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ARTICLE

Echolocation call characterization of insectivorous bats from caves and karst areas in southern Luzon Island, Philippines

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Abstract: Bats are excellent bioindicators and are increasingly used to assess ecosystem health and monitor changes in the environment. Due to increased awareness of the potential transmission of pathogens from bats to humans and recognizing the limitations of traditional bat sampling methods, the use of of non-invasive sampling techniques such as bat recorders were recommended for field-based monitoring studies. In the Philippines, however, bat bioacoustics is still a growing field, and the scarcity of acoustic data hinders the use of echolocation calls to conduct accurate inventories and population monitoring of echolocating bats. Here, we recorded and characterized echolocation calls of insectivorous bats from caves and karst areas located in southern Luzon Island, Philippines. In addition, we compared our results with other studies performed within and outside the country to identify possible regional and local variation in acoustic characters for some species. A total of 441 echolocation calls were recorded from six bat families: Hipposideridae (five species), Rhinolophidae (five species), Vespertilionidae (three species), Miniopteridae (two species), Megadermatidae (one species), and Emballonuridae (one species). Discriminant function analyses (DFA) with leave-one-out cross validation correctly classified bats emitting calls dominated with a constant frequency (CF) component (rhinolophids and hipposiderids) with >97% success and those producing frequency modulated (FM) calls (Miniopteridae and Vespertilionidae) with 88.9% success. We report echolocation calls for Philippine population of two species (*Megaderma spasma* and *Hipposideros lekaguli*) for the first time. Moreover, we present geographical variations in call frequencies for some species by comparing previously reported acoustic data elsewhere across the species' range. This underscores the importance of establishing a readily accessible and comprehensive local reference library of echolocation calls which would serve as a valuable resource for examining taxonomic identities of echolocating bats, particularly those whose calls exhibit biogeographic variations.

Keywords: Bat recorders, call frequencies, call library, discriminant function analysis, echolocating bats, ecotourism, limestone forest.

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Author contributions & **Acknowledgements:** See end of this article.

INTRODUCTION

Bats are of great importance because they maintain ecosystem balance in tropical forests and their sensitivity to anthropogenic disturbances makes them excellent bioindicators in assessing ecosystem health and monitoring changes in the environment (Jones et al. 2009). Traditionally, bats are studied by capturing them using mist nets (Kunz & Kurta 1988; Sedlock 2001), although some studies found that the use of harp traps are more effective in capturing echolocating bats (Tidemann & Woodside 1978; Francis 1989). Sampling bats using mist nets provides a more standardized method of measuring bat abundance; however, this method is prone to sampling biases since mist nets are usually placed below the canopy. This practice underestimates bat diversity of an area since there are relatively more species of bats on the upper forest layers (O'Farrell & Gannon 1999; Larsen et al. 2007; Gonzalez et al. 2020). Moreover, mist nets are more biased towards larger–bodied bats which do not have the ability to evade mist nets like most echolocating bats (Larsen et al. 2007).

There has been a growing global trend in utilizing ultrasonic detectors and recording echolocation calls as an alternative approach for a non-invasive and passive means to document the occurrence of echolocating bats, investigate their ecology, behavior, and responses to various anthropogenic pressures, and identify habitat and important areas for conservation of these species (Rydell 1991; Siemers & Schaub 2011; Pauwels et al. 2019). Species-specific acoustic cues and characteristics allowed the accurate classification of species and the use of automatic classifiers have enabled rapid species identification using computer software programs (Adams et al. 2010; Agranat 2013; Amberong et al. 2021). Further, continuous advancements in ultrasonic bat recorders to cater the need for this growing field have led to improved features that facilitate the collection and analysis of larger datasets. These advancements have contributed to increased inventory completeness in studies focused on bat assemblages and have made long-term monitoring experiments feasible (Lausen & Barclay 2006; MacSwiney et al. 2008). Lastly, considering the recent Covid-19 pandemic and the potential transmission of bat-borne viruses and other zoonotic pathogens, the use of acoustic surveys offers a means of studying bats without direct contact, thereby reducing the risk of zoonotic transmissions (Nuñez et al. 2020; Pekar et al. 2022).

While acoustically monitoring bats ensures researchers' safety and significantly cuts the time and effort in surveying, there is still paucity of comprehensive and reliable bat call libraries in many regions which is an essential component for accurate species identification of echolocating bats (Karine & Kalko 2001). In the Philippines, bat bioacoustics is still in its infancy, and a comprehensive bat call library is still lacking. Relevant studies based on bioacoustics of bats are limited to very few localities and islands such as in Luzon (Sedlock 2001; Sedlock & Weyandt 2009; Esselstyn et al. 2012; Dimaculangan et al. 2019; Sedlock et al. 2019; Amberong et al. 2021; Taray et al. 2021), Panay Island (Mould 2012), Bohol Island (Phelps et al. 2018; Sedlock et al. 2014a), and Siquijor Island (Sedlock et al. 2014b). Previous works have focused on examining taxonomy (Sedlock & Weyandt 2009; Sedlock et al. 2014b) and studying behaviour, cave emergence, and activity (Dimaculangan et al. 2019; Sedlock et al. 2019) using few available acoustic data. Meanwhile, the works of Sedlock (2001), Amberong et al. (2021), and Taray et al. (2021) have delved into the characterization of acoustic calls of echolocating bats, aiming to establish a foundational dataset for creating a local bat call library for the Philippines. The archipelagic nature of the Philippines also provides an avenue to examine possible local echolocation variation or dialects, especially for endemic species with limited population dispersion.

Caves provide specific and stable microclimatic conditions, including temperature, relative humidity, and air quality, along with physical structures that are crucial for the survival of many bat populations. These factors provide a suitable environment for protection, roosting, and feeding (McCracken 1989; Murray & Kunz 2005). Among the 79 bat species present in the Philippines, 49 are known to roost in caves (Heaney et al. 2010). However, threats to these resident cave fauna are still rampant, including hunting, habitat destruction, and disturbances caused by unregulated human visits, leading to roost abandonment and rapid population decline (Mould 2012; Domingo & Buenavista 2018; Alcazar et al. 2020). Moreover, caves are also often overlooked and unprotected due to harsh conditions for proper assessment, research, and mapping of these landscapes (Tanalgo et al. 2022). Out of the 3500 caves identified in the Philippines, only approximately 40% have been adequately assessed and protected (BMB CAWED 2021). The lack of protection exposes these caves to potential exploitation, resulting in adverse long-term impacts on wildlife populations, such as reduced species richness and diversity, as well as the destruction of cave features. To address these challenges, rapid and cost-effective methods for surveying and monitoring cave bat populations, such as acoustic surveys, would be instrumental in assessing more caves in the country and protecting cave bats.

Here we describe echolocation calls of some insectivorous bat species we recorded from caves and karst areas in southern Luzon Island, Philippines and evaluate the potential of utilizing acoustic characters in identifying echolocating bat species. In addition, we want to assess possible geographic variation in echolocation call characteristics of some species by examining other existing acoustic data and studies done within and outside the country. Threats observed in the study areas and conservation implications of our results are also discussed. With this study, we aim to contribute to the building of a comprehensive reference library of bat echolocation calls for the Philippines and provide a non-intrusive and cost-effective tool for monitoring insectivorous bats.

MATERIALS AND METHODS

Study Site and Bat Sampling

Study sites were located in the Calabarzon Region, southern Luzon Island, Philippines. Four caves and surrounding karst forest areas were sampled between 2021 and 2023: (1) Cathedral Cave in Cavinti, Laguna Province, (2) Sungwan Cave in Tayabas City, Quezon Province, (3) Kamantigue Cave in Lobo, Batangas Province, and (4) Pamitinan Cave inside the Pamitinan Protected Landscape (PPL), Rodriguez, Rizal Province (Figure 1). We captured bats from sunset (1800 h) until 2000 h using mist-nets (12 x 2.6 m with 36 mm mesh). Nets were set up in cave openings, forest interior, and across water bodies and were checked at 10 min intervals.

Bat captures were identified to species level using external characters and morphometric measurements such as forearm length (FA), following an identification guide by Ingle & Heaney (1992). Wing biopsy tissue samples from released individuals or muscle tissues from voucher specimens were also collected for molecular analysis. All voucher specimens were deposited at

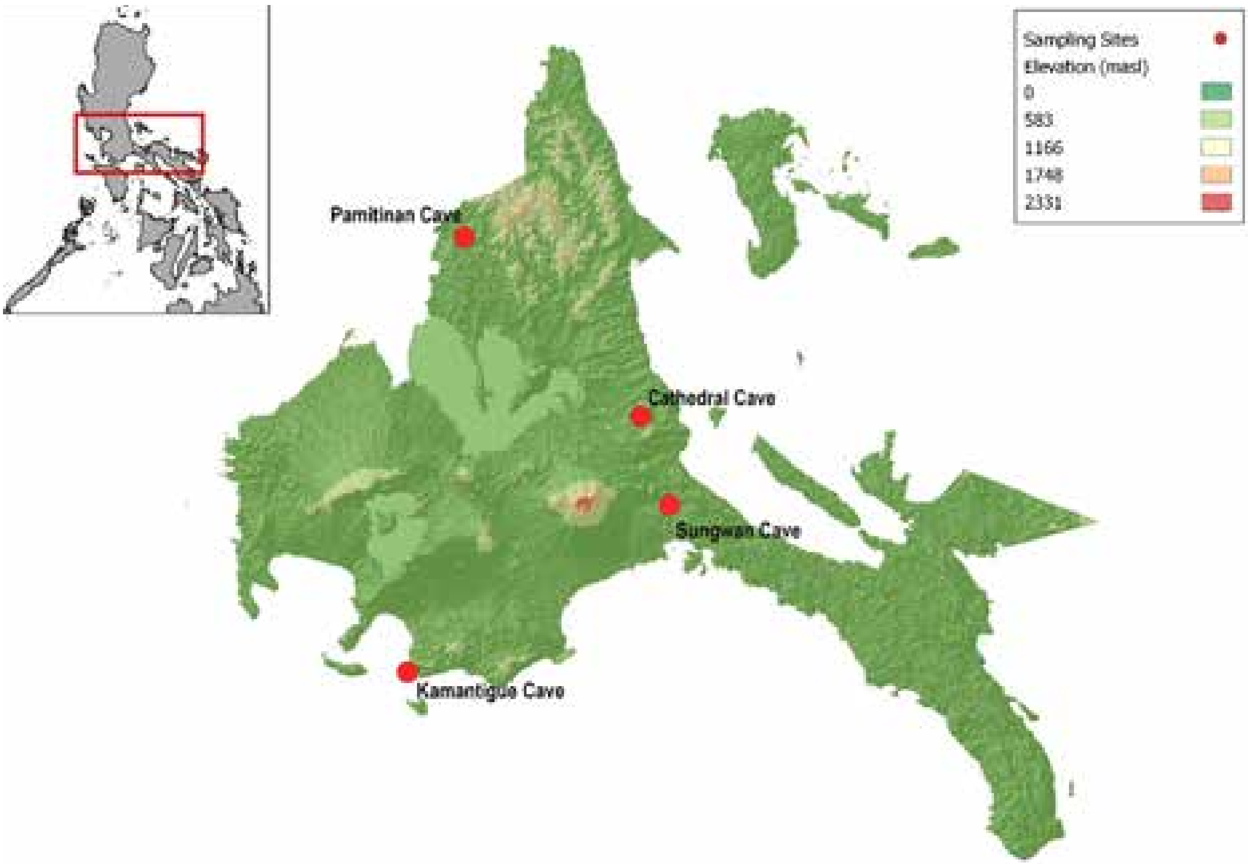

Figure 1. CALABARZON Region depicting locations of caves surveyed for sampling during this study.

University of the Philippines Los Baños-Museum of Natural History Zoological Collection. Field sampling was covered by Wildlife Gratuitous Permit numbers R4A-WGP-2021-LAG-004, R4A-WGP-2021-QUE-005, R4A-WGP-2021-BAT-006, and R4A-WGP-2021-RIZ-010.

Acoustic recording and description of echolocation calls

Echolocation calls were recorded using M500 USB Ultrasound Microphone attached to a laptop PC (Pettersson Elektronic AB, Upsala, Sweden) with a sampling rate of up to 768 kHz and a frequency range of 5–235 kHz. Recordings were made from adult bats released in an enclosure (polyester camping tent with dimensions 2.74 x 2.1 x 1.5 m) to allow recording of echolocation call of bats on free flight for maximum of one minute per individual. Calls were recorded near the sampling site within two hours after retrieval. Call recordings were saved in WAV format on a flash card and call files were displayed as spectrograms using BatSound v. 4.2.1 (Pettersson Elektronik AB) with a sampling rate of 500 kHz with 16 bits/sample. Spectrograms were examined using 512-size fast fourier transformation (FFT) in a Hanning window. Three high quality search calls with high signal-to-noise ratio were chosen for analysis from each individual.

The following call parameters were measured from the spectrograms of each selected call (Figure 2): maximum frequency (Fmax), minimum frequency (Fmin), initial frequency (Fini), terminal frequency (Fter), call duration (D); frequency is given in kilohertz (kHz) while time is expressed in millisecond (ms). In addition, frequency at maximum energy (FmaxE) was measured from the power spectra.

Based on spectrograms, calls were described on the basis of their shape: (1) CF/FM call – consists of a constant frequency component terminated by a frequency modulation, (2) FM/CF/FM – constant frequency

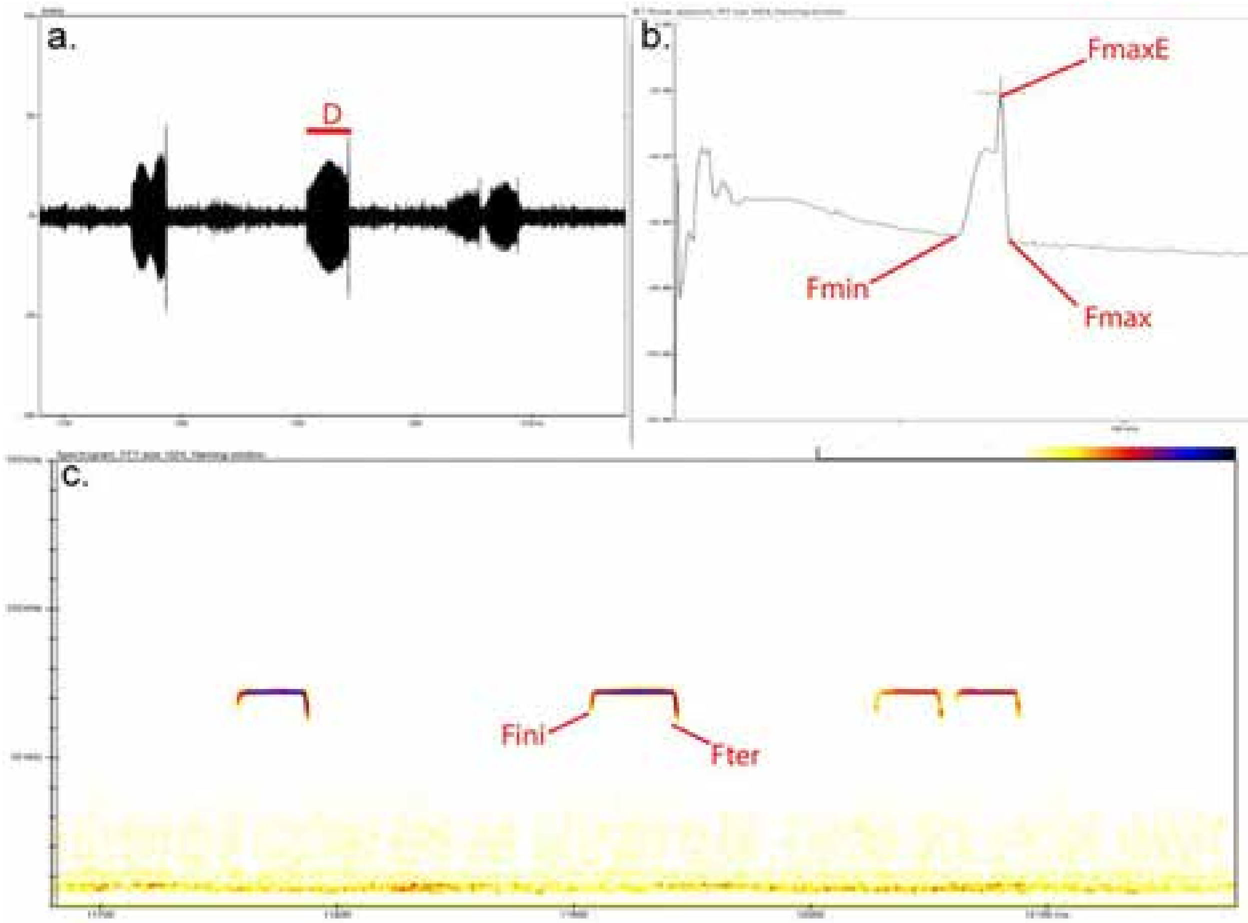

Figure 2. Call parameters extracted from call recordings of bats collected. a. Oscillogram of BatSound software where call duration (D) was measured; b. Power spectrum of the software showing the call variables frequency at maximum energy (FmaxE), minimum frequency (Fmin), and maximum frequency (Fmax); c. Spectrogram where initial frequency (Fini) and terminal frequency (Fter) were measured.

preceded and terminated by frequency modulation component, (3) FM – composed mainly of a steep pure frequency modulated sweep, and (4) Multiharmonic – pulses composed of two or more harmonics.

To investigate inter- and intraspecific variation in echolocation calls for the species we have sampled, we tabulated and analyzed available acoustic metrics reported elsewhere. This includes published research papers, bat acoustic identification guides, and local bat call libraries.

Statistical analysis

Intraspecific variation in call frequency across our samples was first investigated by performing Kruskal-Wallis test with post-hoc Mann-Whitney test. We compared echolocation call parameters between sexes and across the four study areas. No significant difference in the call parameters was observed for all species analyzed ($p < 0.05$), thus, data were pooled in subsequent analyses.

Discriminant function analysis (DFA) with leave-one-out cross-validation was used to determine whether species could be separated in independent groups and to test the extent to which the measured call parameters could be used to identify species (Fils et al. 2018). Except for bats with multiharmonic calls, we carried out DFA separately for each of the three call types identified: CF/FM bats (Hipposideridae), CF/FM/CF bats (Rhinolophidae), and FM-dominated bats (Vespertilionidae and Miniopteridae). Wilk's lambda values were obtained to test for statistical significance of the discriminant functions in discriminating calls between species (Pedro & Simonetti 2013). We also plotted group centroids with 95% confidence limits to present a graphical representation of the separation of species within families based on their discriminant functions.

Lastly, descriptive statistics (mean ± SE) for all call parameters were also computed for each species. All analyses were performed using IBM SPSS Statistics for Windows v 20.0.

RESULTS

Echolocation call descriptions

In total, we recorded and analyzed 441 echolocation pulses belonging to 147 individuals from six bat families: Hipposideridae (five species), Rhinolophidae (five species), Vespertilionidae (three species), Miniopteridae (two species), Megadermatidae (one species), and Emballonuridae (one species) (Table 1).

Hipposideridae calls showed the typical CF/FM call characteristic of the family wherein calls begin with a constant frequency component then terminate with a descending frequency modulated component (Figure 3A). Call frequency values were highest for *Hipposideros antricola*, followed by *Hipposideros bicolor*, and *Hipposideros pygmaeus* (Table 1). Call duration was longest for *H. diadema* (13 ms). All of the call parameters measured did not overlap between the five species.

Rhinolophidae calls were characterized by a long CF component preceded and terminated by an FM component (Table 1, Figure 3B). FmaxE values ranged from 28.2–40.0 kHz in *Rhinolophus philippinensis* to 73.3–76.3 kHz in *R. macrotis*. Most of the call parameters measured showed little to no overlap in values between species.

Vespertilionidae produced predominantly frequency-modulated (FM) calls (Figure 3C). Two species of the genus *Myotis* had calls characterized by a steep FM sweep of short duration (<4 ms). Based on the call parameters analyzed, the two *Myotis* species can easily be distinguished from one another; *Myotis horsfieldii* had lower call frequency values for all the parameters measured than *Myotis muricola* (Table 1). Meanwhile, calls of species within genus *Miniopterus* and *Tylonycteris* have steep FM components terminated by a short narrowband tail (Figure 3C). Between the two *Miniopterus* species, *M. paululus* emitted higher frequency for all call parameters measured (Table 1). Call measurements of *Tylonycteris pachypus* meanwhile overlapped with those of *M. paululus*.

Megadermatidae call was characterized by broadband FM, multi-harmonic signals of short duration. In contrast, calls of *Taphozous melanopogon* are characterized by having long multiharmonic call signals with most energy contained on the first three harmonics (Figure 3D).

Discriminant function analysis (DFA)

Hipposideridae (CF/FM)

In total, 97.8% of the original grouped cases were correctly classified to the five hipposiderid species (Wilk's $\lambda = 0.003$, $p < 0.001$) with Discriminant functions (DF) 1 and 2 explaining 97.1% and 2.9% of the total variance observed, respectively (Figure 4). Among the call parameters used in DFA, FmaxE was the most useful in discriminating between the species (Wilk's $\lambda = 0.042$, $p < 0.001$). Classification rates for the *Hipposideros* species are high based on the results of DFA; all species except *H. antricola* can be identified unambiguously with 100% success classification rate.

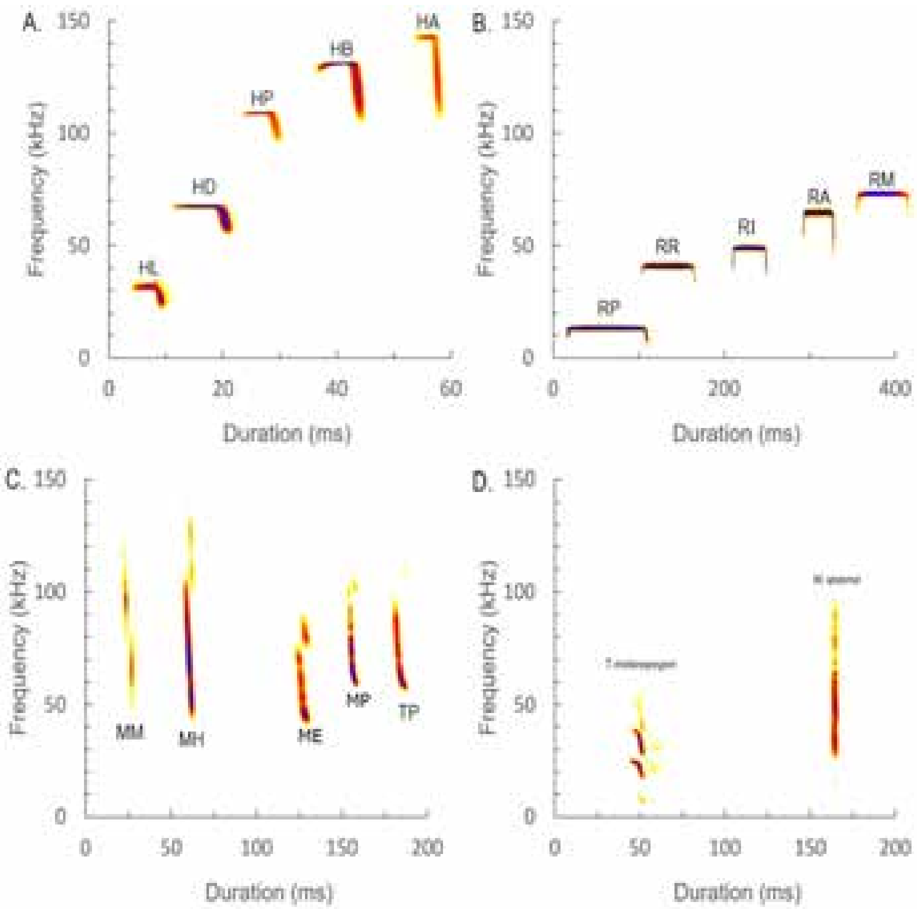

Figure 3. Spectrograms of representative echolocation calls of insectivorous bats recorded from caves and karst areas in southern Luzon Island, Philippines: A. Hipposideridae (HA: *H. antricola*, HB: *H. bicolor*, HD: *H. diadema*, HL: *H. lekaguli* HP: *H. pygmaeus*), B. Rhinolophidae (RP: *R. philippinensis*, RR: *R. rufus*, RI: *R.* inops, RA: *R. arcuatus*, RM: *R. macrotis*), C. Vespertilionidae (MM: *M. muricola*, MH: *M. horsfieldii*, MP: *M. paululus*, ME: *M. eschscholtzii*, TP: *T. pachypus*), and D. Multiharmonic bat calls.

Rhinolophidae (FM/CF/FM)

DFA analysis using the six acoustic parameters gave an overall correct classification of 99.4% of the calls after cross-validation (Wilk's λ = 0.006, p <0.001) (Figure 5). Further, 99.1% of the variation was explained by the first two discriminant functions, with FmaxE being the most important parameter in discriminating between species (Wilk's λ = 0.03, p <0.001). Calls emitted by all rhinolophids were 100% correctly identified and grouped independently of the rest of the species, except for *R. philippinensis* with 93.3% correct classification rate.

Vespertilionidae and Miniopteridae (FM-dominated)

Cross-validated DFA analysis resulted in 86.7% correct classification rate (Wilk's λ = 0.046, p <0.001) (Figure 6). The most important variable in discriminating between the three species was minimal frequency (Wilk's λ = 0.169, p <0.001) and terminal frequency (Wilk's λ = 0.136, p <0.001). Correct classification rates (100%) were achieved for the three vespertilionids: *M. horsfieldii*, *M. muricola*, and *M. eschscholtzii*. Meanwhile, cross validated DFA for *M. paululus* and *T. pachypus* showed <20% misclassification rate to each other.

DISCUSSION

Acoustic identification and DFA Classification success

Reference calls were collected from 147 bat individuals across 17 species and six families. These calls provided additional data and contributed to efforts in building a call library for the acoustic identification of bats in the Philippines (Amberong et al. 2021). Moreover, these acoustic data will also be of great help in developing acoustic classifiers in the future, to utilize passive acoustic monitoring more effectively.

Table 1. List of insectivorous bats collected in four caves sampled in southern Luzon Island, Philippines including their call structure and summary statistics (mean ± SE) for all echolocation call parameters measured (D: Duration, FmaxE: frequency at maximum energy, Fini: initial frequency, Fter: terminal frequency, Fmax: Maximum frequency, Fmin: minimum frequency, n: number of calls analyzed, nInd: number of individual bats recorded). "+" indicates the presence of the species in the study areas (CC: Cavinti Cave, SC: Sungwan Cave, PC: Pamitinan Cave, KC: Kamantigue Cave).

Species	n	nInd	Call structure	D (ms)	FmaxE (kHz)	Fini (kHz)	Fter (kHz)	Fmax (kHz)	Fmin (kHz)	CC	SC	PC	KC
Hipposideridae													
Hipposideros antricola	21	7	CF/FM	6.03 ± 0.25	146.00 ± 1.6	144.21 ± 1.2	124.73 ± 2.5	146.09 ± 0.9	124.15 ± 2.9			+	+
Hipposideros bicolor	42	14	CF/FM	4.93 ± 0.7	133.24 ± 8.4	131.65 ± 9.3	111.64 ± 14.7	133.57 ± 7.2	109.59 ± 15.9				+
Hipposideros diadema	15	5	CF/FM	13.41 ± 2.8	69.05 ± 3.1	67.89 ± 2.7	57.48 ± 4.2	70.21 ± 2.0	55.78 ± 4.6		+		
Hipposideros lekaguli	33	11	CF/FM	8.48 ± 2.6	37.18 ± 1.8	36.15 ± 2.5	31.48 ± 3.0	38.09 ± 1.7	30.56 ± 2.5	+	+		
Hipposideros pygmaeus	24	8	CF/FM	3.93 ± 1.6	110.5 ± 0.8	109.13 ± 1.9	94.27 ± 1.9	112.23 ± 0.7	93.30 ± 0.2	+			
Rhinolophidae													
Rhinolophus arcuatus	108	36	FM/CF/FM	36.82 ± 0.9	65.76 ± 0.2	58.47 ± 0.3	53.51 ± 0.3	66.36 ± 0.9	50.96 ± 0.3	+	+	+	+
Rhinolophus inops	6	2	FM/CF/FM	49.10 ± 2.19	49.55 ± 0.22	43.13 ± 0.88	41.78 ± 0.76	50.37 ± 0.16	37.35 ± 0.51		+		
Rhinolophus macrotis	6	2	FM/CF/FM	36.65 ± 2.07	74.60 ± 0.42	66.40 ± 0.96	64.23 ± 0.74	74.38 ± 0.63	61.56 ± 0.59		+		
Rhinolophus philippinensis	15	5	FM/CF/FM	73.01 ± 3.30	30.73 ± 0.71	26.23 ± 0.44	24.50 ± 0.46	31.47 ± 0.63	22.44 ± 0.56	+	+		
Rhinolophus rufus	30	10	FM/CF/FM	47.74 ± 2.15	42.05 ± 7.1	34.75 ± 1.4	33.03 ± 1.0	42.39 ± 0.9	31.7 ± 1.3	+	+		
Vespertilionidae													
Myotis horsfieldii	9	3	FM	3.65 ± 0.11	70.02 ± 0.77	106.6 ± 0.79	42.37 ± 0.28	109.47 ± 0.71	39.33 ± 0.22	+	+		
Myotis muricola	6	2	FM	3.43 ± 0.18	82.37 ± 0.82	108.22 ± 1.45	44.27 ± 1.28	112.2 ± 2.00	42.17 ± 1.04		+		
Tylonycteris pachypus	6	2	FM/QCF	3.15 ± 0.5	69.37 ± 1.4	111.21 ± 11.6	59.52 ± 3.1	115.96 ± 11.6	55.8 ± 4.1			+	
Miniopteridae													
Miniopterus paululus	57	19	FM/QCF	2.68 ± 0.10	70.77 ± 0.27	109.38 ± 1.70	61.82 ± 0.37	120.42 ± 2.16	60.94 ± 0.31	+	+	+	
Miniopterus eschscholtzii	12	4	FM/QCF	3.26 ± 0.40	53.13 ± 0.39	97.25 ± 1.27	45.88 ± 0.33	99.9 ± 1.38	44.25 ± 0.71		+	+	
Emballonuridae													
Taphozous melanopogon	45	15	Multiharmonic	4.01 ± 0.40	28.4 ± 0.31	29.4 ± 0.22	20.16 ±0.65	29.9 ± 0.21	22.18 ± 0.16				+
Megadermatidae													
Megaderma spasma	6	2	Multiharmonic	2.90 ± 0.21	48.0 ± 0.23	72.0 ± 0.62	40.0 ± 3.01	115.1 ± 2.63	17.2 ± 1.61	+	+		

Our results demonstrate accurate classification of bat calls to families by considering their call structure, and identification to species level to some extent by analyzing several echolocation call parameters using DFA. Among the CF emitting bats, families Hipposideridae and Rhinolophidae could be distinguished from each other with the presence of an FM sweep preceding the CF component in the latter. Calls of *Hipposideros* species are also generally of shorter duration (<20 ms) compared to rhinolophids (Hughes et al. 2010). Most of the call parameters measured showed little to no overlap in values between species, indicating the reliability of utilizing these variables for acoustic identification of these bats in our study site.

