freshwater fishes of Channidae family. *Pallisentis ophiocephali* is characterized with some unique characters of proboscis, hooks, and spines. However, initial description is a little sketchy and yet not updated. The present study communicates the detail morphology of an acanthocephalan species obtained from two host fishes of Channidae family—*Channa striatus* and *Channa punctatus*—during a one-year survey from different places of Hooghly District, West Bengal. The parasite is examined under light microscope as well as under scanning electron microscope. Unique four hook circles with different sizes, collar, and trunk spines, male and female genital organs are described for taxonomic study. Scanning electron microscopic study also provides the detailed information about the surface topography including longitudinal muscle, retractor muscles, and posterior ends. Comparing the studied specimen with other closely related species, the present acanthocephalan specimen has been identified as *P. ophiocephali* from *Channa striatus*. The retractable nature of proboscis has also been studied from the live specimens with the help of light microscope.

Keywords: Acanthocephalan, *Channa punctatus*, *Channa striatus*, retractable proboscis.

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Author contributions: Authors have the equal contributions to do the entire research work.

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INTRODUCTION

Species of the genus *Pallisentis* has been mostly found among the freshwater fishes of Channidae family. The genus *Pallisentis* has been considered in the family of Quadrigyridae Van Cleave (1920) in the subfamily Pallisentinae Van Cleave (1928) (Harada 1935; Yamaguti 1963). Amin et al. (2000) revised the characters of the genus *Pallisentis* under the subgenus *Demidueterospinus*. Thapar (1930)& Baylis (1933) described the species *Pallisentis ophiocephali.* According to Thapar (1930), *Farzandia* is a new genus, whereas, Baylis (1933) considered the genus as synonymous to *Pallisentis*. The type species *P. ophiocephali* was first reported from fresh water fish *Channa marulius* in different places of India (Andhra Pradesh, Odisha, Uttar Pradesh). Soota & Bhattacharya (1982) revised the validity of the species in the genus *Pallisentis* from India. Later Gupta et al. (2012) mentioned two host fishes (family: Channaidae) of the genus *Pallisentis* from Bareilly, Uttar Pradesh. Gupta et al. (2015) described the ultrastructure of another species, *P. punctati*, from Bareilly, Uttar Pradesh from the host *C. punctatus.* The present study has been carried out to understand the structural details of proboscis, hooks, spines, and genital parts from light microscopic as well as from scanning electron microscopic study and also to study the retractable nature of unique thorny proboscis from the live specimens.

MATERIALS AND METHODS

In order to study the prevalence of intestinal acanthocephalan infection among different freshwater fishes from July 2014–August 2015, 50 specimens of *Channa punctatus* (average length 15.4 cm and weight 190 g) and 47 specimens of *Channa striatus* (average length 18.7 cm and weight 320 g) were examined in Chandannagore (22.86^{0}N & 88.36^{0}E) of Hooghly District, West Bengal (Figure 1).

The fish samples were identified after Dey (1996), Talwar & Jhingran (1991). The classification of fishes was done following the keys of Jayaram (1999). White tape-like parasites were found tightly anchored through the proboscis in the intestinal wall of the host fishes. The specimens were carefully removed with a fine brush. Then they were collected and preserved following the methods of Soota (1980). All the parasites were washed by shaking thoroughly in physiological saline and transferred to 70% alcohol for their use in light microscope (LM) study. Pictures were taken with the help of Labomed CXL microscope. Measurements (based on 20 specimens) were recorded in millimetres and represented by mean followed by ranges. Schematic illustrations were drawn using the software CorelDraw 12, a vector graphics editor by Corel Corporation. For scanning electron microscopic study, specimens were preserved in 4% glutaraldehyde. Then they were transferred to 30%, 50%, and 70% alcohol respectively. After that the specimens were passed through a mixture of absolute alcohol and amyl acetate in 3:1, 1:1 for half an hour and then in 1:3 ratio for two hours. Lastly, they were transferred into 100% amyl acetate. After Critical Point Drying (CPD) the specimens were coated with gold and photos were taken under scanning electron microscope (Hitachi; S-530).

RESULTS

The present acanthocephalan species were collected from the intestinal wall of the host fishes. Among the collected specimens of the parasite, 20 male and 25 female were identified after studying under a light microscope. The species showed the structural details under light and scanning electron microscopic study.

General body structure: White in colour, cylindrical and 5.9–19.4 mm in length (Figure 2A; Image 1A). The body is divided into three regions: proboscis, short neck, and elongated trunk region. Proboscis; with recurved hooks, looks globular just erecting from proboscis receptacle and it seems subglobular when fully erected (Figure 2C; Image 1C,3C). Proboscis receptacle is sac like; cylindrical, single walled, and measures 0.39 (0.38–0.42) mm x 0.104 (0.10–0.12) mm (Image 1B). Two unequal, ribbon like, coiled lemnisci are present within the body cavity. They measure 1.562 (1.553–1.575) mm and 1.861 (1.857–1.920) mm (Image 1B). Anterior and middle part of the body is marked by several circlets of characteristics collar spines and body or trunk spines.

Proboscis hooks: The proboscis is armed with four circles (H1, H2, H3, and H4) of recurved hooks and each circlet contains eight hooks (Image 3C). The hooks are similar in shape but different in size (Figure 2F). The hooks are deeply rooted in the proboscis wall and without any striations or grooves. Hooks are dagger shaped (Figure 2G; Image 3D). Initial 0.020–0.028 mm of the hook is projected upwards and the rest bends outwardly. Each hook consists of a backwardly projected blade; a horizontally directed root and a handle embedded into the proboscis wall (Figure 2G; Image 3D).

Neck: A short neck, 0.19–0.29 mm, is present in

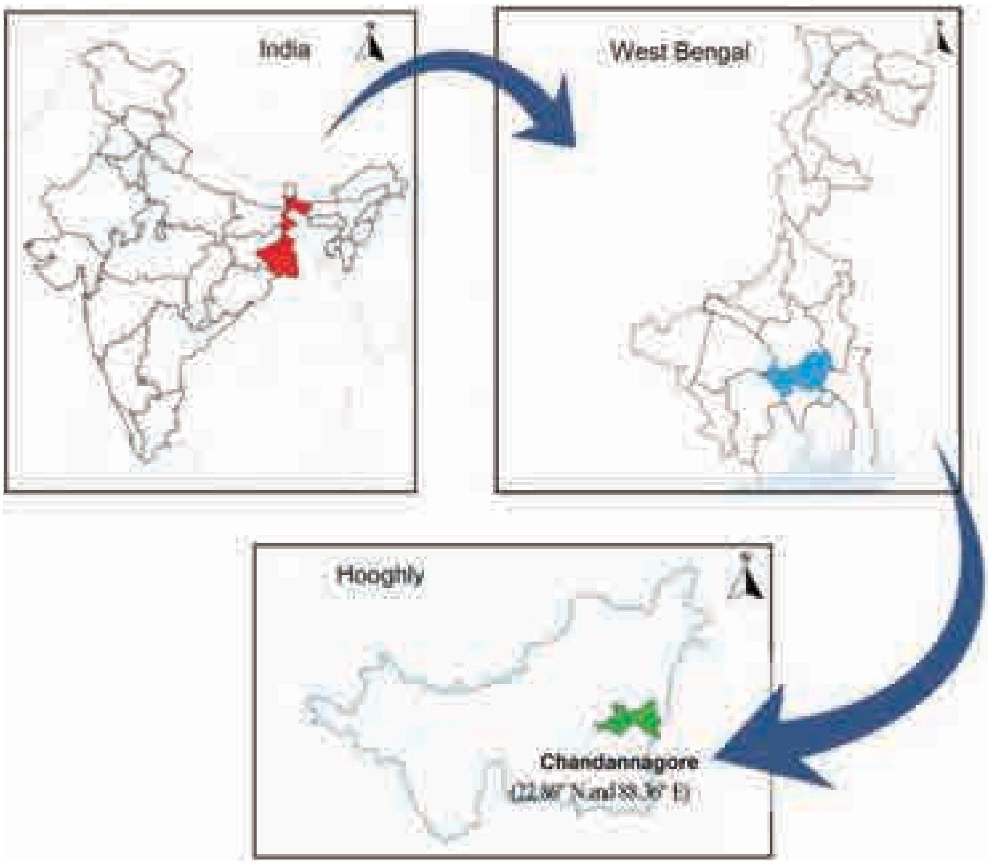

Figure 1. Survey area of of *Pallisentis ophiocephali* (Thapar, 1930) Baylis (1933) Chandannagore (22.86^{0}N & 88.36^{0}E) in Hooghly, West Bengal, India.

between the proboscis and the body. The retractor muscles are seen in the neck region (Image 3B). The parasite retracts the proboscis along with the neck into the proboscis receptacle.

Collar spines: Behind the neck, there are 13–16 circlets of collar spines and measure 0.027 (0.026–0.030) mm (Figure 2A,H). Collar spines are peepal leaf shaped structure, from the base it tapers gradually but at the distal end it tapers abruptly and ends in a tip. The base of the spine is 0.044 mm in average. The middle portion of the spine bulges outwardly and forms convex surface (Figure 2H; Image 3E,F).

Collar spines zone is followed by a spine free zone of 0.11–0.165 mm and body spines zone.

Body or trunk spines: The spine is pointed and projected downwards from a strong rounded base. Body is lined with longitudinal striations. Cement gland is single and syncytial with four–five nuclei. Distance between two spines within a row is 0.075 (0.072–0.078) mm. Distance between two rows is 0.25 (0.244–0.252) mm. Striations are of 0.075 (0.072–0.076) mm distance from each other (Figure 2I; Image 3G,H).

Male (Based on 20 specimens): The male is short and measures 6.1 (5.9–8.2) x 0.32 (0.28–0.33) mm (Figure 2C). Proboscis is 0.16 (0.16–0.18) x 0.21 (0.19–0.23) mm. Hooks in first–fourth circle are in the measure of 0.065 (0.064–0.067) mm, 0.06 (0.059–0.062) mm, 0.044 (0.042–0.047) mm, and 0.032 (0.031–0.034) mm respectively. Neck is 0.203 (0.19–0.21) x 0.209 (0.207–0.21) mm. Collar spines are in 13–14transverse circles, each with 16–17 spines. The length of the collar spines is 0.023 (0.022–0.024) mm. Trunk spines are in 26–29 circles. Numbers of trunk spines range from 14–15 in each circle. The length of the spines is 0.034 (0.032–0.036) mm. Testes are equal and measures 0.621 (0.616–0.643) mm. From each testis a vas deferens emerges and associates with cement gland, cement reservoir, and joins bursa. The bursa measures 0.181 (0.176–0.192) mm in length (Image 1G).

Female (Based on 25 specimens): Females are larger than males. It measures 17.1 (16.7–19.4) x 0.54 (0.52–0.60) mm (Figure 2D). Proboscis measures 0.18 (0.17–0.20) x 0.21 (0.19–0.23) mm. Proboscis hooks measure 0.079 (0.077–0.080) mm, 0.067 (0.065–0.072)

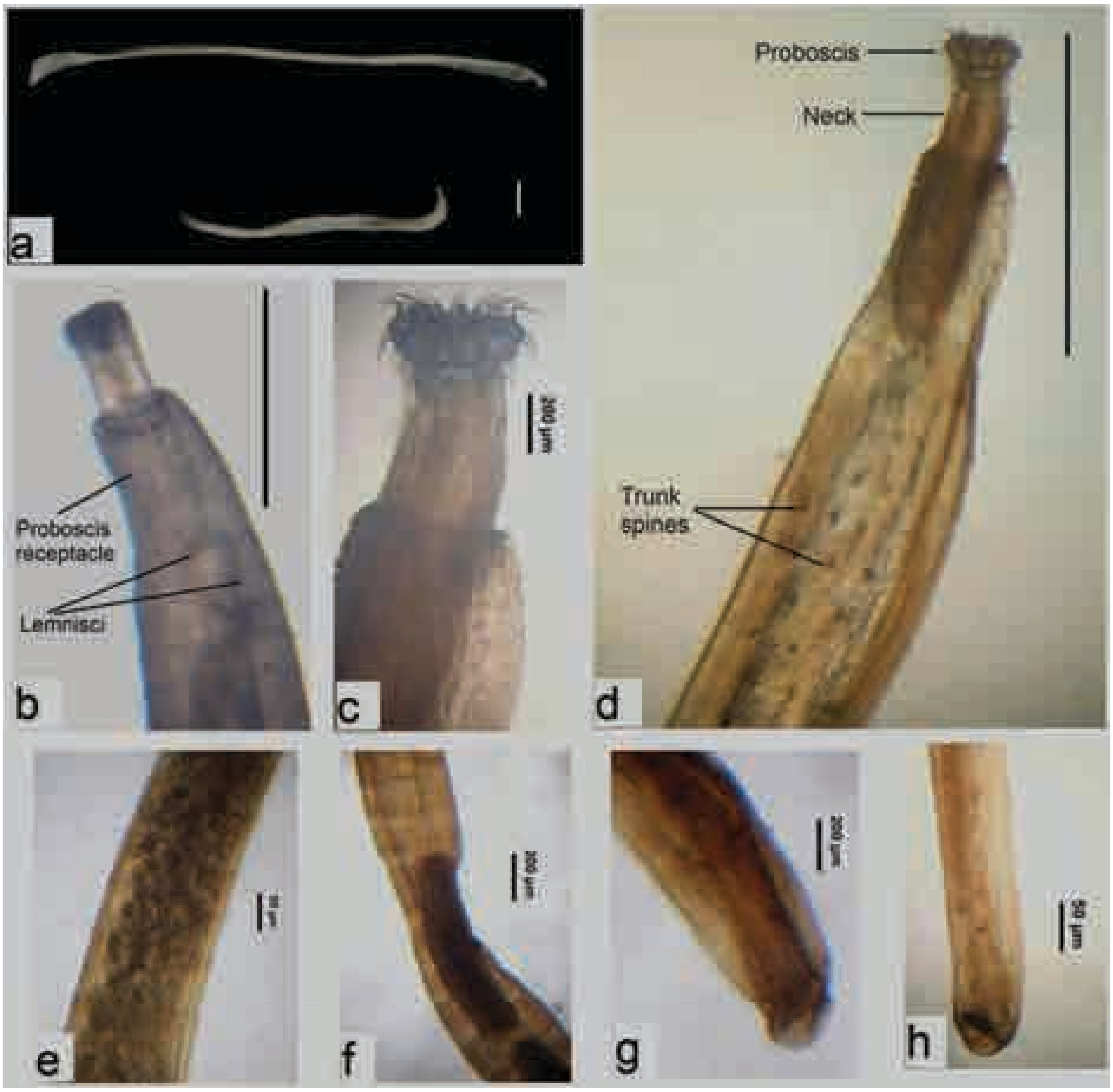

Image 1. Light microscopic pictures of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933: A—Adult specimens | B—Anterior region of male | C—Proboscis | D—Anterior region of female | E—Middle portion of female | F—Middle portion of male | G—Posterior region of male | H—Posterior region of female. (Bar = 1 mm). © Prabir Banerjee.

mm, 0.052 (0.048–0.053) mm, and 0.037 (0.032–0.044) mm respectively in the four successive circlets. The neck measured 0.282 (0.280–0.293) x 0.206 (0.202–0.21) mm. Collar spines are arranged in 14–16 circles. Each circle of collar spines is layered with 16–17 spines. Collar spines are 0.027 (0.026–0.030) mm in length. Numbers of circles of body spines range from 57–60 and, there are 14–15 spines in each circle. Trunk spines are 0.044 (0.042–0.046) mm in length. Whole body cavity of mature worm is filled with large number of eggs (Figure 2J; Image 1H). Eggs are 0.053 (0.051–0.062) x0.022 (0.021–0.033) mm and ovarian balls are 0.107(0.105–0.113) x 0.051(0.050–0.054) mm (Image 1E). The posterior region is rounded with a small gonopore (Figure 2E & Image 1H).

Observations on retractable proboscis

The inward and outward movement of retractable proboscis from the proboscis receptacle was observed on ten live specimens. The time of stretching in and out of the proboscis for each step was also calculated. The total process can be divided into three consecutive steps. First step is the emergence of the proboscis from

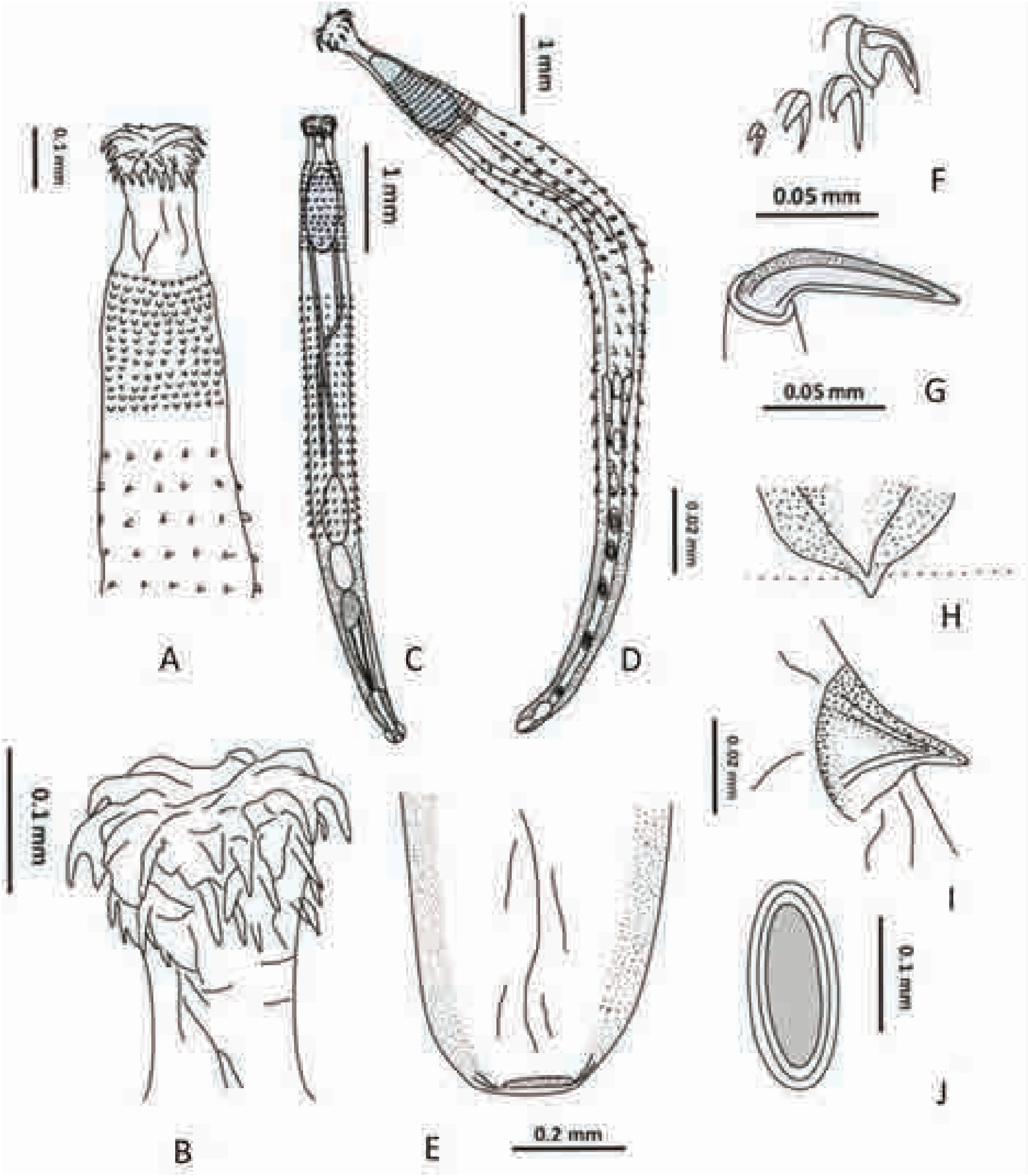

Figure 2. Schematic illustrations of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933: A—Anterior region | B—proboscis| C—Male specimen | D—Female specimen | E—Posterior region of female | F—Different types of proboscis hooks | G—Proboscis hook | H—Collar spine | I—Body spine | J—Egg.

proboscis receptacle and the recorded time was 27.3 (27.12–27.42) seconds (Image 2A–E). After emerging, the fully stretched proboscis paused for 32.52 (32.48–32.58) seconds (Image 2F). Lastly, the species took 12.08 (11.90–12.11) seconds for the inward movement, i.e., retraction was much faster than eversion. The total time for the completion of the whole process was 71.90 (71.84–72.33) seconds. The repetition of the whole process began after a pause of 19.20 (15.55–22.73) seconds.

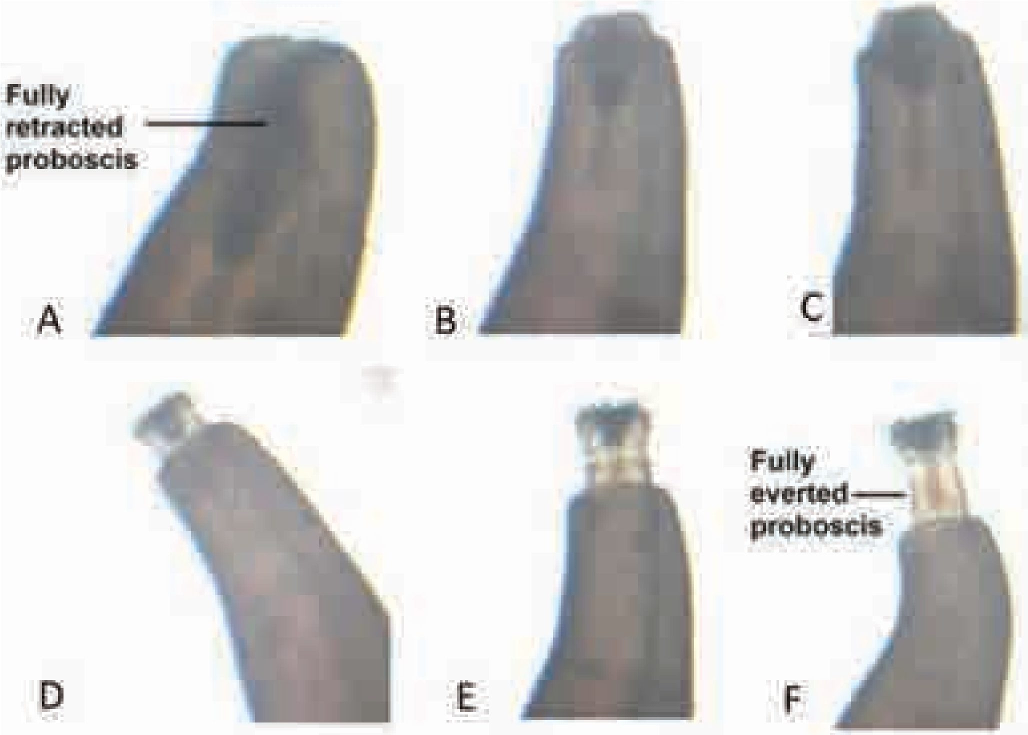

Image 2. Light microscopic pictures of retractable proboscis of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933 in different stages: A—Fully retracted proboscis | B–E—Outward movement of the proboscis | F—Fully stretched proboscis. © Prabir Banerjee.

Type host: *Channa marulius* (Hamilton, 1822).

Other host from different genera: *Nandus nandus* (Hamilton), *Grihiria* (*Cirrhina*) *cupla*, *Ompokpabda* (Hamilton), *Xenentodon cancila* (Hamilton), *Rana trigrina* (Daudin).

Other hosts in this study: *Channa punctatus* (Bloch, 1793) and *Channa striatus* (Bloch, 1793).

Distribution: Andhra Pradesh, Uttar Pradesh, Odisha, West Bengal of India.

Site of infection: Mostly in stomach, in few cases in proximal intestine.

Present collection Locality: Chandannagore (22.86 N & 88.36 E), Hooghly, West Bengal.

DISCUSSION

The present species has been collected from the wall of the intestinal tract of *Channa punctatus* and *Channa striatus*. Prevalence of the infection in two host fishes were recorded 26% and 21.2%, respectively. According to Soota & Bhattacharya (1982) and Bhattacharya (2007), members of the genus *Pallisentis* can be differentiated by the shape and size of the proboscis and hooks. The studied specimens are compared with *P. ophiocephali* (Thapar, 1930) Baylis, 1933, *P. colisai* (Sarkar, 1954) and *P. punctati* (Gupta et al. 2015). Comparing the present specimen with other closely related species, the present acanthocephalan species has been considered as *P. ophiocephali* as described by Thapar (1930) and later Baylis (1933) (Table 1). The species has been reported from Hooghly for the first time. In the description of type species, the geographical distribution was not specifically mentioned. Gupta et al. (2012) mentioned *Channa punctatus* as a host of *P. ophiocephali.* SEM studies reveal the detailed proboscis structure, arrangement of hooks and retractor muscles in the neck region. The scanning electron microscopic pictures also provide the surface structural details with high magnification that facilitates the understanding of the spatial relationships among surface structures and other minute organelle. Hooks and spines are some useful taxonomic tools for differentiating subgenera and species of acanthocephala. Morphometrically identical species generally have been examined and identified with the help of arrangements of hooks and spines. Moreover, some preliminary data has also been recorded from the retractable movements of the proboscis along with the required time span. The inward movement of the proboscis is comparatively faster than the outward movement. Further examinations are required to explore the untouched area of acanthocephalan biology.

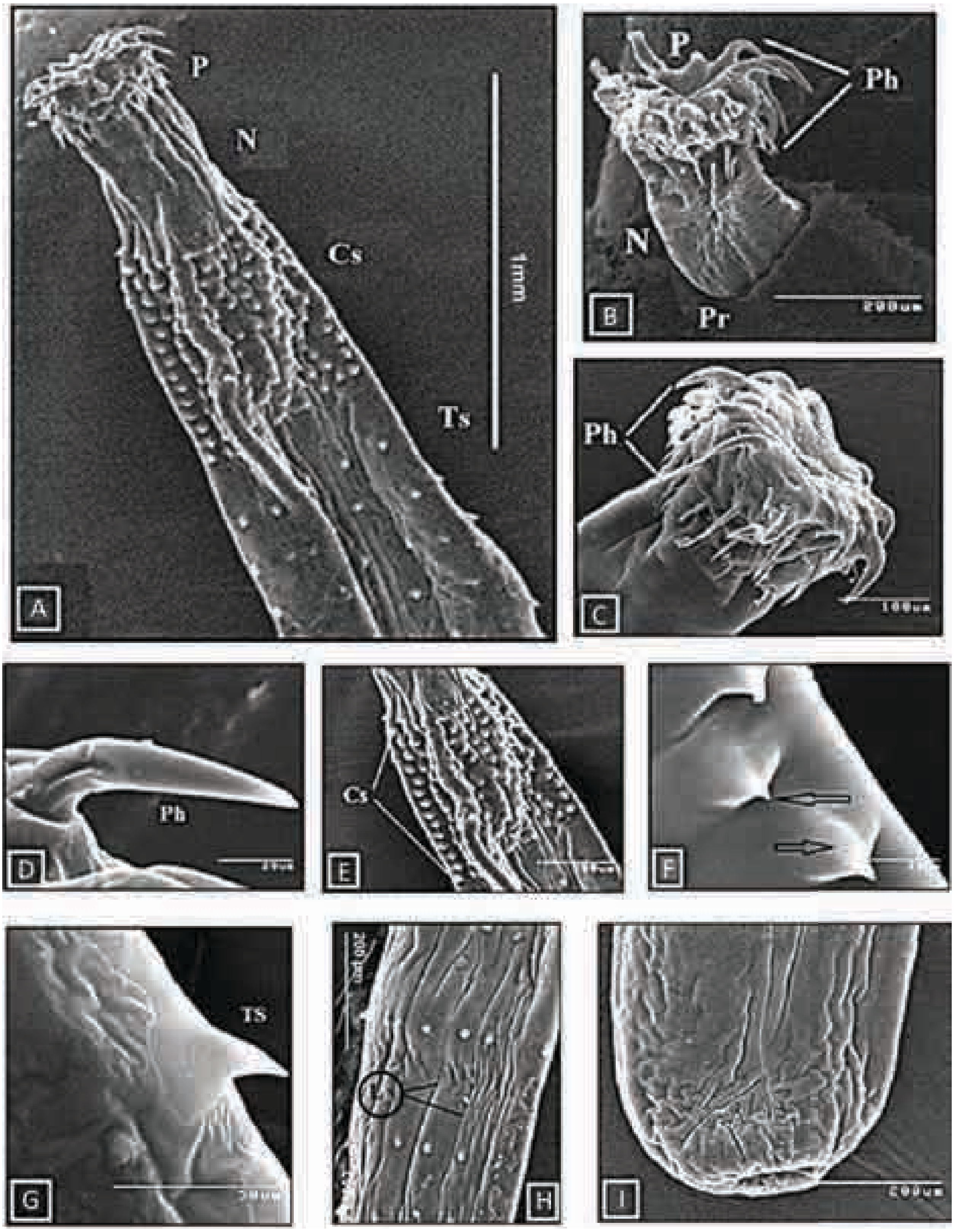

Image 3. Scanning Electron Microscopic pictures of female specimen of *Pallisentis ophiocephali* (Thapar, 1930) Baylis, 1933: A–C—Pictures showing the body with proboscis (*P*—Proboscis, *N*—Neck, *Cs*—Collar spine, *Bs*—Body spine, *Ph*—Proboscis hooks, *Pr*—Proboscis receptacle) | D—One proboscis hook | E–F—Collar spines (←) shows pointed tip and (→) indicates convex surface | G–H—Trunk spine(*Ts*—Trunk spine, *Ls*—Longitudinal striations) | I— Male posterior end (with retracted bursa). © Prabir Banerjee.

Table 1. Morphometric comparison of *Pallisentis ophiocephali* (Thapar, 1930) Baylis (1933) with other closely related species.

	P. ophiocephali (Thapar, 1930) Baylis, (1933) (Present species)	*P. ophiocephali* (Thapar, 1930) Baylis, (1933) (Type species)	*P. colisai* (Sarkar 1954)	*P. punctati* (Gupta et al. 2015)
Host	*Channa punctatus, Channa striatus*	*Channa marulius*	*Colisa fasciatus*	*Channa punctatus*
Location	Hooghly, West Bengal	Different places of India	Delhi	Bareilly, Uttar Pradesh
Size of body (L x W)	♂ 6.1 (5.9–8.2) x 0.32 (0.28–0.33) ♀ 17.1 (16.7–19.4) x 0.54 (0.52–0.60)	♂ 05.99 x 0.34 ♀ 14.3 x 0.495	♂ 4.13 x 0.39 ♀ 5.4–12.9 x 0.610	♂ (3.015–5.899) x (0.307–0.461) ♀ (5.472–14.791) x (0.461–0.820)
Size of proboscis (L x W)	♂ 0.16 (0.16–0.18) x 0.21 (0.19–0.23) ♀ 0.18 (0.17–0.20) x 0.21 (0.19–0.23)	♂ 0.14 x 0.22 ♀ 0.175 x 0.242	♂ 0.13 x .15 ♀ –	♂ (0.104–0.118) x (0.090–0.120) ♀ (0.126–0.180) x (0.140–0.198)
Length of neck (L x W)	♂ 0.203 (0.19– 0.21) x 0.209 (0.207–0.21) ♀0.282 (0.280–0.293) x 0.206 (0.202–0.21)	♂ 0.198 x 0.22 ♀ 0.308 x 0.22	♂ 0.26 x 0.17 ♀ –	♂ (0.190–0.255) x (0.108–0.118) ♀ (0.288–0.558) x (0.162–0.273)
Proboscis hooks length H1	♂ 0.065 (0.064–0.067), ♀ 0.079 (0.077– 0.080)	0.076–0.085	0.08 x 0.007	♂ 0.057 , ♀ 0.073
H2	♂ 0.06 (0.059–0.062), ♀ 0.067 (0.065–0.072)	0.068–0.076	0.07	♂ 0.054 , ♀ 0.063
H3	♂ 0.044 (0.042–0.047), ♀ 0.052 (0.048– 0.053)	0.051	0.03	♂ 0.021 , ♀0.025
H4	♂ 0.032 (0.031–0.034), ♀ 0.037 (0.032–0.044)	0.034–0.0425	0.026	♂ 0.018 , ♀ 0.018
Lemnisci	1.861 (1.857–1.920)	1.925	2.2 x 0.05	–
No. of collar spines	♂ 13–14 x 16–17 ♀ 14–16 x 16–17	♂ 11–13 x 14–16 ♀ 13–14 x 14–16	♂16 x 14–16, ♀–	♂ 14, ♀ 22
Collar spines length	♂ 0.023 (0.022–0.024) x (0.016–0.018), ♀ 0.027 (0.026–0.03) x 0.023 (0.022–0.025)	–	–	♂ (0.021–0.028) x (0.010–0.014) ♀ (0.025–0.046) x (0.010–0.025)
No. of body spines	♂ 26–29 x 14–15 ♀ 57–60 x 14–15	♂ 28–34, ♀ 60–65	♂22 x 12–16, ♀67	♂ 12, ♀ 14–18
Body spines length	♂ 0.034 (0.032–0.036) x 0.013 (0.010–0.016) ♀ 0.044 (0.042–0.046) x 0.016 (0.014–0.022)	–	–	♂ (0.021–0.028) x (0.010–0.018) ♀ (0.036–0.057) x (0.014–0.025)
Testis	0.621 (0.616–0.643)	0.605–0.66	(1) 0.39 x 0.17 (2) 0.35 x 0.17	(0.374–0.684) x (0.133–0.216)
Egg	0.053 (0.051–0.062) x 0.022 (0.021–0.033)	0.068 x 0.025	–	(0.028–0.061) x (0.010–0.025)
Ovarian balls	0.107 (0.105–0.113) x 0.051 (0.50–0.054)	–	–	(0.039–0.064) x (0.025–0.054)

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OPEN ACCESS

COMMUNICATION

A new termite species of the genus *Bulbitermes* (Blattodea: Isoptera: Termitidae) from Meghalaya, India

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Abstract: A new termite species, *Bulbitermes debadiliporum* sp. nov., of the nasutiform genus *Bulbitermes* is described here from the Mawlynnong area of the East Khasi Hills district and Nongkhrah Village of the Ri-Bhoi district of Meghalaya, India. The species is described based on the soldier & worker castes, and a detailed illustration of the diagnostic characteristics of both the castes is provided here. Soldiers of the species are monomorphic whereas the workers are dimorphic with worker major and worker minor. Worker dimorphism is reported hitherto for the first time among *Bulbitermes* species of the Indian region. Furthermore, an updated identification key of *Bulbitermes* species from the Indian region is also provided here.