Meanwhile, calls of FM bats can easily be distinguished from the other families by having calls of short duration and a steep FM component. Within this group, calls could further be classified into those which have pure and steep FM sweep (genus *Myotis*) and with a narrowband tail terminating the FM sweep (genus *Miniopterus* and *Tylonycteris*). Lastly, *Taphozous melanopogon* (Emballonuridae) and *Megaderma*

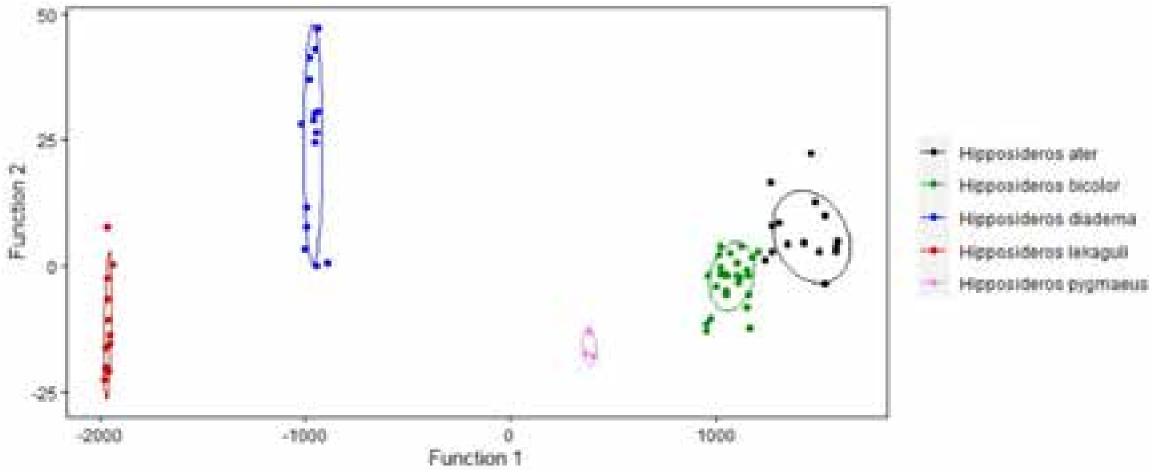

Figure 4. Canonical discriminant function biplots showing groupings of echolocation calls for the five *Hipposideros* species sampled in Luzon Island.

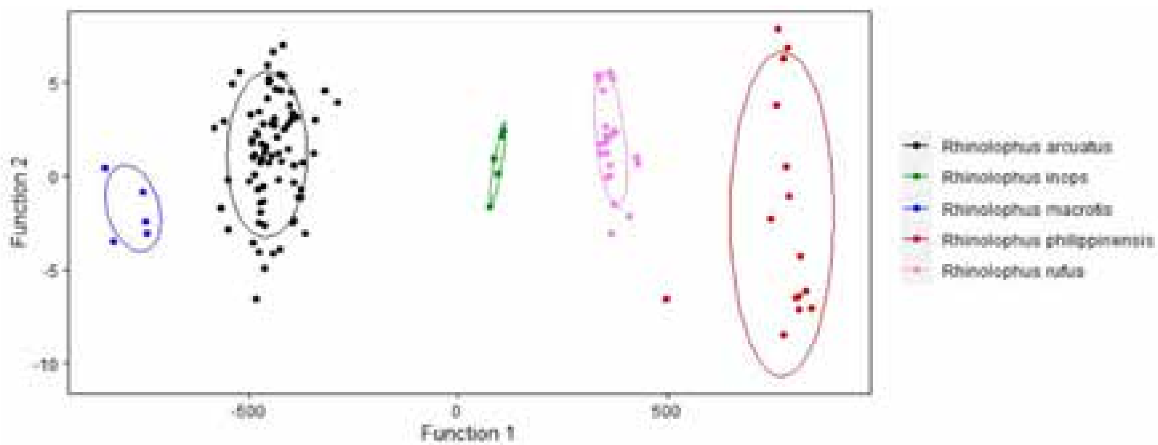

Figure 5. Canonical discriminant function biplots showing groupings of echolocation calls for the five phonic groups of *Rhinolophus* sampled in Luzon Island.

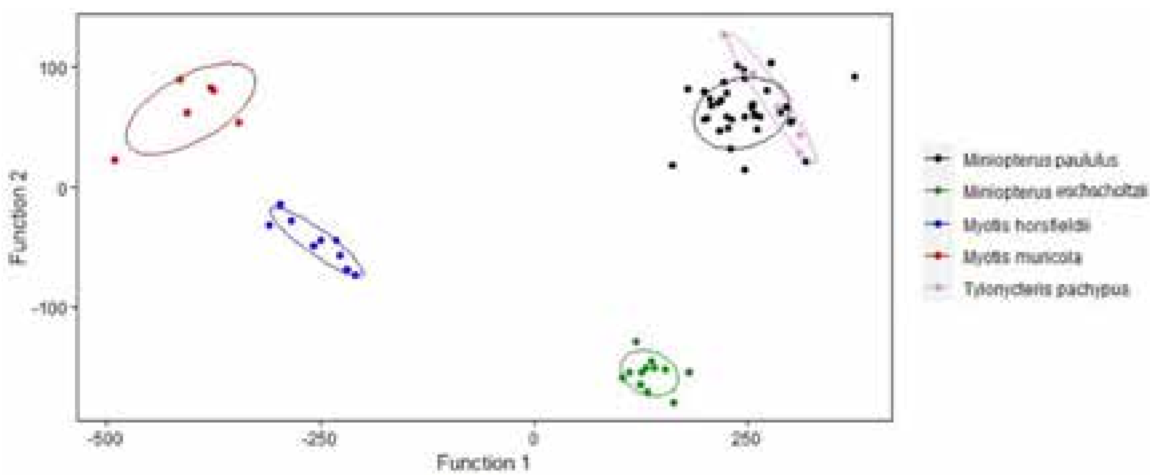

Figure 6. Canonical discriminant function biplots showing groupings of echolocation calls for the FM bats sampled in Luzon Island.

spasma (Megadermatidae) could be unambiguously identified by the presence of multiharmonic calls, the latter emitting broadband FM signals of short duration while the former having longer call duration at lower frequency.

Overall cross-validated DFA resulted in >88% correct classification to species for each family. Calls within each family have high rates (>80%) of classification to species, with most of the species (11 out of 15 species subjected to DFA) classified correctly. However, considering morphology for species discrimination is still important to avoid the risk of misidentification for some species which have overlapping call measurements. For instance, calls of *M. paululus* and *T. pachypus* have relatively lower rate of correct classification to each respective species based on the DFA (80.7% and 83.3%, respectively) but can easily be distinguished based on morphometrics.

Examining geographic variation in echolocation call characteristics based on previous records

As intraspecific variation in call frequency due to geographic location has been observed in many species of echolocating bats, it is essential to collect reference recording from as many locations as possible to reliably identify species whose call parameters overlap with those of others across their known distribution range, determine the accuracy of existing reference call data from different regions and localities, and help in identifying potential novel and cryptic species which have great implications in conservation management (Hughes et al. 2010; Wordley et al. 2014). Based on the compiled list of available acoustic data for the species we recorded, most of the species exhibited variation in their echolocation call frequencies across their range (Table 2), although very few data are publicly available for some species such as *R. inops*, *R. rufus*, and *H. lekaguli*. Further, echolocation call data for most islands and biogeographic regions in the Philippines are virtually absent, with most studies concentrated on Greater Luzon and central Philippine islands. This highlights the need for more acoustic studies in the country to generate a more reliable call library for Philippine bats.

CF bats (Hipposideridae and Rhinolophidae)

This study provided additional acoustic data for some endemic species of CF bats within the Philippines, which is useful for examining possible local variation in their call frequencies across the archipelago. For instance, acoustic data for *H. pygmaeus* are limited to those captured in central Philippine Islands such as Cebu, Bohol, and Siquijor (Sedlock et al. 2014a,b; Phelps et al. 2018). *H. pygmaeus* in these islands have average FmaxE values ranging from 93–102 kHz, which is relatively lower compared to the FmaxE value of 110 kHz recorded in this study. There is still great uncertainty on the taxonomic validity of Philippine hipposiderids which is evident in the recent molecular phylogenetic study done by Esselstyn et al. (2012) which suggested *H. pygmaeus* may comprise of three species.

Meanwhile, the two endemic rhinolophids in this study have little acoustic data reported to date. For instance, call data for *R. rufus* is limited to those collected in Bohol Island; frequency was well within the range with our samples (Sedlock et al. 2014a; Phelps et al. 2018). *R. rufus* is one of the largest insectivorous bats and currently under near threatened category by the IUCN (IUCN 2022). Little is known about its taxonomy due to lack of genetic and acoustic data for this species. Meanwhile, this is the third study to document and measure the echolocation call of *R. inops*; the first was by Sedlock et al. (2014b) in Bohol Island which recorded an average FmaxE value of 54 kHz, while Dimaculangan et al. (2019) recorded an average FmaxE value of 54.3 kHz for this species in Mt. Makiling in Luzon Island. Meanwhile, FmaxE of *R. inops* collected from this study averages at 50 kHz, which is slightly lower than the previous records. Additional acoustic data for these poorly known endemic species recorded from different localities in the country may be needed to further examine possible local dialects.

Meanwhile, the widespread species of CF bats recorded in this study are believed to comprise of species-complexes and may show variation in their call characteristics over their wide range. Cryptic species producing calls at different frequencies have been a recurring theme among CF bats (Kingston et al. 2001). For instance, FA length and FmaxE values of *R. philippinensis* recorded in the Philippines (Luzon Island: 28–30 kHz, Bohol Island: 31–32 kHz) (Sedlock et al. 2014a; Phelps et al. 2018; Amberong et al. 2021; this study) closely resemble the 'large form' (FA length: 52–59 mm) of *R. philippinensis* recorded in Australia (28–34 kHz) than the 'small form' (FA length: 50–53.5 mm, FmaxE: 40 kHz) (Pavey & Kutt 2008). Further, calls of *R. philippinensis* samples from the Philippines is close or well within the range of call frequencies of the species recorded in Borneo (32.8–34.8 kHz) (McArthur & Khan 2021) and in Sulawesi, Indonesia (27.2 kHz) (Kingston & Rossiter 2004) but are significantly lower compared to the other morphotypes discovered for the species: the 'small morph' emitting calls with an average of 53.6 kHz

Table 2. Echolocation call frequencies of bats recorded from this study and in other regions and localities.

Species	*n* individuals	Average FmaxE in kHz (range)	Fmax in kHz (range)	Fmin in kHz (range)	Country/ Region	Locality	Reference	Remarks
Hipposideridae								
Hipposideros antricola	7	146.00 ± 1.6	-	-	Philippines	Batangas	This study	-
	6	134.6 (128.5 – 138.1)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	6	138.6 ± 4.84	-	-	Philippines	Camarines Sur	Esselstyn et al. 2012	-
	9	140.3 ± 2.6 (134–143)	-	-	Philippines	Laguna	Sedlock 2001	-
	1	142	-	-	Philippines	Bohol	Esselstyn et al. 2012	-
	-	138.6	-	-	Philippines	Bohol	Phelps et al. 2018	-
	1	140	-	-	Philippines	Bohol	Sedlock et al. 2014a	-
Hipposideros bicolor	14	133.24 ± 8.4	-	-	Philippines	Batangas	This study	-
	1	136.2	-	-	Philippines	Quezon	Esselstyn et al. 2012	-
	2	126.4 ± 7.9 (119.0 – 133.7)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	2	111.1 ± 2.76	-	-	Philippines	Bohol	Esselstyn et al. 2012	-
	2	109.5 ± 2.1 (108.0–111.0)	-	-	Philippines	Bohol	Sedlock et al. 2014a	-
	-	133.13	-	-	Thailand	-	Hughes et al. 2010	-
	-	138	-	-	Indonesia	Sumatra	Huang et al. 2019	-
	-	133.3–143.1	-	-	Thailand	Satun	Bumrungsri 2010	-
	39	132.4 ± 2.4 (121.5 – 135.5)	-	-	Borneo	-	McArthur & Khan 2021	-
	-	136	-	-	Malaysia	-	Heller & Helversen 1989	-
	-	163.1–169.5	-	-	India	Madurai	Jones et al. 1994	-
Hipposideros diadema	5	69.05 ± 3.1	-	-	Philippines	Quezon	This study	-
	6	67 ± 0.9 (66–68)	-	-	Philippines	Makiling	Sedlock 2001	-
	1	69.5	-	-	Philippines	Quezon	Esselstyn et al. 2012	-
	<10	(68.0–70.0)	-	-	Philippines	Laguna	Sedlock et al. 2019	-
	6	70.0 ± 0.9 (66.5 – 72.0)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	164	69.4±0.1 (66.9–70.8)	-	-	Philippines	Panay	Mould 2012	-
	1	69.3	-	-	Philippines	Bohol	Phelps et al. 2018	-
	12	68.8 ± 1.1 (66.5–70.0)	-	-	Philippines	Bohol	Sedlock et al. 2014a	-
	12	69.5 ± 1.02	-	-	Philippines	Bohol	Esselstyn et al. 2012	-
	5	59.08 ± 0.24 (58.82–59.26)	-	-	India	Andaman Islands	Srinivasulu et al. 2016	-
	-	54.6–55.3	-	-	Thailand	Satun	Bumrungsri 2010	-
	-	61.45	-	-	Thailand	-	Hughes et al. 2010	-
	-	60	-	-	Thailand	-	Robinson 1996	-
	-	57.6	-	-	Indonesia	Sumatra	Huang et al. 2019	-
	2	67.52±2.26 (65.26–69.78)	-	-	Malaysia	Sarawak	Jinggong & Khan 2022	-
	21	67.5 ± 1.2 (65.1 – 69.4)	-	-	Borneo	-	McArthur & Khan 2021	-

Species	*n* individuals	Average FmaxE in kHz (range)	Fmax in kHz (range)	Fmin in kHz (range)	Country/ Region	Locality	Reference	Remarks
	3	65 ± 0.7 (64.5 – 66.7)	-	-	Brunei	-	Aylen 2021	-
	-	54.9	-	-	Australia	-	Fenton 1982	-
	1	56.94 (54–59)	-	-	PNG	Libano Sok	Leary & Pennay 2011	-
Hipposideros lekaguli	11	37.18 ± 1.8	-	-	Philippines	Laguna	This study	-
	-	49.73	-	-	Thailand	-	Hughes et al. 2010	-
	-	45–46	-	-	Malaysia	-	Wilson & Mittermeier 2019	-
Hipposideros pygmaeus	8	110.5 ± 0.8	-	-	Philippines	Laguna	This study	-
	15	111.4 ± 3.3 (105.5 – 115.7)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	17	93.0 ± 1.4 (90.0–95.0)	-	-	Philippines	Bohol	Sedlock et al. 2014a	-
	13	93.0 ± 1.35	-	-	Philippines	Bohol	Esselstyn et al. 2012	-
	-	95.5	-	-	Philippines	Bohol	Phelps et al. 2018	-
	11	102 (90.8–105.4)	-	-	Philippines	Cebu	Sedlock et al. 2014b	-
Rhinolophidae								
Rhinolophus arcuatus	36	65.76 ± 0.2	-	-	Philippines	Southern Luzon	This study	-
	13	71.2± 0.4 (71–72)	-	-	Philippines	Laguna	Sedlock 2001	-
	<10	(46.8–50.0)	-	-	Philippines	Laguna	Sedlock et al. 2019	-
	16	71.9 ± 1.5	-	-	Philippines	Laguna	Dimaculangan et al. 2019	-
	21	69.84 ± 1.70	-	-	Philippines	Quezon	Sedlock & Weyandt 2009	Narrow sella morph
	15	65.92 ± 2.30	-	-	Philippines	Quezon	Sedlock & Weyandt 2009	Wide sella morph
	29	66.81 ± 2.04 (62.17–69.34)	-	-	Philippines	Polilio Island	Taray et al. 2021	-
	23	65.0 ± 1.8 (61.8 –67.0)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	10	69.2±0.1 (68.3–69.3)	-	-	Philippines	Panay	Mould 2012	-
	11	67.48 (66.7–68.5)	-	-	Philippines	Cebu	Sedlock et al. 2014b	-
	-	68.7	-	-	Philippines	Bohol	Phelps et al. 2018	-
	32	68.7 ± 1.4 (67.0–72.0)	-	-	Philippines	Bohol	Sedlock et al. 2014a	-
	1	71.3 (70–72)	-	-	PNG	Libano Sok	Leary & Pennay 2011	-
	62	66.5 (58–69)	-	-	Malaysia	-	Novick 1958	-
Rhinolophus inops	2	49.55 ± 0.22	-	-	Philippines	Quezon	This study	FA length: 53mm
	9	54.3 ± 1.3	-	-	Philippines	Laguna	Dimaculangan et al. 2019	-
	12	54 (52.7– 55)	-	-	Philippines	Cebu	Sedlock et al. 2014b	-
Rhinolophus macrotis	2	74.60 ± 0.42	-	-	Philippines	Quezon	This study	FA length: 40mm
	2	74.0 ± 0.3 (73.7 – 74.4)	-	-	Philippines	Bulacan	Amberong et al. 2021	FA length: 41
	9	52.1 ± 0.80 (51–53)	-	-	Philippines	Laguna	Sedlock 2001	FA length: 44.1 – 46.4
	12	50.9 ± 1.1	-	-	Philippines	Laguna	Dimaculangan et al. 2019	FA length: 43 – 47

Species	*n* individuals	Average FmaxE in kHz (range)	Fmax in kHz (range)	Fmin in kHz (range)	Country/ Region	Locality	Reference	Remarks
	<10	(46.8–50.0)	-	-	Philippines	Laguna	Sedlock et al. 2019	-
	2	50	-	-	Philippines	Bohol	Sedlock et al. 2014a	FA length:44.1 – 46.3 (45.2)
	1	50	-	-	Philippines	Bohol	Phelps et al. 2018	-
	-	48	-	-	Philippines	-	Heller & Helversen 1989	FA length: 46.5
	1	75.1	-	-	Vietnam	Cu Lao Cham and Ly Son Archipelagos	Thong et al. 2019	FA length: 39.1
	-		-	-	Vietnam	Phia Oac	Tu et al. 2017	FA length: 48.6
	11	66.4 ± 0.9 (65.2–67.7)	-	-	Vietnam	-	Furey et al. 2009	-
	10	48.8 ± 0.6	-	-	China	Jiangxi	Sun et al. 2008	"large form", FA length: 45.2 ± 3.7
	2	64.7 ± 0.3	-	-	China	Jiangxi	Sun et al. 2008	"small form", FA length: 39.5–40
	9	57.3 ± 0.6	-	-	China	Yunnan	Sun et al. 2008	FA length: 42–43.5
	28	57.10 ± 0.68 (65.2–67.7)	-	-	China	Yunnan	Shi et al. 2009	FA length:41.8 ± 0.16
	6	66.7 ± 0.6	-	-	China	Guangxi	Sun et al. 2008	FA length: 39–40.5
	6	(47.2–53.9)	-	-	China	-	Zhang et al. 2009	FA length: 46.9–49.9
Rhinolophus philippinensis	5	30.73 ± 0.71	-	-	Philippines	Laguna, Quezon	This study	FA length:54–57mm
	2	28.9 ± 0.6 (28.2 – 29.5)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	5	31.2 ± 0.5 (31.0–32.0)	-	-	Philippines	Bohol	Sedlock et al. 2014a	-
	-	31.2	-	-	Philippines	Bohol	Phelps et al. 2018	-
	6	27.2 ± 0.2	-	-	Indonesia	Sulawesi	Kingston & Rossiter 2004	"large morph" FA length: 56.1 ± 1.5 mm
	3	39.0 ± 0.8	-	-	Indonesia	Buton Island	Kingston & Rossiter 2004	"Buton intermediate" FA length: 50.6 ± 1.4 mm
	1	41.7	-	-	Indonesia	Kabaena Island	Kingston & Rossiter 2004	"Kabaena intermediate" FA length: 48.4 mm
	11	53.6 ± 0.6	-	-	Indonesia	Sulawesi	Kingston & Rossiter 2004	"small morph" FA length: 47.0 ± 0.4 mm
	32	33.8 ± 0.5 (32.8 – 34.8)	-	-	Borneo	-	McArthur & Khan 2021	-
	-	36.6	-	-	Borneo	-	Francis & Habersetzer 1998	-
	-	(28–34)	-	-	Australia	-	Pavey & Kutt 2008	Large form FA length: 52–59mm
	-	40	-	-	Australia	-	Pavey & Kutt 2008	Small form FA length: 50–53mm
Rhinolophus rufus	10	42.05 ± 7.1	-	-	Philippines	Southern Luzon	This study	-
	-	39.5	-	-	Philippines	Bohol	Phelps et al. 2018	-
	9	39.5 ± 1.1 (39.0–41.9)	-	-	Philippines	Bohol	Sedlock et al. 2014a	-

Species	*n* individuals	Average FmaxE in kHz (range)	Fmax in kHz (range)	Fmin in kHz (range)	Country/ Region	Locality	Reference	Remarks
Vespertilionidae								
Myotis horsfieldii	3	70.02 ± 0.77	109.47 ± 0.71	39.33 ± 0.22	Philippines	Laguna, Quezon, Rizal	This study	-
	9	-	91.4 ± 14.1 (67–108)	47.6 ± 5.6 (38–58)	Philippines	Laguna	Sedlock 2001	-
	<10	(47.8–59.5)			Philippines	Laguna	Sedlock et al. 2019	-
	-	47.6	-	-	Philippines	Bohol	Phelps et al. 2018	-
	8	56.93 ± 7.98	134.25 ± 9.60	38.38 ± 3.46	Thailand	-	Hughes et al. 2011	-
	59	53.8 ± 5.14 (37.9–101)	-	-	India	Western Ghats	Wordley et al. 2014	-
	4	64.77 ± 3.91 (58.8–72.4)	104.29 ± 5.13 (94.1–113.5)	42.28 ± 4.29 (37.2–52.1)	India	Andaman Islands	Srinivasulu et al. 2017	-
	3	70.32 ± 17.58 (52.74–87.90)	100.61 ± 11.67 (88.94–112.28)	43.81 ± 6.26 (37.55–50.07)	Malaysia	Sarawak	Jinggong & Khan 2022	-
	5	58.0 ± 6.0 (48.4 – 63.1)	-	-	Borneo	-	McArthur & Khan 2021	-
	5	64 ± 7.2 (54.9–101.8)	103.2 ± 10.6 (78.5–122.9)	43.8 ± 3.7 (37.7–51.7)	Vietnam		Nguyen et al. 2021	-
	10	99 ± 7 (86 – 112)	134	79	Brunei		Aylen 2021	-
Myotis muricola	2	82.37 ± 0.82	112.2 ± 2.00	42.17 ± 1.04	Philippines	Quezon	This study	-
	3		67.2± 3.0 (63–71)	51.8± 0.8 (51–53)	Philippines	Laguna	Sedlock 2001	-
	-		105.2 ± 10.2 (87.8–127.7)	63.3 ± 1.3 (61.1–65.8)	Vietnam	Quang Binh	Thong et al. 2022b	-
	4	66.2 ± 0.9 (62.0–73.6)	59.7 ± 0.9 (57–63.6)	54.5 ± 0.9 (51.5–59.2)	Vietnam	-	Furey et al. 2009	-
	2	64.2–76.5	-	-	Thailand	Satun	Bumrungsri 2010	-
	49	82.27 ± 16.63	137.14 ± 12.79	55.33 ± 6.81	Thailand	-	Hughes et al. 2011	-
	4	66.4 ± 2.6 (63.1 – 69.1)	-	-	Borneo		McArthur & Khan 2021	-
	11	64.39 (63.39 –66.15)	126.07 (119.75 –132.62)	50.29 (45.49–54.08)	Borneo	-	Yoon & Park 2016	-
	-	(40–45)	-	-	Nepal		Csorba et al. 1999	-
	18	57.2 ± 0.0	79.9 ± 1.0	53.7 ± 0.48	Singapore		Pottie et al. 2005	-
	2	51.9 ± 2.51	104.7 ± 2.09	47.8 ± 3.66	India	Uttarakhand	Chakravarty et al. 2020	-
	-	63.5	108.2	40.7	Indonesia	Sumatra	Huang et al. 2019	-
	10	56 ± 1 (54 – 59)	118	48	Brunei		Aylen 2021	-
Tylonycteris pachypus	2	69.37 ± 1.4	115.96 ± 11.6	55.8 ± 4.1	Philippines	Rizal	This study	-
	-	69.8 ± 5.6 (76.7 – 61.1)	124.1 ± 7.1 (111.0 – 137.0)	54.2 ± 5.5 (46.0 – 61.0)	Philippines	Bulacan	Amberong et al. 2021	-
	3	63.38±4.07 (59.31–67.45)	97.03±4.90 (92.13–101.93)	54.75±0.98 (53.77–55.73)	Malaysia	Sarawak	Jinggong & Khan 2022	-
	-	-	-	53.5 (51–56)	Malaysia	-	Novick 1958	-
	1	48.2	-	-	Thailand	Satun	Bumrungsri 2010	-
	5	50.46 ± 13.05	134.4 ± 6.69	39.4 ± 4.39	Thailand	-	Hughes et al. 2011	-
	-	61.8	111.7	52.8	Indonesia	Sumatra	Huang et al. 2019	-
	126	65.1±2.8	129.2±7.4	58.3±1.8	China	Guangxi	Zhang et al. 2006	-
	78	76.5±2.1 (62.4–91.6)	91.6±4.5	62.4±3.8	China	Guangxi	Zhang et al. 2002	-
	4	64.7 ± 1.2 (63.9–66.5)	68.5 ± 3 (65–71)	46.3 ± 1.5 (45–48)	Cambodia		Phauk et al. 2013	-

Species	*n* individuals	Average FmaxE in kHz (range)	Fmax in kHz (range)	Fmin in kHz (range)	Country/ Region	Locality	Reference	Remarks
Miniopteridae								
Miniopterus paululus	19	70.77 ± 0.27	120.42 ± 2.16	60.94 ± 0.31	Philippines	Laguna, Quezon, Rizal	This study	-
	10		76.3± 4.7 (73–80)	61.3± 0.64 (60–62)	Philippines	Laguna	Sedlock 2001	-
	<10	(62.0–73.0)	-	-	Philippines	Laguna	Sedlock et al. 2019	-
	-	(55–80)	-	-	Philippines	Laguna	Sedlock et al. 2021	-
	25	69.68 ± 2.14 (66.24–74.06)	125.35 ± 5.72 (112.14–136.39)	58.29 ± 1.86 (53.71–60.71)	Philippines	Polilio Island	Taray et al. 2021	-
	14	72.9 ± 4.1 (63.7 – 90.0)	115.2 ± 12.1 (76.0 – 134.0)	60.7 ± 2.7 (52.0 – 65.0)	Philippines	Bulacan	Amberong et al. 2021	-
	-	65.18	-	-	Philippines	Bohol	Phelps et al. 2018	-
	4	65.5 ± 4.8 (60.7 – 70.4)	-	-	Borneo	-	McArthur & Khan 2021	-
Miniopterus eschscholtzii	4	53.13 ± 0.39	99.9 ± 1.38	44.25 ± 0.71	Philippines	Quezon, Rizal	This study	-
	2	-	69.6± 3.8 (63–77)	45.6 ± 0.7 (44–46)	Philippines	Laguna	Sedlock 2001	-
	4	51.68 ± 1.08 (50.62–52.90)	100.42 ± 3.63 (97.49–105.31)	41.99 ± 1.47 (39.88–43.29)	Philippines	Polilio Island	Taray et al. 2021	-
	1	53.1 ± 2.9 (49.7 – 55.0)	101.7 ± 1.2 (101.0 – 103.0)	44.3 ± 0.6 (44.0 – 45.0)	Philippines	Bulacan	Amberong et al. 2021	-
	1	48.5	-	-	Philippines	Bohol	Phelps et al. 2018	-
Emballonuridae								
Taphozous melanopogon	15	28.4 ± 0.31	-	-	Philippines	Batangas	This study	-
	<10	(26.0–30.0)	-	-	Philippines	Laguna	Sedlock et al. 2019	-
	-	(20–30)	-	-	Philippines	Laguna	Sedlock et al. 2021	-
	6	29.8 ± 0.9 (28.5 – 31.9)	-	-	Philippines	Bulacan	Amberong et al. 2021	-
	-	29.1	-	-	Philippines	Bohol	Phelps et al. 2018	-
	33	29.71 ± 2.67	76.15 ± 20.18	20.37 ± 6.2	Thailand	–	Hughes et al. 2011	-
	2	28.16 ± 1.70 (25.8–32.5)	34.01 ± 0.54 (32.9–35.2)	26.47 ± 0.95 (25.3–28.6)	India	Andaman Islands	Srinivasulu et al. 2017	-
	10	-	32.5 ± 1.7 (30.1–35.2)	20.6 ± 0.6 (19.7–21.6)	Vietnam	Quang Ninh	Thong et al. 2022a	-
	6	27.9 ± 0.56	28.7 ± 1.24	25.2 ± 0.82	Singapore	–	Pottie et al. 2005	-
	1	28.9	–	–	Thailand	Satun	Bumrungsri 2010	-
	12	30.10 ± 3.41	30.14 ± 2.58	22.72 ± 2.62	China	Guangxi	Wei et al. 2008	-
Megadermatidae								
Megaderma spasma	2	48.0 ± 0.23	-	-	Philippines	Quezon	This study	-
	32	20 (17–22)	-	-	Malaysia	-	Novick 1958	-
	2	47.5–58.8	-	-	Thailand	Satun	Bumrungsri 2010	-
	1	83.2	-	-	Thailand	Rawi Island	Bumrungsri 2010	-
	44	72.99 ± 12.52	108.93 ± 8.24	20.8 ± 12.44	Thailand	-	Hughes et al. 2011	-
	59	55.9 ± 12.3 (38.3–91.4)	99.79 ± 12.37 (65.3–113.1)	38.87 ± 2.30 (34.6–44.3)	India	Western Ghats	Wordley et al. 2014	-
	3	21.87 ± 2.36 (18.1–25.2)	67.03 ± 1.86 (64.0–70.0)	14.90 ± 0.68 (14.0–16.0)	India	Andaman Islands	Srinivasulu et al. 2017	-
	-	63	82.9	47.6	Indonesia	Sumatra	Huang et al. 2019	-
	1	69.0 ± 23.3 (52.5 – 85.5)	-	-	Borneo	-	McArthur & Khan 2021	-
	5	65.4 ± 3.1 (61.6–69.3)	70.8 ± 4.3 (65–74)	62.5 ± 2.1 (60–65)	Cambodia	-	Phauk et al. 2013	-

in Sulawesi, Indonesia and 'intermediate forms' calling at 39.0–42.0 kHz in Indonesia (Kingston & Rossiter 2004).

Initial analysis of morphological and acoustic data of *R. macrotis* available in the Philippines revealed that at least two morphs are present: a small morph (FA: 40–41 mm) and a large morph (FA: 43–46 mm) with dominant frequency at 75 kHz and 50 kHz, respectively (Table 2). An extensive morphological and call frequency variation is present in *R. macrotis* populations in the Philippines and thus considered a species complex (Heaney et al. 2016). The *R. macrotis* samples collected in this study as well as those collected in Bulacan (Amberong et al. 2021) resembles the small morph of this species. Interestingly, the frequency values obtained from *R. macrotis* in this study are also closely similar to those collected in Vietnam (75.1 kHz) (Thong et al. 2019).

Meanwhile, different morphotypes and phonic types of *R. arcuatus* have been observed to occur sympatrically in different localities within Luzon Island. For instance, Sedlock et al. (2019) recorded calls of *R. arcuatus* from Mt. Makiling with FmaxE values ranging from 46.8–50 kHz whereas those recorded by Dimaculangan et al. (2019) and Sedlock (2001) from the same locality ranged from 70–72 kHz. Meanwhile, the FmaxE value obtained from *R. arcuatus* in our study (65.76 kHz) is closely similar to the calls obtained from the 'wide-sella' morph (65.98 kHz), one of the noseleaf morphs observed by Sedlock and Weyandt (2009) to occur sympatrically with 'narrow-sella' morph (FmaxE = 69.84 kHz) in Mt. Banahaw, Luzon Island.

Hipposideros antricola and *H. bicolor* are often misidentified in the field due to similar morphological characteristics and overlapping measurements (Heaney et al. 2016; Amberong et al. 2021). In contrast with the recent survey done by Amberong et al. (2021), this study showed higher correct classification rate of calls to each respective species using DFA but found to emit relatively higher frequencies. Meanwhile, the calls of *H. bicolor* reported from Bohol Island are relatively lower (ca. 111 kHz) compared to those in Luzon Island, and may need additional studies.

For *H. diadema*, there appears to be not much variation in its FmaxE when Philippine populations are considered (Table 2). This is consistent with the molecular phylogeny of *H. diadema* presented by Esselstyn et al. (2012) which suggests that only one species is referred to *H. diadema* in the Philippines. However, the same study suggests that there are three species within *H. diadema* throughout its global range and previous records of the echolocation call for this species outside the Philippines showed variation in terms of FmaxE values. For instance, southern and southeastern Asian populations have average FmaxE values ranging from 58–62 kHz (Robinson 1996; Hughes et al. 2010; Srinivasulu et al. 2016) while those in Australia have FmaxE values ranging from 55–57 kHz (Fenton 1982; Leary & Pennay 2011).

This study is the first to report acoustic data for *Hipposideros lekaguli* from the Philippines. This species is generally poorly known in the country, with only few ecological and distribution records reported to date. Previous records of this species from southeastern Asian countries such as Thailand and Peninsular Malaysia showed FmaxE ranging from 45–50 kHz which is relatively higher than those recorded in this study (37 kHz) (Hughes et al. 2010; Wilson & Mittermeier 2019). Further study is needed to assess the taxonomy of the Philippine population of *H. lekaguli*.

FM bats (Miniopteridae and Vespertilionidae)

Among the vespertilionids with calls characterized by a steep broadband FM, acoustic data for *M. horsfieldii* is closely similar to those recorded from Mt. Makiling, Luzon Island (Sedlock 2001) and Thailand (Hughes et al. 2011). Similarly, our acoustic measurements for *M. muricola* showed similarities with other southeastern Asian forms in Malaysia, Vietnam, and Philippines (Sedlock 2001; Furey et al. 2009; Yoon & Park 2016) (Table 2).

Currently, the distribution of *M. paululus* is limited to Indonesia, Malaysia, the Philippines, and Timor-Leste. In the Philippines, there have been recorded call frequencies (FmaxE) for *M. paululus* on Luzon Island ranging from 62.0–73.0 kHz in Mt. Makiling (Sedlock et al. 2019), 73 kHz in Bulacan (Amberong et al. 2021), and an average of 65.9 kHz on Bohol Island (Phelps et al. 2018). However, no acoustic data has been obtained from other areas where this species is known to occur, although McArthur & Khan (2021) reported an average FmaxE value of 65.5 ± 4.8 kHz for individuals they identified as *M. australis* in Borneo.

Miniopterus eschscholtzii was formerly acknowledged as a subspecies of *M. schreibersii*. However, subsequent molecular studies resulted in its reclassification as a distinct species (Akmali et al. 2015; MMD 2021; Kusuminda et al. 2022; Simmons & Cirranello 2023). FmaxE values of the Philippine endemic *Miniopterus eschscholtzii* recorded in this study were within the range of obtained values recorded from other localities within the Philippines: 48.5 kHz in Bohol Island (Phelps et al. 2018), 45.6 kHz in Mt. Makiling, Luzon Island (Sedlock 2001), and 53.1 kHz in Bulacan, Luzon Island (Amberong et al. 2021).

Multiharmonic bats (Emballonuridae and Megadermatidae)

The calls of *Taphozous melanopogon* can easily be distinguished from other species, containing multiharmonic signals of long duration. The fundamental harmonic of its call is often weakly discerned while the first harmonic is the strongest component (Heller 1989). Calls of this species recorded in this study is well within the range of call measurements recorded from other localities such as in Luzon Island (Amberong et al. 2021), Malaysia (Heller 1989; Kruskop & Borisenko 2013), Vietnam (Pham et al. 2021), and Thailand (Thong et al. 2018).

We report the first echolocation call parameters for *Megaderma spasma* in the Philippines. Except for FmaxE, all call parameters are consistent with those reported before by other studies from Thailand (Hughes et al. 2011) and India (Raghuram et al. 2014). FmaxE values recorded in this study (48 kHz) are relatively lower than those recorded from the abovementioned areas (69–73 kHz). As the measurement of calls from this study is limited to only one individual, more samples are needed to evaluate the observed variation in the FmaxE values recorded.

Bat community of caves and karst areas in southern Luzon: conservation status and current threats

Owing to its unique microhabitat and complex terrains, karst forests are recognized as regions of significant biological significance due to the abundance of unique flora and fauna (Duco et al. 2021). Extensive small to large cave systems are present in these landscapes, making them an important habitat for many cave dwelling species such as bats. However, despite their ecological and economic importance, many caves in the Philippines remain vulnerable and continually being subjected to exploitation. Collection of speleothems, guano mining, vandalism, unregulated visitations, and littering pose significant threats to caves and their inhabitants in the Philippines (Tanalgo et al. 2016).

The present study accounts for 17 species of insectivorous bats (Image 1) from the four caves and surrounding karst forest surveyed in southern Luzon Island. Interestingly, new locality records and species of conservation concern were documented. For instance, three endemic species (*R. rufus*, *R. inops*, and *H. pygmaeus*) were recorded from the study areas while eight species were recorded to be new locality records for Batangas province (*T. melanopogon*, *H. antricola*, *H. bicolor*, and *R. arcuatus*), Cavinti, Laguna (*R. philippinensis*, *H. lekaguli*, *H. pygmaeus*, and *M. spasma*), and Tayabas, Quezon (*M. spasma*). In addition, potential novel or cryptic species of insectivorous bats such as *R. macrotis*, *H. bicolor*, and *H. pygmaeus* were also recorded based on observed acoustic divergence between island populations.

Of the species documented in our study areas, two species (*R. rufus* and *H. lekaguli*) are currently under Near Threatened category by the IUCN (IUCN 2022). Both species are highly associated with caves and limestone areas. The occurrence of these species underscores the importance of the caves surveyed as crucial habitat for species of conservation concern. The caves and karst areas within Calabarzon region are subject to human-induced pressure due to rapid deforestation driven by urban development (Fallarcuna & Perez 2015). Thus, protection and proper management is needed to ensure the availability of suitable habitat for these species.

In addition, the IUCN conservation status assessment for most of the species recorded in this study may require an updated revision. For instance, the last conservation assessment for The IUCN Red List of Threatened Species for 10 out of the 17 species recorded (*T. melanopogon*, *H. bicolor*, *H. lekaguli*, *H. pygmaeus*, *R. macrotis*, *R. rufus*, *R. inops*, *M. horsfieldii*, *M. muricola*, and *T. pachypus*) was done in 2018 (IUCN 2022). Additionally, no evaluation or assessment has been conducted for *H. antricola* and *M. eschscholtzii*. Currently, the Red List assessments are considered outdated after 10 years, although more current assessments (ideally 4–5 years) are recommended to ensure best possible information to conservationists are provided (Rondinini et al. 2014; IUCN 2023). With the rapid deforestation and deterioration of environmental conditions in many critical habitat areas in the Philippines, providing an up-to-date evaluation of population status and conservation assessment for these species is warranted to guide critical conservation management actions.

Majority of the bat community of the caves and karst forest visited in this study are also cave dependent species. In general, bat populations in the Philippines are steadily declining and forest degradation, habitat loss, and hunting are considered primary drivers for this trend (Raymundo & Caballes 2016; Quibod et al. 2019; Tanalgo & Hughes 2019). However, as most of our study areas are locally designated ecotourism areas, the most common threats to bats observed include human disturbance due to frequent human visits as well as

Image 1. Insectivorous bats recorded in caves and karst areas of Southern Luzon. A—*H. antricola* | b—*H. bicolor* | c—*H. diadema* | d—*H. lekaguli* | e—*H. pygmaeus* | f—*R. arcuatus* | g—*R. inops* | h—*R. macrotis* | i—*R. philippinensis* | j—*R. rufus* | k—*M. horsfieldii* | l—*M. muricola* | m—*T. pachypus* | n—*M. paululus* | o—*M. eschscholtzii* | p—*T. melanopogon* | q—*M. spasma*. © RADuco, JCTGonzalez, and JAParcon.

land-use changes resulting from development of these tourism areas. For instance, a project to pave the road going to Cathedral Cave in Cavinti, Laguna has recently been completed resulting in evident fragmentation in the karst landscape. Moreover, the project allowed accessibility resulting in increased tourist visits as well as rapid development and construction of human settlements. Meanwhile, in Pamitinan Cave, low population of bats and roost abandonment is apparent probably due to past human activities (tourist visits, removal of speleothems, hunting, vandalism) done inside the cave. Indeed, ecotourism is a rapidly expanding industry and contributes significantly to economic growth (Clements et al. 2006; Tolentino et al. 2020). Further, the essential role of communities in long–term conservation and protection of caves and its resources is well recognized. Thus, careful planning and proper management of these caves as well as strengthening community involvement are needed for this industry to be sustainable, balancing livelihoods as well as protecting wildlife and cave resources.

CONCLUSION

We successfully described echolocation calls of 17 species of insectivorous bats belonging to five families (Hipposideridae, Rhinolophidae, Verpertillionidae, Emballonuridae, and Megadermatidae). Discriminant function analysis (DFA) was able to correctly identify species with high classification rate, providing a feasible and effective tool for conducting future acoustic surveys in the Philippines.

In addition, we provided evidence of possible regional differences in echolocation calls for some of the species we recorded as well as the presence of unrecognized morphs and potential novel cryptic species. This highlights the importance of conducting more acoustic surveys from as many localities as possible because of the observed geographical variations in call frequencies within a species as well as to confirm the presence of local dialects (Hughes et al. 2010). Acoustic analysis can be utilized in conjunction with morphometric and molecular analysis to accurately determine species' taxonomic identities, especially those which are acoustically divergent and morphologically cryptic species. Our results contribute to the growing field of bat bioacoustics in the Philippines and in the development of a robust and well–developed echolocation call library for the country.

Further, this study identified several anthropogenic activities that may pose threat to the bat population in the study areas. Utilizing bat recorders, this study recommends bat emergence watching as an alternative to conventional ecotourism activities, such as visiting roost sites inside the cave, which could potentially disturb bats during sensitive periods like pregnancy, lactation, and weaning (Sheffield 1992; Tanalgo & Hughes 2021). This recreational night activity occurs at cave entrances, allowing tourists to observe bats emerging from their roosts (Kasso & Balakrishnan 2013). Integrating bat recorders to make bat calls audible to visitors will also enhance the tour experience (Wolf & Croft 2012). These activities present avenues to raise local awareness about bat conservation and the importance of caves and present novel guidelines for managing ecotourism activities in caves and karst landscapes.

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ARTICLE

Seasonality, diversity, and forest type associations of macro moths (Insecta: Lepidoptera: Heterocera) in the Shiwalik landscape of northern India and its conservation implications

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Abstract: A study was carried out to evaluate the seasonal diversity of macro moths across different forest sub-types occurring in the Shiwalik landscape of northern India, mainly Uttarakhand and adjoining states of Himachal Pradesh, Uttar Pradesh, and Haryana. Forty-three field surveys of 59 days were carried out from July 2020 to October 2022 using stratified random sampling in each of the 19 selected study sites. Sampling surveys revealed 321 species of moths belonging to 19 families and 49 sub-families. These new range extensions from central Himalaya and northeastern India indicate the affinity of moths found in the northern Indian Shiwaliks with that of the Oriental region. Seasonal trend of species richness showed two annual peaks, with the first peak occurring in August followed by a smaller peak in October, while the seasonal abundance of moth species was maximum in July followed by a smaller peak in September. One-hundred-and-forty species occurred only during the 'monsoon' season indicating their seasonality, univoltine habit and short flight periods in these tropical forests. Species richness of moths correlated positively with relative humidity ($r^2 = 0.100$; $p = 0.0142$; $n = 59$). The most dominant family was Erebidae (95 species) followed by Geometriidae (61), Crambidae (72), and Noctuidae (28), respectively. Maximum number of moth species were sampled in forest sub-type (i) 3C/C2a Moist Shiwalik Sal Forest, followed by (ii) 5B/C2 Northern Dry Mixed Deciduous Forest, (iii) 3C/C2c Moist Tarai Sal Forest, iv) 5B/C1a Dry Shiwalik Sal Forest, respectively. The study also revealed changing moth communities along with the vegetation structure in the Shiwaliks from east (Nandhaur Willife Sanctaury in Uttarakhand bordering Nepal) to west (Simbalbara National Park in Himachal Pradesh,India) across the landscape. The moth communities of (i) 3C/C2a Moist Shiwalik Sal Forest & (ii) 3C/C2c Moist Tarai Sal Forest being different from that of (iii) 5B/C2 Northern Dry Mixed Deciduous Forest, and (iv) 5B/C1a Dry Shiwalik Sal Forest. Besides, six new range extensions into Shiwaliks of northern India from central Nepal and northeastern India, namely: *Chlorozancla falcatus* (Butler, 1889) (Geometridae); *Cynaeda dichroalis* (Hampson, 1903), *Dichocrocis pyrrhalis* (Walker, 1859) & *Glyphodes canthusalis* Walker, 1859 (Crambidae); and *Acropteris iphiata* (Guenée, 1857) (Uranidae) were recorded.

Keywords: Crambidae, Erebidae, Geometridae, lunar phase, moist Shiwalik Sal forest, monsoon, Mundiapani, Noctuidae, protected area, *Shorea robusta*, Simbalbara, relative humidity.

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Author contributions: The first author carried out the sampling surveys in the field, photographed, identified moths, carried out data analysis and wrote the paper. The second author assisted the first author in sampling surveys, collected, pinned and preserved moth specimens and identified them, compiled the data and species list.

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INTRODUCTION

Shiwaliks are the oldest of the mountain ranges that stretch over ~2,400 km from the Indus River in Jammu & Kashmir in the north-west eastwards close to the Brahmaputra river, located between 28.9544 ^{0}N & 34.1800 ^{0}E and 73.4900 ^{0}N & 80.23972 ^{0}E, spanning the northern parts of the Indian subcontinent. Shiwaliks are also regarded as a distinct zoo-geographical sub-region from the Himalaya and represent the lower or sub-Himalaya with elevation ranging 244–1,500 m, with diverse forest types, i.e., Moist Shiwalik Sal *Shorea robusta* forests, Dry Shiwalik Sal forests, scrubland, raus and grassy banks in northern India (Sivakumar et al. 2010). The Shiwalik region in northwestern part of India is mainly forested and includes many protected areas rich in wildlife, i.e., Rajaji National Park, Corbett Tiger Reserve, Sonanadi Wildlife Sanctuary, & Nandhaur Wildlife Sanctuary in Uttarakhand state; Kalesar Wildlife Sanctuary & the Morni Hills in Haryana; Simbalbara Wildlife Sanctuary in Sirmaur district of Himachal Pradesh; and Sukhna Wildlife Sanctuary in Punjab (Yadav et al. 2015). In recent years, increasing anthropogenic activity such as rapid urbanization and conversion of forest land into agriculture, introduction of exotics, grazing by livestock and lopping of trees by the Gujjar communities—the main inhabitants of this region has caused excessive deforestation and affected the fragile landscape of Shiwaliks which is today witnessing extensive soil erosion in non-forested and degraded tracts that threatens its very existence including that of the native fauna of the landscape (Kukal & Sur 1992; Sharma & Arora 2015).

Lepidoptera is an order of insects that includes moths (Heterocera) and butterflies' (Rhopalocera) that are one of the main phytophagus groups, encompassing as estimated 140,000–157,000 extant species worldwide (Nieukerken 2011; Lees & Zilli 2019), with moths constituting 89% of the known lepidoptera while the rest are butterflies. "Macro-lepidoptera" or "Macro-moth" which is a traditional, non-systematic division of Lepidoptera, largely representing moth families which have fewer traits of the earliest members of the order and tend to include those families with large body size and are usually easier to identify (https://en.wikipedia.org/). Macro-moths have been today used as indicators of environment quality as they depict diversity of plants and health of an ecosystems and may help in conserving microhabitats (Kitching et al. 2002; Summerville 2004). These facts make them interesting organisms for studying their ecology and diversity. They have also been found to be climate change indicators in studies conducted in western countries (Shubhalaxmi 2018).

The moth diversity of Shiwaliks of northern India has been poorly documented. Earlier studies in the region concentrated mainly in the higher western Himalayan ranges rather than the Shiwaliks or focused on particular groups/ families of moths or are not comprehensive in nature, i.e., Dehradun hills (Roonwal et al. 1956); Nanda Devi Biosphere Reserve in Kumaon (Arora et al. 1977); Nainital area in Kumaon (Smetacek 1994, 2008, 2009, 2011). More recently, Sanyal et al. (2013) and Uniyal et al. (2013) studied the entomofauna of the Gangotri landscape, listing 468 moth species. Recently, Sondhi & Sondhi (2016) have compiled a checklist of 248 species of moths from selected areas of Mussoorie and adjoining foothills of Dehradun, excluding the Shiwaliks. 502 species have been listed from New Forest Campus in Dehradun valley (670 m in the tropical moist deciduous forest zone, excluding Shiwalik ranges), in Uttarakhand since 1956 (Singh & Lekhendra 2022). Twenty-four species of Lymantridae have been listed from Punjab and 54 species of the family Arctiidae (Kaleka 2010, 2015). While Kumar et al. (2018) have listed 36 geometrid species, Kirti et al. (2007) and Kumar et al. (2015) have reported over 20 noctuid species of moths, all from Chir Pine *Pinus roxburghii* forest areas of Himachal Pradesh state (Bilaspur, Shimla, & Solan districts). However, none of the authors gave the precise location of any of their records and most of them do not fall in the Shiwalik ranges.

Therefore, the present study was undertaken to evaluate the diversity and seasonality of macro moths across different forest habitats in the Shiwalik landscape of northern India, up till the point which forms the western most limit on the globe (30.5042 ^{0}N & 77.2430 ^{0}E) in the distribution range of tropical moist deciduous Sal forests (a forest type typical in the northern aspect of the Shiwalik mountain ranges), i.e., Simbalbara National Park/Suketi in Himachal Pradesh State.

METHODS

Sampling

Seasonal sampling surveys were carried out to collect data on species richness and abundance of moths from 19 sites across the Shiwalik ranges from east to west in four northern Indian states (mainly in Uttarakhand and adjoining parts of Himachal Pradesh, Uttar Pradesh, and Haryana states; Figure 1) from July 2020 to October 2022. Surveys were conducted

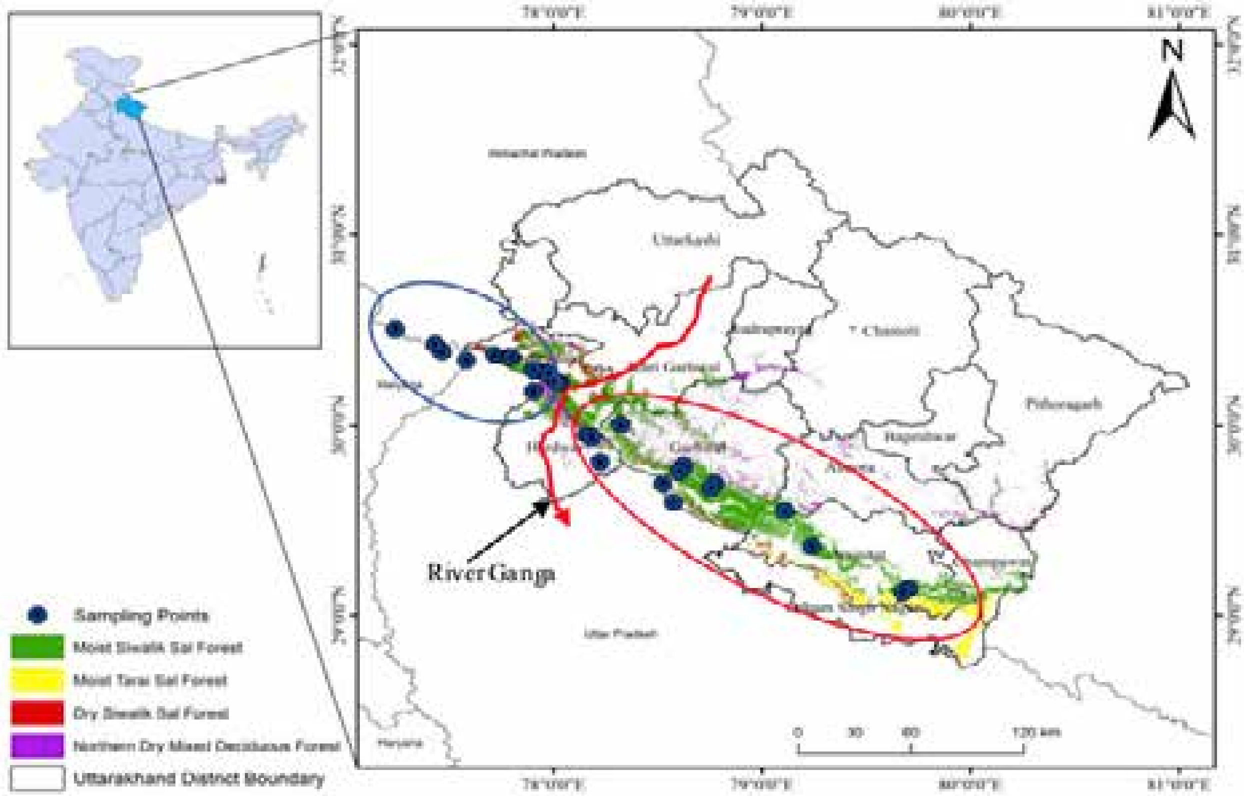

Figure 1. Location of the 19 sampling sites (dark blue dots) along different forest sub-types across the Shiwalik Landscape of Uttarakhand State and adjoining states in northern India with river Ganga intersecting the landscape.

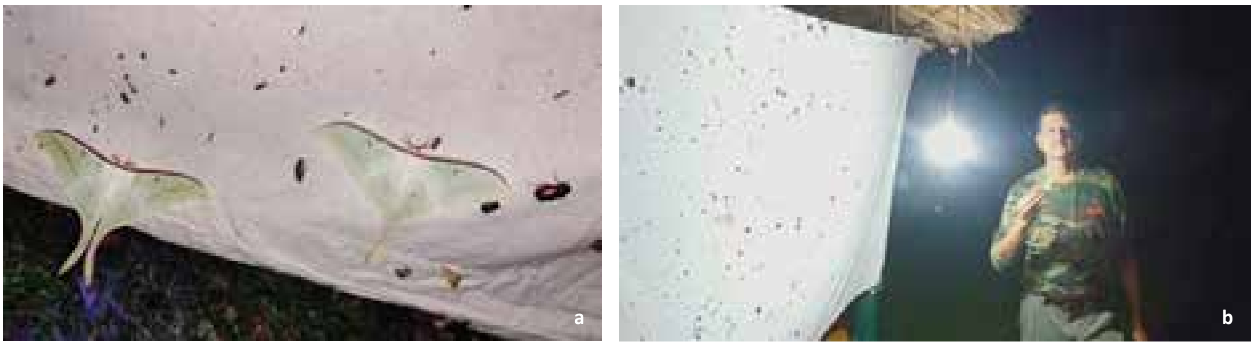

Image 1. a—Lunar moth, *Actias selene* (Saturnidae) attracted to CFL Light on moth screen during sampling (© Arun P. Singh) | b—The first author sampling moths sampling using CFL lamp and moth screen (© Lekhendra).

during different seasons of the year, i.e., pre-monsoon (April–June), monsoon (July–August), post monsoon (September–November), when moths are in flight here except in winter and early spring (December, January, February& March) when moths undergo hibernation in the northern India. A total of 59 days of individual sampling surveys (43 tours) were carried out each night from 1900–2200 h in these sites (Figure 1, Image 1, Table 1), as this is the time most of the moths are attracted to artificial light just after sunset. It was noted that moth species composition on moth screens altered less after mid night. We used CFL—compact fluorescent lamps (27 Watt; 220–240 Volts; Cool Daylight (6,500–7,500K); Light Color: White; 65 Lumen; Philips) hung vertically in front of a white canvas cloth as a moth screen (180 x 120mm) to attract moths (Raimondo et al. 2003). Data was collected on the date of sampling, GPS coordinates of each sampling site, moth species recorded along

(i) 3C/C2a Moist Shiwalik Sal Forest

(ii) 5B/C2 Northern Dry Mixed Deciduous Forest

(iii) 3C/C2c Moist Tarai Sal Forest

(iv) 5B/C1a Dry Shiwalik Sal Forest

Image 2. Four different forest types across the Shiwalik Landscape. © Arun P. Singh.

with the number of individuals present on the moth screen, and the temperature and relative humidity at the time of sampling. Most of the species sampled were photographed live; besides, vouchers specimens were also collected for those species that could not be identified in the field (Table 1). Forest sub-types covered during sampling in these 19 sites were (i) 3C/C2a Moist Shiwalik Sal Forest, (ii) 5B/C1a Dry Shiwalik Sal Forest, (iii) 5B/C2Northern Dry Mixed Deciduous Forest, and (iv) 3C/C2c Moist Tarai Sal Forest (Champion & Seth 1968) (Image 2, Table1).