Keywords: *Bulbitermes debadiliporum* sp. nov., Indian region, key, Mawlynnong area, morphometrics, Nasutitermitinae, soldier, taxonomy, worker major, worker minor.

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Author contributions: KSD—first author was involved in data collection, data analysis and manuscript preparation; SC—corresponding author was involved in supervision of whole study.

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INTRODUCTION

Termites are widely spread group of insects that fall under the infra-order Isoptera and order Blattodea (Inward et al. 2007; Krishna et al. 2013a). Worldwide, around 2,933 living species of termites have been reported under 282 genera that belong to nine families (Krishna et al. 2013a). Family wise Termitidae is the largest among all the families and comprises of 2,077 species globally that belong to seven subfamilies. Among the subfamilies, Nasutitermitinae is the second largest with around 77 genera and 596 species reported from all over the seven biogeographic regions of the world (Krishna et al. 2013a). Nasutitermitinae group inhabits a variety of ecological conditions with diverse nesting and feeding habits (Eggleton 2000).

The genus *Bulbitermes* is the third largest genus (in species diversity) under the subfamily Nasutitermitinae and is endemic to the Oriental region (Chhotani 1997; Krishna et al. 2013a). It is a dead wood or plant material feeding nasutiform genus (Chuah 2005; Syaukani & Thompson 2011). This genus is similar to genus *Hospitalitermes* with constricted head behind antenna, mandibles with spine like processes and with more than 12 antennal segments in soldier castes. But the genus *Bulbitermes* differ from *Hospitalitermes* with comparatively less constricted head behind the antenna, mandibles with slightly shorter spine like processes and with hind legs not extending beyond the abdomen in soldiers (Chhotani 1997). The genus *Bulbitermes* comprises of 33 species. The Indian region (India, Bangladesh, Pakistan, Sri Lanka, Bhutan, Nepal, and Burma) houses four *Bulbitermes* species, viz., *B. pyriformis* Akhtar, 1975 (From Bangladesh), *B. prabhae,* Krishna, 1965 (From Burma), *B. bulbiceps* Maiti & Saha, 2000 (from Assam, India), and *B. parapusillus* Ahmad, 1965 (from Meghalaya, India) (Krishna et al. 2013b).

In this paper, we describe a new *Bulbitermes* species, *Bulbitermes debadiliporum* sp. nov., based on its monomorphic soldier and dimorphic worker (worker major and minor) castes from Meghalaya, India.

MATERIALS AND METHODS

The samples used in this study were collected from the betel nut plantation of Mawlynnong area, Pynursla, East Khasi Hills district and Nongkhrah village, Nongpoh, Ri-Bhoi district of Meghalaya, India. The collection was done using brush and forceps or handpicking and was preserved at 80% alcohol in the laboratory for further studies. All the type specimens (Holotype & Paratypes) of this study are deposited in the national repository of Zoological Survey of India at the North Eastern Regional Centre, Shillong (ZSI, NERCS), Meghalaya, India. Voucher specimens are preserved in the research collection of entomology laboratory, Department of Zoology, North-Eastern Hill University (NEHU), Shillong, Meghalaya, India.

Leica Stereo Zoom microscope S8AP0 fitted with GT 5.0 camera and mosaic V.2 photographic software was used to take the micrographs of the samples. Measurements were taken on the micrographs using Image J software (2018 version) and were also cross checked with the ocular micrometer fitted with the microscope.

Morphological studies of the soldier and worker castes and also all measurements, terminologies and indices were based on Roonwal & Chhotani (1989). Identification of the species was done following the available literature of Ahmad (1965), Chhotani (1997), and Syaukani & Thompson (2011).

RESULTS

Taxonomic account
Infra-order: Isoptera Brullé, 1832
Family: Termitidae Latreille, 1802
Subfamily: Nasutitermitinae Hare, 1937
Genus: *Bulbitermes* Emerson, 1949

Bulbitermes debadiliporum sp. nov.

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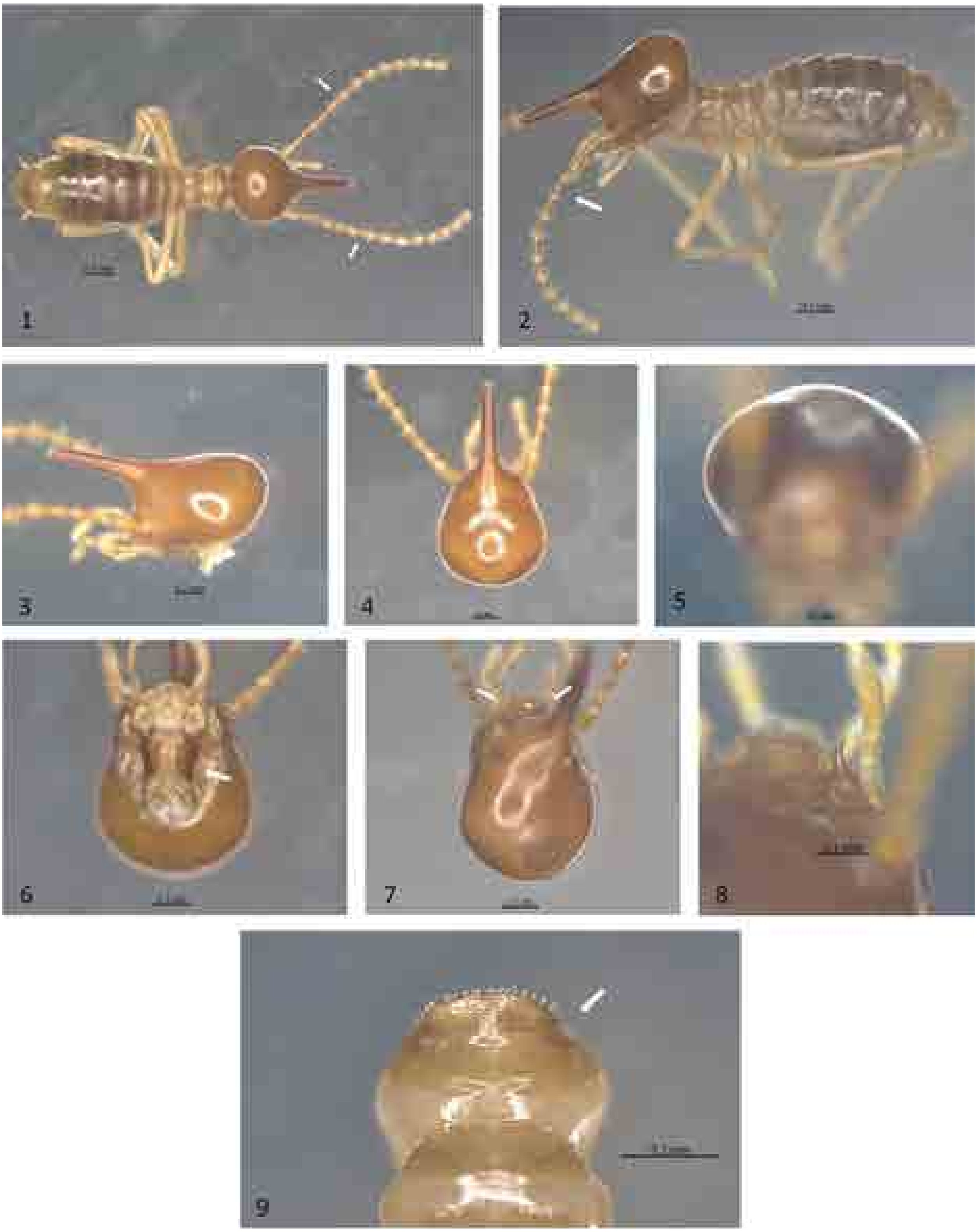
(Images 1–9)

Type materials

Holotype: I/ISOP/ERS/4431, Soldier, 20.xii.2018. The holotype was collected from a carton nest from the betelnut plantation of Mawlynnong area of Pynursla, East Khasi Hills District of Meghalaya, India (25.2047°N, 91.9112°E; elevation 530 m), coll. K.S. Das; deposited in ZSI, NERCS.

Paratypes: I/ISOP/ERS/4432: 17 soldiers and 7 workers (5 workers major and 2 workers minor), same data as holotype.

Other materials studied: ISOP/ZOO/NEHU/100: 18 soldiers and 3 workers major, 20.vi.2018. Collected from dry bamboo plant, Nongkhrah village, Nongpoh, Ri-Bhoi district, Meghalaya, India (25.9270°N, 91.8917°E; elevation 587 m), coll. K.S. Das and party; preserved in the Department of Zoology NEHU, Shillong. ISOP/ZOO/



Images 1–9. Soldier of ***Bulbitermes debadiliporum*** sp. nov. (Holotype—1,3,4,6,7,9; Paratypes—2,5,8): | 1—soldier with 14 segmented antenna (marked) in dorsal view | 2—soldier with 13 segmented antenna (marked) in lateral view | 3—Head in lateral view | 4—Head in dorsal view | 5—long bristles posteriorly on head capsule (marked) | 6—postmentum (marked) | 7—left and right mandibular apical processes (marked) | 8—right mandibular apical process in higher magnification | 9—pronotum (marked). © Khirod Sankar Das.

NEHU/120: 21 soldiers and 8 workers major, 17.x.2018 and ISOP/ZOO/NEHU/121: 12 soldiers, 17.x.2018, samples collected from the same area as mentioned above by same collector and preserved in Department of Zoology, NEHU, Shillong.

Description

Imago: Unknown

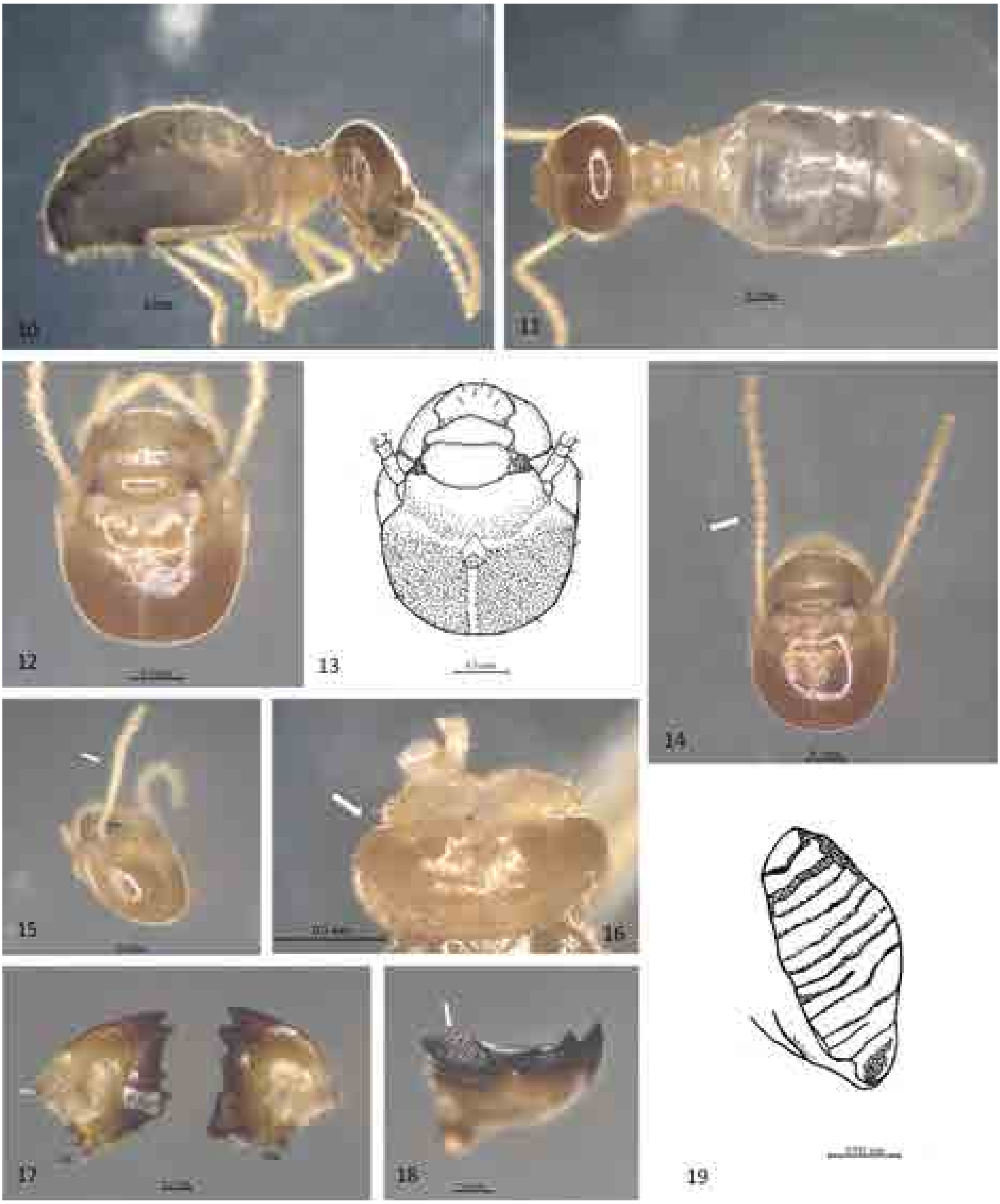
Soldier (Images 1–9, Table 1): Monomorphic. Head is pear shaped without rostrum and slightly constricted behind antennae. In colouration, head brown to rusty brown (in middle & posterior portion) to dark smoky brown (in anterior portion and lateral posterior ends). In dorsal profile, head weakly depressed behind rostrum, faintly concave, posterior margin roundly convex, two long bristles present on posterior end of head capsule. Fontanelle gland and tube visible. Rostrum cylindrical and darker than head with reddish-brown (proximal portion) to dark reddish-brown (distal portion) in colour. Tip portion with four long bristles and somewhat hyaline at end. Rostrum length usually more than the half of head length without rostrum and in some cases less than or equal to ¾ of head length without rostrum. Mandible vestigial with prominent long apical processes. Length of spine like apical processes slightly varies among the soldiers and right mandible without any minute tooth. Antennae generally 14 segmented (sometimes 13 segmented) and yellowish-brown to brown in colour. Segments vary in length. In 13 segmented ones, 2nd shortest, 3rd twice as long as 2nd, 3rd longer than 4th and 5th, 5th longer than 4th. In 14 segmented ones, 2nd shortest or subequal to 3rd, 4th longer than 3rd (sometimes 3rd longer than 4th). Pronotum saddle shaped, indistinctly emarginated anteriorly and posteriorly. In colour, pale brown to brown. Anterior lobe darker than posterior and minute hairs present on anterior margin. Legs pale brown to straw in colour. Abdominal tergites pale brown in colour with long bristles on posterior tergites; sternites brownish-yellow in colour with long and short bristles.

Worker: Dimorphic. Worker major (Images 10–19; Table 2): Head subsquarish in shape; light brown with yellowish tinge to dark brown in colour and fairly pilose. Epicranial Y-suture distinct. Fontanelle plate prominent, oval. Postclypeus swollen, hairy and divided into two equal halves by the median suture. In length, less than half of its width. Antennae pale yellow to pale yellowish brown in colour and 14–15 segmented. In 14 segmented ones, 4th shortest, 2nd subequal to 3rd or longer than 2nd and 3rd. In 15 segmented ones, 3rd shortest or subequal to 4th, 2nd longer or subequal to 4th. Mandibles with apical and 2–3 marginal teeth. Left mandible with prominent apical and 3 marginal teeth. Apical tooth and 1st marginal tooth form an acute angle between them. Posterior margin of 1st marginal slightly sinuate. Second and third marginal teeth small and prominent. Third marginal slightly longer than second and separated by wide U-shaped gap from molar prominence. Molar plate with four prominent ridges. Right mandible with 1 apical and 2 marginal teeth. Apical and first marginal teeth slightly boarder than left mandible ones. Apical tooth and 1st marginal tooth form an obtuse angle between them. Second marginal tooth small, with a blunt apex, posterior edge nearly straight. Molar plate with 10 well developed prominent ridges. Inner layer of molar plate undeveloped and proximal notch of molar plate weakly developed. Thorax pale yellowish in colour. Pronotum saddle shaped, faintly emarginated at anterior and indistinctly emarginated at posterior. In colouration, light brown to brown and slightly lighter than colour of head. Anterior lobe darker than posterior and minute hairs present on anterior margin along with two long bristles on lateral sides each. Legs straw in colour. Abdominal tergites pale brown in colour with long bristles only on posterior tergites; sternites brownish-yellow in colour with long and short bristles.

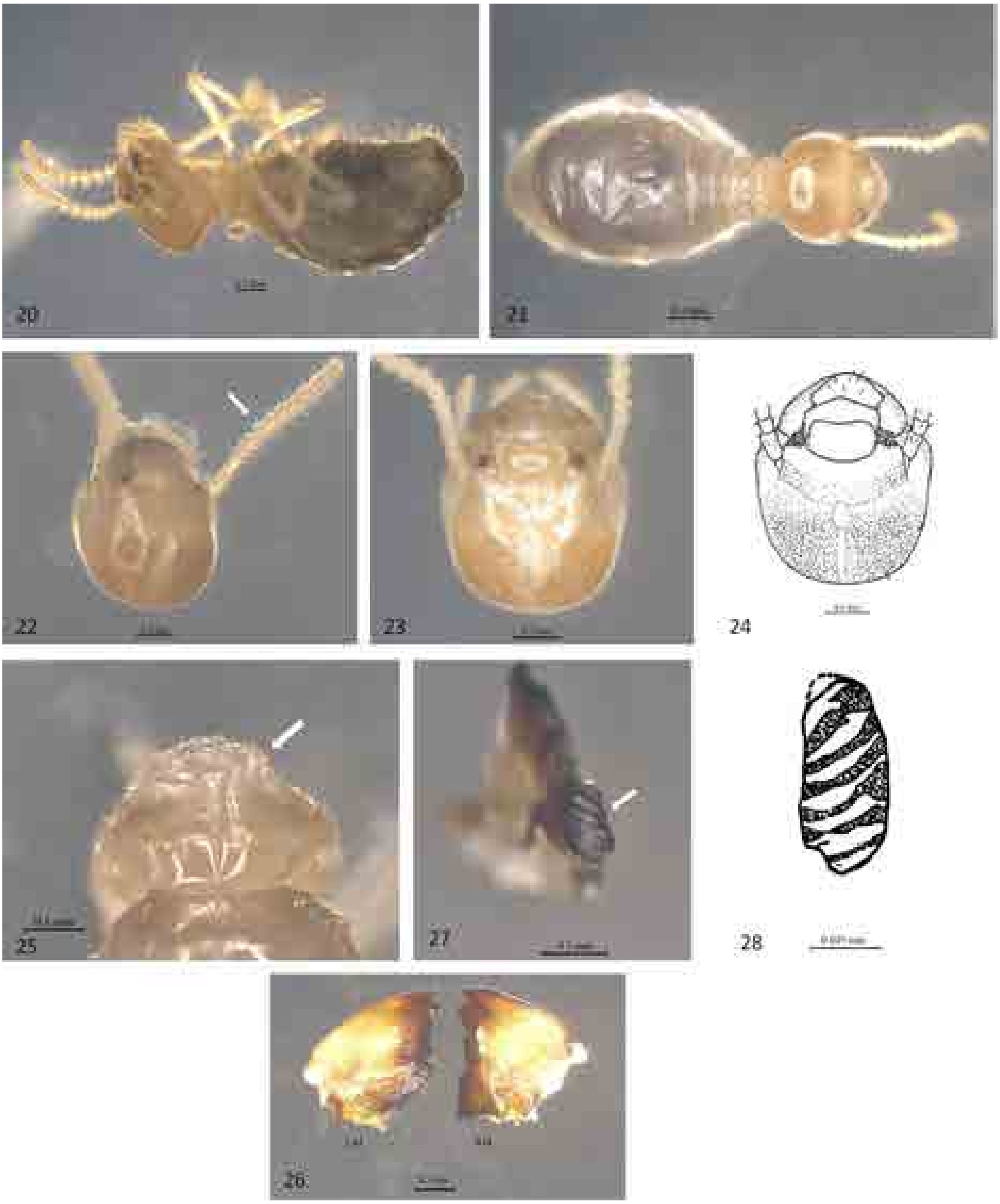
Worker minor (Images 20–28; Table 2): Similar with worker major but smaller in size. Antenna only 14 segmented, 4 shortest, 2 subequal to 3 or longer than 3. Mandibles with apical and 2–3 marginal teeth, smaller in size than worker major. Apical and first marginal teeth less pointed than in worker major. Left mandible with apical and 3 marginal teeth, prominent. Apical tooth slightly shorter than 1st marginal tooth with an acute angle between them. Posterior margin of 1st marginal slightly sinuate. Second and third marginal small and prominent. Second marginal tooth broader than the left mandible ones of worker major. Third marginal slightly longer than second and separated by V-shaped gap from molar prominence. Molar plate with four prominent ridges. Right mandible with 1 apical and 2 marginal tooth. Apical tooth shorter and pointed than the 1st marginal and form an obtuse angle between them. Second marginal tooth small with a blunt apex, posterior edge nearly straight. Molar plate with six well developed prominent ridges.

Comparison

The termite species *Bulbitermes debadiliporum* sp. nov., in its morphological characteristics and morphometrics (Table 1 and 2) of the soldier and worker castes, shows slight similarity with other *Bulbitermes*



Images 10–19. Worker major of *Bulbitermes debadiliporum* sp. nov. (Paratypes): 10—whole body in lateral view | 11—whole body in dorsal view | 12—head in dorsal view | 13—head in dorsal view (line diagram) | 14—worker with 15 segmented antenna (marked) | 15—worker with 14 segmented antenna (marked) | 16—pronotum (marked) | 17—right mandible (RM) and left mandibles (LM) | 18—right mandible molar plate ridges (marked) | 19—right mandible molar plate ridges (line diagram). © Khirod Sankar Das.



Images 20–28. Worker minor of *Bulbitermes debadiliporum* sp. nov. (Paratypes): 20—whole body lateral view | 21—whole body dorsal view | 22—Antenna (marked) | 23—Head dorsal view | 24 —head dorsal view (line diagram) | 25—pronotum (marked) | 26—right mandible (RM) and left mandibles (LM) | 27—right mandible molar plate ridges (marked) | 28—right mandible molar plate ridges (line diagram). © Khirod Sankar Das.

species of Indian region (Chhotani 1997), Thailand (Ahmad 1965) and Sumatra (Syaukani & Thompson 2011) but differ significantly in the key taxonomic characteristics which separate this species from the rest as a new *Bulbitermes* species.

Bulbitermes debadiliporum is closely related to *B. prabhae* in its morphological characteristics. But, the soldiers of *B. debadiliporum* have two long hairs on the head prosteriorly whereas *B. prabhae* without hairs. Antennae are 14 segmented in *B. prabahe* where segment 3rd is longer than 4th whereas 13–14 segmented in *B. debadiliporum* with segment 3rd shorter than 4th in 14 segmented antennae. No minute inconspicuous tooth observed below the tip of the apical process of the right mandibles in *B. debadiliporum* unlike *B. prabhae*. Morphometrics of soldier such as head length with and without rostrum, head width and rostrum length etc. are also vary slightly between the species.

In comparison to *B. parapusillus*, the head length with rostrum (1.17–1.52 mm), without rostrum (0.78–1.08 mm), head width (0.67–0.88 mm), and rostrum length (0.53–0.60 mm) of *B. parapusillus* are less than *B. debadiliporum*. Antennae with only 13 segments in *B. parapusillus* whereas 13–14 segmented in *B. debadiliporum*. Workers monomorphic in *B. parapusillus* with only 14 segmented antenna whereas dimorphic (with worker major and minor) in *B. debadiliporum* and with 14–15 segmented antenna.

In case of *B. pyriformis,* the head length with rostrum (1.28–1.59 mm), head length without rostrum (0.78–0.98 mm) and head width (0.64–0.85 mm) are less in both the upper and lower range values than *B. debadiliporum*. Antennae with 12–13 segments in *B. pyriformis* whereas 13–14 segmented in *B. debadiliporum*. Pronotum not notched or emarginated at the anterior and posterior margins in *B. pyriformis* whereas in *B. debadiliporum* indistinctly emarginated.

In contrast to *B. bulbiceps*, the soldier of *B. debadiliporum* is larger in size than *B. bulbiceps* (2.90–3.15 mm). Antennae are with only 12 segments in *B. bulbiceps* whereas in *B. debadiliporum* antennae are 13–14 segmented. Rostrum is shorter in length in *B. bulbiceps* (0.42–0.46 mm) than *B. debadiliporum.* Mandible without any apical process or tooth in *B. bulbiceps* in contrast to the long spine like apical processes of *B. debadiliporum.* Head length with rostrum (1.10–1.26 mm), head width at constriction (0.43–0.48 mm), head height (0.46– 0.54 mm), head width a constriction/ Head width index (0.58–0.60), postmentum length (0.18–0.21 mm), postmentum width (0.24–0.26 mm), pronotum width (0.30–0.34 mm), pronotum length (0.11–0.12 mm) and head length (0.76–0.80 mm) of *B. bulbiceps* are less than *B. debadiliporum*. Workers are monomorphic with 14 segmented antennae in *B. bulbiceps* whereas dimorphic (workers major and minor) in *B. debadiliporum* with 14–15 segmented antennae. Total body length, head length and width, pronotum length and width of workers also vary significantly between the species.

Table 1. Morphometrics (in mm) of soldiers of the termite species *Bulbitermes debadiliporum* sp. nov.

Characteristics	Holotype	Range (n = 18)
Total body length	3.69	3.14–4.70
Head length with rostrum	1.60	1.51–2.00
Head length without rostrum	0.93	0.87–1.25
Head width	0.85	0.75–1.22
Head width / Head length without rostrum	0.91	0.86–0.98
Rostrum length	0.67	0.61–0.80
Rostrum length / Head length without rostrum	0.72	0.55–0.78
Head width at constriction	0.58	0.57–0.69
Head width at constriction / Head width	0.68	0.58–0.78
Head Height	0.66	0.55–0.81
Pronotum length	0.22	0.18–0.28
Pronotum width	0.46	0.42–0.55
Postmentum length	0.35	0.27–0.35
Postmentum width	0.31	0.28–0.36

Table 2. Morphometrics (in mm) of worker major and worker minor of the termite species *Bulbitermes debadiliporum* sp. nov.

Characteristics	Worker major (n = 5)	Worker minor (n = 2)
Total body length	3.20–5.90	2.65–2.78
Head length	0.87–1.25	0.68–0.74
Head width	1.04–1.40	0.74–0.81
Pronotum Length	0.21–0.43	0.18–0.27
Pronotum width	0.51–0.77	0.41–0.44
Postclypeus length	0.20–0.25	0.18–0.20
Postclypeus width	0.50–0.60	0.38–0.40

Etymology

The name of this species is given in the honor of the first authors' mother Mrs. Deba Das and father Mr. Dilip Das.

Distribution

India, Meghalaya, East Khasi Hills District, Pynursla area, Mawlynnong; Ri-Bhoi District, Nongpoh area.

Keys to *Bulbitermes* species of Indian region based on the soldier caste (modified from Chhotani (1997)

1. Smaller species (Head length with rostrum 1.10–1.59 mm) **2**
\- Larger species (Head length with rostrum 1.37–2.00 mm) **4**

2. Antennae with only 13 segments. Rostrum length 0.53–0.60 mm. Head straight behind rostrum and with a faint hump in profile. Mandibles with short, spine like process .... ***B. parapusillus*** (Ahmad)
\- Antennae with 12–13 segments. Spine like process present or absent **3**

3. Antennae with 12–13 segments. Rostrum length 0.70 mm. Head weakly depressed behind rostrum and with weak hump. Mandibles with long spine like process .... ***B. pyriformis*** (Akhtar)
\- Antennae with 12 segments; head length with rostrum 1.10–1.26 mm. Rostrum length 0.42–0.46 mm. Head weakly depressed behind rostrum and with a weak hump. Mandibles without any spine like process .... ***B. bulbiceps*** (Maiti and Saha)

4. Antennae with 14 segments, 3rd longer than 4th. In profile head depressed behind rostrum appreciably. Right mandible with a minute tooth near the tip .... ***B. prabhae*** (Krishna)
\- Antennae 13–14 segmented; in 14 segmented antennae, segment 4th longer than 3rd. Head weakly depressed behind rostrum. Right mandible without a minute tooth near the tip .... ***Bulbitermes debadiliporum* sp. nov.**

Bioecology

Bulbitermes is primarily an arboreal nester genus that build stercoral carton nest of round or elongate shapes on the tree trunks, branches or in bushes (Weesner 1965; Lommen et al. 2004). They also live in wood nest (Arumugam et al. 2018). In case of the species *B. debadiliporum*, studied samples were collected from stercoral carton nest found inside the trunk of dead standing betelnut and dry bamboo. The trunk of the betelnut plant was eaten up completely from inside where the carton nest was located and it was somewhat elongated in shape. In dry bamboo tree, soldiers and workers were found foraging, but could not locate their nest. Due to their preference to feed on dead wood materials, *B. debadiliporum* may fall under the feeding group II.

DISCUSSION

Bulbitermes debadiliporum sp. nov. stands as a separate species from other *Bulbitermes* species due to its distinct morphological features. The soldier castes of the species have 13–14 segmented antennae along with significant variations in other morphological characteristics such as total body length, head length with and without rostrum, head width, rostrum length, rostrum length and head length index, head width at constriction, head width at constriction and head width index, head height, pronotum length and width etc. with other related *Bulbitermes* species from Indian and other Oriental regions. On the other hand, the worker caste of the species also shows distinct morphological features with 14–15 segmented antennae along with its dimorphic occurrence (workers major and minor). With the differences in number of antennal segments and morphometrics, workers major, and minor also differ in their molar plate ridges (10 molar ridges in workers major and 6 in workers minor) of the left mandibles. Dimorphism among the soldier and worker castes of Nastutitermitinae is not uncommon as many genera comprise of dimorphic soldier and worker caste (Chhotani 1997). *Bulbitermes* species with dimorphic workers also were reported from Malayasia earlier. But, among the *Bulbitermes* species from Indian region, the dimorphic worker caste is reported for the first time in *B. debadiliporum.* Besides, other morphological characters in worker caste such as head length and width, pronotum length and width etc. of *B. debadiliporum* also vary significantly with other related *Bulbitermes* species as discussed above. Thus, we propose this species *Bulbitermes debadiliporum* as a new *Bulbitermes* species from Meghalaya, India.

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COMMUNICATION

First report of the beetle *Henosepilachna nana* (Kapur, 1950) (Coleoptera: Coccinellidae) from Maharashtra with special reference to molecular phylogeny and host plants

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Abstract: A ladybird beetle, collected from different localities of Kolhapur and Satara districts (Maharashtra) was identified as *Henosepilachna nana* (Kapur, 1950). The presence of this species in Maharashtra considerably extends its range to the north-west by about 700 km. Since this species is found on vegetables, Pumpkin (*Cucurbita* spp.), Cucumber (*Cucumis* spp.), and Karit fruit plant (*Cucumis* spp.). It is being recorded for the first time that this species is a pest of these vegetables. Also, molecular phylogeny has been studied for the first time in this species in which, this species is the sister taxon of *Henosepilachna boisduvali*. This species has been described briefly with colour photographs of male genitalia, female coxites and the 6th sternal plate of female, tarsi, & pronotum. *Henosepilachna nana* is being reported for the first time from Maharashtra and now the molecular data of this species is available.

Keywords: Epilachna, Epilachnini, GenBank, genitalia, first record, ladybug, phylogenetic tree, vegetable pest.

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Author contributions: SMG involved in design of the research and write up of the manuscript. PBP collected and dissected specimens and participated in design of the research, digital photography and map preparation.