Data analysis was carried out using the programme 'Bio Diversity Professional Version 2' to draw inferences on (i) alpha diversity – Shannon, species accumulation curve, rarefaction; (ii) beta diversity – species richness estimators (Chao2; Jacknife1 & Jacknife2); (iii) multivariate analysis (correspondence analysis & cluster analysis; correlation coefficient), for individual sites and seasons. Similarity index for moths species sampled in different forest types was calculated using Jaccard coefficient (Jaccard 1901).

RESULTS

Sampling surveys revealed a total of 321 species of macro moths (Appendix I) belonging to 19 families and 49 subfamilies. Amongst these, the family Erebidae (95 species) was the most dominant followed by Crambidae, Geometridae, Noctuidae, Limacodidae, and others, respectively, in terms of number of species (Figure 2).

Species richness

The species accumulation curve (Figure 4) for the entire sampling period suggests that new species were constantly being added till the last sampling and there was still a potential to add many more species when the sampling stopped.

The rarefaction plot for individual sites (Figure 4) suggests that sampling for species was comprehensive for only few sites, i.e., 'Simbalbara' (60 species) and 'Darpur' (20 species) most of the new species were added initially but later only a few species were added until the last sampling. While in Mundiapani, Timli, and Karvapani, new species were added continuously and the plot reached a higher species number (above 80) till

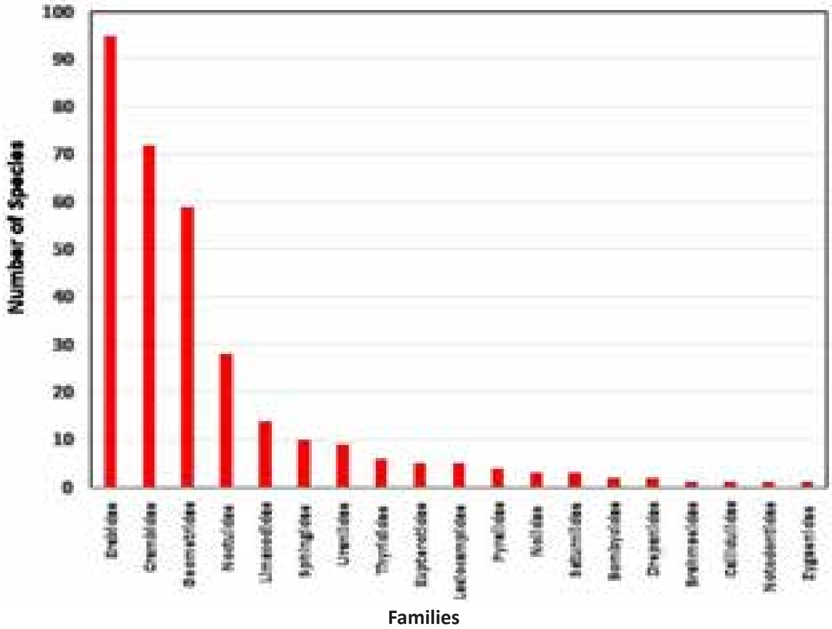

Figure 2. Four different forest types across the Shiwalik Landscape.

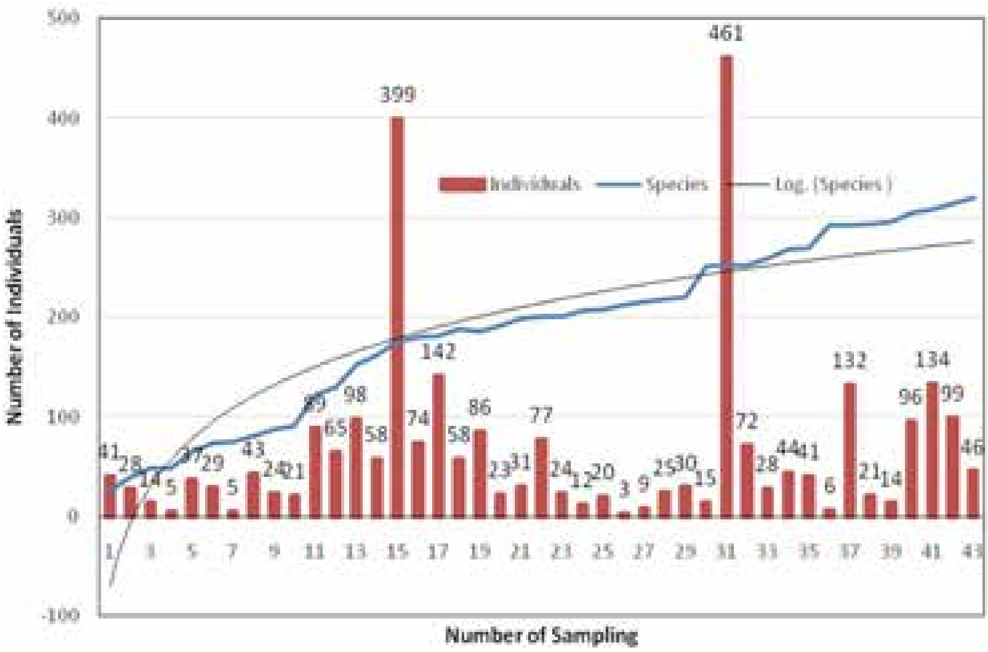

Figure 3. Species accumulation curve for the entire sampling period. 43 tours/surveys covering 59 sampling days.

the last sampling. These sites thus hold greater potential of adding more species to the list. However, sampling at Kalesar, Haridwar Chorgaliya, and Asarori was less frequent and recorded around only 40 species early till the end. In rest of the sites sampling was done only once and only a few species were recorded and more sampling effort was needed in these sites to arrive at any estimate of the number of moth species occurring there. Species richness estimators indicate higher levels of species richness than current number of 321 species during 59 samplings (Chao 2—504; Jacknife 1—473 & Jacknife 2—562) in the study area.

Seasonality

Seasonal trend of species richness showed two annual peaks, with the first peak occurring in August

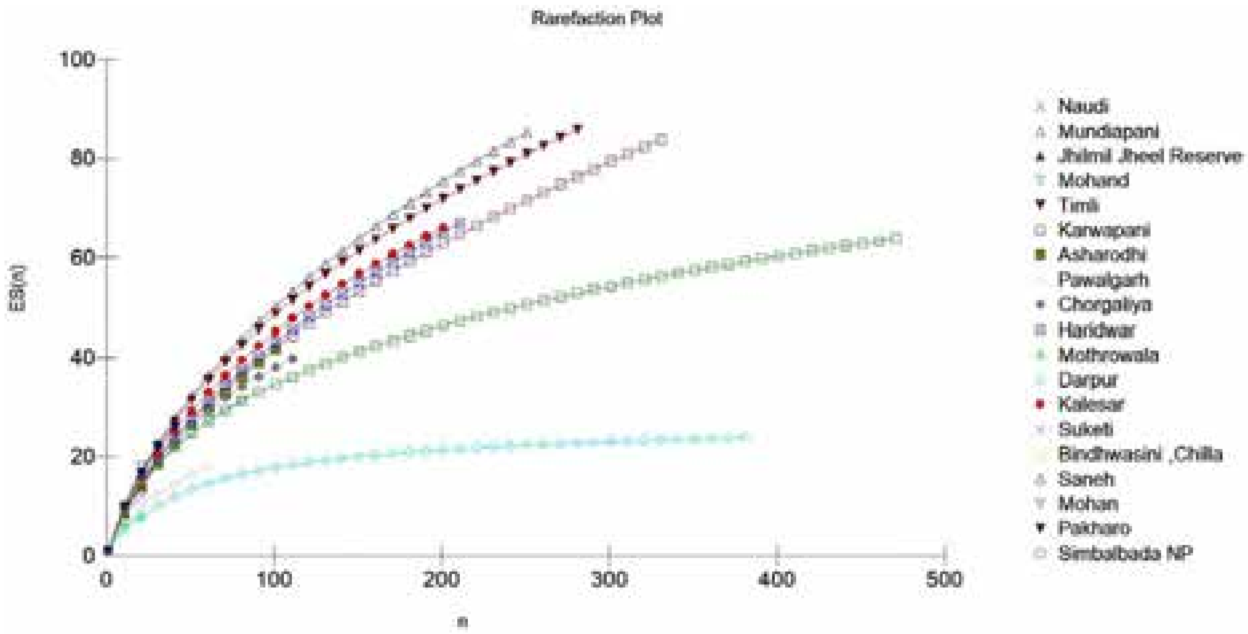

Figure 4. The rarefaction plot for moths sampled in 19 sites in Shiwaliks.

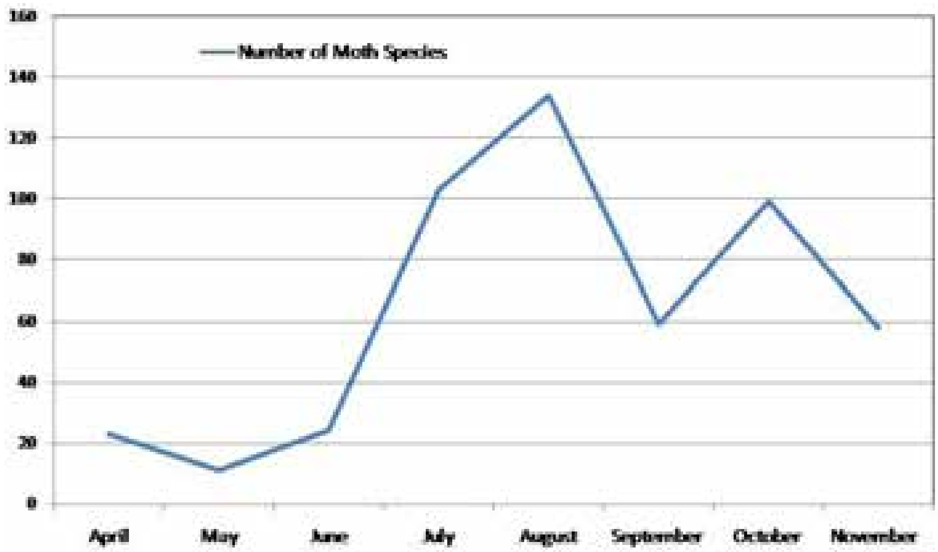

Figure 5. Species richness of moths sampled during different months of the year in Shiwaliks (2020–2022).

(monsoon season) followed by a smaller peak in October (post monsoon season) (Figure 5).

Species similarity of moths was greater between monsoon and post-monsoon seasons than for these seasons with pre-monsoon season (Figure 6), which indicates that species diversity of moths changes more rapidly with the onset of monsoon season as compared to pre-monsoon season.

Species diversity

Seasonal diversity: Shannon diversity index for the three different seasons indicates that species diversity was highest during 'monsoon' followed by 'post monsoon', and 'pre-monsoon', respectively (Figure 7).

Site diversity

The sites with relatively higher diversity index (H'= above 1.5; Figure 8) were identified as Mundiapani, Timli, Karwapani, Haridwar, Kalesar, and Simbalbara.

Site similarity

In the ordination plot (Figure 9), two clusters of sites

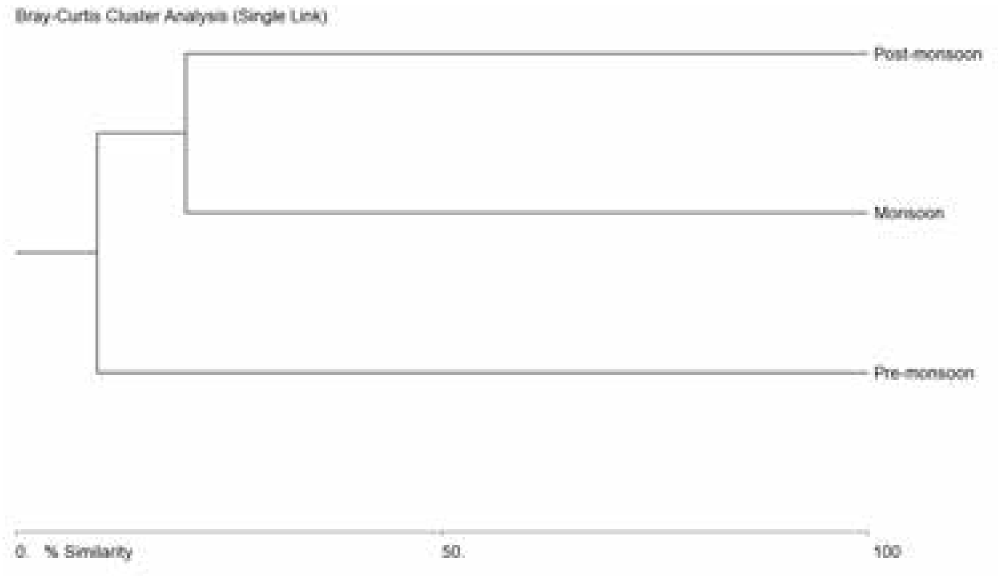

Figure 6. Moth species similarity amongst different seasons.

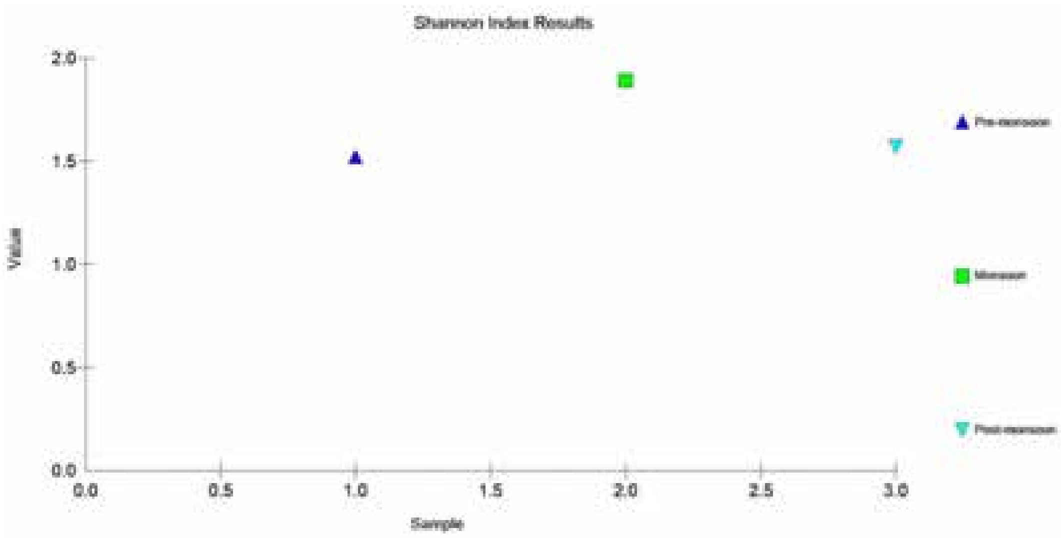

Figure 7. Shannon diversity index of moths during three different seasons of the year in Shiwaliks (2020–2022).

are formed, the first cluster 1 (red circle) consists of seven sites above Axis 2 that lie east of the river Ganga, while another cluster 2 (blue dash circle) of 12 sites lie below or on the Axis 2 that lie west of the river Ganga. The vegetation in the cluster 2 is mainly characterized by forests with high moisture regime (One site with 3C/C2c Moist Tarai Sal Forest & others with 3C/C2a Moist Siwalik Sal Forest) (Figure 10) as compared to cluster 2 (5B/C2 Northern Dry Mixed Deciduous Forest; a few sites having 3C/C2a Moist Siwalik Sal Forest and one site with 5B/C1a Dry Siwalik Sal Forest; Figure 10). This clearly indicates the changing vegetation structure in the Shiwaliks as we move from east to west, hence changing moth communities with changing vegetation. However, only three sites with high diversity index, Mundiapani (3C/C2a Moist Siwalik Sal Forest), Chorgaliya (3C/C2c Moist Tarai Sal Forest) and Simbalbara (5B/C2 Northern Dry Mixed Deciduous Forest) were identified assites with unique moth communities from the rest (Figure 9, 10 & 11).

Forest types similarity

Index of similarity or Jaccard's Coefficient (Jaccard 1901) amongst the four forest sub-types suggests that

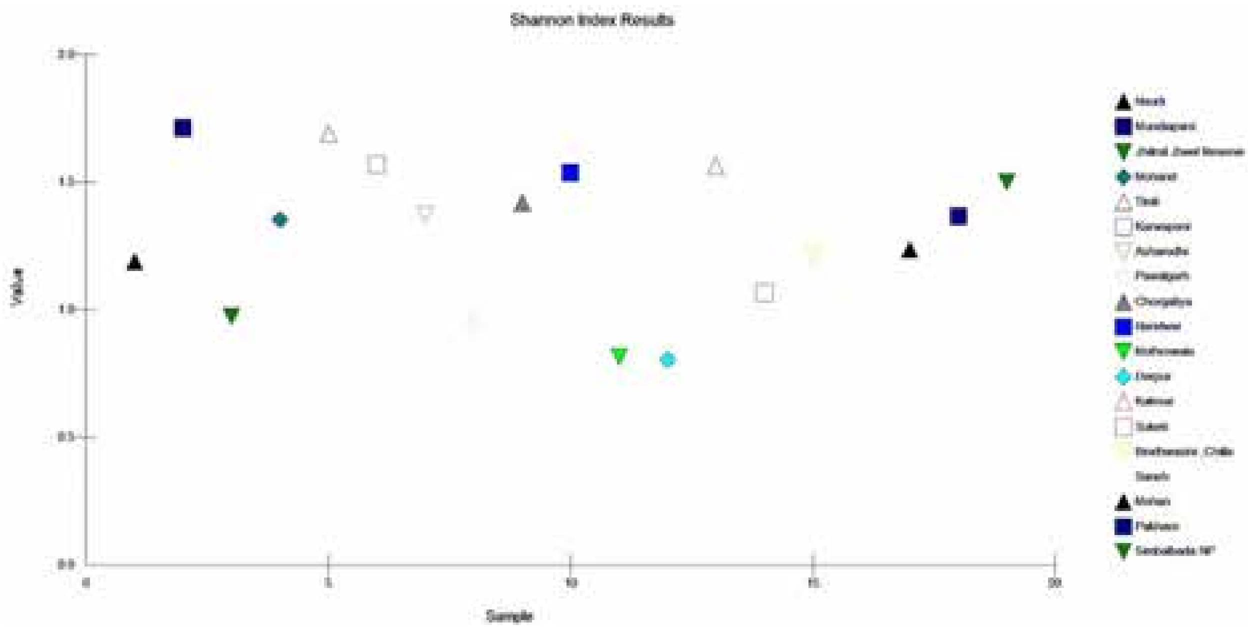

Figure 8. Shannon diversity index of moths for 19 different sites sampled across the Shiwaliks (2020–2022).

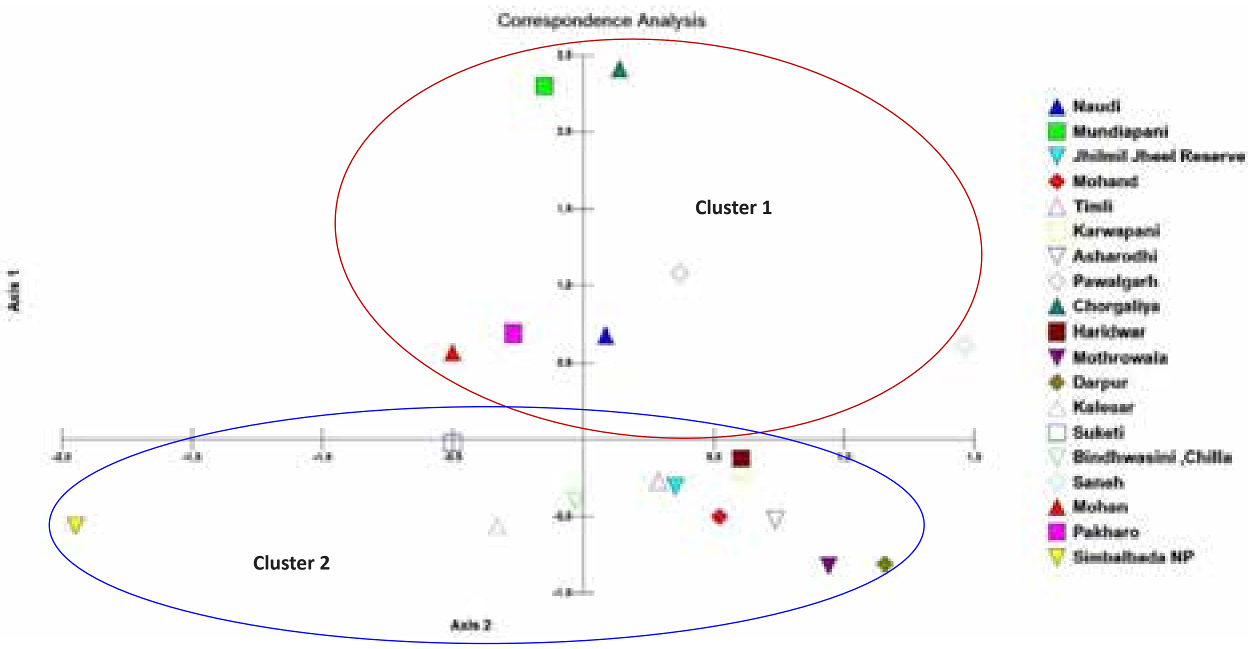

Figure 9. Correspondence analysis ordination graph of plots realised from species abundance data showing relative position of 19 sites.

all forest sub-types show low similarity with each other (less than 0.3157; Table 2) and the maximum similarity exists between 3C/C2a Moist Shiwalik Sal Forest and 5B/C2 Northern Dry Mixed Deciduous Forest. This is an indicator of diverse and distinct moth fauna existing in these four forest sub-types across the landscape.

New range extensions

During recent surveys of moths in the Shiwalik ranges of northern India the authors have already reported *Lymantria tadora* (Erebidae) from Shiwaliks of Haryana State as a new range extension from central Nepal (Singh & Lekhendra 2023). Other five new range extensions from thetwosampling sites, (i) Simbalbara WS, Sirmaur District, H.P., & (ii) Mundiyapani, Kalagarh

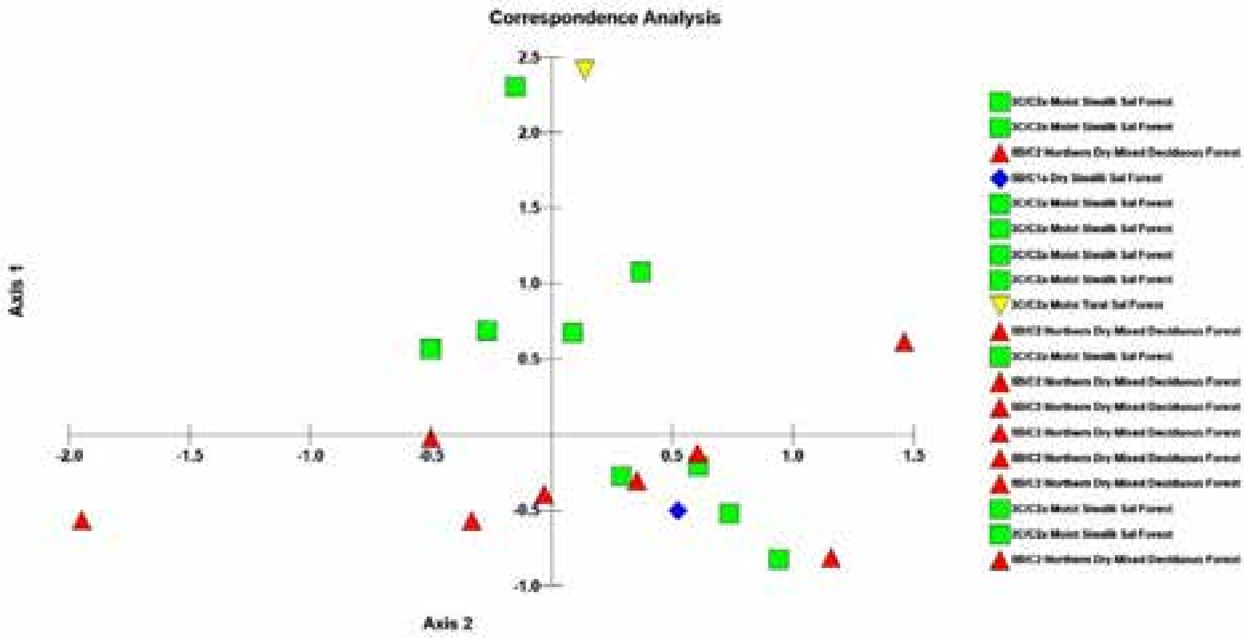

Figure 10. Correspondence analysis ordination graph of plots realised from species abundance data showing relative position of four forest type groups for 19 sampling sites.

Forest Division, Sonandi WS in Uttarakhand (Figure 11, sites marked in red circles) are reported for the first time in this paper for species otherwise known from central Nepal and northeastern India.

1. *Chlorozancla falcatus* (Butler, 1889) (Geometridae: Geometrinae: Geometrini)

One individual of *C. falcatus* (Image 3) was recorded during monsoon season (18.viii.2022; Temp 28.6°C & RH 84%) at Mundiapani [29.6947 °N & 78.7760 °E, 521 m; Forest type - 3C/C2a Moist Shiwalik Sal Forest (Champion & Seth 1968)] in Kalagarh Forest Division, Uttarakhand Shiwaliks, India. *Chlorozancla* genus occurs in Asia [Japan, China, India (Himalaya, Khasi hills)], Sumatra, Java & Borneo (Hampson, 1895). The type species *falcatus* was described from Sikkim, and India (Chhattisgarh, Karnataka, Kerala) (Kirti et al. 2012; Chandra et al. 2019; Anonymous 2023a) and is also known from Nepal (Haruta 1995). The larval food plant of the species is *Lagerstroemia speciosa* (Lythraceae) (Robinson et al. 2010). The moth is dull grey-green. Fore wing with the base yellowish; a pale straight erect post medial line; a yellow fascia below costa towards apex, with a hyaline spot on it. Hind wing with a hyaline medial band, wider and with yellow edges towards inner margin; traces of a sub marginal straight line. Expanse 28 mm (Hampson 1895). This record from Mundiapani is the first confirmed record of this species from the Shiwaliks, which is ~900 km from Mechi Godok (964 m) in eastern Nepal, the nearest known site record of this species.

2. *Cynaeda dichroalis* (Hampson, 1903) (Crambidae: Odontiinae: Odontiini)

One individual of *C. dichroalis* (Image 4) was recorded during monsoon (18.viii.2022; Temp. 28.6 °C & RH 84%) at Mundiapani [29.6947 °N & 78.7760 °E, 521m; Forest type - 3C/C2a Moist Shiwalik Sal Forest (Champion & Seth1968)] in Kalagarh Forest Division, Uttarakhand Shiwaliks, India. The species occurs in Ceylon, Sri Lanka, Sabah, China (Yunnan) in Lowland primary & disturbed forest (Hampson 1903). The species has also been reported from East Karbi Analog District, Assam; South Garo Hills District, Meghalaya; Coimbatore, Tamil Nadu in India (Anonymous 2023b). However, there is no record of this species from northern India. The species is identified by Palpi being slightly marked with black towards extremity; frontal prominence black at sides; abdomen with the first three segments mostly black on dorsum and the next two largely marked with black. Forewings with the inner area fuscous black to beyond middle and confluent with spots on base of costa. Hind wing with the basal area fuscous. Wing expanse 18 mm (Hampson 1903). This record from Mundiapani is the first confirmed record of this species from the Shiwaliks, which is ~1,300 km from South Garo Hills District (349

Table1. Details of sampling sites, dates and weather parameters at the time of sampling.

	Sampling site	Forest status	District	State	Forest type (Champion & Seth 1968)	Altitude (m)	Sampling date	Season	Temperature (°C)	Relative humidity (%)
1.	Naudi FRH	Sonandi Wildlife Sanctuary	Pauri Garhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	765	20.vii.2020	Monsoon	27.0	85
2.	Naudi FRH	Sonandi Wildlife Sanctuary	Pauri Garhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	765	21.vii.2020	Monsoon	26.3	86
3.	Mundiapani FRH	Sonandi Wildlife Sanctuary	PauriGarhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	521	22.vii.2020	Monsoon	27.7	86
4.	Mundiapani FRH	Sonandi Wildlife Sanctuary	PauriGarhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	521	23.vii.2020	Monsoon	26.8	84
5.	Jhilmil Jheel Reserve FRH	Conservation Reserve	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous Forest	303	26.x.2022	Post-monsoon	20.5	75
6.	Mohand FRH	Reserve Forest	Saharanpur	Uttar Pradesh	5B/C1a Dry Siwalik Sal Forest	462	27.x.2020	Post-monsoon	21.5	64
7.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	574	28.x.2020	Post-monsoon	14.5	71
8.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	01.vii.2021	Monsoon	30.8	73
9.	Sabhawala, Malhan FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	520	02.vii.2021	Monsoon	29.1	70
10.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	574	03.vii.2021	Monsoon	29.6	60
11.	Mohand FRH	Reserve Forest	Saharanpur	Uttar Pradesh	5B/C1a Dry Siwalik Sal Forest	462	08.vii.2021	Monsoon	28.1	71
12.	Asharodhi FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	642	09.vii.2021	Monsoon	29.4	78
13.	Mundiapani FRH	Sonandi Wildlife Sanctuary	PauriGarhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	521	10.viii.2021	Monsoon	27.2	87
14.	Mundiapani FRH	Sonandi Wildlife Sanctuary	PauriGarhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	521	11.viii.2021	Monsoon	30.7	93
15.	Pawalgarh FRH	Conservation Reserve	Nainital	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	466	12.viii.2021	Monsoon	27.2	90
16.	Pawalgarh FRH	Conservation Reserve	Nainital	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	466	13.viii.2021	Monsoon	27.3	84
17.	Chorgaliya FRH	Nandhaur WLS edge	Haldwani	Uttarakhand	3C/C2a Moist Tarai Sal Forest	327	14.vii.2021	Monsoon	28.2	92
18.	Kalesar FRH	Reserve Forest	Yamuna Nagar	Haryana	5B/C2 Northern Dry Mixed Deciduous Forest	384	21.ix.2021	Post-monsoon	27.8	83
19.	Darpur FRH	Reserve Forest	Yamuna Nagar	Haryana	5B/C2 Northern Dry Mixed Deciduous Forest	347	22.ix.2021	Post-monsoon	29.3	74
20.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	574	23.ix.2021	Post-monsoon	27	77
21.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	13.x.2021	Post-monsoon	25.4	71
22.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	14.x.2021	Post-monsoon	25.8	67
23.	Mohand FRH	Reserve Forest	Saharanpur	Uttar Pradesh	5B/C1a Dry Siwalik Sal Forest	462	20.x.2021	Post-monsoon	22.6	86
24.	Mohand FRH	Reserve Forest	Saharanpur	Uttar Pradesh	5B/C1a Dry Siwalik Sal Forest	462	21.x.2021	Post-monsoon	20.8	81
25.	Asharodhi FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	685	22.x.2021	Post-monsoon	20.1	78
26.	Asharodhi FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	685	22.x.2021	Post-monsoon	17.8	83
27.	Asharodhi FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	685	22.x.2021	Post-monsoon	16.4	86

	Sampling site	Forest status	District	State	Forest type (Champion & Seth 1968)	Altitude (m)	Sampling date	Season	Temperature (°C)	Relative humidity (%)
28.	Mathrowala FRH	Rajaji National Park edge	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	537	27.x.2021	Post-monsoon	19.1	81
29.	Simbalbada FRH	Simbalbara National Park edge	Sirmour	Himachal Pradesh	5B/C2 Northern Dry Mixed Deciduous Forest	468	22.xi.2021	Post-monsoon	17.9	70
30.	Kalesar FRH	Reserve Forest	Yamuna Nagar	Haryana	5B/C2 Northern Dry Mixed Deciduous Forest	384	23.xi.2021	Post-monsoon	17.8	72
31.	Suketi FRH	Reserve Forest	Sirmaur	Himachal Pradesh	5B/C2 Northern Dry Mixed Deciduous Forest	404	24.xi.2021	Post-monsoon	18.2	76
32.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	25.xi.2021	Post-monsoon	19.2	73
33.	Haridwar -Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous Forest	349	12.iv.2022	Pre-monsoon	28.8	49
34.	Haridwar-Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous Forest	349	12.iv.2022	Pre-monsoon	29.2	52
35.	Mohand FRH	Reserve Forest	Saharanpur	Uttar Pradesh	5B/C1a Dry Siwalik Sal Forest	462	13.iv.2022	Pre-monsoon	27.1	39
36.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	14.iv.2022	Pre-monsoon	24.6	46
37.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	15.iv.2022	Pre-monsoon	23.9	46
38.	Bindhwasini Village	Rajaji National Park Forest Edge	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous	464	22.iv.2022	Pre-monsoon	25.6	40
39.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	574	14.vi.2022	Pre-monsoon	31	59
40.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	574	15.vi.2022	Pre-monsoon	27	66
41.	Haridwar -Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous	349	03.vi.2022	Pre-monsoon	32	35
42.	Simbalbara FRH	Simbalbara National Park edge	Sirmour	Himachal Pradesh	5B/C2 Northern Dry Mixed Deciduous	468	02.vii.2022	Monsoon	28.3	86
43.	Simbalbara FRH	Simbalbara National Park edge	Sirmour	Himachal Pradesh	5B/C2 Northern Dry Mixed Deciduous	468	03.vii.2022	Monsoon	29.8	84
44.	Kalesar FRH	Reserve Forest	Yamuna Nagar	Haryana	5B/C2 Northern Dry Mixed Deciduous	384	03.vii.2022	Monsoon	30.2	80
45.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	574	04.vii.2022	Monsoon	29.8	87
46.	Haridwar -Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous	349	09.vii.2022	Monsoon	31.2	75
47.	Haridwar -Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous	349	10.vii.2022	Monsoon	25.7	87
48.	Pakharo FRH Kalagarh	Sonandi Wildlife Sanctuary	Pauri Garhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	301	16.viii.2022	Monsoon	30.9	72
49.	Pakharo FRH Kalagarh	Sonandi Wildlife Sanctuary	Pauri Garhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	301	17.viii.2022	Monsoon	29.5	80
50.	Saneh FRH	Sonandi Wildlife Sanctuary	Pauri Garhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	310	17.viii.2022	Monsoon	31	79
51.	Mundiapani FRH	Sonandi Wildlife Sanctuary	Pauri Garhwal	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	521	18.viii.2022	Monsoon	28.6	84
52.	Mohan FRH	Reserve Forest	Almora	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	515	19.viii.2022	Monsoon	27.9	86

	Sampling site	Forest status	District	State	Forest type (Champion & Seth 1968)	Altitude (m)	Sampling date	Season	Temperature (°C)	Relative humidity (%)
53.	Chorgaliya FRH	Nandhaur Wildlife Sanctaury edge	Haldwani	Uttarakhand	3C/C2a Moist Tarai Sal F	327	20.viii.2022	Monsoon	28.5	85
54.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	01.x.2022	Post-monsoon	24.3	87
55.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	04.x.2022	Post-monsoon	25.6	89
56.	Haridwar-Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous	349	02.x.2022	Post-monsoon	26.8	78
57.	Haridwar-Chandighat Temple	Reserve Forest	Haridwar	Uttarakhand	5B/C2 Northern Dry Mixed Deciduous	349	03.x.2022	Post-monsoon	26.6	82
58.	Timli FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	596	08.x.2022	Post-monsoon	24.8	88
59.	Karwapani FRH	Reserve Forest	Dehradun	Uttarakhand	3C/C2a Moist Siwalik Sal Forest	583	16.x.2022	Post-monsoon	25.1	83

m) in northeastern India, the nearest known site record of this species.

3. *Dichocrocis pyrrhalis* (Walker, 1859) (Crambidae: Spilomelinae)

One individual of *D. pyrrhalis* (Image 5) was also recorded during monsoon season (02.vii.2022; Temp 29.8 °C; RH-84%) in the Shiwaliks range of Simbalbara NP, [30.4253 °N & 77.4332 °E; 468 m; forest sub-type-5B/C2 Northern Dry Mixed Deciduous Forest (Champion & Seth1968)] Sirmaur District, Himachal Pradesh. The genus *Dichocrocis* is found in Asia, North America, Africa, Australia, and Ceylon (Hampson 1896). *D. pyrrhalis* is known from Borneo, Sarawak, Nilgiri's Ceylon (Wing expanse 26 mm) (Walker 1859; Hampson 1896). In India *D. pyrrhalis* found Maharashtra, Karnataka (Anonymous 2023c). Palpi curved hardly rising above the vertex; second joint with a slight luteous mark on the outer side; abdomen extending rather far beyond the hind wings, with a luteous stripe along each side and with a black apical mark. Fore wings with six luteous bands; Hind wings with four luteous bands, wing expanse 30 mm (Walker 1859). This record from Simbalbara is the first confirmed record of this species from the Shiwaliks, which is ~1,400 km Mauli Hills (710 m), Maharashtra the nearest known site record of this species.

4. *Acropteris iphiata* (Guenée, 1857) (Uraniidae: Microniinae)

One individual of *Acropteris iphiata* (Image 6) was recorded during monsoon season (11.viii.2021; Temp. 28.6 °C; RH 84%) at Mundiapani [29.6947 ^{0}N &78.7760 ^{0}E, 521m; Forest type - 3C/C2a Moist Shiwalik Sal Forest (Champion & Seth 1968)] in Kalagarh Forest Division, Uttarakhand Shiwaliks, India. The genus occurs in Japan and throughout the Oriental and Australian regions (Hampson 1895). *Acropteris iphiata* is found in Japan, Burma, China, Himalaya, Khasi, & eastern Himalaya (Hampson 1895; Sanyal et al. 2018) and northeastern India (Arunachal Pradesh, Assam, Meghalaya, West Bengal) (Anonymous 2023f). The species *iphiata* differs from *striataria* in the cell of the fore wing being suffused with fuscous; the first line single and well defined; the marginal line of both wings almost black. Expanse: 3,236 mm (Hampson 1895). This record from Mundiapani is the first confirmed record of this species from the Shiwaliks, which is ~1,000 km from Ajodhya Hills (699 m) in Purulia District, West Bengal the nearest known site record of this species.

Image 3. *Chlorozancla falcatus* (Butler, 1889)

Image 4. *Cynaeda dichroalis* (Hampson, 1903)

Image 5. *Dichocrocis pyrrhalis* (Walker, 1859).

Image 6. *Acropteris iphiata* (Guenée, 1857)

Image 7. *Glyphodes canthusalis* Walker, 1859

5. *Glyphodes canthusalis* Walker, 1859 (Crambidae: Spilomelinae: Margaroniini)

One individual (Image 7) was recorded during monsoon season (11.viii.2021; Temp 28.6 °C; RH 84%) at Mundiapani [29.6947 °N & 78.7760 °E, 521 m; Forest type - 3C/C2a Moist Shiwalik Sal Forest (Champion & Seth 1968)] in Kalagarh Forest Division, Uttarakhand Shiwaliks, India. The genus is found in tropical and warmer temperate Zones (Hampson 1896). The species *Glyphodes canthusalis* occurs in Sarawak, Borneo, Nepal, Bhutan, Sri Lanka, Myanmar, China, Taiwan, Indonesia (Sumatra, Borneo), Australia, Nigeria,central Himalaya, Bhutan (Walker 1859; Haruta 1995; Irungbam 2016; Sanyal et al. 2018; Chandra et al. 2019). In India, the species is found in Andamans, Sikkim, Assam, West Bengal, Andhra Pradesh, Arunachal Pradesh, Meghalaya, Maharashtra, Chhattisgarh, Jharkhand, Karnataka (Hampson 1896; Shah et al. 2018; Chandra et al. 2019; Anonymous 2023f). The moth is ferruginous red and yellow, spotted and irrorated with white; frons white; abdomen with paired white spots on basal segments. Fore wings with series of indistinct antemedial white spots. Hind wing with a large dark edged hyaline white post-medial patch from costa to vein 2. Wing expanse 30–36 mm. The larval food plant of the species is *Ficus religiosa* (Moraceae) (Hampson 1896; Robinson et al. 2010). This record from Mundiapani is the first confirmed record of this species from the Shiwaliks, which is ~600 km from Devi Ghat Narayangarh (519 m) in central Nepal, the nearest known site record of this species.

Table 2. Moth species similarity amongst forest sub-types.

		Similarity index		
		2.	3.	4.
	Forest sub-type	**5B/C2 Northern Dry Mixed Deciduous Forest**	**3C/C2c Moist Tarai Sal Forest**	**5B/ C1a Dry Shiwalik Sal Forest**
1.	**3C/C2a Moist Shiwalik Sal Forest**	0.3157*	0.091*	0.105*
2.	**5B/C2 Northern Dry Mixed Deciduous Forest**	-	0.095*	0.071*
3.	**3C/C2c Moist Tarai Sal Forest**	-	-	0.042*

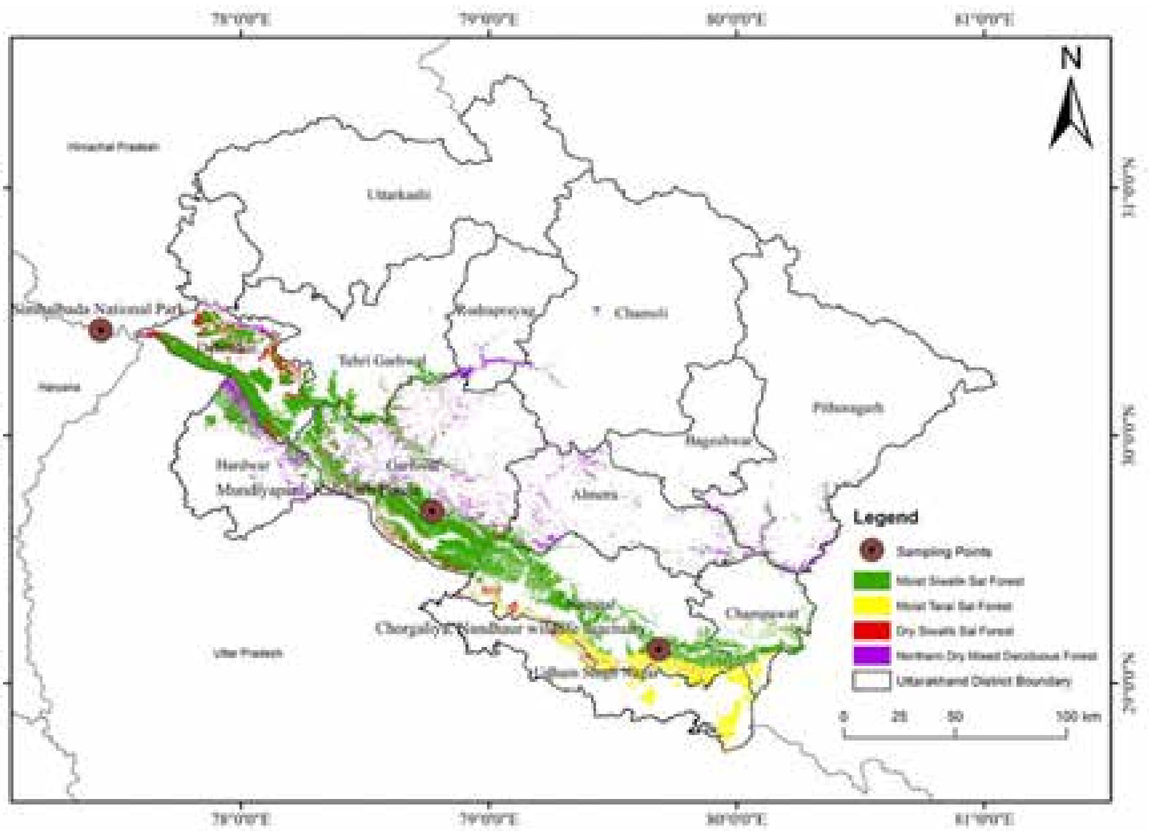

Figure 11. Conservation priority sites (red circles—left to right: i—Simbalbara| ii—Mundiyapani| iii—Chorgalyia) and the locations of new moth range extensions are reported as mentioned in the text.

DISCUSSION

During the present study we were able to sample approximately 2/3 (64–68 %) of the estimated number of moth species occurring in the study area of Shiwaliks of northern India. The flight period of moths was recorded from April–October, but the species richness and abundance were both high only during July–October months, i.e., monsoon & post-monsoon seasons, and a pattern of two peaks (August & October) in species richness of moths annually was visible during this period. A total of 232 species showed occurrence in only one season and 140 species amongst them occurred only during the 'monsoon' season indicating a Univoltine habit or a short flight period of these species. Species richness of moths correlated positively with relative humidity (r^2 = 0.100; p = 0.0142; n = 59) during the sampling period from April–November, with maximum number of species recorded (80–90 % RH) during the 'monsoon' season. Lunar phase correlated negatively with both species richness (r^2 = 0.0508; p = -0.2253; n = 59) and abundance (r^2 = 0.0401; p = -0.1551; n = 59) of moths attracted to light on moth screen.

This trend of seasonality for species richness and abundance of moths in the Shiwaliks is a bit different from that of moths occurring in the higher temperate Himalaya, i.e., Gangotri landscape of Uttarakhand, where catch success per night was recorded to be maximum during April–May (pre-monsoon season) and also during August–September (monsoon & early post-monsoon season), but was lower during June–July (early monsoon season) (Sanyal et al. 2013).

The families Crambidae, Erebidae, Geometridae, and Noctuidae, respectively dominated the northern Indian Shiwalik landscape. In another study carried out on moths limited to the Nandhaur Wildlife Sanctuary, in the Shiwaliks in Uttarakhand state, the peak abundance of moths was recorded during the monsoon season and the family Erebidae (10 species and 174 individuals) was the most dominant followed by Crambidae (four species and 148 individuals) out of the total six families and 347 individuals (Arya et al. 2021). In a recent assessment of the moth diversity of New Forest Campus in Dehradun valley (670 m; in the tropical moist deciduous forest zone, lying between the lower Himalayan ranges and the Shiwaliks), in Uttarakhand, the family Erebidae dominated (165 species), followed by Geometridae (74), Noctuidae (63), and Nolidae (27) respectively, and the maximum number of species was reported during July (monsoon) and October (post-monsoon season) (Singh et al. 2022). Another study carried out in Dehradun District and Devalsari Village in Tehri Garhwal (sub-tropical & temperate), Uttarakhand revealed 246 species with the family Erebidae to be the most dominant (96 species) (Sondhi & Sondhi 2016). Besides, in the higher western Himalaya from Gangotri Landscape, the family Geometridae dominated (113 species) followed by Erebidae (61) and Noctuidae (58), respectively (Sanyal et al. 2013). In the study conducted at Kedarnath WS, Uttarakhand, the family Geometridae (86 species) dominated followed by Erebidae (18) (Dey 2019). A study of moth diversity at various altitudes in the in the Nainital district, Kumaun (temperate & sub-tropical Himalayan region of Uttarakhand) revealed 887 different species of moths with the family Erebidae (260 species) being most dominant followed by the Geometridae (198) and Noctuidae (155), respectively (Smetacek 2008). Most of these studies in the north Indian landscape show a trend of seasonality and dominating families similar to the present study.

There is changing vegetation structure in the Shiwaliks as we move from east to west across the landscape in the study area (Figure 1). Forest sub-type 3C/C2c Moist Tarai Sal Forest (Figure 1, yellow colour) occurs in the extreme eastern part of the Shiwaliks and is characterized by high moisture regime whereas 5B/C2 Northern Dry Mixed Deciduous Forest (Figure 1, purple colour) occurs further west across the river Ganges while 5B/C1a Dry Siwalik Sal Forest (Figure 1, red colour) lies in the extreme western part of the study area. As moths are surrogates of plant diversity with their larval host plants occurring in the habitats where they live, the changing vegetation structure also indicated a changing moth community and this is reflected in the ordination plot (Figure 9,10) amongst the 19 sampling sites being separated into different clusters of forest types and proximity amongst them on the land.

Amongst the forest sub-types, 3C/C2a Moist Shiwalik Sal Forest sub-type holds the maximum number moth species occurring in the north Indian Shiwaliks as compared to the other forest sub-types owing to the large area of occupancy. Moths like *Chabula telphusalis* Walker, [1859] and *Creatonotos gangis* Linnaeus,1762 were two such species whose abundance dominated and showed preference for '3C/C2a Moist Shiwalik Sal Forest' and similarly both *Creatonotos transiens* (Walker, 1855) and *Aegocera venulia* Cramer, 1777 abundance dominated and preferred for '5B/C2 Northern Dry Mixed Deciduous Forest' (Appendix I).

The three sampling locations (Mundiapani, Chorgaliya & Simbalbara) in three different representative forest sub-types in the Shiwalik ranges were identified having unique (Figure 10) and relatively higher moth diversity than other sites, and can be recommended as priority areas for conservation of Shiwalik moth diversity and biodiversity in general. One of the reasons for this uniqueness is the well preserved and intact natural forest habitats here due to their legal protection status (i) Mundiapani-Sonanadi WS; (ii) Chorgaliya-Nandhaur WS & (iii) Simbalbara-Simbalbara/ Col. Sher Jung NP). Besides, these sites revealed the occurrence of some unique and uncommon moths, i.e., *Brahmaea hearseyi* (Brahmaeidae), *Pidorus glaucopis* (Zygaenidae), *Antheraea assamensis* and *Antheraea mylitta* (Saturniidae) in Mundiapani; *Hypolamprus* sp. (Thyrididae) in Chorgaliya and Lunar Moth *Actias selene* (Saturnidae) in Simbalbara NP.ß

The five new range extensions recorded into the Shiwaliks of northern India are from central Himalaya and northeastern India indicate the affinity of these moths to the Oriental tropics, as tropical vegetation and climate over here is also mainly influenced and characterised by the monsoon rains. The pattern of new range extensions indicates the changing/expanding range margins of these moths, typically known from northeastern India /southeastern Asia, which may be associated with the current global climate change.

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Appendix 1. Systematic list of moths (Heterocera) recorded in the Shiwaliks of northern India along with their relative number of individuals recorded on moth screen during the 43 field surveys covering 59 days of sampling at 19 locations (2020–2022) from Nandhaur Wildlife Sanctuary ,Uttarakhand to Simbalbara National Park, Himachal Pradesh.

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
A.	Bombycidae	Bombycinae	1.	*Penicillifera apicalis* (Walker, 1862)****	1	Mon
			2.	*Trilocha varians* (Walker, 1855)	1	Mon
B.	Brahmaeidae	-	3.	*Brahmaea hearseyi* White, 1862*	1	Mon
C.	Callidulidae	Callidulinae	4.	*Tetragonus catamitus* Geyer, 1832*	1	Mon
D.	Crambidae	Acentropinae	5.	*Eoophyla peribocalis* (Walker, 1859)	7	Mon
			6.	*Eoophyla* sp. Swinhoe, 1900	2	Mon
			7.	*Paracymoriza vagalis* (Walker, 1866)	3	Mon
			8.	*Parapoynx bilinealis* (Snellen, 1876) **	1	Post
			9.	*Parapoynx* sp. Hübner, [1825] *	1	Post
		Odontiinae	10.	*Cynaeda dichroalis* (Hampson, 1903) *	1	Mon
		Pyraustinae	11.	*Ategumia adipalis* (Lederer, 1863) *	3	Mon
			12.	*Hyalobathra illectalis* (Walker, 1859) **	2	Post
			13.	*Lamprosema poeonalis* Walker, 1859*	1	Mon
			14.	*Pagyda salvalis* Walker, 1859*	1	Post
			15.	*Pyrausta phoenicealis* Hubner, 1818***	1	Post
		Spilomelinae	16.	*Agathodes ostentalis* (Geyer, 1837) *	2	Mon
			17.	*Agrotera scissalis* Walker, [1866])	4	Mon
			18.	*Agrotera basinotata* Hampson, 1981*	1	Mon
			19.	*Arthroschista hilaralis* (Walker, 1859)	1	Post
			20.	*Bradina admixtalis* (Walker, [1859]) *	1	Post
			21.	*Bradina diagonalis* Guenée, 1854*	9	Mon
			22.	*Chabula telphusalis* (Walker, 1859) *	20	Mon; Post
			23.	*Cirrhochrista brizoalis* (Walker, 1859)	1	Post
			24.	*Cirrhochrista fumipalpis* Felder & Rogenhofer, 1875	1	Mon
			25.	*Cnaphalocrocis medinalis* (Guenee, 1854)	65	Post
			26.	*Cnaphalocrocis rutilalis* (Walker, [1859])	310	Post
			27.	*Cnaphalocrocis trebiusalis* (Walker, 1859)	13	Post
			28.	*Conogethes punctiferalis* (Guenée, 1854)	1	Mon
			29.	*Cydalima lacticostalis* (Guenee,1854) **	2	Mon
			30.	*Diaphania indica* (Saunders, 1851)	9	Post
			31.	*Dichocrocis pyrrhalis* (Walker, 1859)	1	Mon
			32.	*Endocrossis flavibasalis* (Moore, [1868])	1	Mon; Post
			33.	*Glyphodes bicolor* (Swainson, 1821)	19	Mon; Post
			34.	*Glyphodes bivitralis* Guenée, 1854	3	Mon; Post
			35.	*Glyphodes caesalis* (Walker, 1859) *	1	Mon
			36.	*Glyphodes canthusalis* Walker, 1859*	1	Mon
			37.	*Endocrosis flavibasalis* (Moore, [1868])	2	Mon
			38.	*Haritalodes derogata* (Fabricius, 1775) *	1	Post
			39.	*Herpetogramma basalis* (Walker, [1866])	1	Post
			40.	*Herpetogramma bipunctalis* (Fabricius, 1794)	2	Mon
			41.	*Herpetogramma luctuosale* (Guenée, 1854)	6	Mon

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
			42.	*Herpetogramma phaeopteralis* (Guenée, 1854)	3	Pre; Mon; Post
			43.	*Herpetogramma* sp. Lederer, 1863**	4	Mon
			44.	*Herpetogramma* sp. Lederer, 1863	2	Mon
			45.	*Hodebertia testalis* (Fabricius, 1794)	3	Mon
			46.	*Hymenia perspectalis* (Hübner, 1796)	4	Mon
			47.	*Leucinodes orbonalis* Guenée, 1854**	1	Mon
			48.	*Maruca vitrata* Fabricius, 1787	25	Pre; Post
			49.	*Nausinoe perspectata* (Fabricius, 1775) **	2	Post
			50.	*Nomophila noctuella* (Denis & Schiffermüller, 1775)	6	Pre
			51.	*Notarcha aurolinealis* (Walker, 1859)*	3	Mon
			52.	*Omiodes analis* Snellen, 1880	82	Mon; Post
			53.	*Omiodes indicata* (Fabricius, 1775) *	3	Mon; Post
			54.	*Omiodes milvinalis* (Swinhoe, [1886]) *	3	Mon
			55.	*Omiodes* sp. Guenée, 1854	10	Pre; Mon; Post
			56.	*Omiodes* sp. Guenée, 1854	9	Post
			57.	*Orphnophanes eucerusalis* (Walker, 1859)	1	Post
			58.	*Pachynoa sabelialis* (Guenée, 1854) **	5	Mon; Post
			59.	*Paliga damastesalis* (Walker, 1859)	1	Mon; Post
			60.	*Palpita vitrealis* (Rossi, 1794)	1	Post
			61.	*Parotis marginata complex* (Hampson, 1893)	1	Mon
			62.	*Patania caletoralis* (Walker, 1859)	1	Mon
			63.	*Patania* sp. Moore, 1888*	1	Mon
			64.	*Pilocrocis milvinalis* Swinhoe, 1886**	3	Post
			65.	*Poliobotys ablactalis* (Walker, 1859) *	1	Mon
			66.	*Pycnarmon alboflavalis* (Moore, 1888) *	1	Mon
			67.	*Pycnarmon cribrata* (Fabricius, 1794) *	2	Post
			68.	*Pygospila tyres* (Cramer, [1780]) **	1	Post
			69.	*Rehimena phrynealis* (Walker, 1859)****	2	Mon
			70.	*Sameodes cancellalis* (Zeller, 1852) *	1	Mon
			71.	*Scirpophaga excerptalis* (Walker, 1863) *	1	Mon
			72.	*Scirpophaga incertulas* (Walker, 1863)	4	Mon; Post
			73.	*Spoladea recurvalis* (Fabricius,1775)	27	Mon; Post
			74.	*Syllepte concatenalis* (Walker, 1865	39	Mon
			75.	*Tysponodes linealis* (Moore, [1868]) *	1	Mon
			76.	*Udea ferrugalis* Hübner, 1796***	1	Post
E.	Drepanidae	Drepaninae	77.	*Tridrepana albonotata* (Moore, 1879) *	1	Post
			78.	*Teldenia vestigiata* Butler, 1880	5	Mon; Post
F.	Erebidae	Aganainae	79.	*Asota caricae* (Fabricius, 1775)	43	Pre; Mon; Post
		Arctiinae	80.	*Argina astrea* (Drury, 1773)*	1	Post
			81.	*Aloa lactinea* (Cramer, [1777]) **	1	Mon
			82.	*Asura calamaria* (Moore, 1888) **	2	Post
			83.	*Barsine orientalis bigamica* (Cerny & Pinratana, 2009)	8	Mon
			84.	*Brunia antica* (Walker, 1854)	9	Mon; Post
			85.	*Creatonotos gangis* (Linnaeus,1763)*	17	Mon; Post
			86.	*Creatonotos transiens* (Walker, 1855) **	23	Mon; Post