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INTRODUCTION

The tribe Epilachnini (Mulsant 1846) comes under the subfamily Coccinellinae (Slipinski 2013; Seago et al. 2011) but previously it was considered as a separate subfamily Epilachninae of the family Coccinellidae (Szawaryn & Tomaszewska 2014). The members of this tribe are economically important and distributed throughout the world (Szawaryn et al. 2015), comprising 27 genera (Tomaszewska & Szawaryn 2016). There are about 1,000 herbivorous species of this tribe that feeds on plant tissues and fluids (Giorgi et al. 2009; Bustamante-Navarrete et al. 2018).

The genus *Epilachna* is one of the genera of the subfamily Epilachninae in which hundreds of species from all over the world have been described and later many species were subsequently removed to other genera (Jadwiszczak & Węgrzynowicz 2003). Based on basal toothed tarsal claw and longitudinally divided sixth female abdominal sternite, Li & Cook (1961) raised a separate genus *Henosepilachna* from *Epilachna.* To date, there are about 110 described species of the genus *Henosepilachna* from Asia and Australia (Szawaryn 2011). There are 33 species of *Henosepilachna* in India (Poorani 2012; Poorani et al. 2021).

The male specimen collected from Nilgiri Hills, India has been described by Kapur (1950) as *E. nana* and its holotype is in the Zoological Survey of India (No. 3426/13). In the world catalogue of Coccinellidae, Jadwiszczak & Wegrzynowicz (2003) placed *E. nana* in the genus *Henosepilachna,* which is followed by Poorani (2012) in the updated checklist of Coccinellidae from Indian subregion and Borowski (2020) in the inventory of world Epilachninae. In the present study, we describe the detailed morphology of the male genitalia, 6th sternal plate, female genitalia and tarsi of *H. nana* along with coloured photographs. In addition, this species is being reported for the first time in Maharashtra through this communication and its molecular phylogeny has been given.

MATERIAL AND METHODS

Specimens of *H. nana* were collected on different vegetable crops by handpicking method from Halondi (Hatkanangale), Kanthewadi (Radhanagari) and Parite (Karveer) of Kolhapur district and Sajjangad and Yerad (Patan) of Satara district of Maharashtra, India (Image 1). Specimens were photographed on stereo zoom microscope LYNX LM-52-3621 using TCapture software and preserved dry and wet and deposited at the Department of Zoology, Shivaji University, Kolhapur. Captured images stacked in Helicon Focus 7 software. Measurements were taken in ImageJ software. The genitalia was dissected and photographed using the same microscope and software. Species was identified and confirmed based on the detailed taxonomic description provided by Kapur (1950) along with genitalia description.

Molecular analysis

Genomic DNA extraction was done by the manual CTAB method (Boopathi et al. 2020). PCR of extracted DNA was carried out using the primers LepF1-5'-ATTCAACCAATCATAAAGATATTGG-3' and LepR1-5'-TAAACTTCTGGATGTCCAAAAAATCA-3' (Hebert et al. 2004; Wilson 2012). PCR amplified mitochondrial COI gene was sequenced by the Sanger sequencing method. The obtained sequence were submitted to GenBank. To construct the phylogenetic tree, sequences for the COI gene of *Henosepilachna* were downloaded from NCBI. A total of 54 sequences for eleven species of *Henosepilachna* and one for *Epilachna* were downloaded from NCBI GenBank (Table 1) of which *Epilachna* sp. is an outgroup.

Generated COI gene sequences were edited in BioEdit Sequence Alignment Editor Software. Multiple sequence alignment was carried out using MUSCLE (Edgar 2004) in MEGA 7 (Kumar et al. 2016). Phylogenetic analysis was done for COI sequences for 13 taxa.

Phylogenetic analysis was carried out using Maximum Likelihood (ML) method (Felsenstein 1981). MEGA 7 was used to find out the best-fit nucleotide model for the dataset under the corrected Akaike Information Criterion (AICc). The best nucleotide substitution found was T92+G, which is not implemented in the RAxML program, so we used the default model which was GTR + G (Lanave et al. 1984; Gu et al. 1995). ML analysis was performed on RAxML HPC2 (Stamatakis 2014) at the CIPRES portal (Miller et al. 2010). Bootstrap support values were obtained with the rapid bootstrap algorithm with 1000 bootstrap replicates.

RESULTS

Material examined: 1 male, SUKDZLB94, Halondi, Kolhapur (16.7348°N, 74.3016°E) 27.ii.2018, Priyanka Patil; 2 females, SUKDZLB314, SUKDZ317, 3 males, SUKDZLB311, SUKDZLB312, SUKDZLB313, Kanthewadi, Kolhapur (16.4693°N, 74.0329°E), 26.x.2018, coll.-

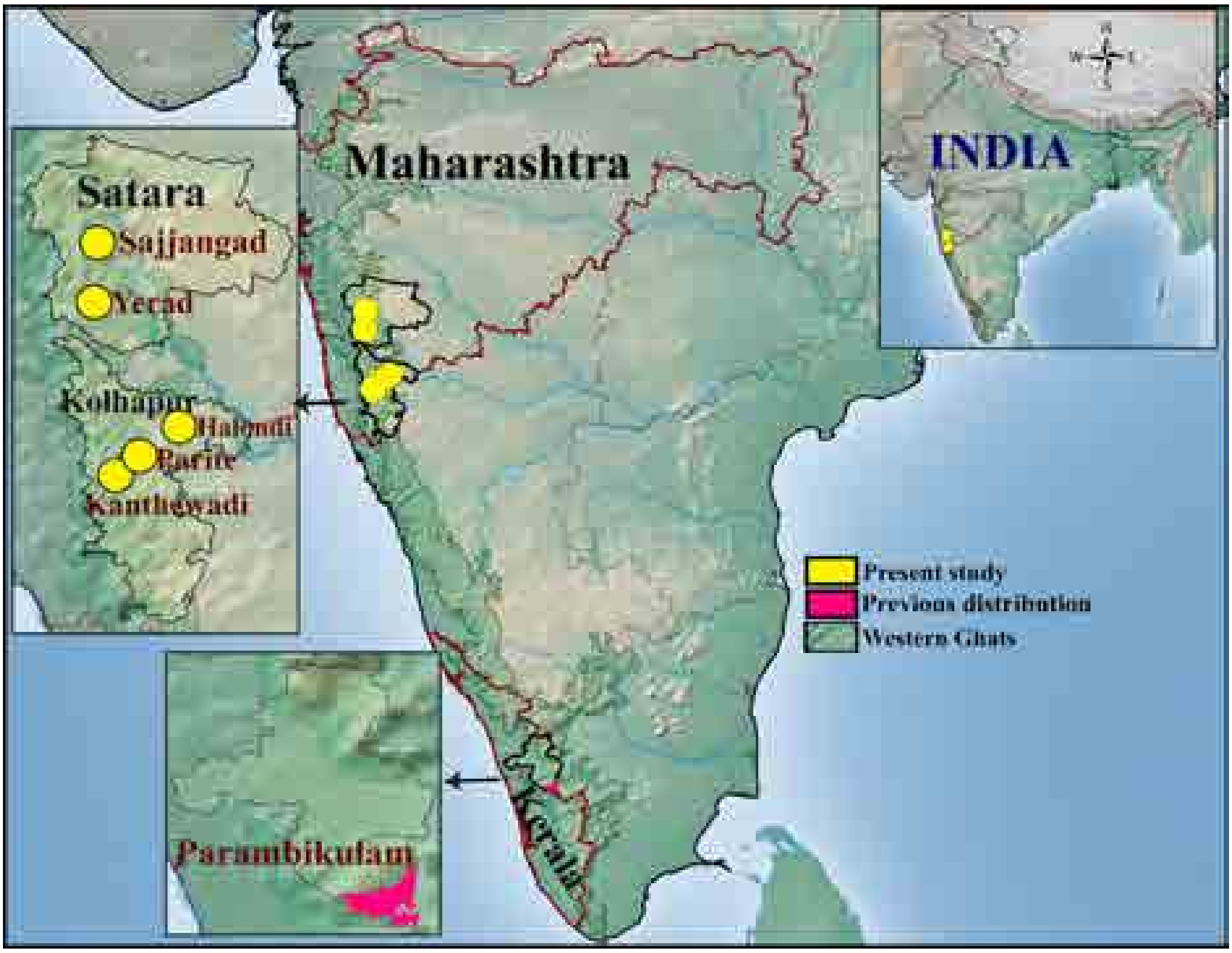

Image 1. Distribution map for *Henosepilachna nana*.

Priyanka Patil; 1 female, SUKDZLB450, 1 male, SUKDZLB453, Yerad, Satara (18.3614°N, 73.3502°E) 15.ix.2019, coll. Priyanka Patil.

***Henosepilachna nana* (Kapur, 1950)**

Diagnostics: Length 6.0–6.6 mm, width 4.6–5.2 mm.

The body is oval with 6 black elytral spots over grey background (Image 2a). The black elytral spots are surrounded by a brown ring. The dorsal body shows grey pubescence except for black spots. Brick red coloured pronotum with four black spots (Image 2m). In many examples, pronotal spots on the same side join to form a quadrate spot, which is nearer to the base than the apex and does not touch to anterolateral margins and pronotal median line (Image 2l). Scutellum brown, triangular, pointed at the elytral side (Image 2i). Ventrally brown except dark patches on metasternum and abdominal sternites. Tarsi are pseudotrimerous having bifid tarsal claws (Image 2o). The tarsal claw is with a basal tooth (Image 2p). Sixth abdominal sternite is divided in females (Image 2h) and emarginated in males (Image 2j).

Male genitalia: Penis is thin, with a slightly broad penis capsule (Image 2f). Penis apex is with V-shaped notch and a small projection at the notch base (Image 2d,e). Tegmen with paramere tip rounded having many setae at the apex and fewer setae on the distal half. Penis guide is uniformly curved at the distal half, curved and pointed at the apex (Image 2b,c).

Female genitalia: Female coxites quadrate with blunt corners, inner margin is straight except for a shallow notch at the base and with a pear-shaped small stylus with numerous setae (Image 2g).

Host plants: The specimens of *Henosepilachna nana* were found to be associated with Cucumber (*Cucumis* spp.), Pumpkin (*Cucurbita* spp.), and Karit fruit plant (*Cucumis* spp.). On the underside of leaves, in a bunch, approximately 20 elliptical, yellow, sparsely laid eggs were observed. Both the larvae and adults of *H. nana* skeletonize the leaves of the above vegetables by eating the chlorophyll and can act as a serious pest

Table 1. Taxa used in phylogeny analysis with their GenBank accession numbers.

	Taxa	GenBank accession numbers
1	*Henosepilachna niponica*	LC228599, LC228594, LC228597, AB002185, LC228600
2	*Henosepilachna pustulosa*	AB300448, LC228587, AB300446, LC228586, AB495213, AB495212, AB495210, AB300447, AB300454, AB300457, AB495211, LC228585, AB300452, LC228588, AB300456, AB002183, AB300451, AB300453, AB300450, AB300449, LC228582
3	*Henosepilachna vigintioctopunctata*	KU234209, KU234206, KU234207, KU234208, KU234204, KU234205, KU234199, KU234210, KU234203, KU234201, KU234200, AB002180, KU234211, KU234202
4	*Henosepilachna yasutomii*	LC228592, AB002184
5	*Henosepilachna enneasticta*	AB002173
6	*Henosepilachna pusillanima*	MH395854, AB002177, MT985168
7	*Henosepilachna implicata*	MT985166
8	*Henosepilachna* sp. 1	MH395855
9	*Henosepilachna* sp. 2	AB002174
10	*Henosepilachna boisduvali*	AB002175
11	*Henosepilachna septima*	KT693136, AB002176, KX503056, MT985165
	Epilachna sp.	AB002179

of cucurbitaceous plants. Under laboratory conditions, conspecific egg predation by this species was observed (Image 2n).

Phylogeny (Figure 1): COI gene sequenses for *Henosepilachna nana* generated during this study were first time submitted to NCBI GenBank under accession numbers ON220741 (for SUKDZLB94) and ON220742 (for SUKDZLB311). The phylogenetic tree is rooted with outgroup *Epilachna* sp. The larger part of the tree is a nested clade that comprises four main sub-clades. Within the first sub-clade, *H. septima* and *Henosepilachna* sp.1 are the sister taxon to all remaining species with high support (bootstrap (BS) 99). The second sub-clade, *H. boisduvali* is sister taxa to *H. nana* with a high bootstrap value (BS 88). Within the second sub-clade, *Henosepilachna* sp.2 and *H. implicata* are sisters to *H. pusillanima* with high support (BS 98). The third sub-clade shows three different *Henosepilachna* sp. named *H. vigintioctopunctata* showing its variation among its congeners. The sub-clade at the top of the tree shows two nodes, one for *H. enneasticta* and *the* other for *H. vigintioctopunctata* complex consisting of *H. niponica*, *H. pustulosa*, *H. yasutomii*, and *H. vigintioctopunctata.* The species *H. nana* is genetically closer to *H. boisduvali* than other congeners.

DISCUSSION

Kapur (1950) wrote a note on *Epilachna ocellata* with a description of three species, viz., *E. nana*, *E. anita*, and *E. manipurensis.* It includes details of identification and genitalia description along with images of all four species. After 25 years, Gordon (1975) revised Epilachninae of the Western hemisphere in which he described one new species named *E. nana.* However, the species which was described by Gordon (1975) is different from *E. nana* having different characteristics than *E. nana* which was described by Kapur (1950). Later, Kapur informed Gordon that the nomenclature *E. nana* is already given to one ladybird species and then Gordon (1985) replaced *the E. nana* name with *E. minuta.* The Holotype of *E. nana* was collected in 1892 and paratypes were collected in 1914 from Nilgiri Hills and Parambikulam (Kerala), respectively (Kapur 1950). The species is distributed over the Nilgiri Hills of southern India (Kapur 1950; Poorani 2012; Borowski 2020). The localities in Maharashtra, which are being reported in the present article, will add *H. nana* to the Fauna of Maharashtra, proving that the range extension of this species towards the north-west by about 700 km.

According to the illustrations and description given by Kapur (1950), the specimens recorded during the study are treated here as *H. nana.* Every specimen of *H. nana* collected during the study show 12 elytral spots without any variation in the count. Based on the number of elytral spots, *H. nana* differs from the other *Henoesepilachna* species. The species *H. boisduvali* is yellowish-red having a median black pronotal spot and 12 elytral spots (Li & Cook 1961; Li 1993). The species *H. nana* is varying from *H. boisduvali* in having two spots present on each side of the median line, there is no median pronotal spot and the median area is always spotless. In the male genitalia, the penis tip of

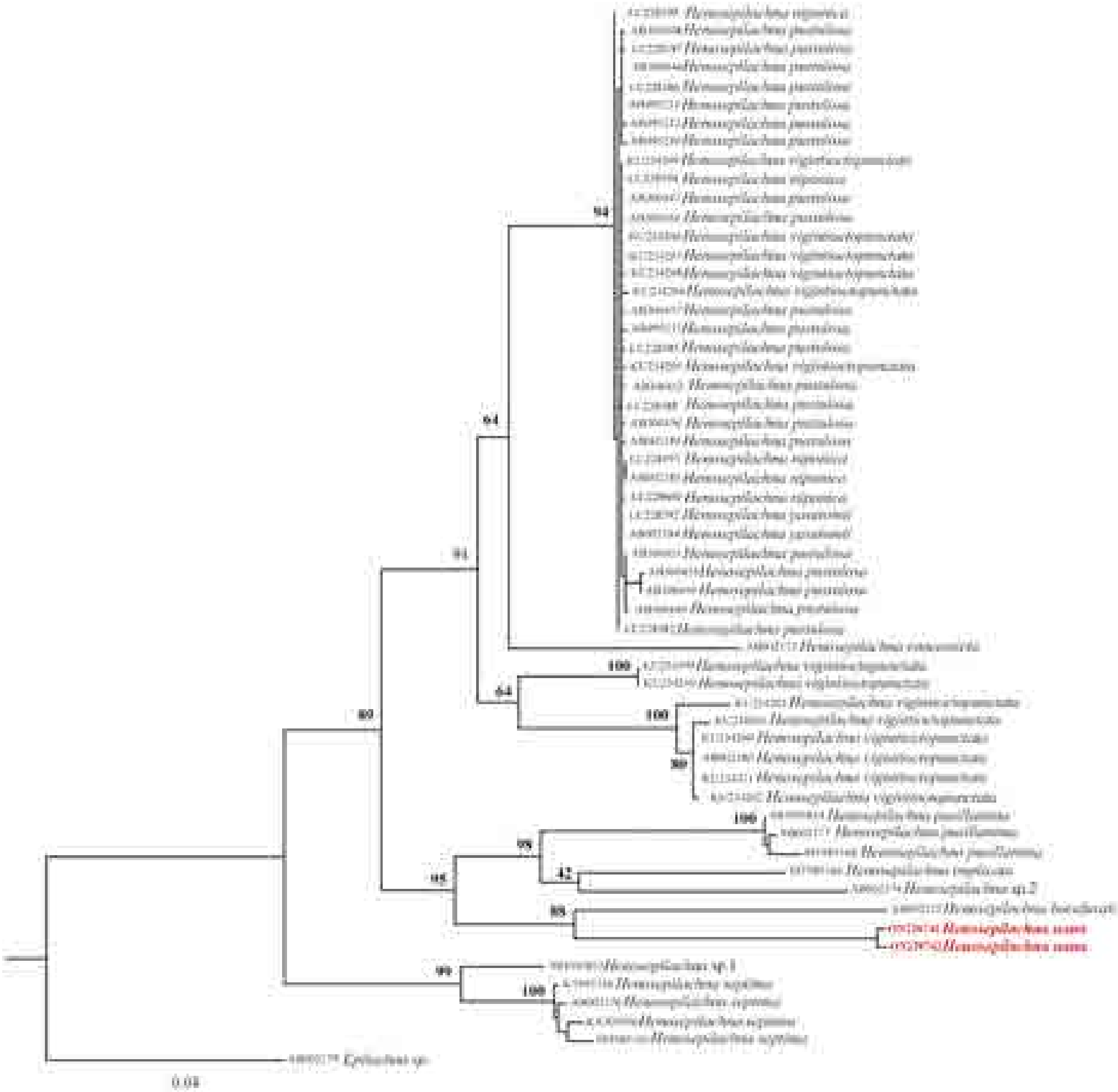

Figure 1. Maximum likelihood phylogenetic tree for *Henosepilachna* species based on COI sequences.

H. nana is similar to *H. boisduvali* but differs in having a small projection at the base of the notch. Poorani et al. (2021) have also mentioned 5–7 pronotal spots in *H. implicata.* In *H. nana,* the total number of pronotal spots is 2–4 and elytral black spots are 12 (6 on each elytron) which agrees with the observations of Kapur (1950) and confirms the identification of *H. nana*. The species *H. implicata* is with 26–28 black spots on the elytra, resembles other 28-spotted species of *Henosepilachna* such as *H. vigintioctopunctata*, *H. septima*, and *H. pusillanima* (Poorani et al. 2021). Although the tegmen of *H. implicata* and *H. nana* look a bit similar but it differs in their penis structure. The penis apex in *H. implicata* is with a shallow notch (Poorani et al. 2021) while the penis apex in the species under study is with a deep notch having a central small projection at the base of the notch which is similar to the penis illustration of *H. nana* given by Kapur (1950). So far, no information is available about the host plant of *H. nana*. Kapur (1950) wrote a note on *E. ocellata* including *H. nana* in which he mentioned that *E. ocellata* is found on potato but he does not mention the host of *H. nana*. According to Katoh et al. (2014), adults and larval stages of all Epilachnini species show phytophagous feeding nature. The record of this species on the Cucurbitaceae plant is providing new host records for the species under study and related species. In the

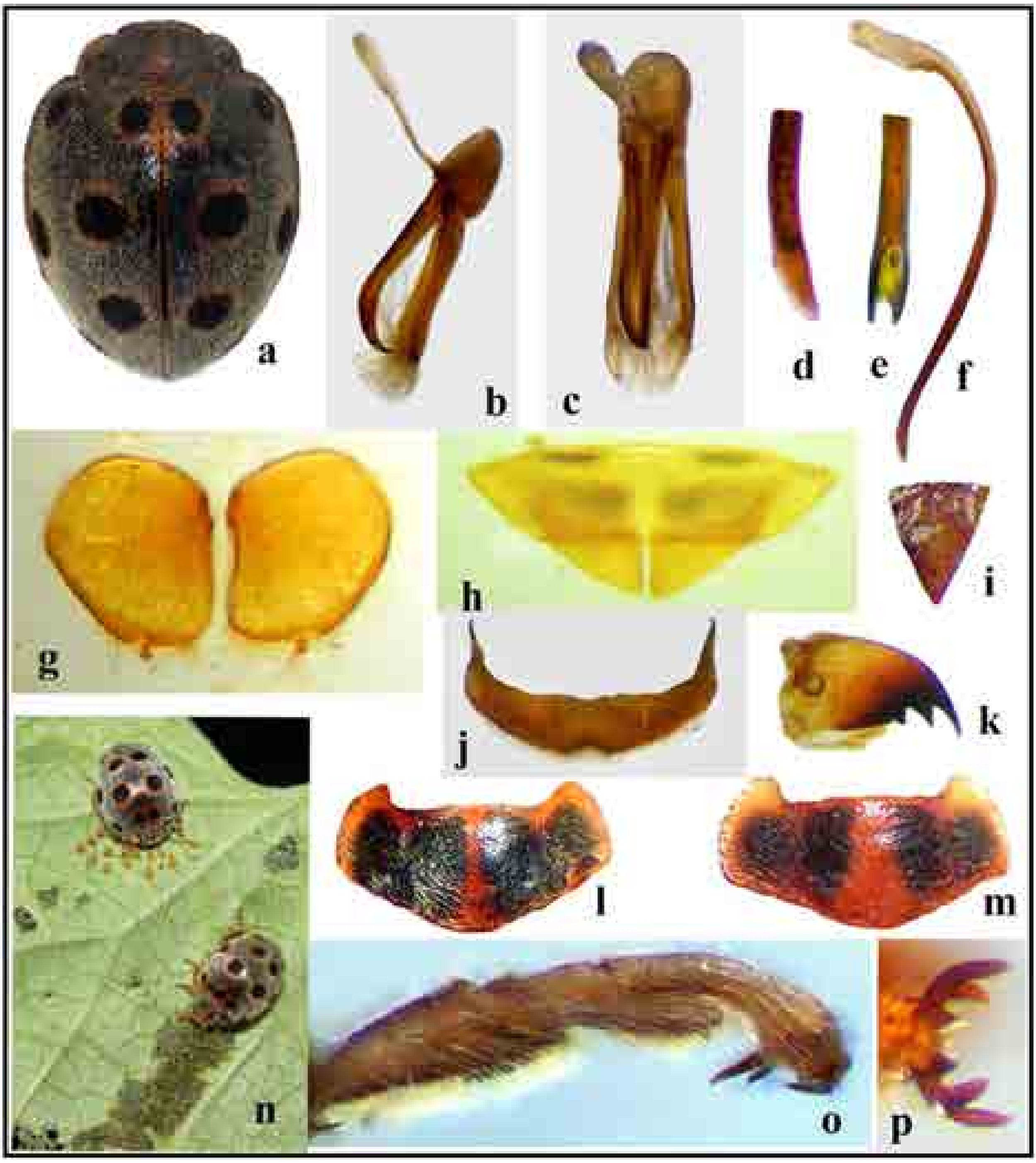

Image 2. a—*Henosepilachna nana* habitus | b—male genitalia: tegmen lateral view | c—tegmen ventral view | d—penis tip- lateral view | e—penis tip- ventral view | f—penis | g—female coxites | h—6th abdominal sternite of female | i— scutellum | j—6th abdominal sternite of male | k—mandible | l,m—pronotum | n—feeding adults | o—tarsi | p—tarsal claw. © Priyanka Patil.

present study, *H. nana* specimens were observed on the leaves of pumpkin, cucumber, and karit fruit plant which are additional host records.

A recent study on DNA sequence data and phylogenetic tree of *Henosepilachna* species includes five species (Poorani et al. 2021) excluding species *H. nana*. Therefore, the COI gene sequence of *H. nana* is becoming available in the NCBI database for the first time. The present consensus tree arrangement shows the co-evolution of species from common ancestors. When the phylogenetic tree of *H. nana* was constructed using COI gene sequences of eleven species of the

Henosepilachna genus and one outgroup *Epilachina* sp. from the same family from NCBI's database. The species *H. nana* shows variation among its congeners.

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COMMUNICATION

Assessment of population, habitat, and threats to *Cycas pectinata* Buch.-Ham. (Cycadaceae), a vulnerable cycad in Bhutan

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Abstract: *Cycas pectinata* Buch.-Ham. is an ancient gymnosperm that is now confined to pockets of habitats in the Indian subcontinent but was once widely distributed. Its decline is attributable to habitat loss, and has reached the point where *C. pectinata* is listed as 'Vulnerable' in the IUCN Red List. *C. pectinata* is the only species of *Cycas* found in Bhutan, and in this biodiversity-rich area it is present as a relic of great scientific and conservation value because of its rarity and long evolutionary history. Although it is well known in India, it has not been studied in detail in Bhutan. This study assessed populations and threats to *C. pectinata* in two places in Bhutan. Field visits were made to document the distribution, habitats, and associated threats to the populations. Plants were observed growing in steep rugged terrain in the open Chir Pine forest. Populations are significantly threatened due to human activities such as habitat destruction and over collection as ornamental plant. Possible expansion of populations is naturally threatened by low seed production and by predators.

Keywords: Conservation, dioecious, gymnosperm, habitat loss, IUCN Red List, ornamental, population, regeneration, threats.

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Author contributions: ST and TW conceptualised and designed the study, including data collection and manuscript writing. KW, JD, and TN contributed in developing field method, field data collection, and manuscript drafting.

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INTRODUCTION

Cycas is an evergreen, palm-like, dioecious tree with a robust trunk (Grierson & Long 1983; Lindstrom & Hill 2007; Khuraijam & Singh 2015). The trunk bears an apical crown of pinnately compound leaves with few spines in the petiolar region. Male cycads bear large, cylindrical ovoid yellow or green male cones having numerous microsporophylls with long apical spines (Lindstrom & Hill 2007; Khuraijam & Singh 2015). Female plants bear compact closed cones with numerous broadly subulate pectinate megasporophylls which bear 2–6 ovules.

The Scottish surgeon and botanist Francis Buchanan-Hamilton first described *Cycas pectinata* in 1824 from "Camrupae orientalis" which is probably part of modern-day Assam in northeastern India (Buchanan-Hamilton 1824; Grierson & Long 1983; Lindstrom & Hill 2007; Nguyen 2010; Khuraijam & Singh 2018). The family Cycadaceae Pers. includes the sole genus *Cycas* L. with 118 accepted species (Lindstrom & Hill 2007; Calonje et al. 2023). Cycads are one of the most ancient gymnosperms, originating in the early Permian period 280 million years ago or possibly in the late Carboniferous period (300–325 MYA). This long evolutionary history makes them of great scientific and conservation value (Zheng et al. 2017). The genetic information contained in cycads is important for palaeontology, palaeoclimatology, and palaeogeography (Feng et al. 2016; Zheng et al. 2017). Additionally, cycads are thought to be the earliest gymnosperm lineage, reaching the greatest diversity during the Jurassic-Cretaceous period, approximately 200–65 MYA (Feng et al. 2016). Cycads are bridges in major evolutionary transition in plants, and remain indispensable for understanding the origin and subsequent evolution of seed plants (Feng et al. 2016; Zheng et al. 2017).

Cycads, once more widely distributed, are now primarily distributed in Africa, Asia, Australia, and South & Central America (Feng et al. 2016; Zheng et al. 2017; Khuraijam & Singh 2020). The current distribution of *C. pectinata* extends from Nepal to northeastern India and Bangladesh (Lindstrom & Hill 2007; Khuraijam & Singh 2020), generally recorded within the elevation range of 600–1,300 m (Khuraijam & Singh 2014, 2020), mostly restricted to subtropical and tropical regions. From Bhutan, *C. pectinata* was first documented by Grierson & Long (1982, 1983) from two districts: Mongar and Trashigang (Now Trashi Yangtse) growing an elevations of 925 and 1,100 m. Subsequently, four additional localities for the species have become known (Tobgay et al. 2019), though none of these populations has been studied in detail.

C. pectinata in Bhutan is mostly confined to small geographically isolated populations in the rugged terrain of river gorges; this isolation may pose threats to genetic viability in the future (Yang et al. 1996). Although more than half of the Bhutan's total land area is protected for conservation (Wildlife Conservation Division 2016), only one known location of *C. pectinata* is in a protected area, making the species less likely to enjoy full protection by law. Habitat suitability modeling under climate change scenarios also does not indicate the protection of the species from known locations (Pradhan & Chettri 2017). In Bhutan species like *C. pectinata* is threatened by developmental activities like the construction of new and widening of existing roads, hydropower projects, and electric transmission lines. Such activities increase threats not only through habitat destruction but by facilitating exploitation through the collection for ornamental purposes and for food by the increasing human population in the localities. In Bhutan, despite knowledge of the localities of *C. pectinata*, detailed studies on these have not been undertaken. Although 68 globally threatened floral and faunal species are legally protected in Bhutan, *C. pectinata* is not included in this list (Biodiversity Statistics of Bhutan 2017).

Today, cycads are by far the most threatened plants (Donaldson 2003; Zheng et al. 2017). Around 62% of Cycad species are listed in IUCN Red List as threatened (Nagalingum et al. 2011). *C. pectinata,* though considered to be one of the most common and widespread cycads, is suffering a significant current population decrease due to habitat loss. The IUCN estimates there to be around 200,000–250,000 individuals of *C. pectinata* in the world; it is listed as 'Vulnerable' in the IUCN Red List (Nguyen 2010). The species is under severe threat with its total population declining due to an estimated 30% of its natural habitat having been destroyed over the past 90 years (Pradhan & Chettri 2017). More of its suitable habitats are very likely to be further reduced under predicted climate change scenarios (Pradhan & Chettri 2007; IPCC 2007).

Important threats to Cycads in general are collection from the wild for various purposes across the globe. *Cycas* has probably been used by people since prehistoric times in some regions and they have been traded for many different purposes (Donaldson 2003; UNEP-WCMC 2009; Williamson et al. 2016). The most common uses have been for food, medicine, and cultural practice (Bhowmik & Datta 2014; Khuraijam & Singh 2015). *Cycas* also has a long history of use as ornamental plants in Asia and they have now become popular garden and specialist collector plants in other parts of the world. By far the greatest

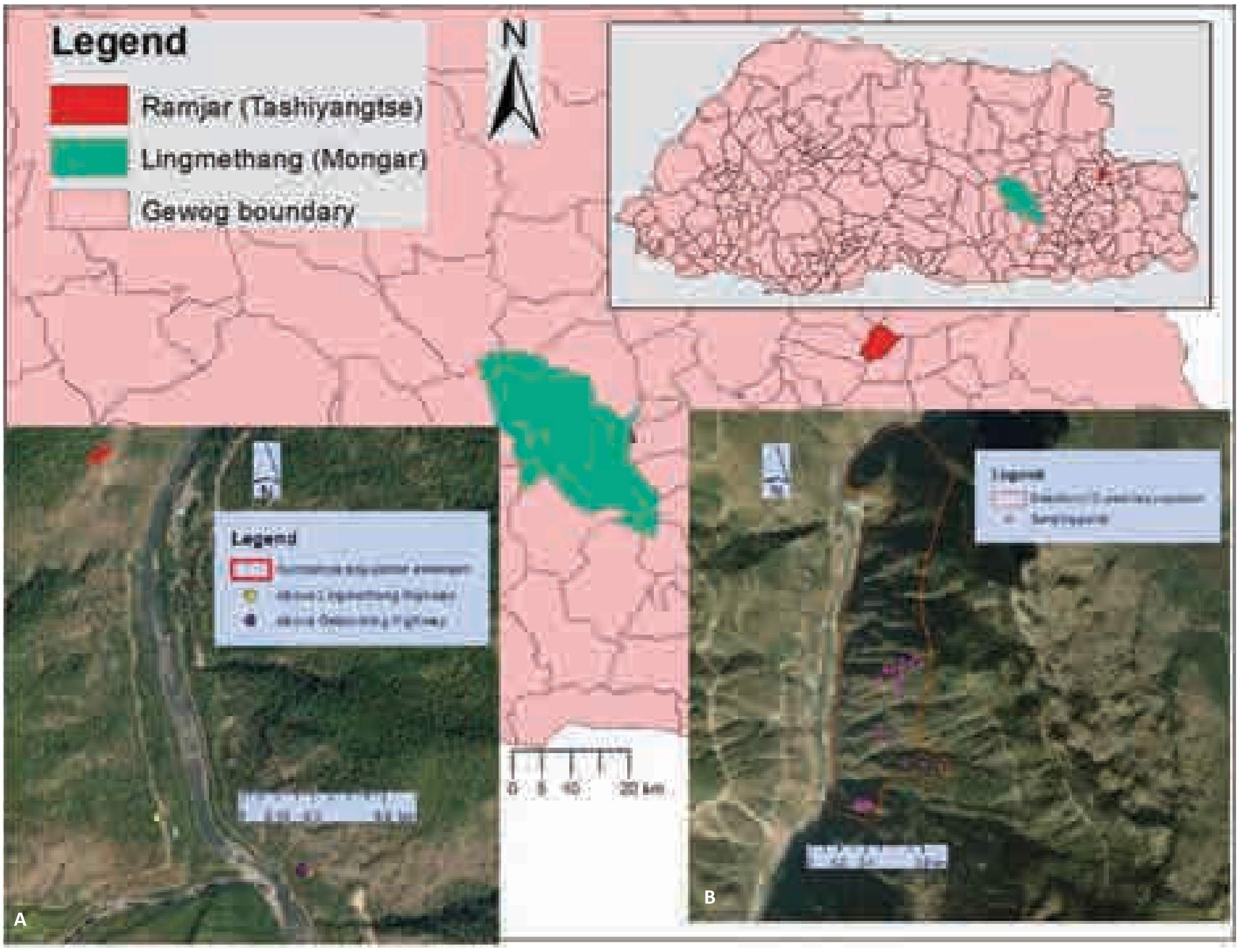

Image 1. Study sites: A—Kurizampa & Lingmethang | B—Ramjar.

global trade of *Cycas* is for ornamental purpose, with more than 30 million plants being traded between 1977 and 2001 (Bhima 2003). This study has assessed natural populations and threats to *C. pectinata* populations from two places in Bhutan.