92.	*Eilema sororcula* Hufnagel, 1766	9	Post

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
	Erebidae	Arctiinae	87.	*Cyana arama* (Moore,1859) *	1	Pre; Mon; Post
			88.	*Cyana coccinea* (Moore, 1878)	8	Post
			89.	*Cyana puella* (Drury, 1773) *	1	Post
			90.	*Cyana* sp. Walker, 1854	1	Mon; Post
			91.	*Eilema cf. costalis* Moore, 1878	15	Mon; Post
			92.	*Eressa confinis* (Walker,1854) *	1	Mon
			93.	*Juxtarctia multiguttata* (Walker, 1855) **	1	Post
			94.	*Macrobrochis gigas* (Walker, 1854)	41	Mon
			95.	*Mangina argus* (Kollar, [1844]) **	1	Mon
			96.	*Miltochrista dharma* (Moore, 1879)	38	Mon; Post
			97.	*Miltochrista* sp. Hübner, [1819]	1	Mon
			98.	*Miltochrista terminospota* N. Singh, Kirti & Joshi, 2015	1	Mon
			99.	*Macotasa nubecula* (Moore, 1879)	15	Mon; Post
			100.	*Nyctemera adversata* (Schaller, 1788) *	2	Post
			101.	*Olepa ricini* (Fabricius, 1775)	8	Mon
			102.	*Phragmatobia fuliginosa* (Linnaeus, 1758)	1	Post
			103.	*Zadadra distorta* (Moore, 1872)	27	Post
			104.	*Rajendra biguttata* (Walker, 1855) **	1	Mon
			105.	*Rema tetraspila* Walker, 1865 **	1	Mon
			106.	*Spilosoma virginica* (Fabricius, 1798) **	2	Post
			107.	*Syntomoides imaon* (Cramer, [1779])	29	Pre; Mon; Post
			108.	*Wittia* sp.	9	Post
		Boletobiinae	109.	*Ataboruza divisa* (Walker, 1862) **	1	Post
			110.	*Diomea lignicolora* (Walker, [1858]) *	5	Mon
			111.	*Enispa* sp. Walker, [1866] **	1	Post
			112.	*Eublemma accedens* Felder &Rogenhofer, 1874)	2	Post
			113.	*Gesonia obeditalis* Walker, [1859] *	2	Post
			114.	*Zurobata vacillans* (Walker, 1864) **	1	Mon; Post
		Calpinae	115.	*Calyptra ophideroides* (Guenée, 1852) **	1	Post
			116.	*Episparis liturata* Fabricius, 1787*	1	Mon
			117.	*Eudocima cajeta* Cramer, ([1775]) **	1	Post
			118.	*Oraesia emarginata* (Fabricius, 1794)	2	Pre; Mon; Post
		Erebinae	119.	*Attatha ino* (Drury, 1782) **	1	Mon
			120.	*Artena dotata* (Fabricius, 1794)	4	Post
			121.	*Chalciope mygdon* (Cramer, [1777])	2	Post
			122.	*Bastilla arcuata* (Moore, 1877) *	1	Post
			123.	*Bastilla crameri* (Moore, [1885])	7	Mon; Post
			124.	*Entomogramma fautrix* Guenée, 1852 *	1	Post
			125.	*Erebus albicincta* Kollar, [1844] *	1	Post
			126.	*Erebus caprimulgus* (Fabricius, 1775)*	1	Mon
			127.	*Erebus hieroglyphica* (Drury, 1773) **	3	Post
			128.	*Fodina pallula* Guenee, 1852	8	Pre; Mon; Post
			129.	*Fodina stola* (Guenée, 1852) **	1	Mon
			130.	*Grammodes geometica* (Fabricius, 1775)	1	Mon

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
		Erebinae	131.	*Homaea clathrum* Guenée, 1852****	3	Mon
			132.	*Hamodes propitia* (Guérin-Méneville, [1831])	1	Mon
			133.	*Ischyja marapok* Holloway, 2005****	3	Mon
			134.	*Mocis frugalis* (Fabricius, 1775)	23	Mon; Post
			135.	*Mocis undata* (Fabricius, 1775)	3	Mon; Post
			136.	*Nagia linteola* Guenee,1852*	1	Mon
			137.	*Pericyma umbrina* (Guenée, 1852)	2	Mon; Post
			138.	*Spirama retorta* Clerck, 1764	2	Pre; Mon; Post
			139.	*Thyas coronata* (Fabricius, 1775) **	1	Mon
		Herminiinae	140.	*Adrapsa ereboides* (Walker, [1863]) *	1	Mon
			141.	*Progonia oileusalis* (Walker, [1859]) *	1	Post
		Hypeninae	142.	*Dichromia pullata* Moore,1885*	4	Post
			143.	*Hypena iconicalis* Walker, [1859]	2	Post
			144.	*Hypena* spp. Schrank, 1802	2	Mon; Post
			145.	*Hypena* spp. Schrank, 1802	1	Mon
			146.	*Hypena* spp. Schrank, 1802	1	Post
			147.	*Hypena* spp. Schrank, 1802	5	Post
			148.	*Hypena laceratalis* Walker, [1859]	18	Mon; Post
			149.	*Hypena obacerralis* Walker, [1859] *	1	Post
		Hypocalinae	150.	*Hypocala deflorata* Fabricius, 1794*	1	Mon
		Lymantriinae	151.	*Artaxa digramma* (Boisduval, [1844])	3	Mon; Post
			152.	*Artaxa guttata* Walker, 1855	11	Pre; Mon; Post
			153.	*Artaxa* sp. Walker, 1855	1	Post
			154.	*Arctornis lactea* (Moore, 1879) *	1	Mon
			155.	*Arctornis comma* (Hutton, 1865) ***	1	Mon; post
			156.	*Arctornis submarginata* (Walker, 1855)**	1	Post
			157.	*Arctornis* sp. Germar, 1810	10	Mon; Post
			158.	*Calliteara* sp. Butler, 1881**	1	Mon
			159.	*Euproctis lunata* Walker, 1855**	1	Post
			160.	*Euproctis magna* (Swinhoe, 1891) *	1	Post
			161.	*Lymantria concolor* Walker, 1855*	9	Post
			162.	*Lymantria detersa* Walker, 1865**	1	Post
			163.	*Lymantria incerta* Walker, 1855	3	Mon; Post
			164.	*Lymantria marginata* Walker, 1855*	4	Pre; Mon
			165.	*Lymantria mathura* Moore, [1866]	8	Mon; Post
			166.	*Lymantria* sp. Hübner, [1819]	1	Pre
			167.	*Lymantria todara* Moore, 1879**	1	Post
			168.	*Olene* sp. Hübner, 1823	1	Post
			169.	*Orgyia postica* (Walker, 1855)	9	Mon; Post
			170.	*Somena scintillans* Walker, 1856	9	Pre; Mon; Post
		Scoliopteryginae	171.	*Anomis flava* (Fabricius, 1775)	1	Post
			172.	*Anomis fulvida* Guenée, 1852	2	Post
		Tinoliinae	173.	*Calesia dasypterus* Kollar, 1844**	1	Pre; Post

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
G.	Eupterotidae	Eupterotinae	174.	*Eupterote bifasciata* Kishida, 1994	55	Mon
			175.	*Eupterote mollifera* Walker, 1865*	1	Mon
			176.	*Eupterote undata* Blanchard, [1844]	16	Mon
			177.	*Ganisa plana* Walker, 1855	7	Mon
			178.	*Ganisa* sp. Walker, 1855**	2	Mon
H.	Geometridae	Ennominae	179.	*Abraxas* sp. Leach, 1815	2	Post
			180.	*Abraxas* sp. Leach, 1815	1	Post
			181.	*Anonychia grisea* (Butler, 1883)	2	Mon; Post
			182.	*Biston suppressaria* (Guenée, [1858]) **	3	Pre; Mon
			183.	*Calletaera subexpressa* (Walker,1861) **	1	Post
			184.	*Chiasmia eleonora* (Cramer, [1780])	10	Pre; Mon; Post
			185.	*Chiasmia emersaria* (Walker, 1861)**	6	Post
			186.	*Chiasmia* sp. Hübner, [1823]	1	Mon
			187.	*Chorodna strixaria* (Guenée, [1858]) **	1	Mon
			188.	*Cleora contiguata* (Moore, [1868])****	1	Mon
			189.	*Corymica* sp. Walker, 1860	2	Post
			190.	*Cusiala boarmoides* Moore, [1887] *	2	Mon
			191.	*Cusiala* sp. Moore, [1887]	1	Mon
			192.	*Dasyboarmia subpilosa* (Warren, 1894)	3	Post
			193.	*Dalima patularia* (Walker, 1860) *	1	Pre
			194.	*Ectropidia shoreae* (Prout, 1934) *	1	Mon; Post
			195.	*Eumelea* sp. Duncan [& Westwood], 1841	2	Pre; Mon; Post
			196.	*Fascellina chromataria* Walker, 1860*	1	Mon
			197.	*Gonodontis clelia* (Cramer, [1780]) **	1	Post
			198.	*Hypochrosis abstractaria* (Walker, [1863])	3	Mon
			199.	*Hypomecis procursaria* (Walker,1860) **	3	Post
			200.	*Heterostegane* sp. Hampson, 1893	1	Mon
			201.	*Heterostegane tritocampsis* (Prout, 1934)	5	Mon; Post
			202.	*Hypomecis cineracea* (Moore, 1888)	52	Pre; Mon; Post
			203.	*Hypomecis separata* (Walker 1860) *	16	Pre; Mon; Post
			204.	*Hypomecis* sp. Hübner, 1821	3	Post
			205.	*Hypomecis* sp. Hübner, 1821	1	Post
			206.	*Hydatocapnia marginata* (Warren, 1893)	1	Post
			207.	*Hydatocapnia* sp. Warren, 1895	1	Post
			208.	*Hyposidra talaca* Walker, 1860	28	Pre; Mon; Post
			209.	*Iridoplecta ferrifera* (Moore, 1888) ****	1	Mon
			210.	*Phazaca* sp. Walker, 1863****	1	Mon
			211.	*Scardamia* sp. Guenée, 1857*	1	Post
			212.	*Zamarada Euphrosyne* Oberthür, 1912	2	Post
			213.	*Zamarada* sp. Moore, [1887]	5	Mon; Post
		Geometrinae	214.	*Agathia lycaenaria* (Kollar, 1848)	3	Mon; Post
			215.	*Chlorozancla falcatus* (Butler, 1889) *	1	Mon
			216.	*Comibaena cassidara* (Guenée, [1858]) *	1	Mon
			217.	*Episothalma robustaria* (Guenée, 1858)	1	Post

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
	Geometridae	Geometrinae	218.	*Hemithea tritonaria* (Walker, [1863]) **	4	Pre; Mon; Post
			219.	*Herochroma* sp. Swinhoe, 1893	1	Pre
			220.	*Maxates veninotata* (Warren, 1894)	4	Post
			221.	*Ornithospila avicularia* (Guenée, 1857) *	3	Mon
			222.	*Protuliocnemis biplagiata* (Moore, [1887])	2	Pre; Post
			223.	*Pelagodes falsaria* (Prout, 1912)	1	Pre; Post
			224.	*Pelagodes* sp. Holloway, 1986	1	Post
			225.	*Pelagodes veraria* (Guenee, 1857)	2	Mon
			226.	*Pingasa ruginaria* (Guenée, [1858])	3	Post
			227.	*Pingasa* sp. Moore, [1887]	6	Pre
			228.	*Spaniocentra lyra* (Swinhoe, 1982)	4	Pre;post
			229.	*Thalassodes quadraria* Guenee, 1857*	1	Post
		Larentiinae	230.	*Eupithecia abisinthiata* Clerck,1759*	2	Mon
			231.	*Photoscotosia miniosata* (Walker, 1862)	1	Post
		Sterrhinae	232.	*Scopula cuneilinea* (Walker, [1863])	1	Pre
			233.	*Anisephyra ocularia* (Fabricius, 1775) *	5	Mon
			234.	*Problepsis albidior* Warren, 1899	3	Mon
			235.	*Scopula cuneilinea* (Walker, [1863])	1	Mon
			236.	*Scopula* sp. Schrank, 1802***	6	Pre; Mon; Post
			237.	*Scopula* sp. Schrank, 1802**	1	Mon; Post
			238.	*Scopula* sp. Schrank, 1802	3	Pre; Post
			239.	*Traminda mundissima* (Walker, 1861) *	4	Mon
I.	Lasiocampidae	Lasiocampinae	240.	*Gastropacha* sp. Ochsenheimer, 1810**	1	Mon; Post
			241.	*Kunugia* sp. Nagano, 1917	3	Mon; Post
			242.	*Metanastria* sp. Hübner, [1820]**	3	Mon
			243.	*Trabala vishnou* (Lefèbvre, 1827)	3	Mon; Post
		Pinarinae	244.	*Lebeda trifascia* Walker, 1855	1	Mon
J.	Limacodidae	Limacodinae	245.	*Aergina hilaris* (Westwood, 1848)	30	Mon; Post
			246.	*Altha subnotata* (Walker, 1865)	33	Mon
			247.	*Cania bilinea* (Walker, 1855)	1	Mon
			248.	*Cheromettia apicata* (Moore, 1879) **	9	Mon
			249.	*Cheromettia* sp. Moore, 1883	1	Mon
			250.	*Miresa argentifera* Walker, 1855*	1	Mon
			251.	*Miresa bracteata* Butler, 1880	25	Mon
			252.	*Miresa fulgida* Wileman, 1910*	1	Mon
			253.	*Parasa lepida* Cramer, 1799*	2	Mon
			254.	*Phocoderma velutina* (Kollar, [1844])	66	Mon; Post
			255.	*Thespea bicolor* (Walker, 1855)*	2	Mon
			256.	*Thosea* sp. Walker, 1855	42	Mon; Post
			257.	*Scopelodes testacea* Buttler, 1886	34	Mon
			258.	*Scopelodes venosa* Walker, 1855**	2	Mon

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
K.	Noctuidae	Agaristinae	259.	*Aegocera venulia* Cramer, [1777]**	17	Mon; Post
			260.	*Episteme adulatrix* (Kollar, [1884])*	1	Mon
		Amphipyrinae	261.	*Callyna jugaria* Walker,1858	10	Pre; Mon; Post
			262.	*Callyna monoleuca* Walker, 1858	12	Mon
			263.	*Callyna* sp. Guenée, 1852	6	Mon
		Bagisarinae	264.	*Chasmina candida* Walker,1865	2	Mon
			265.	*Chasmina* sp. Walker, 1856	1	Pre; Mon
		Condicinae	266.	*Bagada spicea* (Guenée, 1852)	2	Pre; Mon
			267.	*Condica conducta* Walker 1857	1	Pre; Mon; Post
			268.	*Condica* sp. Walker, 1856***	1	Mon
		Eustrotiinae	269.	*Amyna axis* (Guenee, 1852)	19	Mon; Post
			270.	*Maliattha quadripartita* (Walker, 1865)	10	Mon
			271.	*Maliattha separata* Walker, 1863	2	Mon; Post
			272.	*Maliattha* sp. Walker, 1863	1	Post
		Hadeninae	273.	*Athetis delecta* (Moore, 1881) **	1	Pre
			274.	*Leucania* sp. Ochsenheimer, 1816*	1	Mon
			275.	*Mythimna (Aletia) hamifera* (Walker, 1862)	2	Mon
		Heliothinae	276.	*Helicoverpa armigera* (Hübner, [1808]) *	6	Pre; Mon; Post
		Noctuinae	277.	*Axylia* sp. Hübner, [1821]	1	Post
			278.	*Leucania yu* Guenée, 1852 *	1	Post
			279.	*Spodoptera litura* (Fabricius, 1775)	32	Pre; Mon; Post
			280.	*Spodoptera* sp. Guenée, 1852****	1	Mon; Post
		Plusiinae	281.	*Chrysodeixis eriosoma* Doubleday, 1843*	5	Mon; Post
			282.	*Thysanoplusia intermixta* (Warren, 1913)	2	Post
			283.	*Thysanoplusia orichalcea* (Fabricius, 1775)	2	Pre; Post
			284.	*Thysanoplusia reticulata* (Moore, 1882)	1	Post
			285.	*Thysanoplusia* sp. Ichinosé, 1973****	1	Mon
			286.	*Thysanoplusia* sp. Ichinosé, 1973	3	Pre; Mon
L.	Nolidae	Chloephorinae	287.	*Carea angulata* (Fabricius, 1793)	1	Mon
		Eligminae	288.	*Selepa celtis* Moore, [1860]*	2	Post
		Westermanniinae	289.	*Westermannia superba* Hübner, 1823**	3	Mon; Post
M.	Notodontidae	Phalerinae	290.	*Phalera grotei* Moore, 1859	70	Mon
N.	Pyralidae	Pyralinae	291.	*Arippara indicator* Walker, [1864]*	1	Mon; Post
			292.	*Zitha torridalis* (Lederer, 1863)	1	Mon
O.	Sphingidae	Macroglossinae	293.	*Acosmeryx pseudonaga* Butler, 1881*	1	Mon
			294.	*Eupanacra mydon* (Walker, 1856)	1	Mon
			295.	*Macroglossum corythus* Walker, 1856	2	Pre
			296.	*Nephele hespera* (Fabricius, 1775) *	3	Pre
		Smerinthinae	297.	*Anambulyx elwesi* (Druce, 1882) *	1	Mon
			298.	*Marumba cristata* (Butler, 1875) *	1	Mon
			299.	*Marumba dyras* (Walker, 1856) **	1	Mon
			300.	*Marumba indicus* (Walker, 1856) **	14	Mon
			301.	*Marumba* sp. Moore, 1882	1	Mon
		Sphinginae	302.	*Dolbina inexacta* (Walker, 1856)*	1	Mon

	Family	Sub-family		Scientific name	No. of individuals sampled	Flight period/ Seasons #
P.	Saturniidae	Saturniinae	303.	*Actias selene* (Hübner, [1807])	6	Mon; Post
			304.	*Antheraea assamensis* Helfer, 1837*	1	Mon
			305.	*Antheraea mylitta* Drury, 1773*	1	Mon
		Siculodinae	306.	*Herdonia thaiensis* Inoue, 1993*	1	Mon
			307.	*Herdonia* sp. Walker, 1859	1	Mon
			308.	*Hypolamprus* sp. Hampson, [1893]****	2	Mon
			309.	*Opula* sp. Walker, 1869	1	Post
			310.	*Striglina scitaria* (Walker, 1862)	3	Mon; Post
			311.	*Striglina* sp. Guenée, 1877**	1	Mon
Q.	Uraniidae	Epipleminae	312.	*Dysaethria lilacina* (Moore, [1887])*	2	Mon; Post
			313.	*Epiplema fulvilinea* (Hampson, 1891) **	2	Mon; Post
			314.	*Epiplema ruptaria* Moore, 1883*	1	Mon
			315.	*Epiplema* sp. Herrich-Schäffer, [1855]	4	Mon
			316.	*Epiplema* sp. Herrich-Schäffer, [1855]	1	Mon; Post
			317.	*Orudiza protheclaria* Walker,1861	4	Mon
			318.	*Phazaca* sp. Walker, 1863****	1	Mon
		Microniinae	319	*Acropteris iphiata* Guenée, 1857*	2	Mon
			320	Pseudomicronia sp. Moore, 1887	1	Mon
R.	Zygaenidae	Chalcosiinae	321.	*Pidorus glaucopis* (Drury, 1773) *	1	Mon

*Forest Sub-types (Champion &Seth1968): *— 3C/C2a Moist Shiwalik Sal Forest | **—5B/C2 Northern Dry Mixed Deciduous Forest | ***—5B/C1a Dry Shiwalik Sal Forest | ****—3C/C2c Moist Tarai Sal Forest.
Seasons: Pre—pre-monsoon | Mon—monsoon | Post— post-monsoon.

Appendix II. GPS Coordinates of Sampling sites (Figure 1, East to West).

	Site	Coordinates	
		North	East
1	Chorgaliya	29.69797222	79.70508333
2	Pawalgarh	29.36477778	79.23961111
3	Mohan	29.54863889	79.10980556
4	Mundiapani	29.69475000	78.77605556
5	Naudi	29.78497222	78.62586111
6	Pakharo Kalagarh	29.59313889	78.57655556
7	Saneh	29.69316667	78.52483333
8	Bindhwasini	30.00361111	78.32363889
9	Jhilmil Jheel	29.80602778	78.22480556
10	Haridwar-Chandighat	29.93400000	78.18238889
11	Mothrowala	30.22136111	78.03466667
12	Asharodhi	30.26022222	77.98191667
13	Mohand	30.17672222	77.90030556
14	Karwapani	30.28725000	77.91250000
15	Timli	30.36897222	77.71991667
16	Kalesar	30.34075000	77.58341667
17	Darpur	30.38427778	77.46677778
18	Simbalbada	30.42536111	77.43325000
19	Suketi	30.50425000	77.24308333

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ARTICLE

Vertebrate assemblages on fruiting figs in the Indian eastern Himalaya's Pakke Wildlife Sanctuary

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Abstract: Ficus is undeniably one of the most important plants in the tropical forest in the Indian eastern Himalaya. The species composition and assemblages were analysed on fruiting figs on the west bank of Pakke Wildlife Sanctuary (PWS), Arunachal Pradesh. Figs trees are often ecologically significant keystone species because they sustain the population of the many seed-dispersing animals that feed on these fruits. This research endeavors to comprehend the dynamics of vertebrate assemblages inhabiting fruiting figs within the West bank of PWS. Over a span of 60 days and a cumulative 89 hours of observation, both direct sightings and indirect indicators of vertebrate presence were integrated. The outcome revealed a diverse spectrum of 54 vertebrate species, comprising 43 avian and 11 mammalian species, distributed across four Ficus species; concurrently, alternative plant species accommodated 28 avian and four mammalian species. Among these, the pre-eminence of green pigeons within Ficus species underscores their feeding behaviors, underscoring the vital role of figs as a dietary cornerstone within PWS's west bank. Notably, the comparative underrepresentation of vertebrates on the local fig species *Ficus drupacea* offers intriguing insights. The findings substantiate the significance of figs as a nourishment resource and instigate the necessity for extended investigations to fully unravel the intricate reliance of vertebrates on Ficus species within the tapestry of tropical forests.

Keywords: Biodiversity community structure, habitat, keystone resources, species coexistence, tropical forest.

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INTRODUCTION

Ficus is one of the largest genera of woody species in the tropics and subtropics (Janzen 1979; Berg 1989; Harrison 2005) with approximately 750 species occurring globally (Berg 2005). India has 115 *Ficus* taxa belonging to 89 species and 26 intra-specific taxa (Chaudhary et al. 2012); 58 species have been reported from Arunachal Pradesh alone (Buragohain 2014). Tropical forests are a rich source of food for animals dependent on fruit (Fleming et al. 1987; Corlett 1998), where *Ficus* is identified as a vital 'keystone' food resource that attracts tropical frugivorous animals (Kinnaird et al. 1996; Kannan & James 1999; Kissling et al. 2007). Keystone plants play a significant role in setting the carrying capacity of the frugivore community and in the tropics, the diversity and abundance of Ficus (figs) correlate with the diversity or richness of frugivores (Goodman et al 1997). Ficus sustain diverse organisms owing to dense foliage and moisture retention capacity that provides an ideal habitat in terms of nesting, roosting, and perching grounds for vertebrate species (Vanitharani 2006). Although figs are considered keystone species, this concept usually signifies the whole Ficus community rather than a single species (Kinnaired et al. 1999). The existence of different Ficus dispersal guilds implies that fig preference of frugivores is influenced by chemical, and morphological variables such as size, colour, display mechanism and habitat characteristics such as forested, disturbed, and urban. (Sanitjan & Chen 2009; Lok et al. 2013; Daru et al. 2015). Different species of figs differ in their nutrient content nevertheless; a single species is insufficient to provide adequate nutrients to the species that depend on it (Wendeln et al. 2000). Non-bird dispersal Ficus often display their figs in places where it is not convenient for the birds (Lambert 1989a; Shanahan & Crompton 2001). These traits help Ficus species to attract discrete frugivore species which, in return guide frugivores while selecting suitable fruits. A global review of figs and vertebrates revealed that 1,274 bird and mammal species in 523 genera and 92 families are known to eat figs apart from the small number and fish and reptiles (Shanahan et al. 2001).

Studies on fig dependency on vertebrates in India particularly in northeastern India are scanty where the Ficus diversity is higher and usually such studies are species-specific (Datta & Rawat 2008; Krishna et al. 2014). Therefore, the present study was planned to investigate the vertebrate assemblages, inter-species differences among the fruiting Ficus and non-ficus species and Ficus preference of vertebrates over a period of 60 days to understand how Ficus species form the focal points for vertebrate assemblages in Pakke Wildlife Sanctuary of Arunachal Pradesh, India. Thus, providing insights into vertebrates that are dependent on figs in this region.

METHODS

Study Site

This study was conducted in Pakke Wildlife Sanctuary (PWS) 27.430278 N to 93.4025 E and 28.369167 N to 94.360833 E located in the Pakke Kesang district, Arunachal Pradesh. It is one of the best-managed protected areas of the state among the 13 protected areas and is famous for the two major flagship species, viz., hornbills and tiger. PWS shares its boundary with Nameri Tiger Reserve, Doimara Reserve Forest, Papum Reserve Forest, Tenga Reserve Forest, and Sessa Orchid Sanctuary and it is surrounded by Pakke River in the east Kameng River in the west and north. The rugged and hilly terrain encompasses elevational diversity, ranging 150–2,000 m. The forest falls under the classification of Assam Valley tropical semi-evergreen forest 2B/C1 according to Champion & Seth (1969). It has a tropical and subtropical climate where October to February is the coldest month (Birand & Power 2004), and May and June are the hottest. Park receives rainfall from south-west monsoon (May–September) and north-east monsoon (November–April). The average annual rainfall ranges 2,086.9–2,972.7mm (humid subtropical region-cold humid regions) and the average mean maximum and minimum temperatures are 29.5^0 C and 17.7^0 C in the humid subtropical region and 21.4^0 C and 2.4^0C in the cold humid region (Buragohain 2014). The floristic and climatic conditions provide rich faunal diversity in the sanctuary by documenting 60 species of mammals, 282 species of birds (Kumar 2014) and home to around 340 species of butterflies (Sondhi & Kunte 2014). The area holds four species of hornbill and is stated to be one of the best places for frequent sightings of hornbill species in the state (Datta 2001).

There are 19 villages located in the eastern periphery of the sanctuary and the population is dominated by Nyishi; a major ethnic tribe of the state (Vishwakarma et al. 2021). Their livelihood involves the collection of non-timber forest produce, hunting and fishing, shifting agriculture and cultivation of rice (Datta et al. 2008; Hui et al. 2012), maize and millets. The West Bank area (26.938° N, 92.911° E) with an elevation of 150–600 m (Datta & Rawatt 2008) of the sanctuary was selected to document the assemblage of vertebrate species on

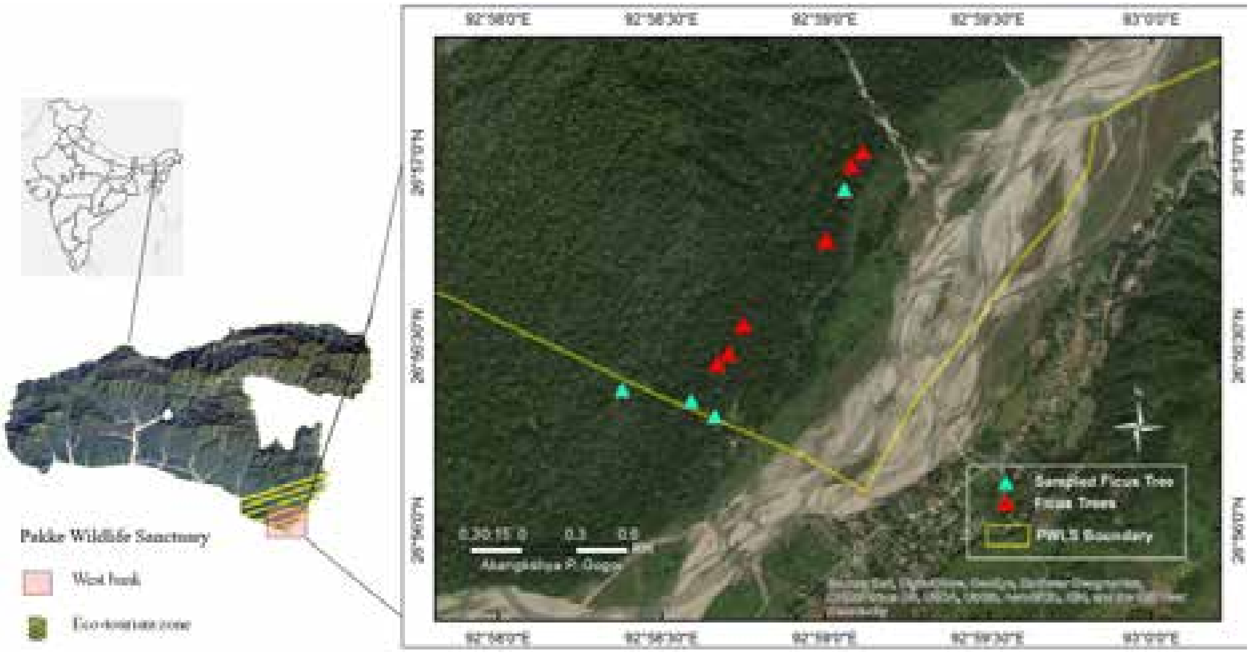

Image 1. Map of west bank showing locations of *Ficus* trees chosen for documenting the vertebrate species, Pakke Wildlife Sanctuary, Arunachal Pradesh.

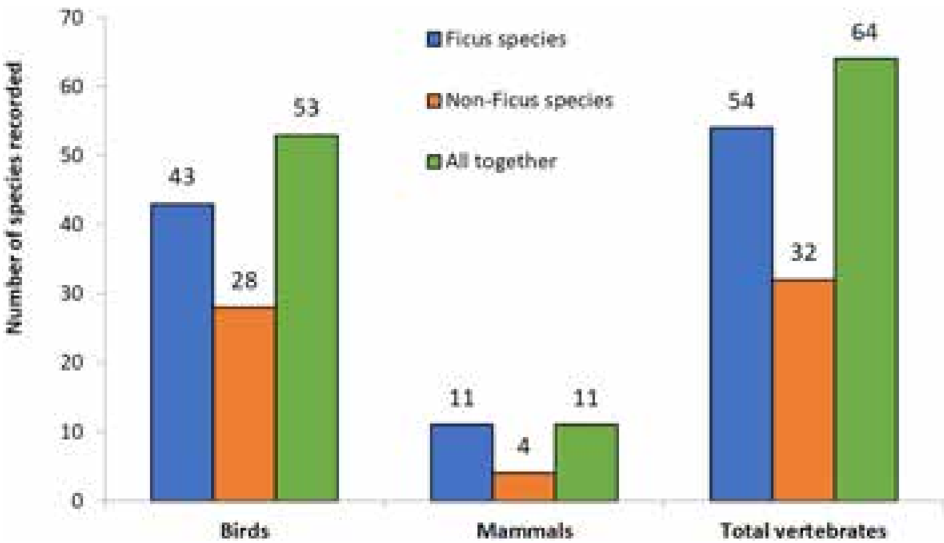

Figure 1. Vertebrate diversity was recorded at the focal Ficus and non-ficus plants in the west bank, Pakke Wildlife Sanctuary.

fruiting *Ficus* species found in the area, as it is located approximately 3 km away from the office of Pakke headquarter (Seijosa) and reachable site for the tourist (Figure 1).

Method and materials

During the study period, the existing nature trail in the study area was first surveyed to locate fruiting *Ficus* species and fruiting and flowering non-ficus plants. Secondly, bird surveys were conducted on the nature trail twice a week (16 days) from 0600–1000 h and 1300–1600 h to record the bird species of the site usually when the vertebrate fauna was active. Four *Ficus* species with over 40% ripe fruits found in the nature trail were chosen and tagged as suitable focal *Ficus* trees to document the vertebrate assemblage. *Ficus geniculata and Ficus altissima* are hemi-epiphytic axillary (inflorescence present in the leaf axis) plants, while *Ficus variegata* is a cauliflorous (inflorescence present in the trunk) tree. Fruiting and flowering non-ficus plants present within a 10-m radius of the focal *Ficus* species were also documented to compare the vertebrate assemblage with *Ficus* plants. *Ficus* species were distinguished by referencing the taxonomic framework established by Buragohain (2014). Avian identification was facilitated through the utilization of established field guides authored by Grimmett et al. (2016), while for mammalian species classification, the field guide 'Mammals of India' by Menon (2014) served as a point of reference.

Scan sampling for vertebrate species, including both mammal and bird assemblages on focal trees and non-ficus plants, was conducted between February and April 2019. The survey encompassed both direct sightings and indications of vertebrate presence. Over the 60-day (89-h) study period, selected focal *Ficus* species were visited biweekly, with observations carried out twice a week during the time intervals of 0600–0900 h and 1330–1630 h. A total of 44 scan sample episodes were performed, each averaging 3-h per scan, and yielding an average of two samples per day. During each scan of focal species, the species name, the total count of visiting individuals, and the overall time spent by the visiting vertebrate species were meticulously documented. In the case of non-ficus plants, the name of the visiting vertebrate species and the total count of species encountered during each scan were recorded.

In this study, the vertebrate species data associated with each focal Ficus species were compared during scanning sessions with the data collected from the neighboring non-ficus fruiting and flowering plants. The recorded vertebrate species counts for both focal *Ficus* and non-ficus species were categorized into four rankings: 1 for counts between 0 and 5, 2 for counts between 6 and 11, 3 for counts between 12 and 17, and 4 for counts exceeding 18. This ranking system aimed to quantify the variation in vertebrate assemblages between *Ficus* and non-ficus plants, with statistical analysis performed using the Mann-Whitney U test. Furthermore, the spatial distribution of species was analyzed by considering the number of vertebrate species visiting each focal species, employing the variance-to-mean ratio (VMR). The VMR, a tool for discerning spatial object distribution, indicated random distribution at VMR = 1.0, clump distribution at VMR > 1.0, and uniform distribution at VMR < 1.0, as per Datta & Rawatt (2008). Additionally, to evaluate the similarity of vertebrate species among different *Ficus* species, the Jaccard similarity index was computed, shedding light on species likeness within the focal *Ficus* species' group.

Vertebrates directly observed feeding on figs were categorized into three groups: frugivorous birds (including Bulbuls, Barbets, Pigeons, Hornbills, Mynas, Orioles, and Asian fairy bluebirds) following Naniwadekar et al. (2019), opportunistic feeders of figs (occasionally consuming figs), and mammals (detailed in Appendix 2). The preference of vertebrate species for specific Ficus species was determined using data on the number of individuals, total time spent, and visit frequency, applying the formula established by Ragusa-Netto (2002). *Ficus variegata* was excluded from Ficus preference analysis due to its infrequent encounters throughout the sampling period Let, Px = {Mean individual/scan * Mean visiting time duration of species/ scan * visiting frequency (no. of time a species visited a focal tree throughout the survey}, P = Presence value of a vertebrate species in a focal Ficus species, x = Ficus species.

TPx = Sum of (Px) of all vertebrate species assembled in the focal *Ficus* species

Tree preference (percentage) = Px / TPx * 100

RESULTS

A total of 15 individuals of five *Ficus* species, viz., *Ficus nervosa* (1), *Ficus drupacea* (7), *Ficus geniculata* (3), *Ficus altissima* (2), & *Ficus variegata* (2) and 13 species (n = 41) *of non*-ficus fruiting and flowering plants representing 10 families*; Canarium resiniferum, Duabanga grandiflora, Sterculia villosa, Sterculia colorata, Tetrameles nudiflora, Shorea robusta, Dysoxylum binectariferum, Artocarpus chaplasha, Polyalthia simiarum, Chisocheton paniculatus, Aglaia spectabilis, Phlogacanthus thyrsiformis* (shrub), *Dilenia indica* (Appendix 3) was recorded. *Ficus drupacea, Ficus geniculata* and *Ficus altissima* are hemi-epiphytic axillary (inflorescence present in the leaf axis) plants while *Ficus variegata* is cauliflorous (inflorescence present in the trunk) tree.

Vertebrate diversity in the West bank area of PWS

During the study, a total of 64 vertebrate species within four focal *Ficus* species and the surrounding non-ficus fruiting and flowering plants were identified Among these, there were 53 bird species belonging to 29 families and 43 genera, as well as 11 mammal species from seven families and 10 genera (Figure 2). It's important to note that across the entire study duration, the nature trail recorded a comprehensive total of 98

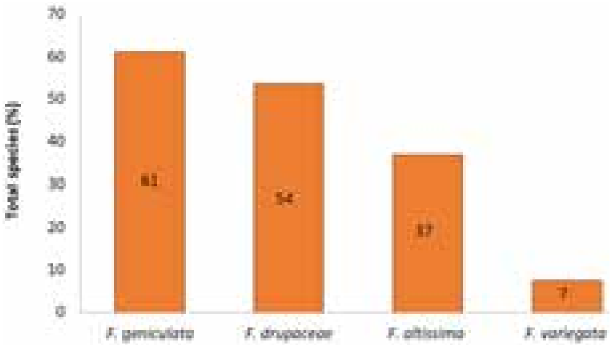

Figure 2. Vertebrate species composition at focal *Ficus* trees in the west bank, Pakke Wildlife Sanctuary out of all the species recorded.

bird species representing 39 families and 76 genera, and this information is provided in Appendix 1.

Vertebrate assemblage

Recorded were 43 species of birds (21 families, 34 genera) and 11 species of mammals (7 families, 10 genera) in focal *Ficus* species, namely, *variegata*, *drupacea*, *altissima*, and *variegata*. Additionally, 28 species of birds (21 families, 23 genera) and four species of mammals (3 families, 4 genera) were found in fruiting and flowering non-ficus plants within a 10 m radius of the focal *Ficus* species (Figure 2). The highest vertebrate assemblage was observed in *Ficus variegata*, accounting for 61% (29 bird species, 4 mammal species), followed by *Ficus drupacea* at 54% (22 bird species, 7 mammal species), *Ficus altissima* at 37% (19 bird species, 1 mammal species). The lowest vertebrate assemblage was recorded in *Ficus variegata*, constituting 7% (3 bird species, 1 mammal species) (Figure 3). The most prevalent vertebrate species within Ficus were green pigeons (4 species, 70.2 individuals/scan), followed by mynas (1 species, 32.3 individuals/scan), bulbuls (6 species, 28.3 individuals/scan), hornbills (3 species, 13.4 individuals/scan), Asian fairy bluebirds (1 species, 12.4 individuals/scan), along with other bird species (19 species, 9.8 individuals/scan), mammals (11 species, 7.3 individuals/scan), opportunists (4 species, 7.1 individuals/scan), barbets (3 species, 5.2 individuals/scan), and orioles (2 species, 2.2 individuals/scan) (Figure 4).

Birds visited the Ficus species more frequently during the different times. The mean vertebrate assemblage (clockwise direction) in fig trees (21.5 ± 12.9), *Ficus geniculate* (11.3 ± 4.9), *Ficus drupacea* (7.3 ± 3.6), *Ficus*

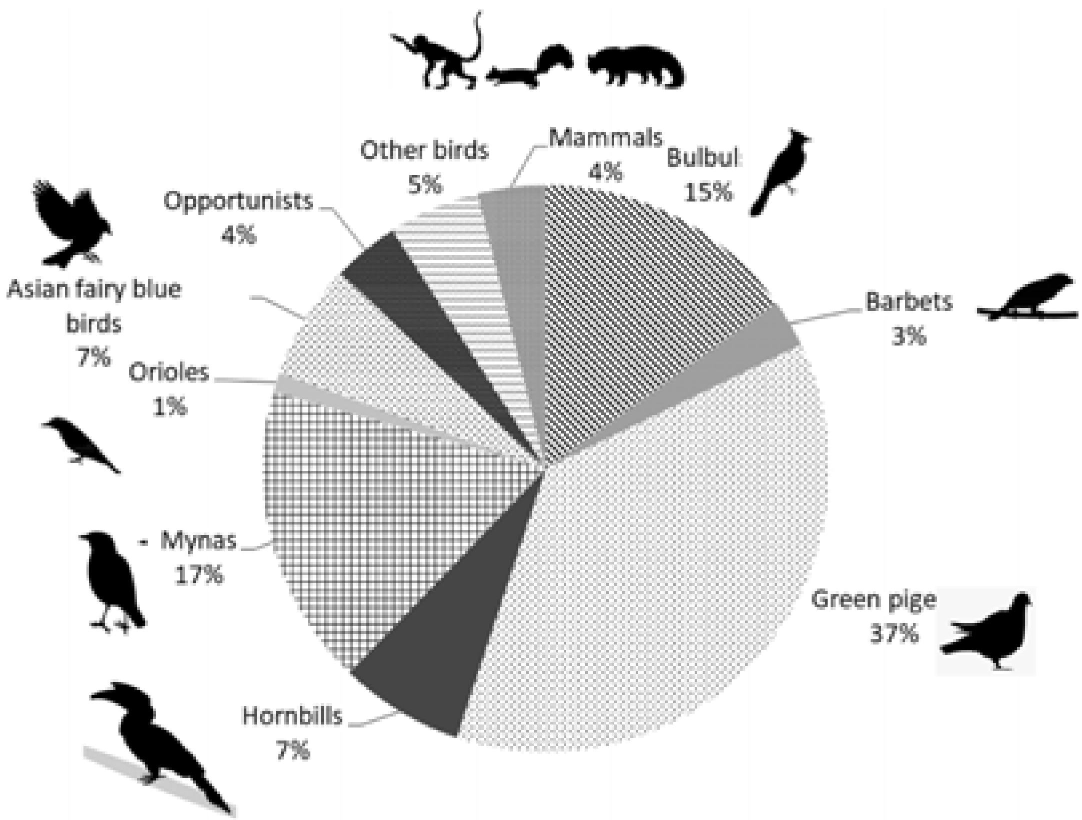

Figure 3. The abundance of different vertebrate taxa (n = 54) was observed in focal Ficus species during the study.

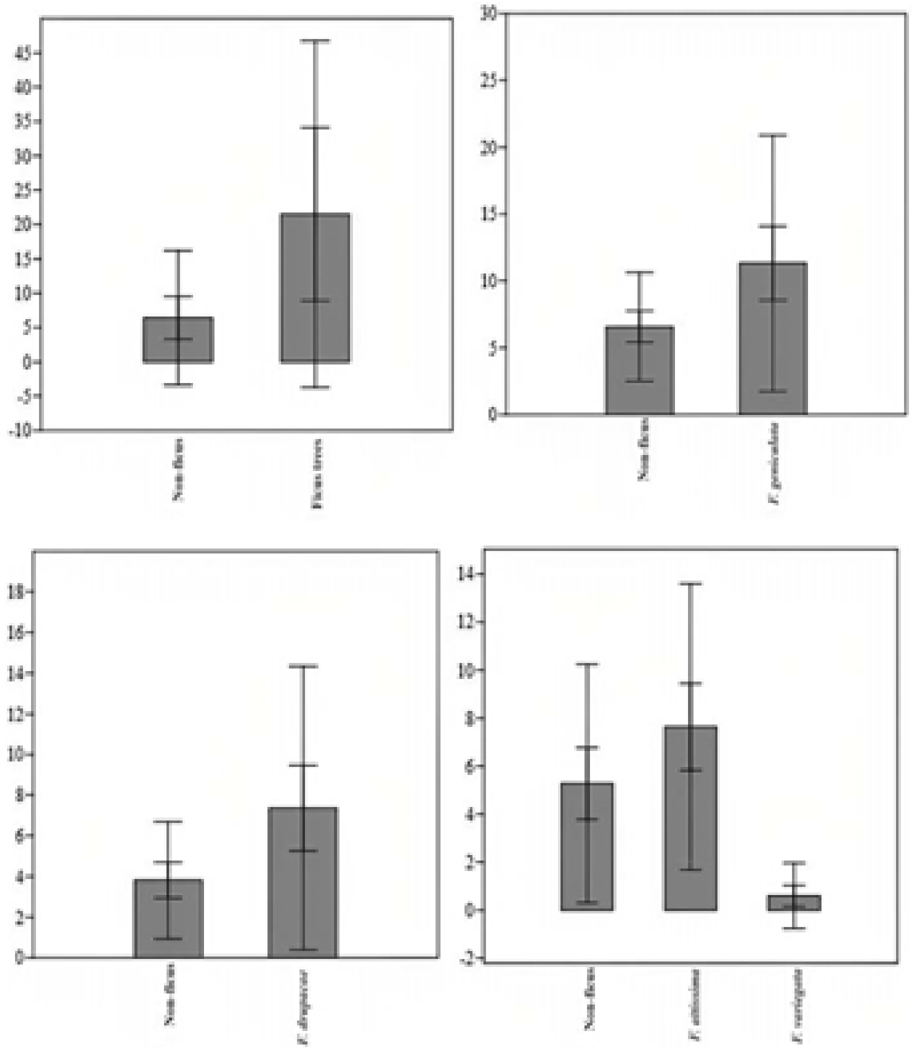

Figure 4. Mean vertebrate assemblage (clockwise direction) in *Ficus* trees (21.5 ± 12.9), *Ficus geniculata* (11.3 ± 4.9), *Ficus drupacea* (7.3 ± 3.6), *Ficus altissima* (7.6 ± 3), *Ficus variegata* (0.6 ± 0.7) and non-ficus fruiting and flowering trees (6.4 ± 5, 6.9 ± 2.07, 5.27 ± 2.53, 3.82 ± 1.5 within 10-m radius).

altissima (7.6 ± 3), *Ficus variegata* (0.6 ± 0.7), and non-ficus fruiting & flowering trees (6.4 ± 5, 6.9 ± 2.07, 5.27 ± 2.53, 3.82 ± 1.5 within 10 m radius) (Figure 5).

Comparison of vertebrate species between Ficus versus non-ficus plants

Both bird and mammal surveys in the study site recorded the maximum number of vertebrate species at focal *Ficus* at 44% and 84.4% respectively as compared to non-ficus plants at 29% and 50% (Figures 2 & 6). On the other hand, 32 vertebrate species in Ficus and 10 vertebrate species in non-ficus recorded during the scan sampling were unique or specifically confined themselves to either Ficus or non-ficus. While 34 vertebrate species were common between Ficus and non-ficus plants (Appendix 1 and 2), the number of

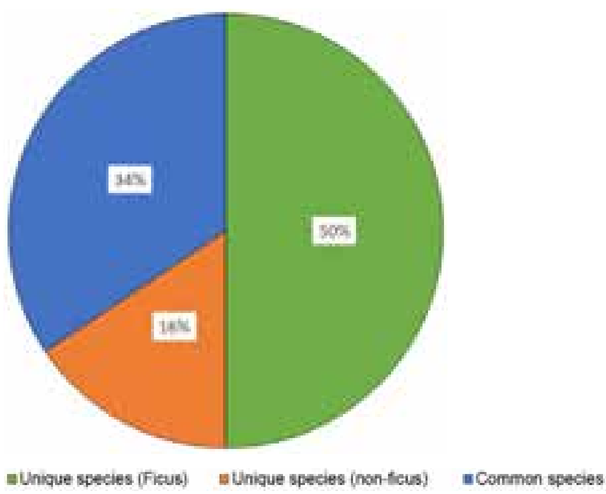

Figure 5. Composition of unique and common vertebrate species recorded in focal *Ficus* and fruiting and flowering non-ficus plants.

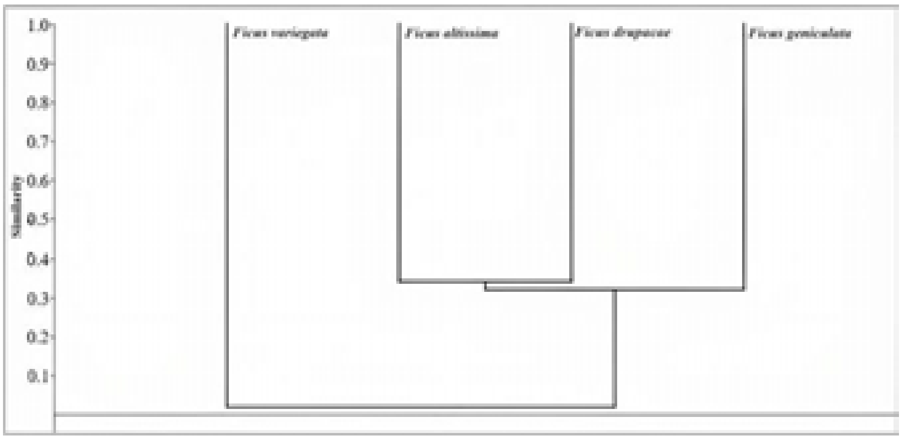

Figure 6. Dendrogram showing the similarity (Jaccard similarity) of vertebrate assemblages in *Ficus* species using cluster analysis.

vertebrate species assembled in Ficus per scan was significantly different from non-ficus plants (U = 830, z = 5.99, p = 0.0001, critical value = 1.96). The average VMR for *Ficus* species is (1.5, Range 0.8–2.1) and for non-ficus plants is (0.8, Range 0.2–2.9).

Ficus tree preference

Less than 35% of the vertebrate species were similar among *Ficus drupacea*, *Ficus variegata* and *Ficus altissima* and almost zero similarity was recorded *between Ficus variegata* and other focal *Ficus* species (Figure 7). Ficus tree preference (percent) of bulbuls, barbets, green pigeons, hornbills, mynas, orioles, Asian fairy bluebirds, opportunists and mammals in the west bank is analysed in (Table 1).

Vertebrate groups observed in the Ficus trees, mean number of individuals, visiting frequency, visiting duration in each tree per scan and preference of vertebrate groups towards Ficus tree (Table 2).

The vertebrate assemblages and the dominant species recorded in Ficus plants in Tropical regions across different time and habitat types were compared. Data was collected from the literature as mentioned in the parenthesis (Lambert 1989a; Shanahan 2000; Sanitjan & Chen 2009; Barua & Tamuly 2011; Lok et al. 2013; Daru et al. 2015). The different parameters like LD = low disturbance, D = disturbed, F = Forest, A = agricultural matrix, and U = urban. (*) = only bird diversity was recorded, (^) = Current study, Jan. = January, Sept. = September, Oct. = October (Table 3) was assessed.

DISCUSSION

This study provides information on distinct vertebrate assemblage in Ficus and non-ficus plants. Large vertebrate assemblage recorded at *Ficus drupacea, Ficus geniculata*, and *Ficus altissima* than non-ficus plants can be attributed to the fewer availability of ripened fruit in the study site (Fleming et al. 1987; Shanahan et al. 2001; Kissling et al. 2007). Majorly, Ficus plants had ripened fruits during the survey. Whereas the neighboring non-ficus plants were either in the flowering stage or had unripe fruits. The larger vertebrate assemblage at Ficus in our results also reflects the dispersal mechanism of the trees at PWS. The sampled hemi-epiphytic Ficus present their crop in the forest canopy and are generally considered bird dispersal species with a wide niche breadth. Therefore, they are capable of attracting a large diversity of birds and mammals including nomadic frugivores such as pigeons and hornbills (Lambert & Marshall 1991; Shanahan et al. 2001; Shanahan & Crompton 2001; Harrison & Shanahan 2005; Dutta & Rawatt 2008). For example, three-year study on frugivore and seed dispersal network in PWS recorded maximum number of frugivore birds in *Ficus* species, such as *Ficus drupacea* (25), *Ficus geniculata* (24), and *Ficus altissima* (20) (Naniwadekar et al. 2019). Consequently, these our findings are in line with the previous studies conducted in PWS which suggested greater vertebrate assemblages in *Ficus* species.

In southern Asian tropical forests, green pigeons (Lambert 1989a,b), bulbuls, barbets, hornbills (Kinnaird et al. 1996), and Asian fairy bluebird species are the primary groups of fig-eating birds (Corlett 1998; Shanahan et al. 2001; Sanitjan & Chen 2009; Barua & Tamuly 2011). The results demonstrated that the green

Table 1. Comparison of species assemblage among Ficus and non-ficus (NF) plants.

Man-Whitney U test	*F. drupacea*	NF	*F. altissima*	NF	*F. variegata*	NF	*F. geniculata*	NF
Mann-Whitney U	23		32.5		3		27	
p (<0.05)	0.0095		0.0635		0.0002		0.0081	
Critical value	23		23		20		30	

Table 2. Vertebrate groups observed in the *Ficus* trees, mean number of Individuals, visiting frequency, visiting duration in each tree per scan and preference of vertebrate groups towards *Ficus* tree in percentage.

Ficus tree preference (%)			Visiting time duration/scan (Mean ± sd)			Visiting frequency			Individuals/scan (Mean ± sd)			
F. geniculata	*F. altissima*	*F. drupacea*	*F. geniculata*	*F. altissima*	*F. drupacea*	*F. geniculata*	*F. altissima*	*F. drupacea*	*F. geniculata*	*F. altissima*	*F. drupacea*	Plant taxa
95	0	5	148.2 ± 79	4	28.88 ± 28.01	1.00	0.09	0.73	40.42 ± 31.64	2	13.5 ± 10.82	Bulbuls
12	79	9	23.4 ± 10.7	78.1 ± 52.8	20.43 ± 23.29	1.00	0.82	0.64	3.8 ± 1.55	7.56 ± 3.97	4.14 ± 4.85	Barbets
18	69	12	116.5 ± 65.1	152.9 ± 108.2	85.9 ± 46.9	0.83	0.82	0.64	48.3 ± 36.2	115.2 ± 74.3	47.3 ± 29	G. pigeons
2	91	7	21.67 ± 14.43	42 ± 35.9	7 ± 6.73	0.67	0.55	0.36	1.67 ± 0.57	19.5 ± 16.78	13 ± 19.34	Hornbills
1	98	1	37.5 ± 10.61	87.3 ± 52.5	10.6 ± 13.13	0.25	0.91	0.45	7.5 ± 2.12	48.9 ± 22.51	9 ± 9.14	Mynas
98	0	2	65.5 ± 62.7	–	8.67 ± 3.06	0.17	0.00	0.27	2.5 ± 1.35	–	1	Orioles
46	33	21	75.8 ± 54.5	62.1 ± 33.3	42.3 ± 41.5	0.83	0.82	0.55	13.11 ± 7.93	10.33 ± 3.35	14.5 ± 12.99	Fairy birds
99	1	0	68.5 ± 82.8	6 ± 3	–	0.75	0.27	0.00	9.83 ± 10.7	1.667 ± 0.57	–	Opportunists
34	54	12	70 ± 43.6	83 ± 52.3	27.33 ± 23.86	0.50	0.18	0.55	9.33 ± 3.51	17 ± 0	3.71 ± 5.02	Mammals

Table 3. Vertebrate assemblages and the dominant species recorded in Ficus plants in tropical regions across different time and habitat types. Data was collected from (Lambert 1989a; Barua & Tamuly 2011; Senitjan & Chen 2009; Shanahan 2000; Daru et al. 2015; Lok et al. 2013). LD = low disturbance, D = disturbed, F = Forest, A = agricultural matrix, U = urban. (*) = only bird diversity was recorded, (^) = Current study, Jan. = January, Sept. = September, Oct. = October.

Location	Year	Sample size	Sampling effort (h)	Sampling period	Species recorded (n)	Dominant species	Site type
Kuala lampat, Malaysia	1984–86	38	750	March–October	60*	Green Pigeons	LD
Borneo, Malaysia	1998–99	34	700	March–September	69	Bulbuls, Pigeons	D, F
China	2004–06	32	816	May–June	30*	Bulbuls	D, F
Nigeria	2007–09	12	–	March–June	48	Bulbuls, Yellow- fronted Tinker-bird	F
Assam, India	2009–10	59	177	September–September	67	Green Pigeons	A
Singapore	2013	43	–	–	104	Pigeons, Barbets	U
Arunachal Pradesh^	2019	4	89	January–May	64	Green Pigeons	F

pigeons dominated the vertebrate assemblage in Ficus in 89 h of observational study. It can be attributed to the voracious feeding nature of green pigeons, which are fig specialists that feed exclusively on figs (Lambert 1989a,b). Despite PWS having a rich faunal diversity, the study still recorded poor mammal assemblages. It might be because due to the presence of observers, which prohibited them from approaching the fruiting trees. Also, the survey did not cover the nocturnal mammals that feed on Ficus (Krishna et al. 2013).

The contention arises that while figs are universally regarded as a crucial tropical resource, not all fig species offer an equal bounty to vertebrate fauna. The findings distinctly unveil variations in the preferences of vertebrate species for different *Ficus* species. Among the focal *Ficus* species, *Ficus drupacea* emerges as the least favored by vertebrates. This trend is likely a result of factors such as the species' smallest crop size (n = 3,240) (Sanitjan & Chen 2009) and differences in nutrient composition, notably calcium, potassium, and magnesium, among the focal Ficus species, despite its larger fig size. These particular nutrients play a pivotal role in eggshell development and bone growth (Kinnaird et al. 1999; Wendln & Runkle 2000; Daru et al. 2015). Minimal distinctions were noted for other Ficus parameters (see Appendix 4).

CONCLUSION

Hemi-epiphytic Ficus trees emerge as significant attractions for vertebrates, boasting a rich diversity of species and distinct appeal compared to fruiting and flowering non-ficus plants. Notably, *Ficus altissima* becomes a favored choice for barbets, green pigeons, hornbills, mynas, and mammals, while *Ficus geniculata* exclusively draws bulbuls, orioles, Asian fairy bluebirds, and opportunistic feeders. Amidst the array of frugivorous bird species within the west bank of PWS, green pigeons, particularly the *Teron* species, stand out as primary beneficiaries. Despite various frugivorous birds present, pigeons dominate the West bank, averaging 70.2 individuals per scan. The findings reveal a tendency for vertebrate assemblages to cluster more in Ficus trees compared to non-ficus plants, indicating intricate interactions between figs and frugivores. This study offers insights into Ficus trees' pivotal role, emphasizing their ecosystem significance, potential for vertebrate-centered tourism, and vital conservation role in an eco-tourism context.