METHODS AND METHODS

Study sites

Bhutan is part of the eastern Himalayan global biodiversity hotspot known for species richness with more than 11,000 known species (National Biodiversity Centre 2019). The high variation in climatic conditions across the small geographic area has supported diverse biodiversity. The IUCN (2019) has listed 99 species from Bhutan as threatened which include 43 plant species, 13 species are vulnerable to extinct including *C. pectinata*. This study has assessed the population, habitat, immediate threats and necessary conservation measures of *C. pectinata* from known locations in two districts of Bhutan (Trashi Yangtse and Mongar). In Trashi Yangtse, the population of the study species grows on the eastern bank of the Dangme Chhu River opposite Gomphu Kora at Ramjar within Trashi Yangtse District. The place name is "Bawoongshing-pek" meaning the hill of *C. pectinata*. Another population was assessed from Lingmethang and Kurizampa in the Mongar district. Both the historical and extant population in this site lies on the bank of Kurichuu River (Image 1A). The population here is easily accessible with a national highway passing nearby. Three populations within the locality have been assessed. The vegetation in both the study locations is dominated by large Chir Pine *Pinus roxburghii* Sarg. forming a canopy and *Cymbopogan flexuosus* (Nees ex Steud., Will. Watson) forming dominant ground vegetation (Table 1). Commonly associated shrubs includee *Rhus paniculata* Wall. ex G. Don., *Phyllanthus emblica* L., *Ficus bengalensis* L., *Asparagus racemosus* Willd. (Wild asparagus), *Zanthoxylum* L., *Grewia* L., and other commonly associated ground vegetation are *Artemisia vulgaris* L. and *Chromolena odorata* (L.) R.M. King & H. Rob. Ramjar study site is steep with hilly terrain and rocky outcrops while in Kurizampa the study site was on a hilltop.

Data collection

Population assessment in Ramjar locality (787–1,394 m) was carried out by laying random quadrats in between approximately 1.5 km^2. A total of 20 quadrats measuring 40 m^2 were laid out. In Kurizampa all the individuals were counted. All the *C. pectinata* plants within the quadrat were counted and morphometric measurements were taken along with the geocode for the quadrat. Male and female plants were identify based on cone morphology. Each plant was measured in height, girth, and base. Plants with stem well-formed above ground measuring >5 cm in height were recorded as individual plant and plants with less than two leaves and stem not seen above ground were categorized as saplings. The total number of leaves on each plant was counted along with the measurement of the length of selected leaves from each tree and leaf samples were collected from each tree and the number of pinna counted along with a length of petiole measurement. Those small plants height less than 5 cm were categorized as seedlings. All the quadrats and site of the population were assessed for any signs of threats.

Population structure determination

Cycads do not produce annual growth rings which in other trees are generally used to estimate age. For this species, the age of the plant can be estimated by its height, as in other *Cycas* species (Jian et al. 2006).

RESULTS

Population structure

A total of 566 individuals were recorded from Ramjar, Trashi Yangtse; 25 were identified as female and 43 as male, with the remainder lacking cones at the time of field sampling (Table 2). Most of the female plants recorded were mature with fully mature ovules, and male plants bore remnants of male cones. The shortest male cone-bearing plant measured 50 cm, and shortest female 55 cm.

The average density of plants measuring more than 5 cm in stem height within a quadrat was 27 (SD ± 13.42). In an estimated 1.5 km^2 of *C. pectinata* habitat, the total number of individuals were estimated to be around 25,000. The density distribution of plants show a significant correlation with elevation (p-value 0.045 and R^2 = 0.51); with a gradient of increasing density towards lower elevation.

In Mongar study area, three different populations were identified. The entire population in Kurizampa comprise 62 recorded individuals with stem height measuring >5 cm, and 74 saplings. Of these were three plants with female cones and seven with male cones. The male cone bearing plant has a minimum height of 80 cm and female plats 56cm. Another site in this region which includes an area above Lingmethang highway had recorded only one male plant, and another site above Gyelpozhing highway recorded three individuals all without any sign of coning. Age classification of individual plants measured in the field shows *C. pectinata* population has an opposite pyramid age structure in Ramjar site. There are older plants but fewer younger ones. In contrast, the population from Kurizampa has more young individuals than older plants (Figure 2).

Coning and regeneration

In both populations, the number of plants bearing cone is very low compared to the total number of adult and old individuals which all have the potential of bearing one type of cones. Further, the percentage of plants bearing a female cone is lower than that of the male cone bearing plants. Population in Ramjar site have 2% and 11.75% of female and male coning individuals, respectively (Table 1). Regeneration from bulbils is common in *Cycas* species. A total of 36 individuals were recorded with a total of 105 bulbils, mostly associated with fallen trunk remains. One fallen trunk was recorded with three bulbils in the Kurizampa site.

Threats to the populations

Socio-economic developmental activities are common in both study sites. These activities do not show an imminent threat to *Cycas* population in Ramjar. Activities for a hydropower project on the other side of the river at Dangmechu are completely separated by the river. Other activities like road construction took place away from the area where *Cycas* was recorded. Threats to *C. pectinata* population in Kurizampa and nearby places are directly associated with modern socio-economic development activities. This includes human settlement, construction & widening of roads, and hydropower related activities. The site above Lingmethang Highway and Gyelpozhing Highway were represented by only one and three individuals respectively. Small populations above Kurizampa were observed to be frequented by cattle and occasionally by human.

Individuals in both populations had symptoms of pest infection which includes drying of leaves or cutting off pinnules on rachis (Table 3). The young leaf rachis

Table 1. Location and characteristics of *Cycas pectinata* habitat.

Site name	Latitude	Longitude	Elevation range (m)	Habitat	Area (m²)
Ramjar	27.417	91.56827	791–1,296	Open Chir Pine forest and broad leave forest with lemongrass	1,514,856
Kurizampa	27.276	91.19106	844–872	Open Chir Pine forest with lemongrass and *Chromolena odorata*	2,285
Lingmethang Highway	27.263	91.19059	641	Open Chir Pine forest and lemongrass	
Gyelpozhing Highway	27.26	91.19595	597	Open Chir Pine forest with rocky areas	

bored by larvae (belonging to Lepidoptera) were common in Ramjar site, resulting in death of the crown of young leaves (Image 2A,B).

Additionally, leaf litter accumulating in the crown of leaves has been observed to become inhabited by millipedes resulting in deformed leaf sprouts (Image 2C,D). In Ramjar, Wild Boar is also seen as a threat to *Cycas* populations, leading to seed damage and uprooted young individuals (Image 2G,H), but not observed in the population from Kurizampa and nearby areas. The adult individuals are resistant to fire incidences protected by thick scaly bark. However, frequent disturbances have resulted in growth of invasive species. This was evident in Kurizampa area where plants were overgrown by weed species like *Chromolaena odorata* (L.) R.M.King & H.Rob. (Image 2I,J).

Collection of the plants by humans was not evident in the Ramjar site, except for a few translocated to gardens by local people. Individuals at Kurizampa and nearby areas are threatened by collection. The presence of more *Cycas* plants above Gyelpozhing Highway a couple of years back was confirmed by locals. However, during the present study visit only three individuals were recorded and the other plants had been removed for their ornamental value (Image 2K,L).

Table 2. Age structure of the two natural population of *Cycas pectinata*.

Site	Ramjar			Kurizampa		
Tree height class (Stem height)	No. of plants	Cone		No. of plants	Cone	
		M	F		M	F
>100 cm	201	32	19	1	1	0
51–100 cm	148	9	7	18	5	3
26–50 cm	102	0	0	15	0	0
6–25 cm	85	0	0	28	0	0
<5 cm	30	0	0	74	0	0

Table 3. Level of threats as determined by the characteristics of the leaves.

Leaf characteristics	Kurizampa	Ramjar
Pest	62 (100%)	158 (62.69%)
Few leaves, not integrated	13	93
Many leaves, some integrated	2	55
Many leaves, integrated	45	64
Nibble	7	22

DISCUSSION

Population structure

Grouping plants by height approximating their age, the *C. pectinata* population in Ramjar site shows an inverted pyramid age structure where more individuals are in the adult and old categories compared to saplings and seedlings. Similar declining recruitment of seedlings has been observed in populations of other *Cycas* species (James et al. 2018). Low seedling recruitment directly threatens the viability of the population, as fewer individuals remain to replace old plants, which in the long term can reduce the number of plants in the population (Shen et al. 2009; Dian-pei et al. 2012). In contrast, the population from Kurizampa showed more sapling recruitments than older plants, a positive sign for sustainability of the population in the area. However, the number of individuals in the different populations must greatly contribute to the viability of the population. Only 62 adult plants constitute the population in Kurizampa.

A population from the Ramjar site was observed with increasing density of plants at a lower elevation with density decreasing with increasing elevation. However, this relation from a Pearson correlation test indicates a weak association P >0.05. (r = -0.73). The increasing density at the lower elevation can be associated with the steepness of the habitat and larger seed size, which coupled would facilitate easy roll down by gravity thus concentrating seed germination at lower elevations. Other mode of seed dispersal was also evident in the field, seedling recruitment at elevation ranges higher than the adult trees affirms that seeds dispersal was mediated

Image 2. *Cycas pectinata*: A & B—Young leaves bored and eaten by larvae (Lepidoptera) | C & D—Millipede inhabiting bases of crown of leaves | E—Remnants of leaf pinnule | F—Plant with leaves showing symptoms of gradual drying of leaves | G—Small plants uprooted by boars | H—Female cone with seed formed being damaged by boar | I & J—Extensive growth of *Chromolaena odorata* in the habitat | K & L—Planted as ornamental plant. © A–E: Tshering Nidup | F–L: Sonam Tobgay.

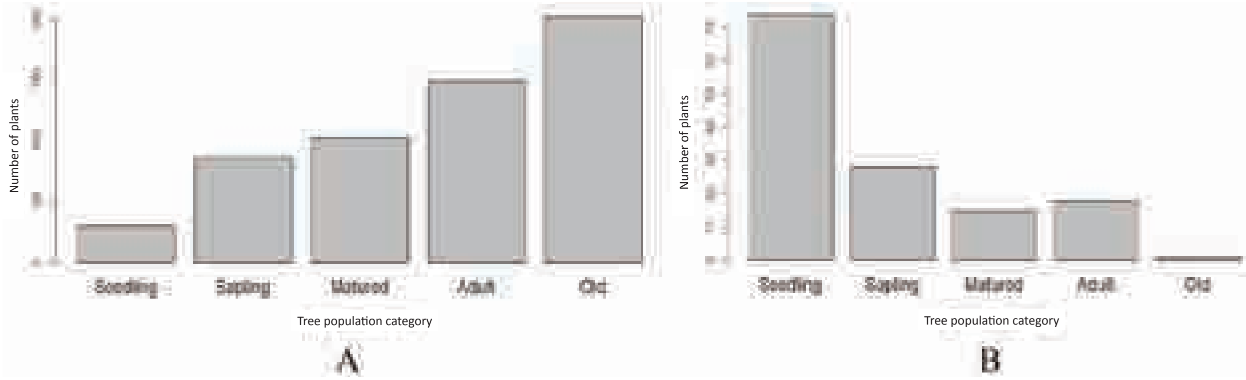

Figure 2. Population structure of *Cycas pectinata* plant form: A—Ramjar in Trashi Yangtseand | B—Kurizmapa in Mongar.

by small mammals such as rodents and fruit-eating bats (Dehgan & Yuen 1983; Yang & Meerow 1996).

Coning and regeneration

Stability of a population will be determined by the number of seed-producing individuals present. In both populations there are fewer coning individuals, particularly female. Only 2% and 16% of adults bore a female cone, compared to 12% and 32% of adult plants bearing a male cone, in respective population from Ramjar and Kurizampa. The trend of declining coning is common in populations of other *Cycas* species (Jian et al. 2006). Determination of sex composition within a population is important, particularly for a dioecious species like *Cycas,* to understand population stability. Many works of literature support a 1:1 ratio as being a theoretical and evolutionarily stable sex ratio for maintaining a dynamic population under natural selection (Yu & Lu 2011; Tarsi & Tuff 2012; Shin et al. 2019). Without comparable numbers of males and females, reproduction opportunities may be limited and population growth stunted (Tarsi & Tuff 2012). Similar observations of a dwindling sex ratio in cycad populations have been made resulting in a reduction of seed production and a reduction of population size (Singh & Singh 2010). Nonetheless, Ueno et al. (2007) claim that in most dioecious plants, either the population sex ratios do not depart from unity, as expected by Fisher's Law (Fisher 1930), or males are more numerous than females, as observed in this study. This difference is attributed to the higher reproductive cost for females producing seeds, resulting in reduced growth rates, higher mortality rates, delayed flowering, lower frequency of future reproduction, and less extensive clonal growth. The ultimate result of reduced seed production means a lower number of recruitments and population showing a decreasing trend.

Threats to the population

Human activities and habitat destruction continue to be the major threats to species diversity including the loss of plants from wild (Zheng et al. 2017; Hossain et al. 2021). Historical evidence confirms *C. pectinata* population in the area above Lingmethang Highway, formally thriving very well, is now found to be represented by only a single male individual. Threats to the *Cycas* population in the area are clearly from anthropogenic disturbances. Population re-settlement driven by developmental activities and associated economic activities have contributed to the loss of population. Activities such as road widening, establishments of gravel grinding machineries, and tunnel construction for hydropower projects have contributed significantly resulting in habitat disturbance.

Loss of *Cycas* populations from one of the study sites near Gyelpozhing Highway is a very recent event. The decrease of *Cycas* population in this location can be evidently attributed to the over-collection of plants for ornamental use. Being easily accessible through roads, the plants were removed by plant collectors. Locals collected the plants as priced specimen for use as ornamental plants in their gardens, a common threat to *Cycas* (Bhima 2003; Chowdhury et al. 2011) beside being culturally associated in using as sources of nutrition (Khuraijam & Singh 2015; Hossain et al. 2021).

Natural threats to the population include those from pest infestation, in particular affecting the young leaves. Such damage to the foliar part of the plants can cause direct deformation of the plant parts and limit the capacity of photosynthesis (Guest 2017).

CONCLUSION

It is clear from this study and others that *C. pectinata* populations are severely threatened and therefore require priority conservation plans. As anthropogenic activities continue to be a major threat, education and awareness are some of the possible ways to conserve and save species in their natural habitat.

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COMMUNICATION

Ecological niche modeling to find potential habitats of *Vanda thwaitesii*, a notified endangered orchid of Western Ghats, India

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Abstract: Conservation planning of a threatened species essentially requires information on its present population and extent of distribution. Ecological niche modeling (ENM) is a suitable machine learning technique to predict potential distribution of a species enabling to identify suitable habitat for conservation action. The present study undertook distribution modeling of *Vanda thwaitesii*, a notified endangered orchid species of the Indian Western Ghats and Sri Lanka using maxent software. Geographical coordinates of 54 occurrence points at 1 km resolutions gathered during the study were utilized for modeling. A total of 37 variables including bioclimatic, topographical, and seasonal climate subjected to principal component analysis extracted into three components based on temperature and precipitation. Four representative variables from each component in all possible combinations resulted consistent output showing distribution of the species extending from Gavi in Periyar Tiger Reserve of Kerala to Chikkamagalur of Karnataka. Habitat suitability was confined to the cooler regions receiving an average 3,400 mm annual mean precipitation, 22.7^{0}C annual mean temperature, and 290 mm summer precipitation. A total of 2,557 km^2 in Kerala and Karnataka mostly outside protected forests demonstrated as the highly suitable habitats. Silent Valley National Park, Idukki Wildlife Sanctuary, Periyar Tiger Reserve, and Brahmagiri Wildlife Sanctuary in addition to a few reserve forests hold sufficient area for reinforcement of diversity of *V. thwaitesii* from vulnerable locations. The present study revealed niche modeling as a useful tool to find suitable habitats for *V. thwaitesii* in the Western Ghats.

Keywords: Bioclimatic variables, co-linearity, conservation, endangered species, jack knife test, maxent modeling, Orchidaceae, QGis, summer precipitation, Wayanad.

Malayalam: വംശനാശ ഭീഷണി നേരിടുന്ന സസ്യങ്ങളുടെ സംരക്ഷണ ആസൂത്രണത്തിന് അവയുടെ വിതരണപരിധിയും എണ്ണവും നിർബന്ധമായും അറിയേണ്ടതായിട്ടുണ്ട്. സസ്യ ജാതികളുടെ സാദ്ധ്യമായ വിതരണ പരിധി പ്രവചിക്കുവാനും സംരക്ഷണ പ്രവർത്തനങ്ങൾക്ക് അനുയോജ്യമായ വാസസ്ഥലങ്ങൾ തിരിച്ചറിയുവാനും യാന്ത്രിക പഠനവിദ്യയായ എക്കോളജിക്കൽ നിഷ് മോഡലിങ് പ്രാപ്തമാക്കുന്നു. ഭാരതത്തിലെ കേന്ദ്ര പരിസ്ഥിതി വനം മന്ത്രാലയം, വംശനാഷ ഭീഷണി നേരിടുന്ന സ്പീഷീസായി വിജ്ഞാപനം ചെയ്തിട്ടുള്ള, പശ്ചിമഘട്ടത്തിലും ശ്രീലങ്കയിലും മാത്രം കണ്ടെത്തിയി-ട്ടുള്ള, വാണ്ട ത്വയ്റ്റീസി എന്ന ഓർക്കിഡിൽ മാക്സെൻറ് എന്ന സോഫ്റ്റ്‌വെയർ ഉപയോഗിച്ച് ഇവിടെ പഠനവിധേയമാക്കി. വിപുലമായ പഠന യാത്രകളിലൂടെ ശേഖരിച്ച, 54 വാസസ്ഥാനങ്ങളുടെ ജോഗ്രഫിക്കൽ കോർഡിനേറ്റർ ഉപയോഗിച്ച് വിതരണ മാതൃക തയാറാക്കി. ബയോക്ലൈമറ്റ്, ടോപോഗ്രഫി, സീസണൽ ക്ലൈമറ്റ് എന്നിവയിൽപ്പെടുന്ന 37 വേരിയബിൾസ് പ്രിൻസിപ്പൽ കമ്പോണൻറ് അപഗ്രഥനത്തിലൂടെ താപനില, മഴ ഇവയെ അടിസ്ഥാനമാക്കി 3 ഘടകങ്ങളായി ചുരുക്കി. എല്ലാ ഘടകങ്ങളെയും പ്രതിനിധീകരിച്ചുള്ള 4 വേരിയബിൾസ് സാധ്യമായ എല്ലാ കോമ്പിനേഷനിലും അപഗ്രഥിച്ചപ്പോൾ, സ്ഥിരതയുള്ള വിതരണ മാതൃക ലഭിച്ചു. മാതൃക പ്രകാരം, കേരളത്തിലെ പെരിയാർ കടുവാ സങ്കേതത്തിൽ ഉൾപ്പെട്ട ഗവി മുതൽ കർണാടകയിലെ ചിക്കമഗല്ലൂർ വരെ വിതരണസാദ്ധ്യതയുള്ളതായി തെളിഞ്ഞു. പൊതുവെ തണുത്ത കാലാവസ്ഥയിൽ, ശരാശരി 3400 മി.മി. വാർഷിക വൃഷ്ടിയും 22.7°C വാർഷിക താപനിലയും, 290 മി.മി. വേനൽ മഴയും ലഭിക്കുന്ന സ്ഥലങ്ങൾ വാണ്ട ത്വയ്റ്റീസിയുടെ വളർച്ചക്കും നിലനില്പിനും വളരെ ഉചിതമെന്നു കണ്ടെത്തി. കേരളത്തിലും കർണാടകയിലുമായി ആകെ 2557 ച.കി.മി. പ്രദേശം വളരെ അനുയോജ്യമെന്ന് സമർത്ഥിക്കുവാൻ കഴിഞ്ഞു. സുരക്ഷിതമല്ലാത്ത പ്രദേശങ്ങളിൽനിന്നും വാണ്ട ത്വയ്റ്റീസിയുടെ ജനിതകവൈവിദ്യം പുനരധിവസിപ്പിക്കുവാൻ സൈലൻറ് വാലി, ഇടുക്കി വന്യജീവി സങ്കേതം, പെരിയാർ കടുവ സങ്കേതം, ബ്രഹ്മഗിരി വന്യജീവിസങ്കേതം കൂടാതെ ഏതാനും സംരക്ഷിത വനങ്ങൾ അനുയോജ്യമായ വാസസ്ഥലങ്ങൾ ഉൾക്കൊള്ളുന്നതായി തെളിയിക്കുവാൻ നിഷ് മോഡലിങ്ങിന് കഴിഞ്ഞു.

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INTRODUCTION

Orchids are a group of plants belonging to Orchidaceae with 29,335 accepted species worldwide (POWO 2021). They have wide climatic preferences ranging from tropical to alpine habitats. However, due to habitat loss, habitat fragmentation, over-exploitation, and unrestrained illegal activities, many orchids have limited distribution range and population strength as other threatened plant species (Agustini et al. 2016; Warghat et al. 2016; Bachman et al. 2019; Lughadha et al. 2020). The existence of biodiversity have prime importance for the stability of an ecosystem and thus developing effective strategies for their conservation is the serious concern of conservation biologists (Singh et al. 2017). Besides, conservation planning of a threatened species essentially requires information on its present population and extent of distribution (Radosavljevic & Anderson 2014; Štípková et al. 2020). Mapping the identified geographical locations and predicting potential distribution of a species out of that is known to be useful in identifying critical regions that may need conservation action (Warren & Seifert 2011).

Vanda thwaitesii Hook.f. is one among the 81 species of *Vanda* reported worldwide (POWO 2021). It is endemic to the southern Western Ghats and Sri Lanka and is endangered mainly due to habitat loss and fragmentation. It was first collected by Thwaites from Sri Lanka in 1898 and remained elusive for over a century which forced to declare the species as extinct in 1981 (Sathishkumar & Sureshkumar 1998). During the period from 1982 to 1997, the species was collected from Silent Valley and Wayanad, Kerala thus confirming its presence in India (Sathishkumar & Sureshkumar 1998). The latter authors could also locate reference sample of *V. thwaitesii* collected in 1885 from Mananthavady in Wayanad District of Kerala. As per reports, the species have distribution in seven localities in Kerala. The species is distributed in narrow pockets with restricted numbers and later under section 38 of the Biological Diversity Act 2002, the Central Government notified that *V. thwaitesii* is on the verge of extinction and prohibited/regulated collection (MOEF 2009). The ministry also called for studies on all aspects of the notified species for holistic understanding and propagation of the species for the purpose of in situ and ex situ conservation and rehabilitation.

Plant distribution modeling/ ecological niche modeling (ENM) is recognized as an efficient tool to understand potential distribution of a plant/animal species and maximum entropy method (MEM; Phillips et al. 2006) is widely used for the purpose (Elith et al. 2011; Peterson et al. 2011). These models establish relationships between occurrences of species and biophysical and environmental conditions in the study area thus predicting suitable habitat for survival and existence of a species. This technique was successfully applied to find potential distribution and identify environmental niches for several plant species including orchids *Vanda wightii* (Decruse 2014), *V. bicolor* (Deb et al. 2017), *Paphiopedilum javanicum* (Romadlon et al. 2021), and *Habenaria suaveolens* (Jalal & Singh 2017). Based on the current understanding and the requirement of devising conservation action, the present study is framed to understand the habitat suitability of the species in localities other than that described earlier and the reported localities in Western Ghats region of Kerala, Tamil Nadu, and Karnataka states in India.

MATERIALS AND METHODS

Study site and field survey

As per the cited literature, Wayanad, Silent Valley, and Periyar Tiger Reserve in Kerala are the reported localities of *V. thwaitesii* in India (Agustine 1995; Sathishkumar & Sureshkumar 1998). The species have distribution at altitudes 500–1,060 m in moist deciduous to evergreen forests. Therefore, similar habitats in Idukki District of Kerala to Coorg District of Karnataka were surveyed to record geographical coordinates of occurrence points and score population status. Periyar Tiger Reserve, Idukki WS, Thirunelli, and Silent Valley are the protected forests surveyed. The inhabited land mainly covered is along roadways in North and South Forest Division of Wayanadu and adjoining places in Coorg District of Karnataka and Nilgiri District of Tamil Nadu. The surveys were conducted during 2011–2014 periods. The presence of *V. thwaitesii* was confirmed through close observation of the morphological feature (Image 1) of the species including fruit and flowers with the help of binoculars. Geographical co-ordinates of the presence location were recorded using Garmin GPSMap 60CSx.

Distribution modeling

A total of 37 environmental variables including 19 bioclimatic, six topographic, 11 seasonal climatic, and one vegetation variable (Table 1) influencing survival of a plant species were analyzed for their importance in modeling studies. As peninsular India receive monsoon in four distinct season, i.e., January–February (Dry period),

Image 1. Habit of *Vanda thwaitesii* Hook.f. © S. William Decruse

March–May (Summer precipitation), June–September (South-west monsoon), and October–December (North-east monsoon) the monthly precipitation data obtained from world climate (https://www.worldclim.org) was reconstructed to obtain seasonal precipitation variables of the region. All extraction, clipping, and recalculation were undertaken using QGIS software version 3.10

The climatic variables are derived from temperature and precipitation data and thus multi co-linearity always exists among different variables. Therefore, principal component analysis (PCA) is often recommended (Junior & Nóbrega 2018) to control the negative effects of co-linearity and as a more objective solution for the problem of variable selection. The data points of all the 37 variables corresponding to the geo-reference points of *V. thwaitesii* occurrence in 1-km spatial resolution were extracted using point sampling tool in QGIS and PCA analysis undertaken in SPSS 16 software to sort out the variables having significant contribution to the model. Accordingly, three major components were extracted (Table 2) and one important variable representing each component and all those with VIF less than 10 (Naimi et al. 2014) in each component were selected. Thus the variables Bio_1, Bio_9, Bio_12 and summer precipitation (PMM) were included in the final model.

The reported localities are confined to southern Indian peninsula and Sri Lanka and thus the region falling under longitudes (E) 67.5 & 89 and latitudes (N) 5.5 & 24.5 was extracted from the world climate to prepare distribution model for peninsular India and Sri Lanka. Maxent software version 3.40 (Philips et al. 2006) was used to build a habitat suitability model. In this modeling, 75% of the encounter data was used for the training set and the rest for the test set. The modeling used auto features with 500 iterations and other default values. For validating model robustness, 10 replicated model runs was executed with a threshold rule of 10 percentile training presence and employed bootstrapping method for dividing the samples into replicate folds. The output of the Maxent software predicts habitat suitability in the range 0 (not suitable) to 1 (appropriate) (Phillips & Dudik 2008). For selection of most important environmental variable, Jack knife test was performed. The output was

Table 1. Climatic and topography variables utilized for analysis of their contributions in plant distribution modeling.

Bioclimatic Variables[a]	Topography [b]	Specific Climate (Western Ghats)	Vegetation[c]
Bio_1–19	Elevation Slope Topical wetness index Vertical distance from channel network Convexity Aspect	*Precipitation[e]:* Jan–Feb Mar–May (PMM, Summer) Jun–Sep (SW monsoon) Oct–Dec (NE monsoon) Precipitation humid months (PHM, Apr–Nov) Precipitation dry months (Dec–Mar) *Evapotraspiration[d]:* Annual (aieto) Humid months (Apr–Nov) Dry months (Dec–Mar) *Temperature[e]* Average humid months (THM) Average dry months	Normalized digital vegetation index (NDVI)

[a] https://www.worldclim.org/data/worldclim21.html.
[b] Digital Elevation Model (DEM) data downloaded from http://www.earthexplorer.usgs.gov/ and derived the topographic variables in QGIS 3.10.7
[c] Buchhorn et al. (2020) Copernicus Global land Service, http://www.land.copernicus.eu/g;pbal/products/ndvi
[d] CGIAR, FAO, http://www. cgiar-csi.org. Monthly data downloaded and computed the seasonal value in in QGIS 3.10.7
e https://www.worldclim.org/data/worldclim21.html). Monthly data downloaded and computed the seasonal data in QGIS 3.10.7

imported to QGIS and a distribution map was created and prediction area calculated.

RESULTS

Field Survey

Extensive field surveys revealed the distribution of *V. thwaitesii* altitudes from 489 m to 1,168 m mostly on evergreen trees exposed to sunlight. A total of 93 occurrence points were recorded in Kerala, Tamil Nadu, and Karnataka. The surveys in Periyar Tiger Reserve (PTR), Idukki Wildlife Sanctuary, and Wayanad revealed inhabited land in Wayanad Plateau as the dominant distribution area and their population near forest segments is meager. Therefore, it is clear that the dominant population in the most ideal habitat was lost due to habitat loss and extension of their population was withheld due to habitat fragmentation.

Modeling studies

Out of the 93 occurrence points recorded during our field surveys, 54 geo-reference points were at 1-km spatial resolution (Table 3). Hunnasgiria in Sri Lanka the reported type locality retrieved from Google Earth was also included for modeling. Thus 55 occurrence points were available for modeling. The 37 variables (19 bioclimatic, six topography, 11 seasonal, and one vegetation) subjected to principal component analysis, extracted into three components explaining 96.5% variance (Table 2). The final model based on the four selected variables revealed summer precipitation in the months of March to May (PMM), annual precipitation (Bio_12), and annual mean temperature (Bio_1), have significant contribution to the model (Table 4). Jack Knife test (Figure 2) revealed PMM as the environmental variable with highest gain and significant drop when it is omitted. The species flowers during April–May when receives summer showers, after a dry period and thus precipitation during March–May may be critical for the survival and spread of the species. In the distribution model generated (Figure 3), one occurrence point at 489 m altitude in Kannur District was in the least probable region. This is the only one occurrence record below 700 m still establishing a solitary colony with 15 individuals with the incidence of fruit set and new recruit. However, 41% of the points fall in high probable (0.8–1) region and 32.6% in the 0.6–0.8 region (Figure 4). The whole peninsular India was modeled where only 0.59% area (11,561 km^2; Figure 4) confined to the Western Ghats region of Kerala, Karnataka, and Tamil Nadu emerged as suitable habitats for *V. thwaitesii*. Out of the total area (2,557 km^2) in the high probable region (0.6–1.0), Wayanad District of Kerala and Coorg District of Karnataka together constitute 1,461 km^2 (57%). Idukki District (353), Nelliyampathy (195), and Sholayar (137) of Kerala are the other regions having habitat suitability of 0.6–1.0 class. Suitable habitats extend from cooler regions in Thirunelveli districts of Tamil Nadu to Chikkamagalur district of Karnataka (Figure 3) covering protected forests as Idukki Wildlife Sanctuary, Silent Valley National Park, Periyar Tiger Reserve, and Brahmagiri Wildlife Sanctuary. In addition, a small area in Central Province of Sri Lanka also has habitat suitability.

Different variables extracted in the two principal components have equal variance of temperature and precipitation in the occurrence localities and thus the distribution models appeared very similar; *V. thawaitesii* inhabits cooler regions receiving annual mean temperature in the range 19.75–24.3 ^{o}C. Similarly, annual precipitation in the occurrence points are in the range 2020–4794 (Table 5). The occurrence points also

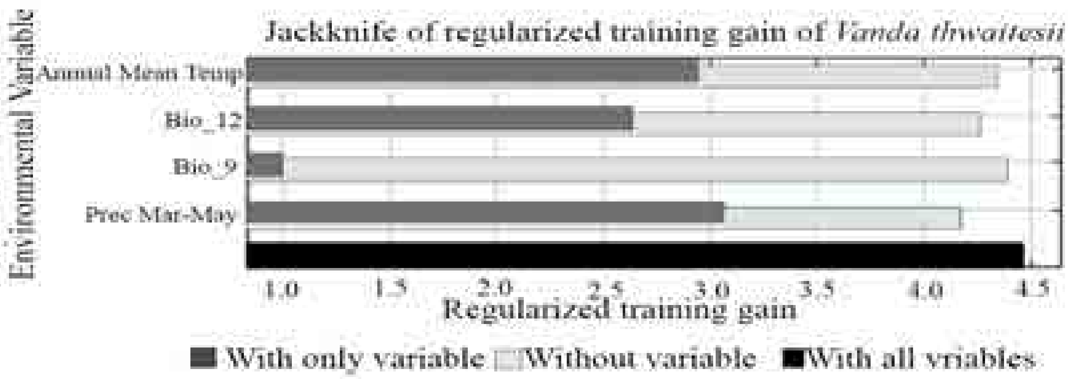

Figure 2. Jackknife of regularized training gain of *Vanda thwaitesii*.

Table 2. Output of principal component analysis (PCA) and variance inflation factor (VIF) among the variables.

Total variance explained and component variables			Principal components and VIF against one variable taken as constant				
Component	PCA output		PC1		PC2		PC3
	Eigen values	% of Variance	Variables	VIF	Variables	VIF	Variable
1	7.14	59.5	Bio_1		Bio_12		PMM
2	3.62	30.3	Bio_8	17.7	Bio_13	199.0	
3	0.8	06.7	Bio_9	5.4	Bio_16	443.9	
Total		96.5	Bio_11	57.3	PHM	61.6	
			THM	32.0	aieto	52.2	

Table 3. Geo-reference points (~1 km spatial data) of Vanda thwaitesii occurrence in Kerala, Tamil Nadu, and Karnataka used for distribution modeling.

Population	Latitude	Longitude	Altitude (m)	Location	District
1	9.4826166	77.144733	910	PTR	Idukki
2	9.46885	77.142266	931	PTR	Idukki
3	9.4653	77.143766	931	PTR	Idukki
4	9.4552	77.1426	1004	PTR	Idukki
5	11.098583	76.442203	920	Silent Valley	Palakkad
6	11.105	76.421345	1149	Silent Valley	Palakkad
7	11.079016	76.441608	1168	Silent Valley	Palakkad
8	11.46208	76.413495	839	Nadugani	Nilagiri
9	9.7479333	76.9709	986	Idukki WLS	Idukki
10	9.83825	76.92895	830	Kulamavu	Idukki
11	9.79245	76.8809	788	Kulamavu	Idukki
12	11.735766	75.9297	990	Vellamunda	Wayanad
13	11.71355	75.919216	991	Vellamunda	Wayanad
14	11.721683	75.935333	751	Pulinjal	Wayanad
15	11.9098	75.989583	793	Thirunelli	Wayanad
16	11.911316	75.98155	787	Thirunelli	Wayanad
17	11.827433	76.03125	774	Onedayangadi	Wayanad
18	11.83295	75.853733	727	Periya	Wayanad
19	11.835716	75.834616	772	Periya	Wayanad
20	11.838516	75.8295	727	Periya	Wayanad
21	11.8385	75.880516	772	Peria Peak	Wayanad
22	11.839583	75.891266	741	Varayal	Wayanad
23	11.84	75.902283	721	Varayal	Wayanad
24	11.838866	75.912816	781	Boys town	Wayanad
25	11.842583	75.868833	781	Peria Peak	Wayanad
26	11.802316	75.865283	754	Aalattil	Wayanad
27	11.76565	75.8666	721	Kunhome	Wayanad
28	11.7638	75.855216	727	Kunhome	Wayanad
29	11.753316	75.855716	720	Kunhome	Wayanad
30	11.7401	75.8398	720	Niravilpuzha	Wayanad
31	11.5139	76.019233	739	Lakkidi	Wayanad
32	11.526066	76.023116	739	Lakkidi	Wayanad
33	11.541	76.037516	735	Vythiri	Wayanad
34	11.83995	75.849278	735	Periya	Wayanad
35	11.839071	75.8535	735	Periya	Wayanad
36	12.154783	75.569166	489	Paithalmala	Kannur
37	12.35375	75.6586	925	Karugunda	Coorg
38	12.3545	75.652566	925	Karugunda	Coorg
39	12.3554	75.649316	925	Karugunda	Coorg
40	12.370266	75.621983	957	Cherambane	Coorg
41	12.376433	75.569266	901	Chettimani	Coorg
42	11.595216	76.01625	727	Achooranam	Wayanad
43	11.619966	76.001316	770	Edathara	Wayanad
44	11.62865	75.989016	817	Thariode	Wayanad
45	11.718533	75.92335	918	Mangalas-sery mala	Wayanad
46	11.69515	75.9385	863	Valaram-kunnu	Wayanad
47	11.712516	75.94255	772	Pulinjal	Wayanad
48	11.63262	76.01695	756	Thariode	Wayanad
49	11.54169	76.22731	898	Vaduvanchal	Wayanad
50	11.53397	76.24425	869	Vaduvanchal	Wayanad
51	11.49433	76.33644	992	Pandallur	Nilagiri
52	11.84624	76.02874	776	Thrissileri	Wayanad
53	7.294328	80.85475	880	Hunnasgiria	Srilanka
54	11.6621	75.94205	764	Kuttiyam-vayal	Wayanad
55	11.676533	75.935517	914	Meenmutty	Wayanad

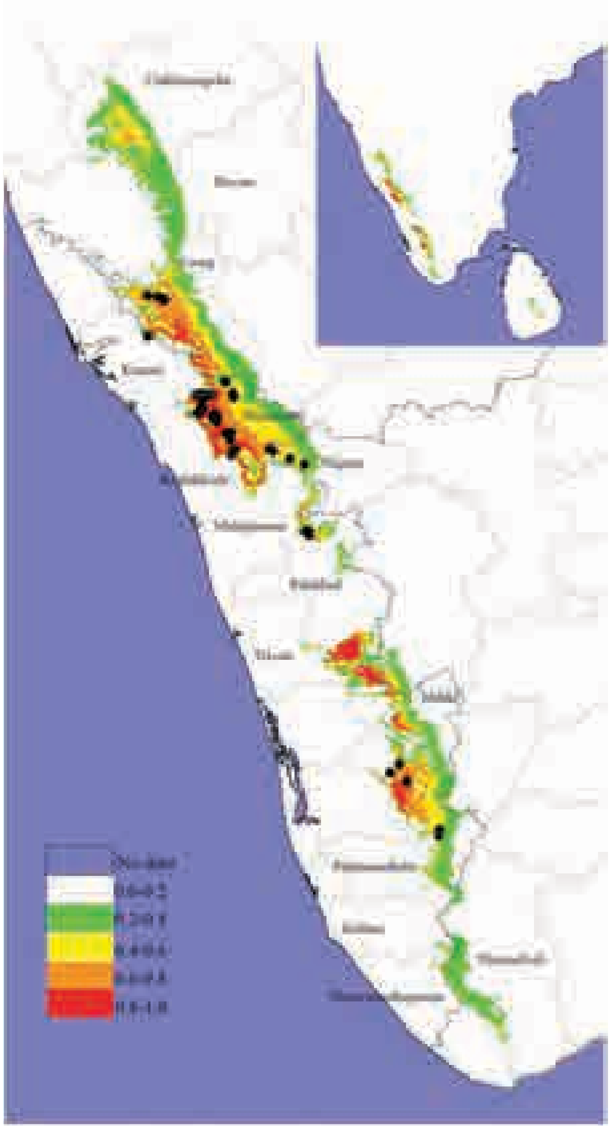

Figure 3. Habitat suitability model of *Vanda thwaitesii* in peninsular India.

Table 4. Contributions of variables in the model.

Bioclim as variables	Percent contribution	Permutation importance
Annual mean temperature (Bio 1)	30.6	34.8
Annual precipitation (Bio 12)	18.6	5.1
Precipitation Mar–May (PMM)	48.4	52.7
Mean temperature of driest quarter (Bio 9)	2.4	7.4

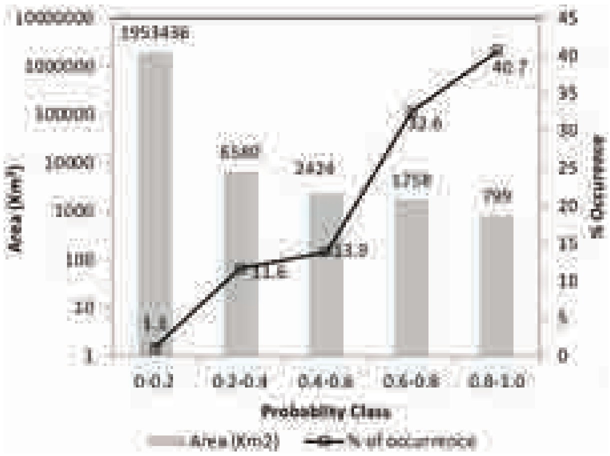

Figure 4. Area of prediction and percentage of occurrence in the habitat model.

received 293.5±28.4 mm rain fall (233–461 mm) during the summer months (March–May). Both annual mean temperature and summer precipitation are contributing significantly to the model as revealed in the model output (Table 4) and jackknife test (Figure 2).

DISCUSSION

Conservation assessment essentially requires sufficient field surveys and gathering of primary data on the distribution of species and their population attributes. This is an exhaustive process requiring substantial effort and investment of human and financial resources which has not been taken seriously in India and therefore the availability of primary data is limited. Conservation planning, action monitoring and evaluation of a species initially require prioritization through threat assessment (Mace & Lande 1991; Master 1991; Moran & Kanemoto 2017). *Vanda thwaitesii* is an epiphytic orchid endemic to Indian peninsula and Sri Lanka hitherto unknown for over 100 years until 1998 when a few populations were discovered from Kerala (Sathishkumar & Sureshkumar 1998). Based on the information on herbarium data, the Government of India notified the species as endangered and invited studies on all aspects of the notified species for propagation and conservation. Based on the extensive field surveys, we found significant populations of the species outside protected forests and the present habitats are highly fragmented. Thus conservation through rehabilitation or translocation into safer localities appeared very essential, preventing further loss before their economic value is deciphered. In spite of its unique aromatic and exquisite flowers it is underutilized in breeding as it is lesser known to orchid breeders. The present study delivers a habitat suitability model for conservation of the species prepared as part of a sponsored project supported by DBT, Government of India during 2010–16.

Table 5. Temperature and precipitation data at occurrence points of *V. thwaitesii*. The data were retrieved from world climatic data using point sampling tool in QGis.

Temperature (°C)			Precipitation (mm)		
Variable	Mean±SD	Min–Max in occurrence points	Variable	Mean±SD	Min–Max in occurrence points
Annual mean temperature	22.27±0.68	20.60–24.3	Annual	3398.98 ±608.4	2020–4794
Mean wettest quarter	21.3±0.6	20.20–23.1	Wettest Period	1153.65±325.4	376–1713
Mean driest quarter	22.5±0.86	20.85–22.4	Wettest quarter	2429.37±650.9	873–3680
Mean coldest quarter	21.2±0.69	19.75–23.2	Humid months	3307.67±687.1	1197–4744
Mean humid months	22.27±0.67	20.75–23.2	Summer (Mar–May)	292.67±30.1	218–461

Niche modelling is an economical and effective tool to prepare guide maps for intended plant survey and delineation of conservation areas for selected species (Adhikari et al. 2012) thus improving the availability of primary data on the distribution of species and their population attributes for improved threat assessment and more accurate categorization of endangered species (Adhikari et al. 2018). Georeferenced occurrence points and environmental data pertaining to the distribution area are the two prerequisites for habitat modeling. The 55 occurrence points used are more than sufficient to undertake such study. Environmental variable as 19 bioclimatic variables and digital elevation are observed as biologically meaningful to define ecophysiological tolerances of a species (Graham & Hijimans 2006; Murienne et al. 2009) and generally utilized for modeling studies. Topographic variables are derived from digital elevation model from satellite data and are also known to influence the distribution of plant species (Wang et al. 2014). However, those variables and normalized digital vegetation index, another remote sensing data variable are least influencing the distribution of *V. thwaitesii* as they did not extract as a significant factor in PCA. Nevertheless, the bioclimatic variables and seasonal climatic variables are derived from temperature and precipitation data and thus often expresses multi co-linearity and thus often difficult to select the decisive environmental variables and their contributions. Principal component analysis and regression analysis executed on them reduced the number of factors into three still explaining 96.5% variance. Environmental variables showing VIF less than 10 are also included as the existing co-linearity is less significant (Naimi et al. 2014). In this study, we could extract three components with six, four, and one variable and excluding variables with VIF greater than 10, retained four variables (Table 2) to determine habitat suitability for *V. thwaitesii* in peninsular India.

The distribution map thus obtained showed high resolution with AUC 0.997 and therefore is having high prediction efficiency. The AUC ranges from 0.5–1.0 for models that are no better than random to perfect predictive ability. It is also clear that 73.3% of the occurrence records falls in the high suitability region (0.6–1.0) and only 1% in the least suitable region (0–0.2). Besides, a few occurrence records in Chikkamagallur, Hassan and Nelliyampathy later gathered from online resources and not used for modeling fall in the suitable area predicted. Thus, the presence of *V. thwaitesii* could be confirmed in most of the prediction area proving the robustness of the model.

Elevation and temperature are often the most determining variable in habitat modeling as revealed in the terrestrial orchids as *Dactylorhiza hatagirea* (Wani et al. 2021) and *Oeceoclades maculata* (Kolanowska 2013). Precipitation is also an important variable that influences habitat modeling in some species, such as *Habenaria suaveolens* (Jalal & Singh 2017) and *Zanthoxylum armatum* (Xu et al. 2019). However, it seems that the determining factor in habitat modeling is species-specific and not exclusively confined to any of the variables. Work undertaken in epiphytic orchids as *Vanda wightii* from Western Ghats, India (Decruse 2014) and *Vanda bicolor* from northeastern region of India (Deb et al. 2017) indicate that precipitation warmest quarter (Bio_18) is the most influential factor in the model. While *Polystachya concreta,* a pantropic epiphytic orchid (Kolanowska et al. 2020), is reported to prefer different temperature and precipitation factors as far as Asian, African, and American regions are concerned. As reported, temperature seasonality (Bio_4), isotheramality (Bio_3), and precipitation seasonality (Bio_15) have significant contribution in Asian region while temperature factors alone (Bio_2, 4, 1) in American region and precipitation factors alone (Bio_12, 18, 14) in African region. Therefore, a single factor can't be considered important for global distribution of a particular species. The model output for peninsular India and Sri Lanka in the present study revealed that temperature and precipitation contribute significantly to the distribution of *V. thwaitesii* providing a robust model with high prediction efficiency. Therefore, the predicted areas in protected forests are highly suitable for conservation of *V. thwaitesii*. The generated model is also a guide map to find new populations from locations other than those reported earlier.

CONCLUSION

Vanda thwaitesii can sustain in the regions receiving 3,400 mm average annual precipitation and 290 mm in summer. In addition, they prefer cool climate with an average annual mean temperature 22.27°C. The most ideal climatic conditions (0.6–1.0 class) prevail mostly in Wayanad, Idukki, and Palakkad districts of Kerala in addition to Coorg District of Karnataka. However, most of the modeled area in Western Ghats is outside protected forests. Still, there are sufficient locations in reserve forests in Kerala and Karnataka in addition to the sanctuaries as Silent Valley National Park, Idukki Wildlife Sanctuary, Periyar Tiger Reserve, and Brahmagiri Wildlife Sanctuary for reinforcement of diversity from vulnerable locations as inhabited land, plantations, and wayside trees.

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COMMUNICATION

Occurrence of opportunistic invasive macroalgal genus *Caulerpa* and *Halimeda opuntia* in coral reefs of Gulf of Mannar

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Abstract: Investigations on indigenous and non-indigenous invasive flora and fauna of a certain geographical area are always important to assess their impact on native biodiversity and control their spread by making conservation strategies to protect biodiversity. In this study, we have encountered overwhelming growth of six seaweed species, *Caulerpa racemosa*, *C. serrulata*, *C. sertularioides*, *C. taxifolia*, *C. peltata,* and *Halimeda opuntia* on the reef flat. These algal covers on reef area appear to compete with coral polyps and restrict their settlement and resilience. Our concern on invasive species distribution in Gulf of Mannar revealed these overgrowing invasive species, which can become potential threats to coral reefs, benthic diversity, and reef assemblage. These observations will help us to develop multidisciplinary management approaches related to the expansion, mitigation, and control of these opportunistic invasive species. Further seasonal monitoring on these algal species is under process to investigate their spatial shift patterns on various coral forms.

Keywords: algal blooms, algal cover, coral-algal interactions, reef conservation, reel flat, reef resilience, reef restoration, reef threats, seaweed, seaweed utilization.

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Author contributions: RC conceived the study, conducted the fieldwork, and prepared the manuscript. KS helped in feild work, wrote and edited the manuscript. TS designed the study, assisted in the field study, corrected the draft manuscript, and supervised the project. MVRM coordinated the study and gave technical advice.

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INTRODUCTION

Invasive species belonging to flora and fauna can lead to adverse effects such as altering the biodiversity and community structure (Antolić et al. 2008), and can even invade or cause mortality to flora (de Villèle & Verlaque 1995) and fauna (Žuljevic et al. 2011). A study on the impact of invasive *Caulerpa taxifolia* on seagrass beds of *Posidonia oceanica* has revealed degradation of seagrass beds in the west of Menton, France (de Villèle & Verlaque 1995). Various authors have also reported the distribution of indigenous invasive species, including *Ulva fasciata*, *Cladophora sericea*, and *Dictyosphaeria cavernosa* in the Hawaiian Islands (Smith et al. 2002), and non-indigenous invasive algae, including *Caulerpa verticillata* in the Gulf of California (Perez-Estrada et al. 2013), *Lophocladia lallemandii* (Kersting et al. 2014), *Caulerpa racemosa* var. *cylindrica* and *C. taxifolia* in Mediterranean region (Žuljevic et al. 2011), and *C. racemosa* in the Gulf of Arzew, Algerian coast (Bouiadjra et al. 2010). Currently, the genus *Caulerpa* has been indicated as an invasive species by the International Union for Conservation of Nature (IUCN) Centre for Mediterranean Cooperation (Otero et al. 2013).

The invasive *Caulerpa* species spread rapidly all along the Mediterranean countries (Klein & Verlaque 2008). Since then, the issue of invasive algae in other parts of the world was also undertaken in different countries, as mentioned above. Recently, studies on invasive flora from the Gulf of Mannar region are gaining more importance due to their reef damaging nature. A few studies showed the invasion of *Kappaphycus alvarezii* on coral reefs in Krusadai and Mulli Islands (Edward et al. 2015), *Caulerpa* sp. and *Halimeda* sp. in the Gulf of Mannar and Palk Bay coral reefs (Manikandan & Ravindran 2016), and *Turbinaria* in the Gulf of Mannar corals (Ramesh et al. 2019). However, there is very little information on the current distribution patterns of invasive seaweeds around the Gulf of Mannar Islands. Therefore, during coral reef monitoring, we investigated invasive species distribution in the Gulf of Mannar group of Islands.

MATERIAL AND METHODS

Reef monitoring surveys were carried out during August to November 2018, from Manoli Putti and Hare Islands under the Gulf of Mannar region, southeastern coast of Tamil Nadu, India. Several underwater dives on fringing reefs of these two islands revealed moderate bloom forming and overwhelming growth of four green seaweeds *Caulerpa serrulata*, *C. racemosa*, *C. sertularioides*, and *C. peltata* on live and dead corals. Underwater photographs of these bloom-forming species were documented using Nikon Coolpix underwater camera. Species identification was made using standard identification keys and AlgaeBase (Guiry & Guiry 2018).

RESULTS AND DISCUSSION

Caulerpa racemosa, *C. serrulata*, *C. sertularioides*, *C. taxifolia*, *C. peltata*, and *Halimeda opuntia* are usually seen in smaller size on corals and rubbles. Their usual occurrence on reefs or rubbles used to be within the size range <5–>30 cm. However, their bloom forming nature identified by recording their overwhelming spread on corals ranged >30 cm–>1 m. Blooms of these species overwhelming on the coral reefs of Mandapam group of Islands were recorded during our study (Image 1a–h; Image 2a–f). The favourable physicochemical conditions that promote their spread in the reef area are poorly understood. These algal species have displayed a distinct distribution in the reef environment, where *C. sertularioides* and *C. racemosa* grew on rubbles, rocky substratum, and *Porites* sp.; *C. serrulata* on dead *Acropora* corals and live *Porites* species; and *C. peltata* on live *Acropora* corals. *C. taxifolia* has formed small patches on rubbles and near reef slopes, and is also found to grow mostly on *Montipora digitata* and *Porites solida rather* than on *Acropora*. Interestingly, *C. racemosa* was observed to grow on multiple species of corals such as *Porites*, *Acropora*, and *Montipora*. *C. racemosa*, *H. opuntia*, and *C. taxifolia* grew together with no inhibition activity against each other. *H. opuntia* was found to cover a few massive *Porites* coral colonies in Manoliputti Island. While its spread in seagrass beds was also observed (Image 2a–f), its actual impact on seagrass beds is yet to be studied.

We did not observe any grazers that preferred to feed on these *Caulerpa* species. Although *C. taxifolia* was found in the reef flats but were not seen to occur profusely. These algal blooms appear to be unsuitable for the development of corals due to their proliferation, smothering the corals. Also, overwhelming algal growth inhibits light penetration required for zooxanthellae to generate energy for polyps. These reasons appear to inhibit the growth of coral polyps and restrict juvenile corals' settlement on the dead reefs or rubble in the Gulf of Mannar reefs.

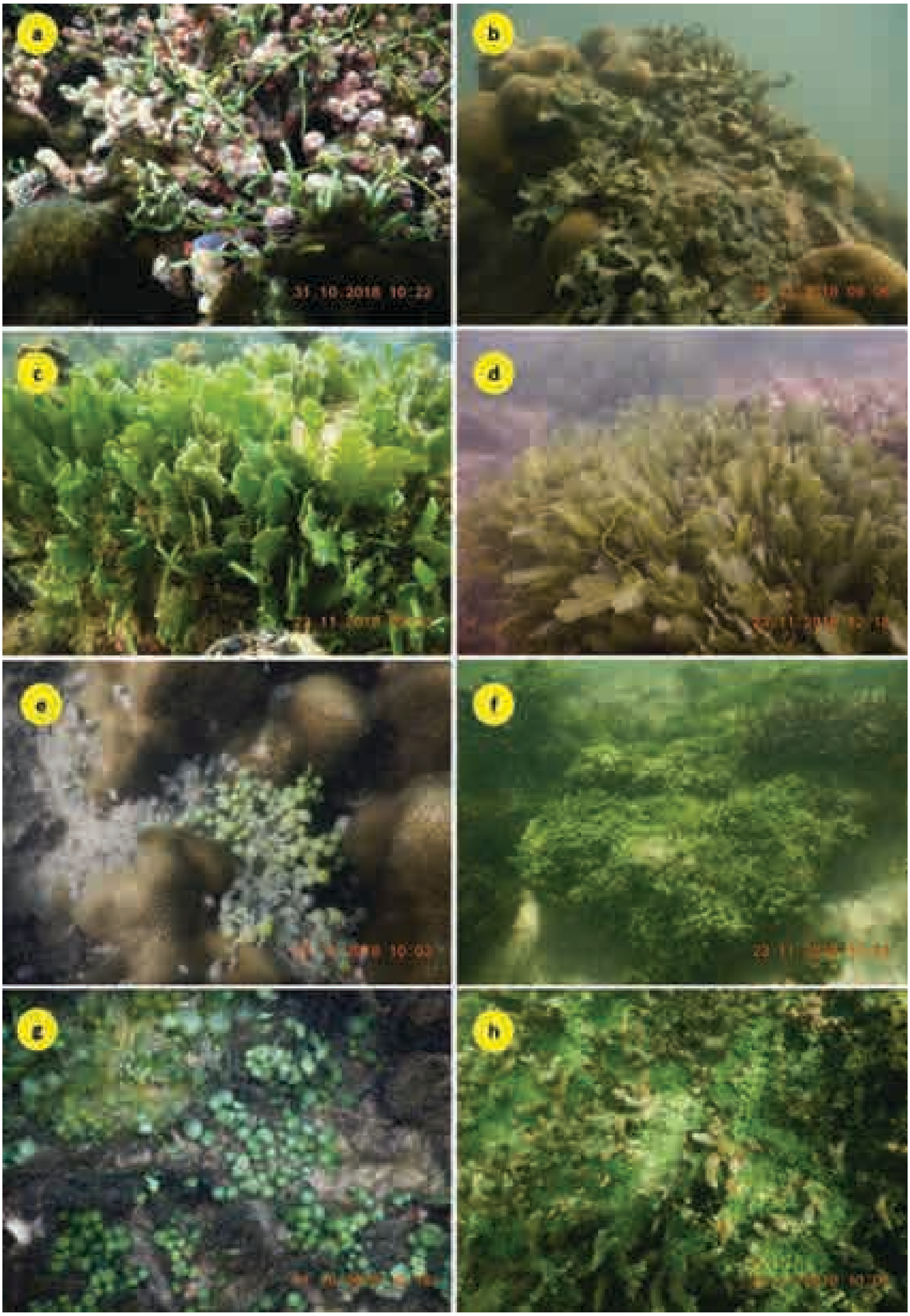

Image 1. a—Invasive and bloom-forming *Caulerpa serrulata* on dead *Acropora* reef | b—its overwhelming growth on *Porites* | c—*Caulerpa sertularioides* usual occurrence | d—canopy formation on the upper surface of *Porites* sp., dead corals and coral rubbles | e—*C. racemosa* in normal condition | f—its algal mat formation on *Porites* sp., dead corals and rubbles | g—*C. peltata* on dead *Acropora* coral | h—its algal mat formation on *Acropora* coral. © Ramesh Ch.

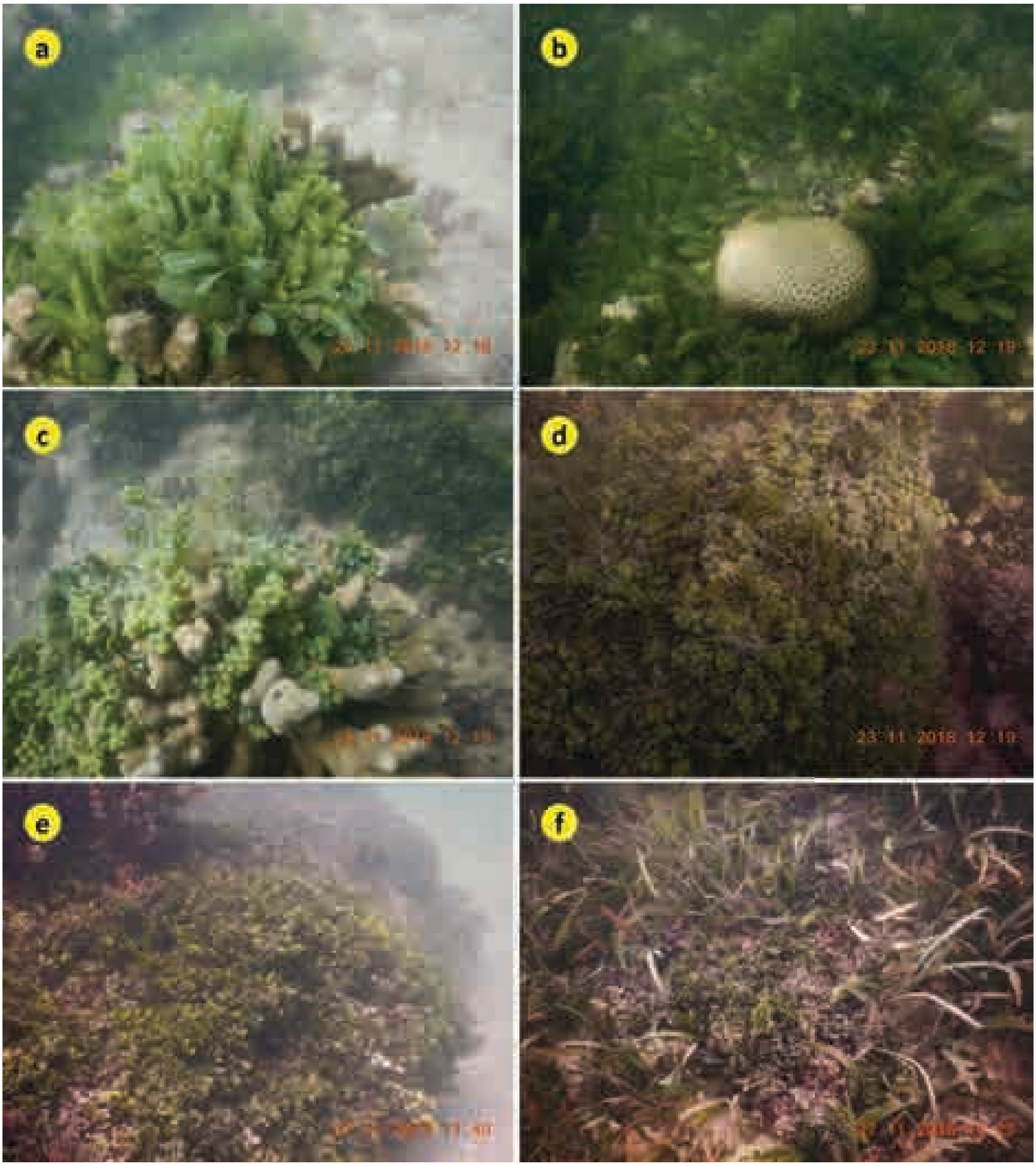

Image 2. a—*Caulerpa taxifolia* invasion on *M. digitata* | b—around a newly recruited *P. solida* | c—*C. racemosa* invading on *M. digitata* | d—its interaction with *Halimeda opuntia* and *C. sertularioides* (top left) | e—profuse growth of *H. opuntia* on *P. luteus* | f—its propagation in seagrass bed. © Ramesh Ch.

Previous reports indicated that *C. racemosa* and *C. taxifolia* could inhabit the entire reef area, which would ultimately undermine the resilience of corals (Hoey et al. 2011). The impact of these *Caulerpa* species on Gulf of Mannar's reef environment is yet to be studied in detail. However, the present study observations are challenging the issues related to the algal destruction and conservation of corals and coral reef biodiversity from invasive algae.

A recent study revealed that a feeding deterrent activity assay on green algae *C. peltata*, *C. sertularioides*, and *C. taxifolia* species display highest deterrent activity of >80% (Rajan & Padmakumar 2017). While *C. cupressoides* and *C. fergusonii* showed moderate deterrent activity, *C. scalpelliformis* and *C. microphysa* exhibited negligible deterrent activity (Rajan & Padmakumar 2017). Experimental studies also show that grazing on *C. taxifolia* resulted in the loss of spines and less gonosomatic ratios in sea urchin *Paracentrotus lividus* (Boudouresque et al. 1996). Later it was observed that high production of toxic and repellent chemical metabolite 'caulerpenyne' in summer causes sea urchins to avoid *C. taxifolia* (Lemee et al. 1996). The deterrent activity exhibited by *C. taxifolia* might be the reason that restricts grazers not to feed on it.

Whereas the same compound produced by *C. racemosa* had not displayed any deterrent activity towards herbivorous surgeon fish *Zebrasoma flavescens* (Wylie & Paul 1988), and it was highly preferred by both juvenile and adult rabbitfish *Siganus argenteus* (Paul et al. 1990). In this study, we have not found any grazers feeding on these *Caulerpa* species during our underwater observations. A recent study also indicated that *C. racemosa* distribution could drastically change over time by spreading from deep water to the shallow waters, as observed in the Columbretes Islands, Mediterranean Sea (Kersting et al. 2014). A study also suggested that overgrowth of *C. racemosa* via chemotropism results in smothering or even death of sponge *Sarcotragus spinosulus* (Žuljevic et al. 2011).

Caulerpa species are well known as edible seaweeds in eastern Asian countries such as China, Japan, Korea, Philippines; southeastern Asian countries like Indonesia, Vietnam; and Indo-Pacific regions (Kaliaperumal & Chennubhotla 2017). In India, *C. lentillifera* is cultured on the Gulf of Mannar coast (Mary et al. 2009). But utilization of these six species in India has not been implemented so far. Hence, they remain an untapped and unutilized food resource from the Indian Coast. Thus, *Caulerpa* species can be considered a potential natural edible seafood to the increasing population in India.

Crustose coralline algae are well known as they support the settlement of coral polyps (Tebben et al. 2015). Although we have found very few coralline algae in our surveys at Hare Island and Manoli Island, the coral recruitment was appreciable on the dead corals free from these algae. However, there is high competition for substrata between algae and corals, similar to previous reports (McCook et al. 2001). Therefore, monitoring the bloom-forming invasive seaweeds in terms of their reproductive strategies and favourable conditions that promote their propagation is essential for developing remedies. Reports evidenced that ease dispersion and spread of *Caulerpa* species is mostly due to fishing nets, anchors, boats, and aquaria (Otero et al. 2013). Studies from the Mediterranean Sea have shown the rapid spread of invasive *C. taxifolia* by fragment mechanism (Ceccherelli & Cinelli 1999; Smith & Walters 1999). Therefore, further investigations are important to understand the temporal and spatial expansion of *C. racemosa*, *C. serrulata*, *C. sertularioides*, *C. taxifolia*, and *C. peltata* in these Islands. Also, multidisciplinary approaches are essential to address issues related to invasive species distribution dynamics, impacts, management, and utilization in the Gulf of Mannar. Also, the impact of environmental factors such as light, temperature, and water quality in different seasons should be analysed in future studies to understand bloom dynamics.

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SHORT COMMUNICATION

Diversity of bees in two crops in an agroforestry ecosystem in Kangsabati South Forest Division, Purulia, West Bengal, India

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Abstract: The investigation study assesses the diversity of bees in Brinjal *Solanum melongena* L. and Ridge Gourd *Luffa acutangula* L. crop field from agroforestry ecosystem in South Kangsabati Forest Division, India. The study was carried out in May 2021 to May 2022 that based on transect, focal observation and pan trap samplings. A total of 1,085 individuals were identified during the field work, belonging to three family seven genera (*Apis*, *Tetragonula*, *Xylocopa*, *Ceratina*, *Amegelia*, *Nomia*, and *Megachile*) and seventeen species, the non *Apis* bees (63.78%) were most abundant than *Apis* bees (36.22%). In brinjal, Shannon diversity index of bees is 2.12 and Shannon evenness index is 0.35, whereas, Shannon diversity index in ridge gourd was 1.94 and Shannon evenness index is 0.3. The observations signify greater diversity and population of wild bees. The natural habitat close to agricultural land helps to sustain the diversity and population of wild bees, which enhance the crop quality and yield.

Keywords: Agro forestry, *Apis* bees, eggplant, non *Apis* bees, pollinator, Ridge Gourd, *Tetragonula*, *Xylocopa.*

Now-a-days, agroforestry is an important ecosystem especially in a tropical country and it is an intensive land management system. It consists of agriculture systems and have potential biodiversity conservation sites. The agroforestry ecosystem provide rural livelihood alongside biodiversity conservation in a sustainable land use system. This system is a transitional process from conventional agricultural practices to agro-ecological agricultural practices (Souza et al. 2014). Combination of crops and diverse plants species in forest provide a rich insect diversity due to increased niche diversity than any agro-ecosystems (Stamps & Linit 1998). Heterogeneous agroforestry ecosystem provides floral resources for pollinators (Sinu & Shivanna 2007). Habitat loss and intensification of agricultural practices threaten wild as well as domestic pollinators. Agroforestry ecosystem provides them suitable nesting sites and floral resources, enhancing their pollination services to crops at a landscape level (Sutter 2017; Kay et al. 2019). Bees are the primary pollinators and roughly cover 90% of world plant population (Winfree 2010).

In agriculture land bees are the essential pollinators for pollination as well as crop production. Non *Apis* species are effective pollinators than honey bees (Javorek 2002; Kreman et al. 2002), but they both can together enhance crop production (Greenleaf & Kreman 2006). Brinjal *Solanum melongena* L. and Ridge Gourd *Luffa acutangula* L. are important and widely cultivated crops across the studied area and also bee-attracting vegetable crops. Buzz pollinators are effective pollinators for solanaceous (Brinjal) and cucurbitaceous (ridge gourd) crops. Brinjal flowers have abundance of pollen but to expel the pollen requires vibration by insects called ‘buzz pollination’. Wild bees are efficient in buzz pollination than honey bees (Buchmann 1983; Herren & Ochieng 2008). Natural forest is a suitable habitat for wild bees but due to extensive deforestation they are

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in threat. This investigation was carried out to generate information about the diversity of bees in pollination dependent crops in an agroforestry ecosystem.

Study Area

The study was conducted in an agroforestry ecosystem in western part of West Bengal, India. The studied area is in Kangsabati South Forest Division in Purulia. The forest division is situated between 23.166–22.833 N & 86.666–87.000 E, covering 310.27 km^2 areas, which are continuations of the Chotanagpur Plateau (Figure 1). Mixed cropping system is practiced dominantly in the studied area. Fourteen plots were selected randomly throughout the South Kangsabati Forest Division on the basis of easy accessibility and densely blooming flowering plots. These fourteen plots were equally divided into seven plots for each crop. The experimental study was conducted in various farm lands from May 2021 to May 2022 in the eggplant and ridge gourd crops fields.

Methods

All bee surveys were conducting from 0830 h to 1630 h, split in three time hours: 0830–0930 h, 1130–1230 h, & 1530–1630 h. Bees are active in warm, sunny days so rainy and cloudy days were avoided for the unbiased data. Three methods—transect, focal observation (15 mins), and pan traps (yellow, white, blue colored pan traps)—were followed throughout one year of survey. The transect length was 100 m with 2-m breadth on each side (Sutherland 1996). In focal observation (Gibson et al. 2011), a 1 m^2 flowering plot was selected randomly and bees were observed for 15 mins. Pan traps of three different color sets were used for passive sampling (Westphal et al. 2008). Yellow, white, blue pan traps were used which were painted with UV-bright colors. Five clusters of pan traps were installed where each cluster was separated from another by a distance of 15 m. Each cluster contained three sets of pan traps filled with 400 ml soapy water. The species not identified in the field were collected through sweep net, killed by ethyl acetate, and preserved in 70% ethanol for future

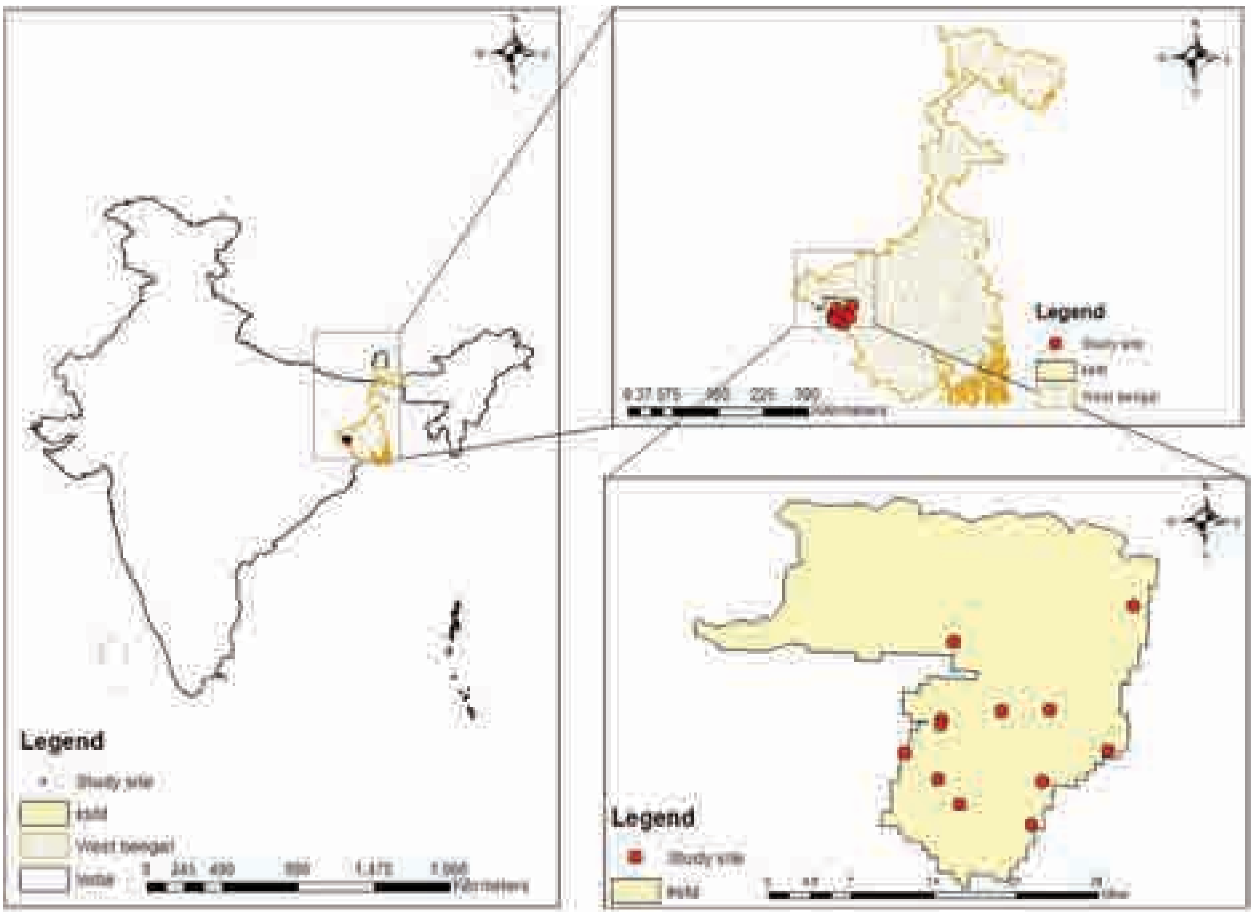

Figure 1. The study site Kangsabati South Forest Division (KSFD), Purulia, West Bengal, India.

reference. We followed Bingham (1897) and Michener (1974, 1994, 2007) for bee identification.

We observed diversity and abundance of bees by observing bees visiting Brinjal and Ridge Gourd flowers. These data were used for analyzing bee diversity by Shannon diversity index (H') and Shannon evenness index (J'). The number of bees data (sampled via transect, focal observation & pan trap) were pooled in each crop independently for analysis of richness and abundance. The data were analyzed using Past Software 3.4.

Shannon Diversity Index ($H' = -\Sigma p_i * ln(p_i)$)

Shannon evenness index (J) = H/lnS

Relative Abundance= (N_i/N)*100

Where, S is the total number of species and p_i is the proportion of the entire community made up of species i. N_i is the abundance of species i and N is the total of all species encountered.

Results and Discussion

A total of 1,085 bee individuals were encountered belonging to three families (Apidae, Halictidae, Megachilidae), seven genera and 17 species during the survey. *Tetragonula iridipennis* was most dominant species with 262 individuals followed by *Apis florea* (182 individuals) and *Nomia elliotii* (169 individuals). Most of the bee species belonging to the family Apidae were observed during the study. During the survey time, non *Apis* bees (63.78%) were dominant in abundance than *Apis* bees (36.22%).

Among these two vegetable crops, Brinjal had most diverse and abundant number of bee visitors. Shannon diversity index of bees in eggplant crop is 2.12 and Shannon evenness index is 0.35; the most abundant bee species was *Tetragonula iridipennis* (33.97%) followed by *Megachile lanata* (14.83%), *Nomia elliotii* (14.59%), *Xylocopa fenestrate* (9.09%). Some rare bees like *Megachile hera* (0.7%), *Nomia westwoodii* (1.7%), and *Ceratina hieroglyphica* (1.2%) were also encountered. The pollinator fauna of Brinjal consist of two species from Megachilidae, three species from Halictidae, and six wild bee species from Apidae family.

In the Ridge Gourd, the Shannon diversity index was 1.94 and Shannon evenness index was 0.3, *Apis florea* was the most abundant species with 23.48%, followed by *Apis dorsata* (21.25%) and *Tetragonula iridipennis* (17.83%). *Amegilla zonata* (0.74%) and *Megachile lanata* (0.3%) were rare visitors of ridge gourd flower. *Apis* bees were the most dominant visitors of ridge gourd flower followed by *Nomia elliotii* of Halictidae family, bees from subfamily Xylocopinae (*Xylocopa* & *Ceratina* bees) were frequently observed from Apidae family. Among wild bees, *Xylocopa fenestrata* and *Nomia elliotii* were dominant throughout the survey in both the crops.

Patricio et al. (2012) recorded the role of bees as

Table 1. List and abundance of the bee species individuals encountered during the survey of Brinjal and Ridge Gourd fields.

Order	Family	Species	Brinjal	Ridge Gourd	Total
Hymenoptera	Apidae	*Apis dorsata* Fabricius, 1793	8	143	152
		Apis cerena Fabricius, 1793	10	49	59
		Apis florea Fabricius, 1787	24	158	182
		Tetragonula iridipennis Smith, 1854	142	120	262
		Xylocopa fenestrate Fabricius, 1798	38	25	63
		Xylocopa aestuans Linnaeus, 1758	7	16	23
		Xylocopa magnifica Cockerell, 1929	0	8	8
		Xylocopa sp.	3	0	3
		Ceratina smaragdula Fabricius, 1787	0	39	39
		Ceratina hieroglyphica Smith, 1854	5	0	5
		Amegilla zonata Linnaeus, 1758	16	5	11
		Amegilla calceifera Cockerell, 1911	13	0	13
	Halictidae	*Nomia elliotii* Smith, 1875	61	108	169
		Nomia crassipes Fabricius, 1798	22	0	22
		Nomia westwoodi Gribodo, 1894	7	0	7
	Megachilidae	*Megachile lanata* Fabricius, 1775	62	2	64
		Megachile hera Bingham, 1897	3	0	3

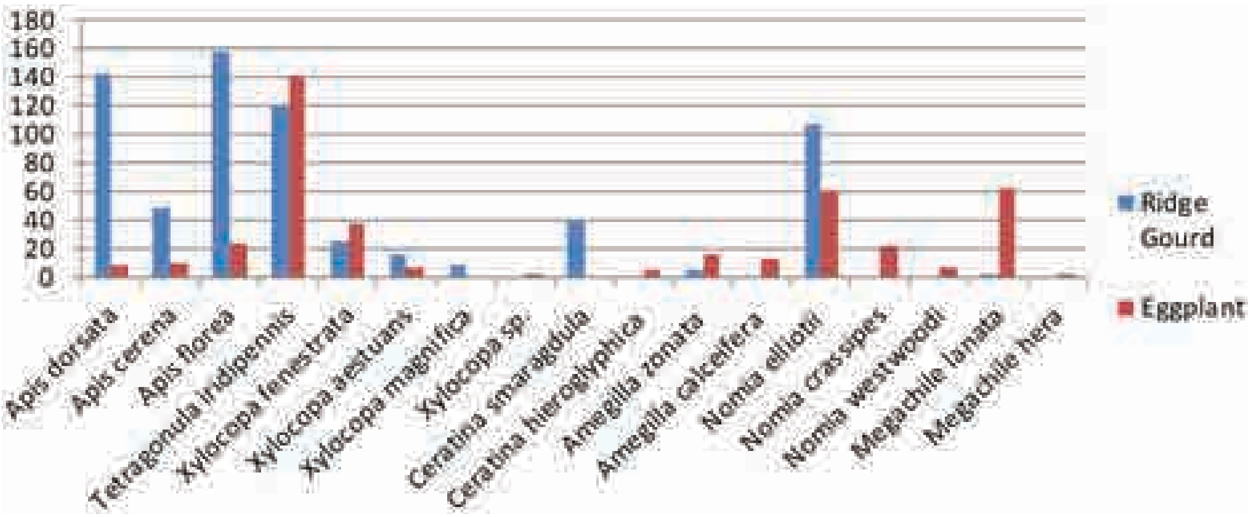

Figure 2. Relative abundance of bees in Ridge Gourd and Brinjal crop fields, where Y axis indicates individual number of bee species & X axis indicates bee species.

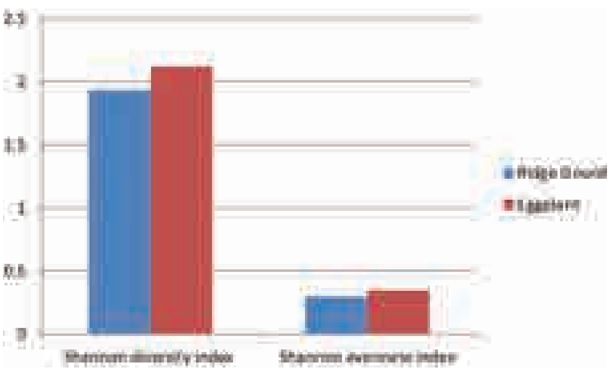

Figure 3. Shannon diversity index & Shannon evenness index of bee species in the Ridge Gourd & Brinjal crop fields.

very significant in promoting good yields in Brinjal. Our results exhibit that stingless bees *Tetragonula iridipennis* are effective pollinators for both the crops in fields and similar results observed in greenhouses by Silva et al. (2013). In an earlier study, Herren & Ochieng (2008) observed that *Xylocopa caffra* was an effective pollinator of Brinjal crop and its visitation rates significantly reduces with the distance from wild habitat. Land management is one of the factors which determines the efficiency of pollination in agriculture (Patricio et al. 2012) as flowers of wild plants was important foraging source for bees. Agro-ecosystem, close to high proportion of natural habitat is benefited by bee diversity, foraging movement, and their mutualistic behavior (Hagen & Kraemer 2010; Balachandran et al. 2017). Agroforestry not only provides niche diversity, it also reduces pest problems (Stamps & Linit 1998).

Conclusion

The experimental study proves that there is a great diversity and abundance of non *Apis* bee species along with *Apis* bee species present in Brinjal and Ridge Gourd fields, as surrounding natural habitat provide them alternative habitat and floral resources which enhance the diversity and population of wild bees.

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SHORT COMMUNICATION

An extended distribution and rediscovery of *Rhynchosia suaveolens* (L.f.) DC. (Fabaceae) for Maharashtra, India

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Abstract: *Rhynchosia suaveolens* (L.f.) DC. was reported for the first time from the Vidarbha region of Maharashtra. It is rediscovered after 144 years from Maharashtra. Previously, this species was recorded only from the southern part of India. In the present article detailed description, illustration, digital photographs, and relevant notes are provided to facilitate its easy identification.

Keywords: Phaseoleae, sacred grove, variety, Vidarbha, Yavatmal.

The genus *Rhynchosia* Lour. belongs to the subtribe Cajaninae, tribe Phaseoleae, in the family Fabaceae. The genus is the largest in the subtribe, comprising about 232 species, distributed throughout the tropics and subtropics and extending to North America from Mexico to some parts of the United States as well as Africa and Madagascar where it is most diverse (Schrire 2005). From the Indian subcontinent Baker (1879) reported 22 species of *Rhynchosia*. Presently, in India the genus is represented by 28 species, one variety and one subspecies (Sanjappa 2020) of which seven species are endemic to India and around 60%, are found in Eastern Ghats (Gamble 1928; Pullaiah & Ramamurty 2000). Recently, one new species has been described from India, *Rhynchosia ravii* K.Prasad & A.Naray (Prasad & Swamy 2014). Additionally, a replaced name for *Rhynchosia fischeri* P. Satyanar. & Thoth., i.e., *Rhynchosia ganesanii* Kottaim. & Vasud. has been provided (Kottaimuthu & Vasudevan 2015)

During the field survey in the Yavatmal District of

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Maharashtra, the authors collected one interesting specimen of *Rhynchosia*. Upon critical studies, scrutiny of authentic literature (Baker 1879; Haines 1916; Gamble 1928; Rao & Razi 1981; Matthew 1981; Vajravelu 1983; Satyanarayana 1993) and study of the herbarium material from Indian as well as foreign herbaria (BLAT, BSI, BM, CAL, BSD, DD, K, LWG, MH, NL, P, UMPU) the identity of the species was confirmed as *Rhynchosia suaveolens* (L.f.) DC. In India the species have previously been reported from Andhra Pradesh, Tamil Nadu, Karnataka, and Kerala. As per (Satyanarayana 1993) the species was previously reported from Maharashtra in the year 1854 and 1878. However, through the visits to the relevant herbaria (CAL, DD) and literature survey (Kamble & Pradhan 1988; Karthikeyan & Kumar 1993; Almeida 1998; Diwakar & Sharma 2000; Singh & Karthikeyan 2000; Bhogaonkar & Dhole 2019) our attempts to locate the specimen studied by Satyanarayana (1993) were unsuccessful. A solitary specimen from CAL with information on place of collection (Bombay) and the collector (Kurz.) belonging to this species was located. In the recent checklist of Fabaceae (Sanjappa 2020), the distribution of this species from Maharashtra is not mentioned. All these point out to doubtful distribution of this species from Maharashtra State. Therefore, our collection of *Rhynchosia suaveolens* (L.f.) DC. from Kesurli sacred grove from Yavatmal District establishes its extended distribution to Vidarbha region of Maharashtra. Moreover, this record becomes the rediscovery of the species from Maharashtra state after a gap of 144 years. A detailed description along with field images, locality map (Figure 1), and notes on taxonomy are provided herewith to facilitate its easy identification.

Material and Methods

Flowering specimens of the species were collected from Kesurli sacred grove of Yavatmal District (20.0158N & 79.0047E), Maharashtra in November 2019 (Figure 1). The specimens were then processed and mounted on herbarium sheets following the methods of Jain & Rao

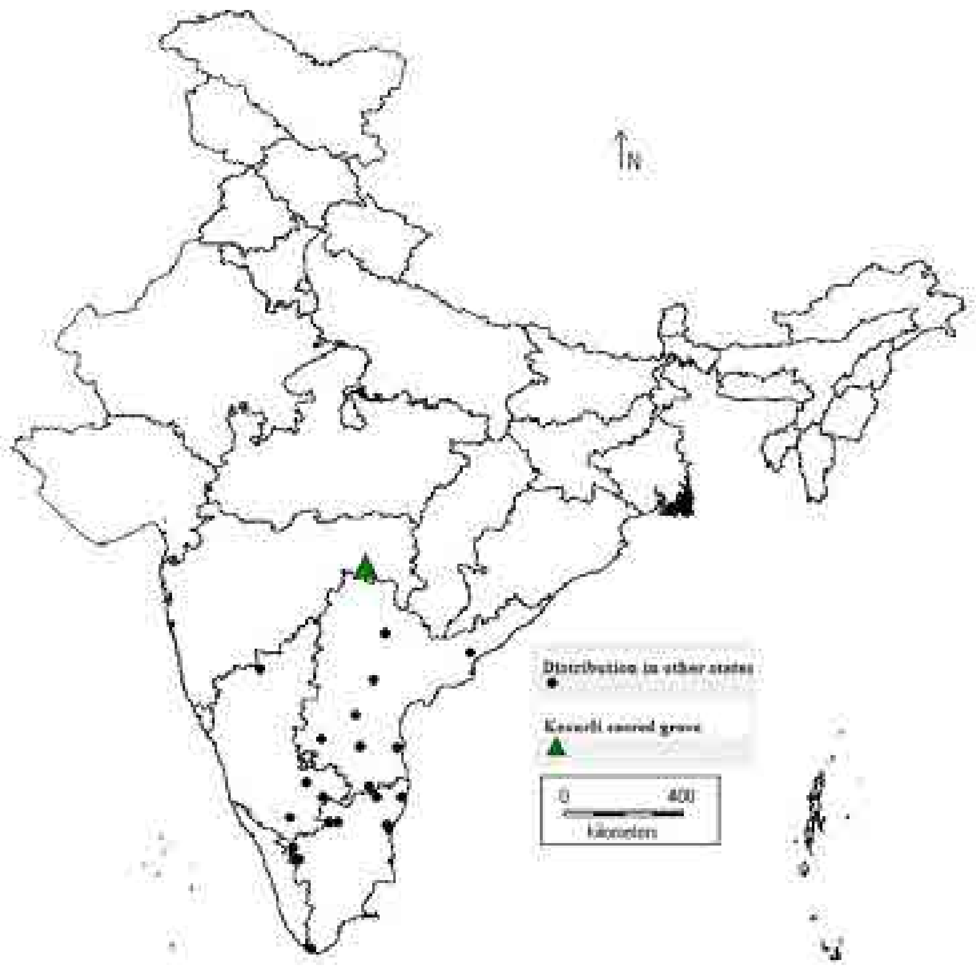

Figure 1. Distribution of *Rhynchosia suaveolens* (L.f.) DC. in India.

(1977) . The floral parts were dissected and observed under the stereo zoom microscope (Leica S8APO) for detailed macro and micro-morphology. The digital field photographs were taken by using Canon EOS80D camera. The co-ordinates of locations were recorded using a global positioning system device (Garmin Montana 680). The distribution map is prepared using recorded geo-coordinates, verified using DIVA GIS Ver. 7.5 (Hijmans 2021).

Taxonomic Treatments

Rhynchosia suaveolens **(L.f.)** DC. Prodr. 2:387. 1825 (Figure 2, Image 1,2)

≡ *Glycine suaveolens* L.f. PI. 326. 1781; Spreng. Syst. 3:196 (1826).

Type: India, Tamil Nadu, Madras, Koenig J.G. s.n. (LINN barcode 901.18); isolectotype (C barcode C10012343) (Designated by Mishra et al. 2021)

Terrestrial, shrub. Stem twining, cylindrical, green/yellowish-green, older branches woody, young one golden yellow with black spot, pubescent with eglandular and as well as glandular hairs (gland at base). Leaves pinnately trifoliolate, alternate, 4–10 cm long; stipules free lateral 3–8 × 1–1.3 mm, lanceolate finely pubescent; petioles round, 4–7 cm long, pubescent with eglandular and glandular hairs; stipels small, pyramidal; petiolules ca. 15 mm long; leaflet not dimorphic, 5–8 x 2.5–5 cm, ovate-elliptic, acuminate at apex, rounded at base, margin entire, terminal leaflet distinctly petioluled, 2/3 leaflet asymmetrical, 1/3 symmetrical. Inflorescence solitary, axillary, usually longer than petiole, pubescent; peduncles slender 2–4 cm long, finely brownish pubescent; bracts 0.8–1.3 cm long; bracteoles 3.4–4.7 × 0.7–1.7 mm; pedicels 1.2–1.5 mm long. Flower zygomorphic. Calyx 10.2 mm long, teeth 5 unequal, campanulate tube 3–4 mm long, teeth 2.45–4.25 mm long. Corolla yellow, longer than calyx, petals unequal; standard 9.37–9.40 × 7.4 mm long, orbicular with 1.5 mm long claw; wings yellow oblong-obovate, 8.5 × 2.28 mm, round at apex, claw ca. 2 mm long; keel yellow, 8.25 × 2.74 mm, boat shaped; Stamens 10, diadelphous, 9+1, unequal; bundle 7.8 × 1.1 mm; individual filament ca. 6.5 mm long; anthers yellow, oblong, ca. 1 × 0.6 mm. Ovary sessile oblong-elliptic ca. 2.1 × 0.9 mm, with white hairs; style 5–7 mm long, glabrous; stigma capitate. Pod compressed, longer than calyx, septa between seed, oblong 1.5–2 × 0.8–1 cm. Seed 2, arillate, ca. 4.14 × 3 mm.

Flowering: November–December; Fruiting: November–February (–March).

Note: *Rhynchosia suaveolens* is characterized by its axillary, solitary, rarely paired flowers and monomorphic leaflets. *R. suaveolens* shows some similarity with *R. ganesanii* and *R. hainesiana* in having a solitary flower but differs in having dimorphic leaflets from *R. ganesanii,* while raceme inflorescence and pod length with *R. hainesiana* (pod 1 cm and raceme 1 cm) *vs.* (pod 1.5–2 cm and raceme more than 1 cm) in *R. suaveolens*.

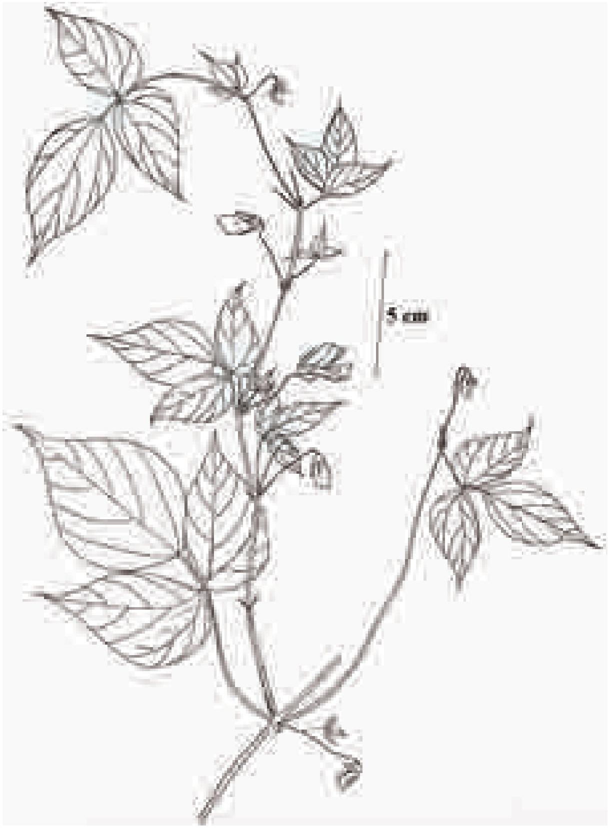

Figure 2. A flowering twig of *Rhynchosia suaveolens* (L.f.) DC.

Distribution and Habitat: This species has been reported to be distributed only in the southern part of India, i.e., Andhra Pradesh, Karnataka, Kerala, and Tamil Nadu (Figure 1) and Sri Lanka (Manjunatha et al. 2004). It grows in rocky areas, especially near boulders. We have collected this species twining on *Gymnosporia emarginata* (Willd.) Thwaites. The other associated species are *Dodonaea viscosa* Jacq.*, Biancaea decapitala* (Roth) O. Deg.*, Azadirachta indica* A. Juss.*, Butea monosperma* (Lam.) Kuntze*, Senna tora* (L.) Roxb.*, Evolvulus nummularius* (L.) L., *Ichnocarpus frutescens* (L.) W.T. Aiton, and *Ehretia microphylla* Lam.

Specimens examined: Asia, India, Andhra Pradesh; Chittoor R.F., 6.iii.1956, S.K. Wagh 5425 & 5426 (BLAT); Cuddapah district, Guvvalacheruvu, 1500 ft., Feb. 1883, J.S. Gamble 10818 (CAL); Chelama, 8.xi.1955, G.H. Madhuram (CAL); Nellore district, Sangam, 23.vii.1914,

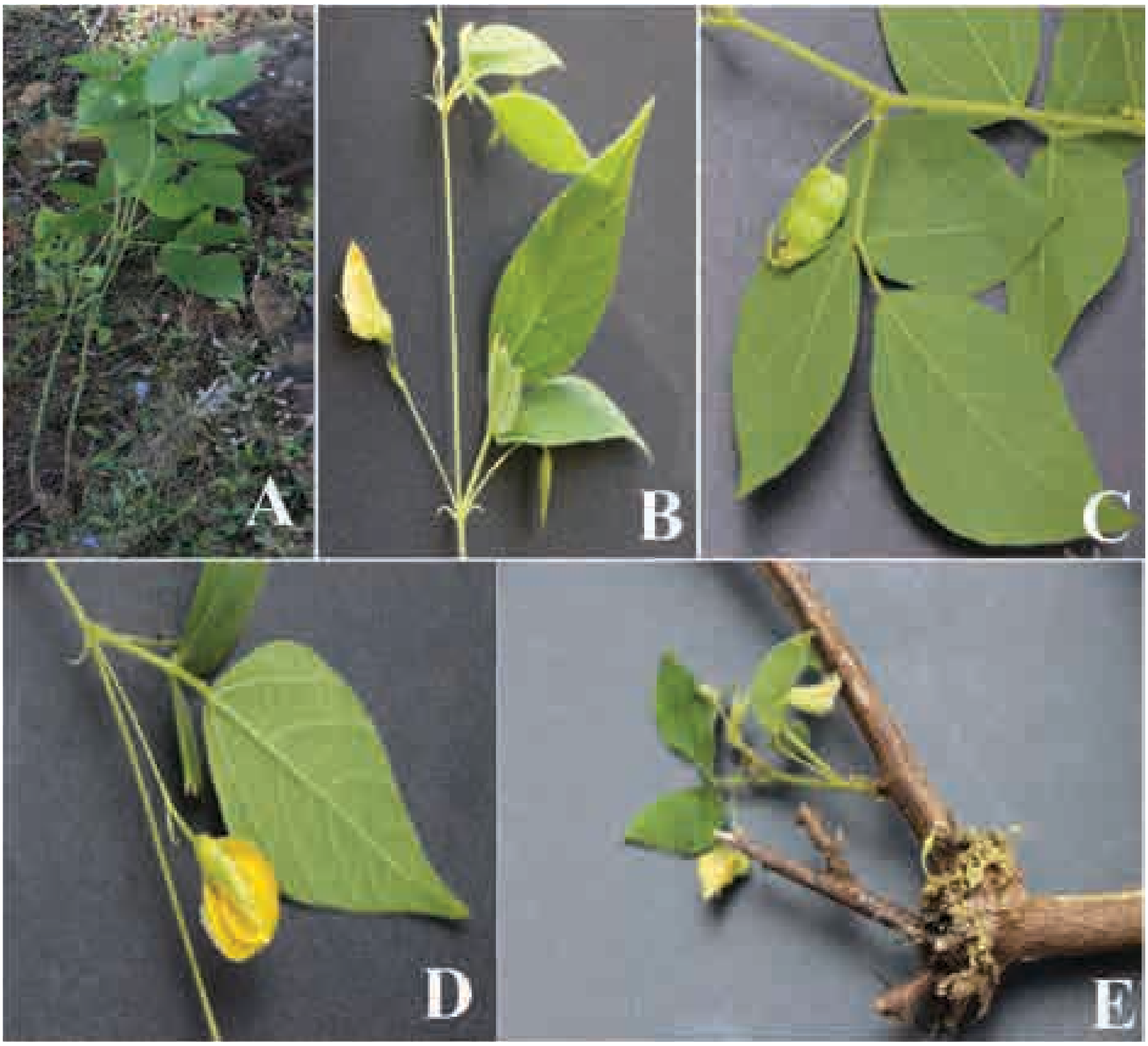

Image 1. *Rhynchosia suaveolens* (L.f.) DC: A—Habit | B—Twig with floral bud | C—Twig with a pod | D—Twig with an open flower | E—Stem. © Vijay Wagh.

M.S. Ramaswami 1152 (CAL); Nalgonda district, Nagarjunasagar, 100 m, 14.xii.1959, K.M. Sebastine 9775 (CAL, MH); Nagarjunakonda valley, 600–900 ft., 26.xi.1961, K.J. Thothathri 9760 (CAL); Karnataka: Mysore district, Dubare, 6.ix.1961, R.S. Rao 73653 (BSI); Mamadachilume Forest R.H., 30.viii.1961, R.S. Rao 73458 (BSD); Bijapur district, Badaarai, 17.x.1892, Talbot 2792 (BSI); Kerala: Tiruvakkaltheri R.F., 3.xii.1961, K.M. Subrahmanvam 77405 (BSI); Travancore district, Courtallum, M. Rama Rao 1888 (CAL); Maharashtra: Bombay, Kurz s.n. (CAL); Yavatmal district, Wani tehsil, Kesurli sacred grove, 20.0158N & 79.0047E, 212 m, 14.xi.2019, V. Gupta, A.K. Mishra & V.V. Wagh 331973 (LWG); Tamil Nadu: Coimbatore district, Aliar submergible area, 350 m, 24.xi.1962, K.M. Sebastine 15343 (CAL); Gundal valley, 3000 ft., 12.xii.1905, C.E.C. Fischer 604 (CAL).

Specimens image viewed: India, Madras, Wight 5579 (K001121339, K001121340), Madras, 1918, Wight & Gamble J.S. 5579 (K000900364), Kambakam, 1938, E. Barnes 218 (NL2044891), Coromandel, Willam Roxburgh, 26 (BM012564191), Dharmapuri district, Renkanikotta taluk, Manjakkondapalli, Sokkabetta hill, 750 m, 18.xii.1978, K.M. Matthew & N. Venugopal 20448 (CAL); Madras, 1854, Wallich 5579B (K) (K000900362, K000900363, K000900364); Dharmapuri Dist, Harur, Chitteri hills, Alangalmalai slopes, 950 m a.s.l, 12.i.1979, N. Venugopal 20826 (NL2044892), Tambram, E. Barnes s.n. (DD); Kurnooll, Srisailam project site, 16.083333 E, 78.866667 N, 28.ix.1978, L.J.G van der Maesen, 3281

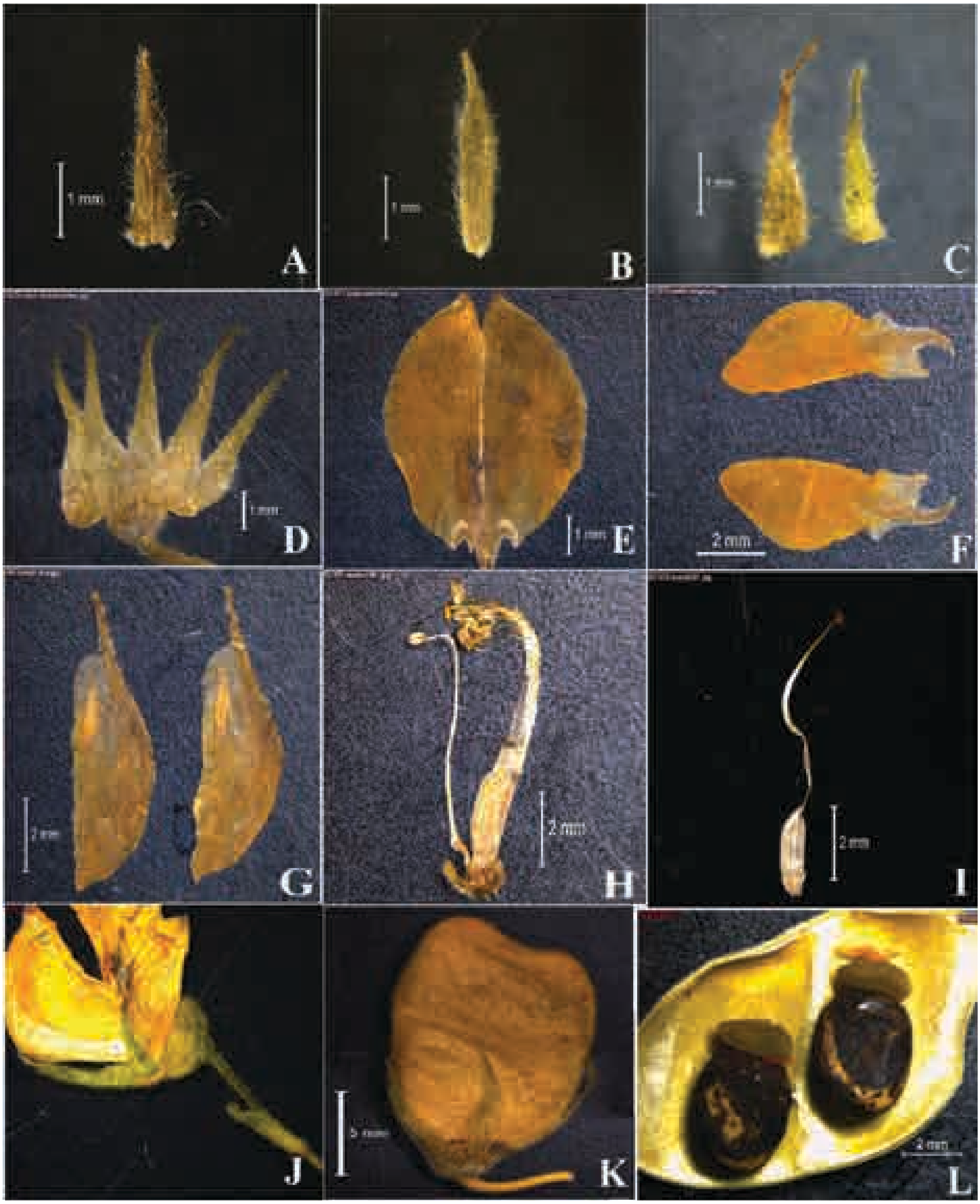

Image 2. ***Rhynchosia suaveolens*** **(L.f.) DC: A—Bract | B—Bracteole | C—Stipule | D—Calyx | E—Standard petal | F—Wing petals | G—Keel petals | H—Androecium | I—Gynoecium | J—Flower | K—Pod | L—Pods opened to show seeds. © Vijay Wagh.**

(NL) (WAG0250919), Sri Lanka: George Henry Kendrick Thwaites , 3851 (BM012564187), Burma, Upper Burma, Sagauig, 04.xi.1892, Abdul Huk 1078 (BM01256419); Koulou, eschenault, 876 (P02748310); Thousaoin Meilen, 1902, Shaik Mokim 617 (UMPU685216); Upper Burma, Nov 1890, Abdul Huk, s.n. (P02748314); Upper Burma, Kyoukse, 02.ii.1893, King's Collector G6 (NL) (L.2044893).

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NOTE

New distribution records of two uncommon microhylid frogs, *Melanobatrachus indicus* Beddome, 1878 and *Mysticellus franki* Garg & Biju, 2019 from Nelliyampathy, Kerala, India

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Melanobatrachus indicus Beddome, 1878 or the Galaxy Frog was first described from the Anamalai Hills of Tamil Nadu (Beddome 1878). This southern Western Ghats endemic belonging to the monotypic genus *Melanobatrachus* is the sole representative of the subfamily Melanobatrachinae (Frost 2023). The IUCN has categorized this species as 'Vulnerable' (IUCN SSC Amphibian Specialist Group 2022), and it has been ranked as a high-priority EDGE species by the Zoological Society of London (Isaac et al. 2012). It is a rarely observed frog and has been reported from select locations in South India by only a handful of researchers since its discovery (Table 1, Figure 1). Another microhylid species *Mysticellus franki* Garg & Biju 2019, or the Mysterious Narrow-mouthed Frog was described from the Suganthagiri region (11.5386^{0}N, 76.0538^{0}E, 852 m) of the central Western Ghats in Wayanad district, Kerala. This genus is also monotypic; interestingly, its closest relative is the southeastern Asian genus *Micryletta* (Garg & Biju 2019). Since its discovery, this little-known frog has not been reported from any other location. In the current note, we report an additional distribution record for these elusive frogs from the southern Western Ghats.

While surveying for skinks and ants around Nelliyampathy Forest Reserve on 16 October 2022, at 1050 h, we encountered two adult individuals [34 mm and 32 mm snout-vent length (SVL)] of *M. indicus* (Image 1); sex undetermined). The individuals were found inside a patch of secondary evergreen forest around a reclaimed cardamom plantation (10.4730°N, 76.6738°E according to World Geodetic System WGS84; 970 m; Image 3). Both individuals were found under rotten logs along a forest pathway, approximately 10 meters from one another. Except for Nixon & Bhupathy (2007), prior records have found this species closely associated with water bodies (Rajkumar et al. 2021) whereas our location point was 150 m away from a perennial stream. The frogs showed typical external characters such as body

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slender; dorsal surface granular; ventral smooth; black body with numerous bluish-white spots and scarlet-orange blotches near the groin, belly, and between forelegs. When handled, the frogs appeared motionless with limbs tucked close to the body, indicating classical defensive behavior while under stress (Kanagavel & Tapley 2015).

On the same day, fieldwork in the vicinity of Kesavanpara (10.5236°N, 76.6708°E according to World Geodetic System WGS84; 950 m) at 1310 h yielded a single adult individual (22 mm SVL) of *M. franki* (Image 2; sex undetermined) under a medium-sized fallen log. The frog was encountered in a disturbed patch of stunted evergreen forest alongside a trail leading to Kesavanpara viewpoint (Image 4). The individual was identified based on overall coloration and external phenotypic characters mentioned in Garg & Biju (2019). These included lack of webbing between fingers; rudimentary webbing between toes; lateral surfaces from the tip of the snout up to the groin prominently dark blackish-brown; two prominent dark blackish-brown 'false-eye' spots on either side of the groin extending just above the hind

Table 1. Previous locality records of *Melanobatrachus indicus*.

	Locality	Year	References
1	Anamalai, Tamil Nadu	1878, 2013	Beddome (1878); Kanagavel & Tapley (2013)
2	Walaghát (present day Walakkad), Kerala	1880	Beddome (1880)
3	Valparai, Tamil Nadu	1928, 2000	Roux (1928), Ishwar 2000
4	KMTR, Tamil Nadu	1997	Vasudevan (1997)
5	Periyar Tiger Reserve, Kerala	1997	Daltry & Martin (1997)
6	Matthiketan Shola, Kerala	2007, 2021	Nixon & Bhupathy (2007); Rajkumar et al. (2021)
7	Marayoor, Kerala	2013, 2017	Rajkumar et al. (2021); Palot & Sureshan (2017)
8	Chinnar Wildlife Sanctuary, Kerala	2015	Rajkumar et al. (2021)
9	Eravikulam National Park, Kerala	2015	Rajkumar et al. (2021)
10	Wayanad, Kerala	2016	Rajkumar et al. (2021)
11	Parambikulam Tiger Reserve, Kerala	2021	Newspaper article (https://www.thehindu.com/news/national/kerala/18-new-amphibian-species-found-in-parambikulam/article38111812.ece)

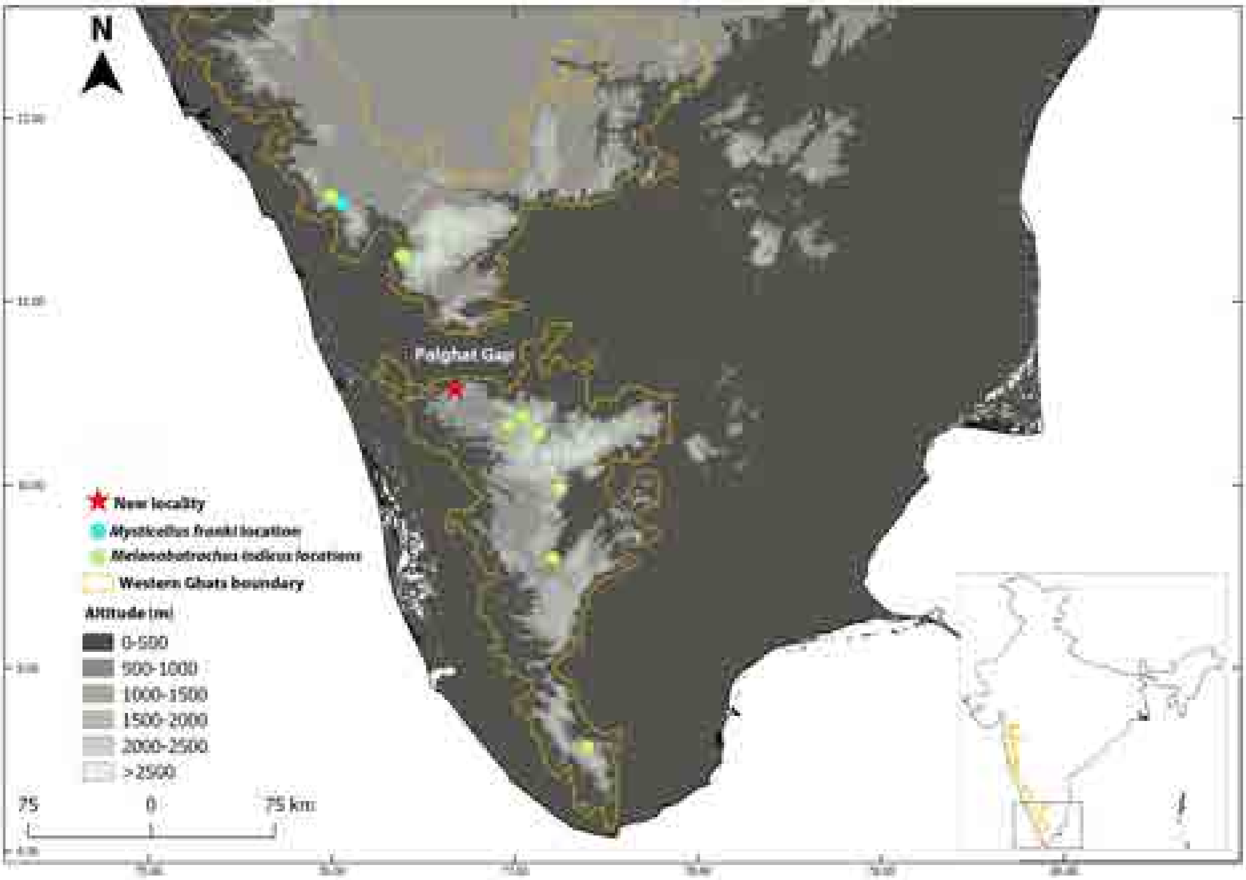

Figure 1. Map of southern peninsular India showing distribution points of *Mysticellus franki* and *Melanobatrachus indicus*.

Image 1. *Melanobatrachus indicus* from Nelliyampathy.

Image 2. *Mysticellus franki* from Nelliyampathy showing its characteristic black groin spots and the mid-dorsal stripe.

Image 3. Habitat of *Melanobatrachus indicus* from Nelliyampathy.

Image 4. Habitat of *Mysticellus franki* from Nelliyampathy.

legs; a thin mid-dorsal line extending from the tip of the snout up to the vent; ventral surfaces of throat, belly, arms, and legs dark with multiple greyish-white blotches and speckles. Other species of anurans recorded in sympatry were *Clinotarsus curtipes*, *Duttaphrynus* sp., *Raorchestes* sp., and frogs of the family *Ranixalidae.*

The current distribution point for *M. indicus* is approximately 37 km north-west from its closest reported locality at Valparai, Tamil Nadu State. Most other distribution points for the species occur south of the Palghat gap (PG from here on) (Figure 1), a significant biogeographic barrier for amphibians in the Western Ghats (Biswas & Karanth 2021). Only two distribution locations north of the PG are known for this frog. While Beddome (1880) mentions its occurrence in Walaghát (present day Walakkad, around Silent Valley National Park), Rajkumar et al. (2021) report its presence from Wayanad without any precise locality. These two localities are ca. 80 km and 140 km north of Nelliyampathy, respectively. The distribution of *M. franki* in Nelliyampathy is also notable for a similar reason. Suganthagiri, the only other known locality for this species in the Wayanad plateau, is ca. 132 km north of Kesavanpara, surmounting the PG. Thus, Nelliyampathy is now the only place south of PG where these two species of anurans occur in sympatry. Further field investigations might also yield these species in sympatry/syntopy in the Wayanad-Walakkad region north of PG. It would be interesting to look at the ecology and genetic structure of *M. indicus* north of the PG and *M. franki* south of PG to understand if these populations are indeed homogenous. Alternately, it is likely that the populations of *M. indicus* and *M. franki* on either side of the gap might represent cryptic allopatric species forming a part of a larger species complex. Another interesting observation is the presence of *M. franki* in the disturbed patch of forest near Kesavanpara viewpoint. It further supports the existence of this enigmatic species around human settlements (Garg & Biju 2019).

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NOTE

First record of Brilliant Flash *Rapala melida nicevillei* (Swinhoe, 1911) (Lepidoptera: Lycaenidae: Theclinae) to Meghalaya, India

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Rapala Moore, [1881] is a genus in the sub-family Theclinae and family Lycaenidae of the insect order Lepidoptera. The genus accounts for about 19 species in India (Varshney & Smetacek 2015) with distribution from west to east Himalaya including northeastern India, peninsular & central India, and Andaman & Nicobar Islands. They are found throughout southern and southeastern Asia, with a few species extending to Australia and into the eastern Palaearctic region, worldwide.

Rapala melida was first described by de Nicéville, 1895 as *Rapala rhoecus* with description of two subspecies and their distribution – *Rapala rhoecus nicevillei* (Northeastern India and Rangoon in Myanmar) and *Rapala rhoecus rhoecus* (Shan states in Myanmar). Fruhstorfer (1912) described *Rapala rhoecus* as *Rapala melida*. In 1932, Evans redescribed this species as *Rapala sphinx* where subspecies *nicevillei* described by de Nicevillei was replaced by the synonym *sphinx* (*Rapala sphinx sphinx*). In 1962, this species was redescribed by Cantile as *Rapala elcia*. At present *nicevillei* is considered as a subspecies of *Rapala melida* following Swinhoei, 1912 (Van Gasse 2013).

We planned an opportunistic survey on 21 March 2017 to document the butterflies of Khasi Hills District near Dawki (25.1900^{0}N, 92.0073^{0}E; elevation about 130 m). As usual we were searching butterflies near hills streams, forest edges, grasslands, and supporting vegetations. After surveying about four hours in Dawki, we spotted a *Rapala* on a sunny patch of the nearby vegetation at about 14.22 h. we took a few photographs of the species including the underside of the butterfly. While searching literature for identification of the species, it exactly matched the original description of *Rapala melida* (Evans 1932; *Rapala sphinx*). The species can be easily distinguished from *Rapala varuna* in having: (a) underside bands on both wings very dark brown, and (b) bands on underside forewing not white-edged. The underside bands of *Rapala varuna* are not dark brown and bands on forewing are white-edged.

The precise localities from which *Rapala melida nicevillei* was previously collected is Sylhet, Bangladesh. This species is recorded for the first time in Meghalaya,

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Image 1. Sidewise comparison of a—*Rapala melida* with the most similar species | b—*Rapala varuna*.

Image 2. A view of the habitat where *Rapala melida* was recorded at Dawki, Meghalaya (21/03/2017).

eastern Himalaya, and is not found in the major historic and taxonomic works done by various authors in the state (Butler 1879; Swinhoe 1893, 1896; Parsons & Cantlie 1948; Cantlie 1952; Radhakrishnan et al. 1989; Alfred 1999; Hatter et al. 2004; Sondhi et al. 2013). We present here the first photograph of the live butterfly of the Indian subspecies.

At present, the exact habitat of *Rapala melida* is not known in India. But our field observation suggests that the species might be a forest dweller and can be found perching on shrubs and bushes (Image 2). The males like other *Rapala* species can be seen flashing their bright upperside to mark their presence.

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NOTE

A note on the occurrence of *Cremnochonchus conicus* (Blanford, 1870) in Mumbai, India

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Members of the genus *Cremnoconchus* are known to be restricted to montane streams on the western escarpment of the Western Ghats, at altitudes between 300 m and 1,400 m (Rao 1989; Reid et al. 2013; Tripathy & Sajan 2020).

Four species and several varieties were described in the 19th century (Blanford 1869). In 2013, only three described species—*C. syhadrensis*, *C. conicus*, *C. canaliculatus*, were considered valid and recognized from the northern Western Ghats in Maharashtra state, where all can occasionally be found sympatrically out of the nine recognized species from the genus *Cremnoconchus* (Reid et al. 2013; Tripathy & Sajan 2020).

The gastropod genus *Cremnochonchus* is endemic to the Western Ghats. The species being habitat specialist are exclusive to spray zones of waterfalls. *C. carinatus* was first recorded by Layard in the spray zones of waterfalls in Mahabaleshwar and is the only known range for the species. The species has since been known to be extirpated from that locality (Molur et al. 2011). In 2013, *C. carinatus* was no longer considered a valid species and has been clubbed with *C. conicus,* which were considered sister species (Reid et al. 2013).

C. conicus has been observed along the streams and cascades of Conservation Education Centre (CEC), Goregaon in good populations. Apart from CEC, *C. conicus* have been observed in the localities of Mulund, Airoli, Thane, Maharashtra (19.1763^{0}N, 73.0122^{0}E), Anushaktinagar, Mumbai, Maharashtra (19.0358^{0}N, 72.92333^{0}E), Manpada, Thane, Maharashtra (19.2407^{0}N, 72.9639^{0}E), and Karnala Bird Sanctuary, Raigad, Maharashtra (18.8860^{0}N, 73.1125^{0}E).

The specimens were identified on the field and by shell specimens using the identification key by Reid et al. (2013) - turbinate shell shape; one shell rib at the shoulder (2 ribs observed in a specimen); wide columella; absence of pseudumbilicus; satin sheen shell surface with microstriae; weakly calcified operculum with no internal ridge.

The current known habitat preference of *C. conicus* is the spray zones of waterfalls and shallow pools of freshwater (Aravind et al. 2011). Our observations in CEC add on to this known habitat preference. *C. conicus* have been observed along the flowing, freshwater streams of CEC. They are found on the moist rocks present in between or along the flowing streams. They also lay eggs in clutches of 10–25 on these boulders. The egg-laying starts in June and lasts up to late July.

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Image 1. *Cremnochonchus conicus* laying eggs in a flowing stream at C.E.C., Mumbai. © Naman Kaji.

Figure 1. External Morphology of *Cremnochonchus conicus*. Dorsal view showing turbinate shell structure. © Mrinmayi Dalvi

Figure 2. External Morphology of *Cremnochonchus conicus*. Ventral view depicting identification characters observed in the field. © Mrinmayi Dalvi

Discussion: *C. conicus* is a habitat specialist gastropod found in the spray zones of waterfalls of Mahabaleshwar Hills (Molur et al. 2011). In the light of this new record, *C. conicus* not only inhabits the waterfalls but also the freshwater streams originating from it. It even lays eggs on the rocks present on these streams just above the flowing water surface. The ecology of eggs needs to be studied further as the fate of the eggs in this new scenario is unknown. There may be two possibilities – either the eggs hatch underwater once the stream overflows due to heavy rains in the month of July or due to change in the habitat conditions, it has to alter its ecology and spawn in such streams that may overflow and destroy the eggs.

The current conservation status of *C. conicus* is Vulnerable (VU) (Aravind et al. 2011; Reid et al. 2013), but may require a re-evaluation as this specialist species, in a rapidly vanishing freshwater habitat, currently faces anthropogenic pressures (Shubham Yadav, pers. comm. August 2021). *C. conicus* as well as other snail species are harvested by the locals for consumption. Apart from that, the freshwater streams are quickly being polluted due to waste disposal, effluent and organic discharge along and directly in these streams. The streams in Mahabaleshwar, from where Blanford collected his specimens, have since been polluted and hence, no species of *Cremnocochus* were found during a survey in 2010 (Aravind et al. 2011). Southern Coucal and Starling sp. predation has been observed on *C. conicus*. Furthermore, its range considering its niche can be estimated to be abundant in the pristine streams of northern Western Ghats. The species needs to be studied further to understand its ecology and importance as an indicator of freshwater habitat.

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NOTE

Jasminum angustifolium (L.) Willd. var. *angustifolium* (Oleaceae): a new distribution record for West Bengal, India

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Jasminum L. is the largest genus of the Olive family Oleaceae, containing about 200 species and distributed from tropical to sub-tropical areas of the world (Green & Miller 2009; Jeyarani et al. 2018). In India, the genus comprises 40 taxa (35 species, two subspecies, and three varieties) (Srivastava 2020). Of these, 16 species are endemic to the Indian Himalayan region, Deccan Peninsula, and Andaman Islands (Srivastava 2002; Green 2003; Jeyarani et al. 2018). The native Indian jasmines are distributed in the eastern (West Bengal), northeastern (Assam, Meghalaya, and Sikkim), and northwestern Himalaya (Kashmir), the western part of Deccan peninsula, and southern Andaman in the tropical forests (Kalaiyarasi et al. 2018). They are represented by 15 species in West Bengal (Chandra 2015).

While exploring the Oleaceae taxa of West Bengal, a *Jasminum* species was collected from four locations in Gorumara forest division (Jalpaiguri, West Bengal). After a thorough literature survey (Clarke 1882; Prain 1903; Kanjilal et al. 1939; Gamble 1967; Manilal & Sivaranjan 1982; Deb 1983; Green 1985, 2003; Haridasan & Rao 1987; Srivastava 1987; Nair et al. 1997; Watson 1999; Gastman & Balachandran 2006; Giri et al. 2008; Sinha et al. 2012; Chandra 2015; Balachandran & Rajendiran 2016; Mallick 2020; Gogoi et al. 2021) and consulting different herbaria (ASSAM, AURO, CAL, IVH, K, MH, NY) and digital repositories (iNaturalist, POWO), it was identified as *Jasminum angustifolium* (L.) Willd. var. *angustifolium.* The taxon was hitherto not reported from West Bengal.

Jasminum angustifolium var. *angustifolium* can be easily distinguished from *J. angustifolium* var. *hirsutum* P.S.Green by the absence of domatia on abaxial surface of leaf; indistinct leaf venation on both surface; presence of linear, puberulent (on inner side) to sparsely ciliate calyx with filiform, thin, and comparatively larger (4–10 mm) sepals; minutely puberulent (not tomentose) young branches and glabrous matured stem (Table 1).

J.a. var. *angustifolium* and *J.a.* var. *sessiliflorum* (Vahl) P.S.Green are slightly close to each other, and both are devoid of domatia on vein axils and hairs on the matured stem. *J.a.* var. *angustifolium* is distinct from *J.a.* var. *sessiliflorum* by the presence of comparatively larger leaf size; minute puberulence on adaxial midvein and petiole; calyx lobes sparsely ciliate, calyx non-ribbed, fruiting sepals shorter than fruit; flower bud with pinkish tinge at apex; pedunculate inflorescence and ellipsoidal fruit (Table 1).

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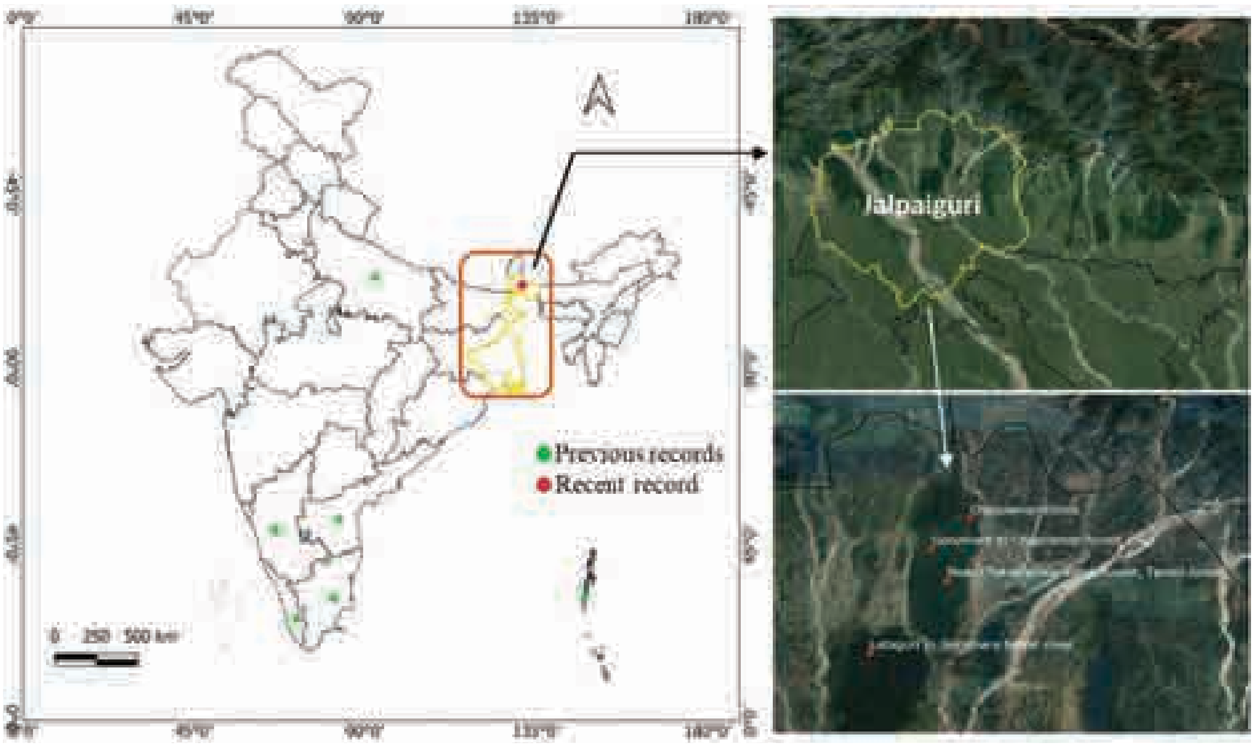

Figure 1. Distribution of *Jasminum angustifolium* (L.) Willd. var. *angustifolium* in India.

There have been no reports of the natural distribution of these three varieties in West Bengal. During the literature survey and herbarium consultation, we found one herbarium specimen (CAL0000031068) of *J. sessiliflorum* (now accepted as *J.a.* var. *sessiliflorum*) at CAL, which was a cultivated specimen from HBC (now AJC Bose Botanical Garden, West Bengal). The species is distributed mainly in the Eastern and Western Ghats of India. The natural distribution of *J.a.* var. *hirsutum* is recorded in India (Tamil Nadu) and Sri Lanka. *J.a.* var. *sessiliflorum* is reported from India (Andhra Pradesh, Kerala, Tamil Nadu) and Sri Lanka.

Jasminum angustifolium (L.) Willd., Sp. Pl. 1(1): 36. 1797; C.B.Clarke in Hook.f., Fl. Brit. India 3: 598. 1882; Gamble, Fl. Pres. Madras 2: 790(555). 1967; P.S.Green, Kew Bull. 40(1): 225–227. 1985; P.S.Green, Kew Bull. 58(2): 283–293. 2003; Muthumperumal & Parthasarathy, Check List. 5(1): 92–111. 2009; Sunil & Sivadasan, Fl. Alappuzha Dist. 413. 2009; Sinha & Shukla., Fl. Uttar Pradesh 123. 2020. *Nyctanthes angustifolia* L., Sp. Pl. 1: 6. 1753. Bengali: Ban mallika

var. *angustifolium*

Aromatic, wild, scandent, and straggling shrubs up to 2 m long. Matured stem woody and glabrous, young branchlets pubescent with whitish hairs. Leaves simple, opposite, petiolate, entire, ovate-lanceolate, shape variable in same branch, 13–51 × 9–25 mm, obtuse to rounded base, apex sub-acute to acuminate or obtuse, glabrous (margins and mid vein regions minutely puberulent), adaxial surface glossy and smooth; 3–5 pairs of lateral veins, vein articulation indistinct (specifically at apical part); domatia absent; petiole 3–5 mm, minutely puberulent. Terminal cyme with 1–5 flowers (mostly 3 flowers in each cluster on axillary shoots), bracteate, pedicel 2–5 mm, peduncle 5–13 mm; bracts 2.5–3 mm, persistent, linear; calyx persistent, linear, filiform, calyx tube 2–3 mm, sepals 5–7, puberulent (on inner side) to sparsely ciliate, 5–9 mm long; corolla white or white with exterior pink tinge at bud apex, fragrant, petals 6–9, linear-elliptical with acute apex, 11–20 mm long, tube 14–19 mm long, glabrous. Stamens 2, anthers 3–5 mm long, apiculate apex, dorsifixed, filaments 2–4 mm. Style straight, stigma slightly bifid, 2–3 mm, ovary 1–2 mm. Green berries turn purplish-black on ripening, ellipsoidal, 7–14 × 15–30 mm, single or paired, glabrous (Image 1).

Phenology: Flowering starts in November and continues till December. Fruit development begins in January and can be found up to July.

Specimens collected: Near Chandrachur watch tower (Tendu forest) (26.8447 N, 88.8630 E), roadside of Gorumara to Chapramari forest (26.8726 N, 88.8500 E), Chapramari forest (26.8990 N, 88.8824 E), Lataguri to Gorumara (26.7815 N, 88.7987 E), Gorumara forest

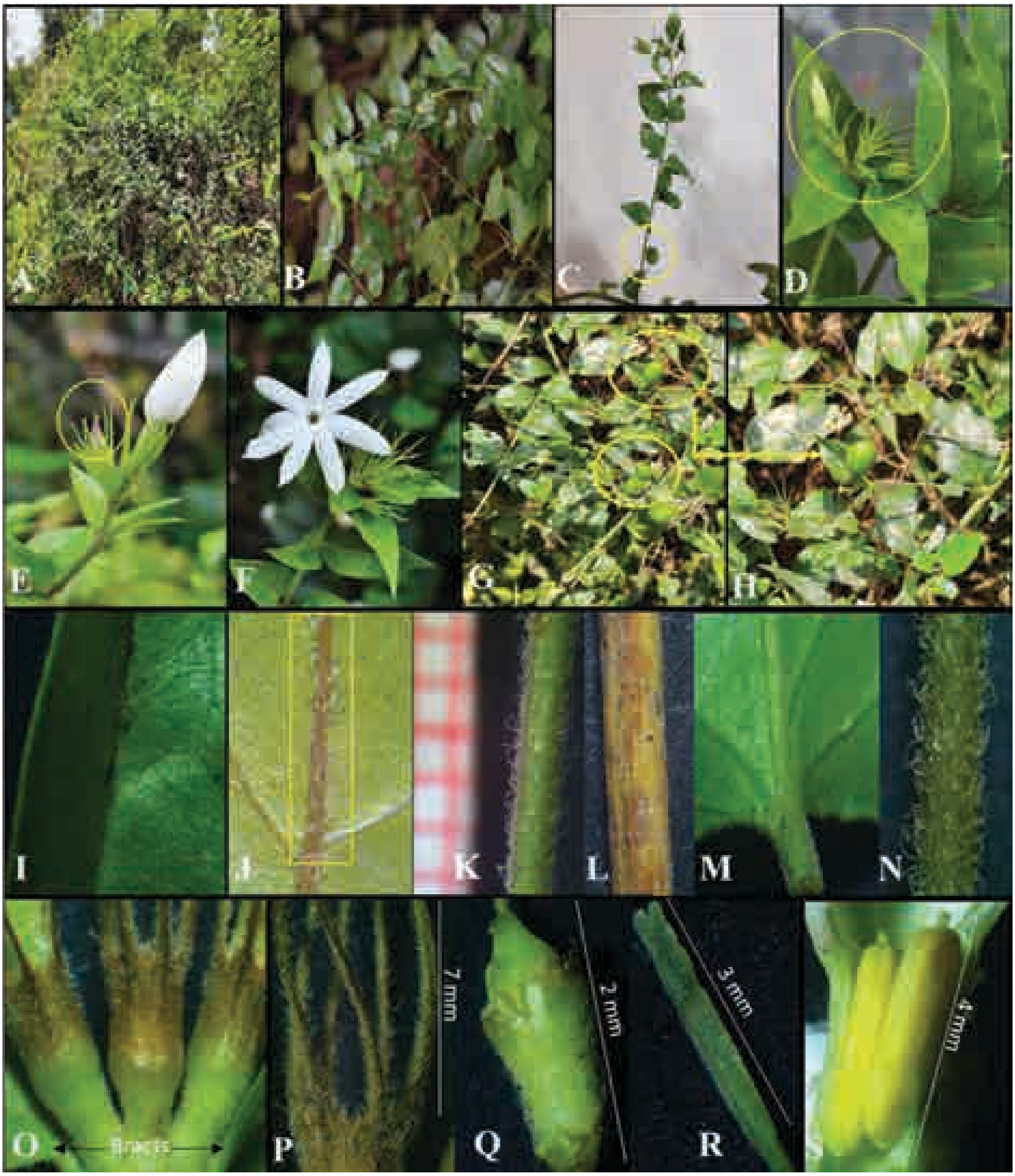

Image 1. *Jasminum angustifolium* (L.) Willd. var. *angustifolium*: A—Habit | B—Plant in vegetative condition | C—A leafy twig showing variations in leaf shape | D—Terminal inflorescence containing three diagnostic calyx and pink tinge bud | E—White bud with pink tinge on young shoot | F—Open flower | G—Single and paired fruit | H—Fruit with magnified view | I—Glabrous lamina surface (except midrib) | J—hairs along the midrib | K—Young shoot hairs | L—Magnified view of a matured, glabrous branch | M—Puberulent petiole | N—Magnified view of petiole hairs | O—Persistent calyx | P—Hairy sepals | Q—Ovary | R—Bifid stigma | S—Apiculate anthers. © Keya Modak.

division, Jalpaiguri, West Bengal, K. Modak & M. Chowdhury NBU 11773, NBU 12638, NBU 12640, NBU 12641.

Specimens examined: *Jasminum angustifolium* var. *angustifolium*: India, Andaman Islands: AURO0212 (AURO); Peninsular India: AURO8618, AURO9351,

Table 1. Comparative characteristics of three varieties of *Jasminum angustifolium*.

Characteristics	*J. angustifolium* var. *angustifolium*	*J. angustifolium* var. *sessiliflorum*	*J. angustifolium* var. *hirsutum*
Stem	Young branches minutely puberulent, matured stem glabrous	Young branches and matured stem glabrous	Young branches and matured stem tomentose
Lamina	Ovate-lanceolate to narrowly lanceolate; 10–70 × 7–40 mm; not recurved; glabrous but midrib minutely puberulence; 3–5 pairs of lateral veins, first pair is obscure	Lanceolate; 6–30 × 4–15 mm; not recurved; glabrous; venation obscure	Broadly ovate to elliptic-lanceolate or orbicular; 10–60 × 7–40 mm; recurved; glabrous; secondary veins prominent on both surfaces
Domatia	Absent	Absent	Present
Petiole	Minutely puberulent	Glabrous	Glabrous
Flower	Petals white with pinkish streaks at least in bud condition; pedunculate, length up to 13 mm; petals number 8–9	White; sessile; petals 8–10	White; pedunculate, length up to 11 mm; petals usually 5, rarely 7
Calyx	Sparsely ciliate, not ribbed, linear and thin; sepals 4–10 mm long	Glabrous, ribbed, linear and thick; sepals 8–12 mm long	Puberulent, not ribbed, ovate-triangular, not as thin as other two; sepals 1–2 mm long
Fruit	Ellipsoidal, 7–11 mm long, purplish or purplish-black; fruiting sepals shorter than fruits (sepals 4–10 mm long)	Globose, 7–8 mm long, purplish-black; fruiting sepals longer than fruits (sepals 8–12 mm long)	Globose, black, shiny, 7–8 mm long; fruiting sepals much shorter than fruits (sepals 1–2 mm long)

AURO10513, AURO11741 (AURO); NY03146644 (NY). Sri Lanka: NY03146636, NY03146638, NY03146639 (NY); *J. angustifolium* var. *hirsutum*: India, Tamil Nadu: AURO8796, AURO9477 (AURO), K000545676 (K); Sri Lanka: K000901409 (K). *J. angustifolium* var. *sessiliflorum*: India, Tamil Nadu: AURO9912, AURO10037, AURO10567 (AURO); BSID0014566 (DRC), CAL0000031069 (CAL), MH16 (MH); West Bengal: CAL0000031068 (CAL).

Natural distribution: India (Andaman, Andhra Pradesh, Kerala, Karnataka, Tamil Nadu, Uttar Pradesh and now from West Bengal), Sri Lanka; growing on the floor of the sub-tropical and tropical forest. Figure 1 shows the distribution of the taxon in India.

Notes: At each location, two–four individuals of *J. angustifolium* (L.) Willd. var. *angustifolium* were found growing in association with *Chromolaena odorata* (L.) R.M.King & H.Rob., *Mikania micrantha* Kunth in the marginal part of partially shaded Sal and Teak forest of Lataguri, Chapramari to Gorumara, West Bengal. According to our sample characteristics, observation of different herbarium sheets and relevant literature, a taxonomic key was prepared for the three closely related varieties of *J. angustifolium*. These key characters will be helpful for the identification of the plant up to varietal rank:

1. Lamina rounded or ovate to elliptical; calyx teeth 1–2 mm, sepals always 5, ovate-triangular; stem hirsute, slender; domatia present .... *J. angustifolium* var. *hirsutum*

1. Lamina lanceolate or ovate-lanceolate; calyx teeth 2–12 mm, sepals 5–7, linear; stem glabrous or minutely puberulent, profusely branched; domatia absent 2

2. Young branches minutely puberulent, matured stem glabrous; inflorescence pedunculate; calyx not ribbed; fruiting sepals shorter than fruits; fruit ellipsoidal .... *J. angustifolium* var. *angustifolium*

2. Young branches and matured stem glabrous; inflorescence sessile; calyx ribbed; fruiting sepals longer than fruits; fruits globose .... *J. angustifolium* var. *sessiliflorum*

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NOTE

Cyrtosia falconeri (Hook.f.) Aver. (Orchidaceae): an addition to the flora of Jammu & Kashmir, India

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The genus *Cyrtosia* Blume consists of six species mainly found in Asia, up to southern China, Japan, and Madagascar (Govaerts et al. 2022). In India, the genus is represented by four species namely, *cathcartii* Hook.f., *falconeri* (Hook.f.) Aver., *lindleyana* (Hook.f. & Thomson) Rchb.f., and *nudifolia* Lour. (Singh et al. 2019), out of which two species— *Lindleyana* and *falconeri*—occur in western Himalaya. During the present biodiversity exploration visit in the forest of Koteranka of Rajouri district in Jammu & Kashmir (J&K), the first author found an interesting mycoheterotrophic orchid growing in an evergreen mature oak forest. After proper morphological study in the field, the plant was collected for the herbarium specimen (Image 1). Collected specimen was submitted to the herbarium of Department of Botany, KL DAV PG College Roorkee. After a comprehensive literature study, it was identified as *Cyrtosia falconeri* (Hooker 1890; King & Pantling 1898; Deva & Naithani 1986; Pearce & Cribb 2002; Misra 2007; Akhtar et al. 2011; Jalal & Jayanthi 2013, 2015). Rai et al. (2017) reported this orchid for the first time from western Himalaya in Uttarakhand. However, the present report extends its distribution further west to Jammu & Kashmir, which consists of a total of 48 species of orchids (Akhtar et al. 2011), and current report also marks the new generic record of the genus *Cyrtosia* for the state. Taxonomic description, photo plate, and herbarium specimen are provided in the manuscript.

***Cyrtosia falconeri* (Hook.f.) Aver.** Turczaninowia 14(2): 38 (2011); *Galeola falconeri* Hook.f., Fl. Brit. India 6: 88. 1890; King & Pantl., Ann. Roy. Bot. Gard. (Calcutta) 8: 265, t. 353. 1898; Duthie in Ann. Roy. Bot. Gard. (Calcutta) 9(2): 156. 1906; Raizada, Naithani & Saxena, Orch. Mussoorie: 46. 1981; Seidenf. & Arora in Nord. J. Bot. 2: 17. 1982; Deva & Naithani, Orch. Fl. N.W. Himalaya: 47, t.15. 1986; Chowdhery, Orch. Arunachal Pradesh 405, t. 243. 1998; Pearce & Cribb, Orch. Bhutan 64. 2002; Lucksom, Orch. Sikkim North East Himalaya: 59, t. 40. 2007; Chowdhery& Agrawala, Cen.W. Himalayan Orch.: 212, t.74. 2013. 2–3.

Type: India, Gharwal, Falconer s.n. (syntype, K).

Plants 1–3 m, in height. Rhizome branched, 3–5 cm in diam., covered with triangular scales at nodes, 2.0–4.2 cm in diam. in size. Stem brownish, glabrous lower part, while upper sparsely & short rusty hairs, dull brownish to reddish, erect, covered with some lanceolate to ovate scales sizing 2.5–4.5 cm. Inflorescence raceme, 5–33 cm, peduncle, and rachis shortly tomentose. Floral bracts narrowly elliptic or ovate, 1.1–3.5 mm, found vertical to rachis, abaxially shortly rusty tomentose. Pedicel and ovary, densely rusty tomentose, 1– 3.5 cm long. Flowers 4–5 cm in diameter, bright yellow. Sepals elliptic to oblong, densely rusty tomentose and smooth outside, 2–3 cm in length and 1–1.6 cm in width. Petals

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Image 1. *Cyrtosia falconeri* herbarium specimen.

are slightly narrower than sepals but as long as sepals. Lip cup–shaped, entire, ovate to orbicular, ca. 2.5 cm in diameter, basal part loosely embracing column, a small basal sac formed with transversal fold, inner side densely papillose, and margins fimbriate. Column erect, stout, slightly curved forward, 2.5–3.5 mm long, basal portions consist of two tufts of long papillae, anther cap nearly glabrous or papillose. Fruit oblong, red to purple, cylindric, 20– 25 cm long, 3 cm in diameter, surface finely verruculose. Seeds dark brown, 1–2 mm in diameter, with a narrow-encircled wing (Image 2).

Specimen examined: KL DAV 092197, 10.iv.2021, India. Jammu & Kashmir, Rajouri, Koteranka, Kalyian forests, coll. Mushtaq Ahmed & Manjul Dhiman.

Habitat: Terrestrial, grows in humus-rich soil in evergreen oak forests at an elevation of 1,765 m, 33.351^{0}N & 74.535^{0}E.

Phenology: May–June.

Distribution: Globally it is distributed in Vietnam, Bhutan, India, China, and Thailand. In India, it was mainly reported from eastern Himalaya ranging from West Bengal to Arunachal Pradesh. However, this species was

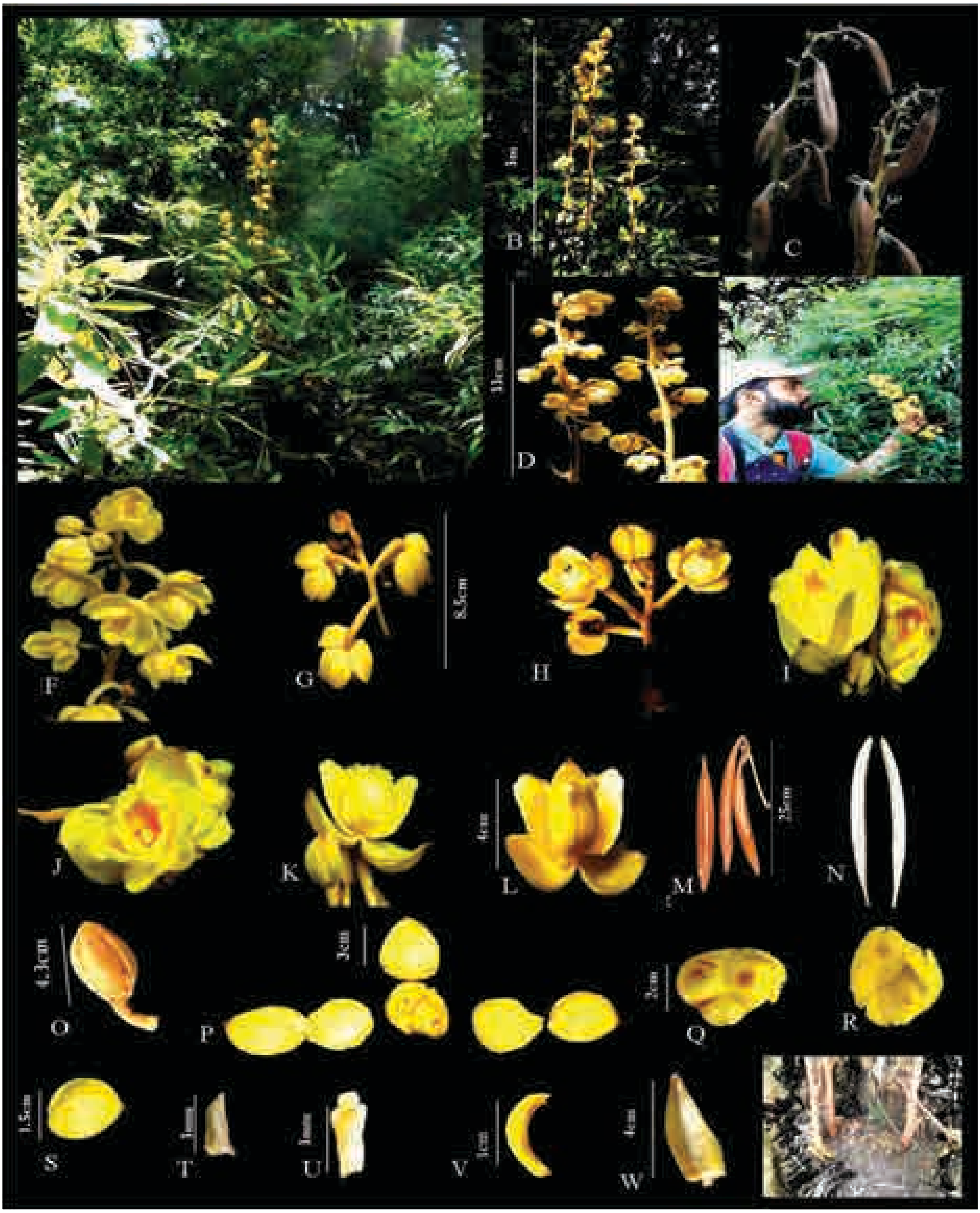

Image 2. *Cyrtosia falconeri*: A,B—Habitat | C—Plant with fruits | D—Inflorescence | E—Collection in the field | F—Close up of inflorescence | G,H—Single branches of inflorescence | I–L—Different views of the flower | M—Fruits | N—Dissected fruit | O—Flower bud | P—Sepals and petals | Q,R—Dorsal and ventral views of the lip | S—Sepal | T—Floral bract | U—Column | V—Pedicel | W—Scale | X—Rhizome. © Mushtaq Ahmed.

also reported from Uttarakhand of the eastern Himalaya.

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NOTE

New distribution record of *Roridomyces* cf. *phyllostachydis* (Agaricales: Mycenaceae), a bioluminescent fungus from Namdapha National Park, Arunachal Pradesh, India

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Bioluminescence is the biochemical reaction in living organisms where chemical energy from a complex compound such as luciferin is converted to light energy through oxidation under the action of luciferase which acts as a catalytic enzyme (Pandey & Sharon 2017). The phenomenon of bioluminescence is randomly reported across 17 phyla and more than 700 genera, both in marine and terrestrial environments (Lee 2015). Bioluminescence in fungi has been observed globally across several species belonging to four distinct monophyletic lineages, namely, *Armillaria* (Fr.) Staude, *Mycenacae* Overeem, the *Lucipentes* lineage of *Mycena* (Pers.) Roussel s.l., and a lineage consisting of *Omphalotus* Fayod & *Neonothopanus* R.H.Petersen & Krisai (Matheny et al. 2006; Desjardin et al. 2008, 2010, 2016; Vydryakova et al. 2011; Aravindakshan et al. 2012; Chew et al. 2013, 2015; Shih et al. 2014; Mihail 2015; Cortés-Pérez et al. 2019).

Being a biodiversity hotspot, India also hosts a variety of fungal species but the documentation on bioluminescent fungi is still deficient. Over the past few years, there have been a few reports on bioluminescence from fungi such as *Nothopanus eugrammus* and *Omphalotus olearius* (Vrinda et al. 1999), followed by a unique taxon from Kerala, *Mycena deeptha* (Aravindakshan & Manimohan 2014) & *Armillaria mellea* (Patil & Yadav 2022). Recently, a new species of bioluminescent fungi- *Roridomyces* cf. *phyllostachydis* (Karunarathna et al. 2020) has been described from Meghalaya (Mawlynnong in East Khasi Hills & Krang Shuri, West Jaintia Hills) respectively at altitude 560 m and 1021 m (Figure 1). In this article, we report the new distribution of *Roridomyces* cf. *phyllostachydis* (Agaricales, Mycenaceae) from Kamala

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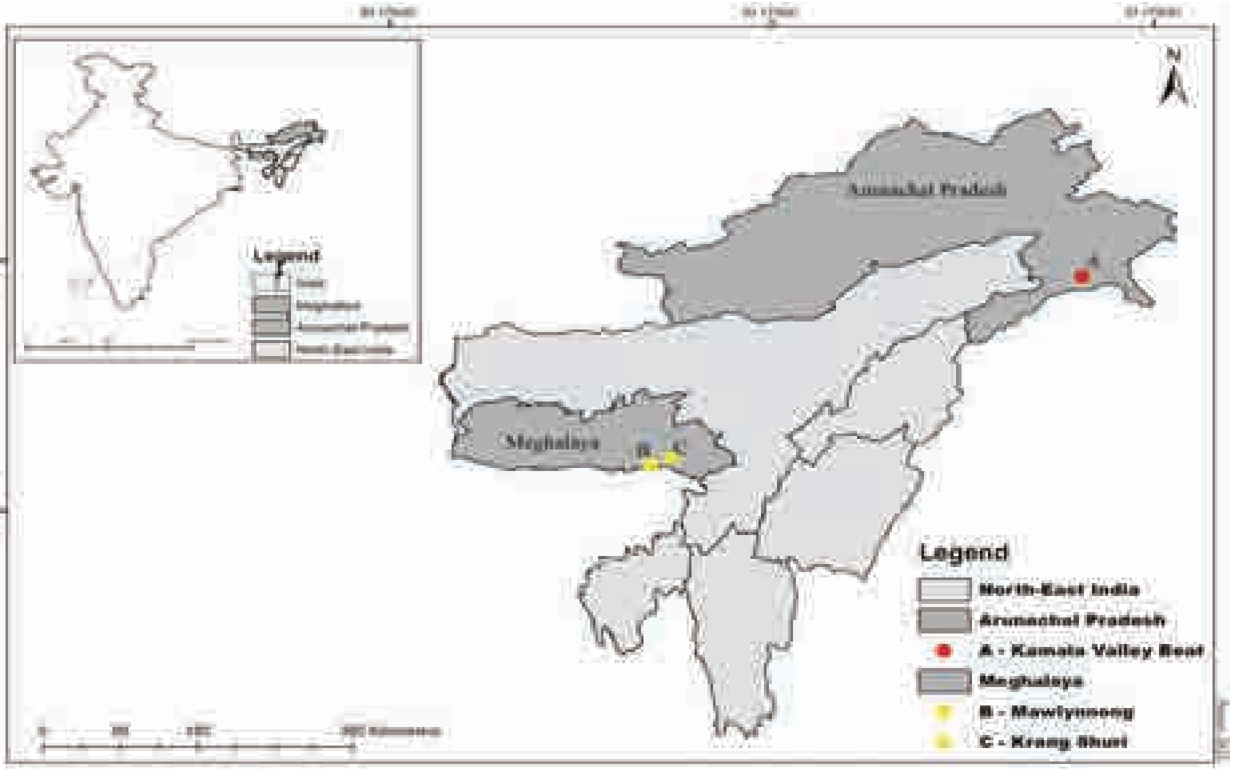

Figure 1. Distribution Map of *Roridomyces* cf. *phyllostachydis*: A—Shows the new distribution record from Kamala Valley beat, Namdapha National Park, Arunachal Pradesh | B & C—Showing the previous record from Mawlynnong & Krang Shuri, Meghalaya.

Valley Beat (27.462036°N, 96.426933°E, altitude, 611 m) in Namdapha National Park (NNP) (Figure 1), which is the easternmost national park, located in Changlang District of Arunachal Pradesh.

On 7 April 2022 around 19:35 hours, while conducting a nocturnal survey as a part of a study on small mammals, we opportunistically encountered a small glowing mass of *Roridomyces* cf. *phyllostachydis* by the side of the road on a wet muddy substratum. On further inspection, we noticed fresh fruiting bodies of the mushroom growing out, from the edge of a fragmented piece of dead, rotten bamboo belonging to the genus *Phyllostachys*, embedded in the soil. We were unable to determine the exact species of the host bamboo from the small fragmented piece. The surroundings were characterized by riparian vegetation consisting of short grasses, scattered with pebbles and ephemeral pools. On encountering the fungal species, we noted down the macro-morphological characters that were visible and photographs were taken in situ under both light and dark conditions (Image 1a).

The species was identified based on physical characteristics like small-sized, pileus 2.5 to 4.5 mm, obtusely conical, subumbilicate to umbilicate centrally depressed with pale brown striations in the middle; decurrent and distant lamellae and central, cylindrical stipe with tapering at the pileus and slightly swollen at the base; pileus margin acute. Bioluminescence was only observed within the stipe, which emits bright luminous green light evenly in the dark (Image 1b,c). These morphological characters lead us to assign the observation as *Roridomyces* cf. *phyllostachydis*, later confirmed by one of the authors (Karunarathna S.C. pers. comm. 31 May 2022).

Earlier observation of *Roridomyces* cf. *phyllostachydis* showed its distribution to be gregarious and scattered, growing on dead bamboo sticks of *Phyllostachys mannii* (Karunarathna et al. 2020). Our observation also supports this description except that we found only a single group of fruiting bodies in the surveyed area. Previously there have been a few studies on the fungal species of Arunachal Pradesh (Sharma et al. 2015; Tabin et al. 2014) but to date, there is no record of any bioluminescent fungi from the state. Hence our observation of *Roridomyces* cf. *phyllostachydis* is the new distribution record from Namdapha, Arunachal Pradesh which is ~493 km (aerial distance) away from the previous record. There have been several hypotheses regarding the evolutionary significance of bioluminescence in fungi facilitating reproduction

Image 1(a–c). Photographic evidence of *Roridomyces* cf. *phyllostachydis*: a—Habitat shot with fruiting bodies of *Roridomyces* cf. *phyllostachydis* on fragmented bamboo within a muddy substrate under the light | b—Stipes emitting bright luminous green light under complete darkness | c—Dorsal view of the pileus. © a & c—Sourav Gupta; b—Arijit Dutta.

by attracting insects for the dispersal of basidiospores (Bechara 2015) or as a defensive mechanism to reduce predation (Karunarathna et al. 2020; Dauner et al. 2021) but no thorough study has been conducted on its ecological effects. Hence our observation further demands more research on its ecological aspect and its effect on associated flora and fauna.

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NOTE

Photographic evidence of bioluminescent mushroom *Mycena chlorophos* (Mycenaceae) from Goa, India

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The first recorded scientific description of the *Mycena chlorophos* Miles Joseph Berkeley & Moses Ashley Curtis in 1860 occurred in the year Berkeley & Curtis 1860. The mushrooms grows in subtropical regions of Asia, especially Western Ghats in India, Sri Lanka, Indonesia, and even recorded in Japan.

The mushroom contains an enzyme called as luciferase, causing substrate luciferin which catalyzes in presence of oxygen. During this chemical reaction, excess energy is released, which is visible as light/ luminescence (Kaskova et al. 2017; Patil & Yadav 2022). This light is visible to naked eye in complete darkness in the form of pale green light. Studies suggest (Fleiss & Sarkisyan 2019; Patil & Yadav 2022) that luminescent nature of the mushroom is for spore dispersal by attracting insects.

The present scientific study is the first of its kind to provide photographic evidence of *Mycena chlorophos*, a bioluminescent mushroom from Goa, the only previously known record of the same is from a newspaper article, reporting its presence in Mhadei Wildlife Sanctuary in North Goa (Times of India travel 2019). The fungus was identified based on the current literature available (Kushwaha & Hajirnis 2016).

On foot surveys were conducted during June 2022, as it was observed that the glowing mushroom grows during initial stages of monsoon. The surveys were conducted in the buffer area of Bhagwan Mahavir Wildlife Sanctuary (15.390 N 74.226 E) where the mycelium was observed during the monsoons. The area has a scant canopy, compared to the protected zone as it is open for wood logging and sand mining. As we are looking to enrich the habitat, this proved to be a trigger point for the study.

The surveys were divided into two stages: Stage 1—preliminary surveys, where the team recorded the presence of bioluminescence on decomposing wood. After narrowing down the potential sites, stage 2 was initiated. Stage 2—a focused search was carried out to identify and establish the presence of bioluminescent mushrooms. Wherever the bioluminescent mushroom was observed, photographs with GPS co-ordinates were taken. A total of 658 grids of 10 × 10m were placed using google earth and ArcGIS were placed.

The team kept regular records and certain

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Image 1. *Mycena chlorophos* in light. © Arcane Conservancy.

Image 2. Bioluminescent mushroom *Mycena chlorophos* emitting pale green light. © Arcane Conservancy.

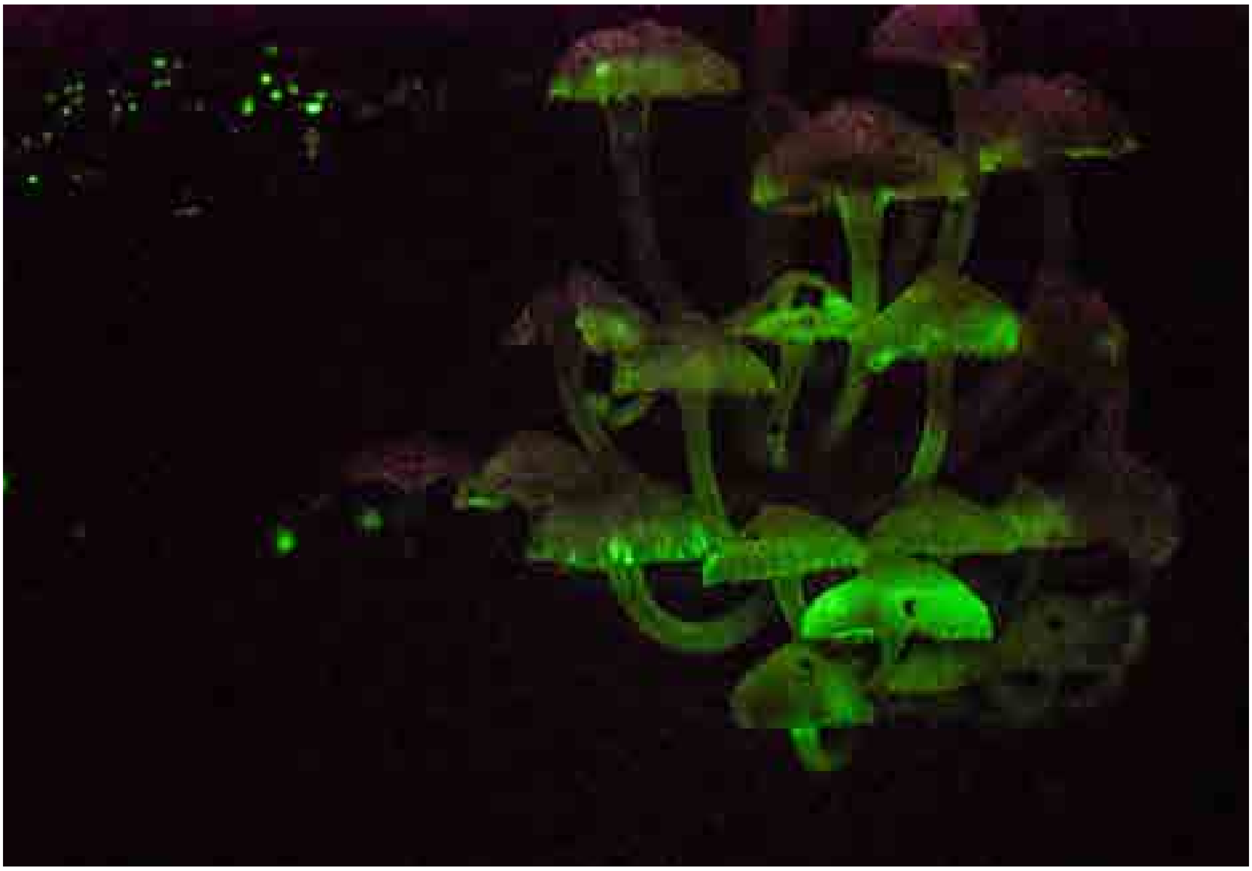

Image 3. Cluster of bioluminescent mushroom *Mycena chlorophos*. © Ramesh Zarmekar - Nature's Nest

observations were made.

· In the presence of an external light source (day light or torch) the mushrooms appear as brownish-white in color.

· The bioluminescence is present for up to four days from the time of first appearance of the fruiting bodies.

· In areas with denser canopy cover and high humidity, the intensity of the glow is higher.

· During the nocturnal surveys, it was observed that the mushrooms released spores in air and wind appears to the primary dispersal agent.

The mushroom appear in groups of - individuals. The furiting body or cap is conical at early stages and as it gets matured flatens out. Hymenium has gills.

The bioluminescent fungus was observed growing on dead and decaying wooden logs, branches and even twigs. This is the first photographic record of the fruiting bodies of the species in the Bhagwan Mahavir Wildlife Sanctuary, Goa, India.

The frequency of occurrence of glowing mushrooms is about 1.97% within the study area. The functions of these bioluminescent mushrooms are still unknown. Further study needs to be conducted to identify the microclimatic conditions essential for the growth of the species.

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