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Appendix 1. Fruiting and flowering non-ficus plants near focal *Ficus* trees.

Fruiting and flowering trees	Family	Phenology	No. of trees observed
Canarium resiniferum	*Burseraceae*	Fruiting	1
Duabanga grandiflora	*Lythraceae*	Flowering	5
Sterculia villosa	Sterculiaceae	Flowering	1
Sterculia colorata	Sterculiaceae	Flowering	4
Tetrameles nudiflora	*Datiscaceae*	Flowering	1
Shorea robusta	*Dipterocarpaceae*	Flowering	3
Dysoxylum binectiriferum	*Meliaceae*	Fruiting	3
Polyalthia simiarum	*Annonaceae*	Fruiting (unripe)	2
Phlogacanthus thyrsiformis (shrub)	*Acanthaceae*	Flowering	20
Dillenia indica	*Dilleniaceae*	Fruiting	1

Appendix 2. Vertebrates were recorded at focal *Ficus* with their vertebrate group based on feeding observations. Indirect observations (*), Common vertebrates between Ficus and non-ficus plants (**), Indirect observations as well as common vertebrates between Ficus and non-ficus plants (*^). F—frugivore | OP—opportunists | O—other birds.

Family	Name	Scientific name	V. group
Birds			
Bucerotidae	Great Hornbill	*Buceros bicornis*	F
	Wreathed Hornbill**	*Rhyticeros undulatus*	F
	Oriental Pied Hornbill**	*Anthracoceros albirostris*	F
Campephagidae	Large Cuckoo Shrike**	*Coracina macei*	OP
	Scarlet Minivet**	*Pericrocotus speciosus*	O
Chloropsidae	Golden-fronted Leafbird**	*Chloropsis aurifrons*	OP
	Orange-bellied Leafbird**	*Chloropsis hardwickii*	OP
Columbidae	Pin-tailed Green Pigeon	*Treron apicauda*	F
	Wedge-tailed Green Pigeon	*Treron sphenurus*	F
	Mountain Imperial Pigeon	*Ducula badia*	O
	Thick-billed Green Pigeon	*Treron curvirostra*	F
	Yellow-footed Green Pigeon	*Treron phoenicoptera*	F
	Barred Cuckoo Dove**	*Macropygia unchall*	O
Dicaedae	Plain flowerpecker**	*Dicaeum minullum*	O
Falconidae	Common Kestrel	*Falco tinnunculus*	O
Irenidae	Asian Fairy-bluebird	*Irena puella*	F
Leiothrichidae	Blue-winged Minla	*Actinodura cyanouroptera*	O
	Silver-eared Mesia	*Leiothrix argentauris*	O
Megalaimidae	Blue-throated Barbet**	*Psilopogon asiaticus*	F
	Blue-eared Barbet	*Psilopogon cyanotis*	F
	Lineated Barbet**	*Psilopogon lineatus*	F
Muscicapidae	Grey-headed Canary Flycatcher**	*Culicicapa ceylonensis*	O
	Little Pied Flycatcher	*Ficedula westermanni*	O
Nectariniidae	Streaked spiderhunter**	*Arachnothera magna*	O
Oriolidae	Black hooded Oriole**	*Oriolus xanthornus*	F
	Maroon Oriole**	*Oriolus traillii*	F
Paridae	Sultan Tit	*Melanochlora sultanea*	O
Phasianidae	Red junglefowl	*Gallus gallus*	O
	Khalij Pheasant	*Lophura leucomelanos*	O
Picidae	Greater yellow-napped Woodpecker**	*Chrysophlegma flavinucha*	O
	Grey-caped Pygmy Woodpecker**	*Yungipicus canicapillus*	O
Pycnonotidae	White-throated Bulbul	*Alophoixus flaveolus*	F
	Black-crested Bulbul**	*Pycnonotus flaviventris*	F
	Black Bulbul	*Hypsipetes leucocephalus*	F
	Red-vented Bulbul	*Pycnonotus cafer*	F
	Mountain Bulbul	*Ixos mcclellandii*	F
	Red-whiskered Bulbul	*Pycnonotus jocosus*	F
Sittidae	Chestnut-bellied nuthatch	*Sitta cinnamoventris*	O
Sturnidae	Common Hill Myna	*Gracula religiosa*	F
Timalildae	Greater Necklaced Laughingthrush	*Pterorhinus pectoralis*	O
Vangidae	Large Woodshrike**	*Tephrodornis virgatus*	O
Zoosteropidae	Oriental White-eye	*Zosterops palpebrosus*	O
	Whiskered Yuhina	*Yuhina flavicollis*	OP
Mammal			
Bovidae	Gaur*	*Bos gaurus*	
Cervidae	Sambar deer*^	*Rusa unicolor*	
	Barking deer*^	*Muntiacus muntjak*	
Cercopithecidae	Rhesus macaque	*Macaca mulatta*	
	Assamese macaque	*Macaca assamensis*	
Pteropodidae	Indian flying fox*	*Pteropus giganteus*	
Sciuridae	Malayan giant squirrel	*Ratufa bicolor*	
	Hoary bellied squirrel*^	*Callosciurus pygerythrus*	
	Palla's squirrel	*Callosciurus erythraeus*	
Suidae	Wild boar*^	*Sus scrofa*	
Viverridae	Common palm civet	*Paradoxurus hermaphroditus*	

Appendix 3. Checklist of birds recorded during scan sampling including vertebrates recorded in the west bank, Pakke Wildlife Sanctuary. Vertebrates recorded in non-ficus (*), Unique to non-ficus (**).

Family	Name	Scientific name	IUCN
Accipitridae	Oriental Honey-buzzard	*Pernis ptilorhynchus*	LC
	Crested Serpent-Eagle	*Spilornis cheela*	LC
Artamidae	Ashy Woodswallow	*Artamus fuscus*	LC
Bucerotidae	Great Hornbill	*Buceros bicornis*	VU
	Wreathed Hornbill*	*Rhyticeros undulatus*	VU
	Oriental Pied-Hornbill*	*Anthracoceros albirostris*	LC
Campephagidae	Large Cuckooshrike*	*Coracina macei*	LC
	Scarlet Minivet*	*Pericrocotus speciosus*	LC
	Long-tailed Minivet	*Pericrocotus ethologus*	LC
Cettiidae	Grey-bellied Tesia	*Tesia cyaniventer*	LC
Chloropsidae	Golden-fronted Leafbird*	*Chloropsis aurifrons*	LC
	Orange-bellied Leafbird*	*Chloropsis hardwickii*	LC
Ciconiidae	Black-necked Stork	*Ephippiorhynchus asiaticus*	NT
Cisticolidae	Common Tailorbird	*Orthotomus sutorius*	LC
Columbidae	Pin-tailed Green Pigeon	*Treron apicauda*	LC
	Wedge-tailed Green Pigeon	*Treron sphenurus*	LC
	Mountain Imperial-Pigeon	*Ducula badia*	LC
	Thick-billed Green Pigeon	*Treron curvirostra*	LC
	Yellow-footed Green Pigeon	*Treron phoenicoptera*	LC
	Asian Emerald dove	*Chalcophaps indica*	LC
	Barred Cuckoo-dove*	*Macropygia unchall*	LC
	Oriental Turtle Dove	*Streptopelia orientalis*	LC
Coraclidae	Indian Roller	*Coracias benghalensis*	LC
	Oriental Dollarbird**	*Eurystomus orientalis*	LC
Cuculidae	Square-tailed Drongo-cuckoo	*Surniculus lugubris*	LC
	Banded Bay Cuckoo	*Cacomantis sonneratii*	LC
	Green-billed Malkoha	*Phaenicophaeus tristis*	LC
Dicaedae	Plain Flowerpecker*	*Dicaeum minullum*	LC
Dicruridae	Ashy Drongo**	*Dicrurus leucophaeus*	LC
	Spangled Drongo**	*Dicrurus bracteatus*	LC
	Greater Racket-tailed Drongo	*Dicrurus paradiseus*	LC
	Lesser Racket-tailed Drongo	*Dicrurus remifer*	LC
Eurylaimidae	Long-tailed Broadbill	*Psarisomus dalhousiae*	LC
Falconidae	Eurasian Kestrel	*Falco tinnunculus*	LC
Irenidae	Asian Fairy-bluebird	*Irena puella*	LC
Laniidae	Brown Shrike**	*Lanius cristatus*	LC
	Long-tailed Shrike	*Lanius schach*	LC
Leiothrichidae	Red-tailed Minla	*Minla ignotincta*	LC
	Blue-winged Minla	*Actinodura cyanouroptera*	LC
	Silver-eared Mesia	*Leiothrix argentauris*	LC
	Rufous-backed Sibia	*Heterophasia annectens*	LC
Megalaimidae	Blue-throated Barbet*	*Psilopogon asiaticus*	LC
	Blue-eared Barbet	*Psilopogon cyanotis*	LC
	Lineated Barbet*	*Psilopogon lineatus*	LC
Meropidae	Chestnut-headed Bee-eater**	*Merops leschenaultia*	LC
	Blue bearded Bee-eater	*Nyctyornis athertoni*	LC
Monarchidae	Black-naped Monarch	*Hypothymis azurea*	LC
Muscicapidae	Grey-headed Canary-Flycatcher*	*Culicicapa ceylonensis*	LC
	Little Pied Flycatcher	*Ficedula westermanni*	LC
	Verditer Flycatcher	*Eumyias thalassinus*	LC
	Pale blue Flycatcher	*Cyornis unicolor*	LC
	Chestnut-bellied Rock-Thrush	*Monticola rufiventris*	LC
	Blue Rock-Thrush	*Monticola solitarius*	LC
	Small Niltava**	*Niltava macgrigoriae*	LC
	Hodgson's Redstart	*Phoenicurus hodgsoni*	LC
	Grey Bushchat	*Saxicola ferreus*	LC
	White-rumped Shama	*Copsychus malabaricus*	LC
	Blue-Whistling Thrush	*Myophonus caeruleus*	LC
Nectariniidae	Streaked Spiderhunter*	*Arachnothera magna*	LC
	Little Spiderhunter**	*Arachnothera longirostra*	LC
Oriolidae	Black-hooded Oriole*	*Oriolus xanthornus*	LC
	Maroon Oriole*	*Oriolus traillii*	LC
Paridae	Sultan Tit	*Melanochlora sultanea*	LC
Phasianidae	Red junglefowl	*Gallus gallus*	LC
	Khalij Pheasant	*Lophura leucomelanos*	LC
	Grey Peacock-Pheasant.	*Polyplectron bicalcaratum*	LC
Phylloscopidae	Tickell's Leaf Warbler**	*Phylloscopus affinis*	LC
	Greenish Warbler	*Phylloscopus trochiloides*	LC
Picidae	Greater Yellownape Woodpecker*	*Chrysophlegma flavinucha*	LC

Family	Name	Scientific name	IUCN
	Grey-capped Pygmy Woodpecker*	*Picoides canicapillus*	LC
	Greater flame back Woodpecker	*Chrysocolaptes guttacristatus*	LC
	Fulvous breasted Woodpecker	*Dendrocopos macei*	LC
	Grey-headed Woodpecker	*Picus canus*	LC
	Lesser Yellownape Woodpecker	*Picus chlorolophus*	LC
	Rufous Woodpecker	*Rufous woodpecker*	LC
Pellorneidae	Abbott's Babbler	*Malacocincla abbotti*	LC
Psittaculidae	Red-breasted Parakeet**	*Psittacula alexandri*	NT
Pycnonotidae	White-throated Bulbul	*Alophoixus flaveolus*	LC
	Black-crested Bulbul*	*Pycnonotus flaviventris*	LC
	Black Bulbul	*Hypsipetes leucocephalus*	LC
	Red-vented Bulbul	*Pycnonotus cafer*	LC
	Mountain Bulbul	*Ixos mcclellandii*	LC
	Red-whiskered Bulbul	*Pycnonotus jocosus*	LC
Rhipiduridae	White-throated Fantail	*Rhipidura albicollis*	LC
Sittidae	White-tailed Nuthatch	*Sitta himalayensis*	LC
	Chestnut-bellied Nuthatch	*Sitta cinnamoventris*	LC
Stenostiridae	Yellow-bellied Fantail**	*Chelidorhynx hypoxantha*	LC
Strigidae	Asian Barred Owlet	*Glaucidium cuculoides*	LC
	Spotted Owlet	*Athene brama*	LC
	Collared Owlet	*Glaucidium brodiei*	LC
Sturnidae	Chestnut-tailed Starling	*Sturnia malabarica*	LC
	Common Hill Myna	*Gracula religiosa*	LC
Timaliidae	Lesser Necklaced laughingthrush	*Garrulax monileger*	LC
	Greater Necklaced laughingthrush	*Pterorhinus pectoralis*	LC
Vangidae	Large Woodshrike*	*Tephrodornis virgatus*	LC
	Common Woodshrike	*Tephrodornis pondicerianus*	LC
Zoosteropidae	Oriental White-eye	*Zosterops palpebrosus*	LC
	Whiskered Yuhina	*Yuhina flavicollis*	LC
Mammal			
Bovidae	Gaur	*Bos gaurus*	VU
Cervidae	Sambar deer*	*Rusa unicolor*	VU
	Barking deer*	*Muntiacus muntjak*	LC
Cercopithecidae	Rhesus macaque	*Macaca mulatta*	LC
	Assamese macaque	*Macaca assamensis*	LC
Pteropodidae	Indian flying fox	*Pteropus giganteus*	LC
Sciuridae	Malayan giant squirrel	*Ratufa bicolor*	LC
	Hoary-bellied squirrel*	*Callosciurus pygerythrus*	LC
	Palla's squirrel	*Callosciurus erythraeus*	LC
Suidae	Wild boar*	*Sus scrofa*	LC
Viverridae	Common palm civet	*Paradoxurus hermaphroditus*	LC

Appendix 4. Fig characteristics of focal *Ficus* species. L = length, B = breadth.

Characteristics	*Ficus drupacea*	*Ficus geniculata*	*Ficus altissima*	*Ficus variegata*
Growth form	Hemiepiphyte	Hemiepiphyte	Hemiepiphyte	Tree
Crop size	3240	2058000	300000	11790
Fruit shape	Globular	Round	round	Pear shape
Fig size (mm)	L = 33, D = 25	L = 9, D= 9	L = 21, D = 20	L = 34, D= 45
Fruit colour	Black-yellow	Green	Yellow-red	red
Fruit placement	Axiaiary	Axiallary	Axiallary	Cauliflory
GBH (m)	6	9.1	7.2	2.43
Height (m)	23	32	26	16
Phenology	Fruiting (ripe)	Fruiting (ripe)	Fruiting (ripe)	Fruiting (ripe)

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COMMUNICATION

From the Arabian Peninsula to Indian shores: Crab Plover *Dromas ardeola* Paykull, 1805 (Aves: Charadriiformes: Dromadidae) breeding at Point Calimere, India

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Abstract: Crab Plover *Dromas ardeola* is endemic to the subtropical and tropical coastlines of the Indian Ocean. It breeds along the eastern coasts of Africa, the Persian Gulf, and southern coasts of the Arabian Peninsula; occurs also in the western Madagascar and most islands northwards to Seychelles. It is a winter visitor to Pakistan, Gujarat, and peninsular India, Andaman & Nicobar Islands, Lakshadweep, northern Sri Lanka, Maldives, and a vagrant to Bangladesh. The objective of this study was to assess the breeding records of the Crab Plover in Point Calimere. After some preliminary surveys and interactions with local birders, between June and August 2023, boat surveys and foot surveys were carried out in the Great Vedaryanam Swamp (GVS) and nearby islets to document the presence of Crab Plover and locate its nests. The presence of five nests of *D. ardeola* was recorded in Manaaran Theevu islet near Siruthalaikkadu of GVS. This observation marks the first documentation of breeding of Crab Plover in the Indian subcontinent. In the context of species conservation within the peninsular Indian region, there is a need for comprehensive and continuous monitoring of breeding sites.

Keywords: Bird migration, breeding colony, nesting site, shorebirds, winter visitor.

Editor: Anonymity requested.

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INTRODUCTION

The Crab Plover *Dromas ardeola,* belonging to the family Dromadidae, is a distinctive and enigmatic species endemic to the subtropical and tropical coastlines of the Indian Ocean. Crab Plovers are winter visitors on the coasts of Pakistan, Gujarat, and peninsular India, Andaman & Nicobar Islands, Lakshadweep, northern Sri Lanka, Maldives, and Bangladesh. Crab Plovers are known to breed on islands in the Arabian Gulf and Africa including the United Arab Emirates, Masirah Island in Oman, Kuwait, Iran, Saudi Arabia, Yemen, Eritrea, Egypt, Sudan, and the islets of northern Somalia (Cramp et al. 1983; De Marchi et al. 2006; Scott 2007; Delany et al. 2009; Jennings 2010; Tayefeh et al. 2011; Javed et al. 2012; Tayefeh et al. 2013; Bom & Al-Nasrallah 2015; Abdelhafez et al. 2020). These birds form colonies and engage in nesting activities from April to August. They lay a single egg within a self-excavated burrow located in a flat sandbank (Morris 1992; Hockey 1995; Rands 1996). The nest is an unlined chamber at the end of a burrow 100–250 cm long excavated into the sandy substrate (Rands 1996). Within these burrows, the female lays a single egg. This nesting strategy provides the necessary protection and safety for the egg during the incubation period and the burrows appear to be useful for partial solar incubation (De Marchi et al. 2008) and extended foraging on the tidal food resource (De Marchi et al. 2015a). The chicks are semi-nidifugous (Cramp et al. 1983; Hockey & Aspinall 1997), and remain within the nest burrow until fledging. It has been found that Crab Plover nests shift annually due to various reasons, like the suitability of the sand bank for digging burrows (Hockey & Aspinall 1997; Chiozzi et al. 2011); easy access to and departure from colony sites that explain the preference for nest sites near to the sea (Cramp et al. 1983) and burrows near the base of shrubs to avoid collapse of nests in loose soil (Bourgeois et al. 2008; Chiozzi et al. 2011). The breeding biology of the Crab Plover incorporates features that are unique among shorebirds. It is the only shorebird that nests in self-dug burrows, the only shorebird that lays a white egg (Rands 1996; Hockey & Aspinall 1997), and the waterbird species known to provide food to its chicks well after the post-reproductive migration and over-wintering (De Sanctis et al. 2005).

While the species was never recorded as a breeder in the Indian subcontinent, a hint of possible breeding has come from the preliminary results of research with satellite-tagged crab plovers. On 16 November 2022, in a talk given by Ms. Guyomini Panagoda, University of Colombo, in the Asian Flyways Collaborative for Waterbirds (AFCoW) platform about the Central Asian Flyway (CAF) and bird migratory pathways in Sri Lanka, mentioned about five Crab Plover individuals being satellite tagged and colour banded for migratory studies (Appendix 1). The findings that were shared on the platform shed more light on the fact that one of the green colour flagged (International colour coding used for Sri Lanka for migratory studies in CAF) and satellite-tagged Crab Plover stayed in the Point Calimere region from June to August 2022 after moving through the Gulf of Mannar (GoM) region of India from Sri Lanka. An attempt was made by the Bombay Natural History Society (BNHS) team in 2022 to trace the same bird, which turned out to be futile. Subsequently, the same male bird was reported with a juvenile on 30 August 2022, in Talai Mannar, Sri Lanka, which further emphasized that the Crab Plover has a potential breeding location at Point Calimere even if earlier studies by the BNHS reported that there has been no Crab Plover record from Point Calimere since 2003 (Balachandran & Thirunavukarasu 2009). The objective of the present study was to find the potential breeding grounds of Crab Plover in the Palk Bay region of Point Calimere.

STUDY AREA AND METHODS

The Great Vedaryanam Swamp (GVS) and its adjacent areas, covering approximately 350 km^2, are situated along the Bay of Bengal, specifically on the low promontory of the Coromandel coast within the Nagapattinam, Thiruvarur, and Thanjavur districts of Tamil Nadu, India. The GVS stretches for about 48 km from east to west, parallel to the Palk Strait and is separated from it by a sandbank. Its extents are about 10 km from north to south, and it is broadest in the east, narrowing to about 8 km in the central part and 6 km at the western end. On the east of the GVS lies the Point Calimere Wildlife Sanctuary known for both migratory and resident bird species. GVS is a coastal wetland featuring a rich diversity of habitats and ecological attributes. These include inter-tidal salt marshes, forested wetlands, mangroves, and brackish to saline lagoons (Image 2 & 3). Additionally, six freshwater inlets connected to the Cauvery River flow into the swamp, contributing to its unique ecosystem, complete with islands and mangrove forests. Notably, the eastern sand dunes within the GVS have largely been stabilized by *Prosopis* vegetation, while the higher dunes in the northeastern region have dense tropical dry evergreen forests. The climate in GVS is characterized by hot and humid summers and rainy winters. Most of

Image 1. The breeding site of Crab Plover in Great Vedaryanam Swamp.

the precipitation occurs during the northeast monsoon season, typically from October to December, contributing to an average rainfall of 1,280 mm in the area. The study area had most of the prey species for Crab Plovers including mudskippers, juveniles of *Portunus portunus,* and Fiddler Crabs (*Uca* spp.) (Balachandran 1995; Byju 2020).

We did some preliminary surveys with birders around the area about frequent visits, sightings, and the presence of Crab Plover from the area for the last two decades. The Crab Plover was regularly documented from Manoli and Hare islands of GoM (Balachandran 1995; Byju 2020), the nearby wintering site on the southeastern coast. With the collected information, we narrowed down the search with three expeditions from June to August 2023 to three islets in the GVS near Siruthalaikadu. Initially, bird surveys were done by boat. Later when the species was located, foot surveys were performed to locate the precise nesting location.

RESULTS

Our observations confirm the breeding records of Crab Plover on Manaaran Theevu (one of the small islets of GVS; 10.2792°N, 79.7264°E), next to the Chellakanni River canal on the southeast coast of India (Image 1). This area is a flat, sandy island with large mudflats extending

Image 2. Habitat of Manaaran Theevu islet in Great Vedaryanam Swamp where Crab Plover breeding was recorded with temporary huts of fishermen.

towards the landmass of Siruthalaikadu. We recorded that the vegetation is entirely composed of grasses and herbs less than 100 cm tall on the edges of the islets towards the landside with intermittent halophytes on the mudflat, along with *Avicennia* mangroves and the invasive *Prosopis juliflora.* On our first survey on 30 June 2023, we counted 13 individuals on the mudflats but could not find any nests. Since as earlier cited, the bird has not been reported from GVS or Point Calimere for more than three decades and this sighting happened during the breeding period. We continued the monitoring to the next month too. The Crab Plover colony was first discovered on 14 July 2023, followed by the next visit on 8 August 2023. On this survey, close to the sea, we identified and documented five nests as a small colony covering a small area of 100–150 m^2 (Image 4 & 5). As we did not want to disturb the nesting, observation was carried out from a safe distance for birds using binoculars (Nikon Monarch7, 10 x 42) and spotting scope (Vanguard HD 82 A).

We could not find any adult-juvenile interaction. We could only spot adult birds near the burrows at times. On 8 August, we recorded some broken egg shells, possibly of Crab Plovers, on the soil surface amid the burrows indicating egg laying (Abdelhafez et al. 2020). We also recorded one green colour flagged bird from Sri Lanka (Image 6 & 7) along with a single juvenile in the August survey on the islet. We could not find any other nesting sites on the nearby islets during the boat surveys.

DISCUSSION

Despite the notable records of Crab Plover breeding in the Arabian Peninsula, the finding of the first confirmed breeding ground in India, well outside the species' primary range, marks a significant discovery. This colony is likely a new one as suggested by the lack of previous records and by the small number of nests, compared to the number of nests in the colonies in the Middle East (Bom & al-Nasrallah 2015). It is interesting to note that the new colony is located in an area with a comparatively high rainfall (on average 1,366 mm) and is therefore more vegetated that the colonies around the Arabian Peninsula (Manikannan et al. 2011; Sathishkumar et al. 2023), but breeding is anyway during the dry season as elsewhere (De Marchi et al. 2015b).

Image 3. The intertidal area and vegetation of the Manaaran Theevu islet with Crab Plovers.

Image 4. Nest of Crab Plover on Manaaran Theevu in Great Vedaryanam Swamp.

Image 5. Closer look of the burrow.

Image 6. Bird (flagged in Sri Lanka, depicted by Green Flag) from the Manaaran Theevu Islet of Great Vedaryanam Swamp observed on 8 August 2023 during the 2023 breeding season.

Image 7. Juvenile bird from Manaaran Theevu Islet observed on 8 August 2023 (during the 2023 breeding season).

While Crab Plover is a 'Least Concern' species as per the IUCN Red List (BirdLife International 2023), their breeding range is notably limited, consisting of only a few colonies, the majority, if not all, of which are located in an area facing rapid exploitation, significant coastal alterations, and pollution (Sheppard et al. 2010; Sale et al. 2011). This environmental context renders the species highly susceptible to threats, as the destruction of a single breeding colony can impact a substantial portion of the overall breeding habitat for the entire population.

Due to our intermittent presence in sight of the breeding colony, we did not record any direct threat, as the collection of eggs by fishermen, which was reported from other nesting sites, like in the Arabian Peninsula (Brown et al. 1991; Rands 1996). We did not record on the breeding islet the presence of dangerous rats and cats (De Marchi et al. 2006; Javed et al. 2012) but we found indirect evidence of the presence of Wild Boar *Sus scrofa* and Golden Jackal *Canis aureus*, which could be a threat to the breeding of this species. An indirect threat we perceived, after informal interaction with the local fishermen community, is the access to outside people for tourism, especially during the hottest months of June and July, which coincide with the breeding time of the Crab Plover. As most of the GVS is not a protected area and the breeding island can't be reached on foot, most of the tourists certainly rent local fishermen's boats in order to illegally spend the whole day on the islets. Moreover, islands are used by fishermen for drying the nets as well as staying for the night for early morning fishing activities. The Crab Plover nesting colonies are found in the vegetated area near the shores where fishermen also land their small fishing vessels, hence the chances of walking over the burrows and the breakages of eggs could happen. Last, as only the eastern tip of the GVS is protected by the Point Calimere Wildlife Sanctuary, the largest part of the insular and intertidal ecosystems of the GVS is threatened by hunting, by the development of salt pans and prawn farms, and by the spread of invasive species like *Prosopis juliflora.* The result is a significant decrease in avian species diversity and abundance compared to historical records (Rashiba et al. 2022).

Considering the location of the Crab Plover colony of the GVS in relation to the main breeding range of the species, it is important to follow the fate of the colony in the coming years and monitor its increase in size or its eventual demise. If possible, access to this area to tourists and fishermen should be curtailed during the breeding season by the concerned authorities. The information collected will be instrumental in enhancing our understanding of the species' breeding strategies and furthering conservation efforts to ensure the preservation of this unique avian species in its limited range.

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COMMUNICATION

Assessing avian diversity and conservation status in Dighal Wetlands, Haryana, India

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Abstract: Birds are considered sensitive indicators of ecosystem health and functionality in freshwater wetlands. Assessment of bird assemblages in wetland habitats is, therefore, emphasised from a sustainable management perspective. Bird surveys were conducted from October 2020 to September 2022. These surveys aimed to assess the community composition and status of avifauna in Dighal wetlands, an important bird area in the Jhajjar District of Haryana, India. Data collection employed point counts and opportunistic encounter methods. A total of 154 bird species belonging to 108 genera, 47 families, and 18 orders were recorded. Of these, 75 species were residents, 60 were winter migrants, and 10 were summer migrants. The greatest species richness was observed for the order Passeriformes (54), followed by Charadriiformes (22), Anseriformes (17), and the rest of the 15 orders. Anatidae was the most dominant family with 17 species, constituting 11% of the bird community in the study area. Data on local abundance revealed that 10 species were common, 23 were fairly common, 83 were uncommon, and 38 were rare in the study area. Among the recorded avifauna, one species is classified as Endangered, three as Vulnerable, and eight as Near Threatened in the IUCN Red List of Threatened Species; 17 species are listed in the Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES); and 11 are included in Schedule I of the Indian Wildlife (Protection) Act, 1972. These wetlands also support 40 species of birds, which have a declining population trend globally. The occurrence of migrants and species of global conservation priority underscores the importance of these wetlands as a conservation site and wintering ground for avifauna due to the extensive food resources and rich biodiversity they support. The present study provides baseline information for future research on monitoring bird assemblages and proper management of the Dighal wetlands of Haryana.

Keywords: Assemblages, biodiversity, birds, community composition, ecosystem, indicator, migrants, point counts, species richness, threatened species.

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Author details: Dr. Parmesh Kumar is professor of Zoology, Department of Zoology, Institute of Integrated & Honors Studies, Kurukshetra University. He has published dozens of papers in the journals of national and international repute, and few book chapters on biodiversity specifically on avifauna of Haryana. His field of research includes wildlife ecology and animal behaviour. Parul is a research scholar and pursuing her Ph.D. from Department of Zoology, Kurukshetra University, Kurukshetra.

Author contributions: P. Kumar conceived and designed the study as well as wrote the final draft of the manuscript. Parul performed the field surveys, analysed the data and prepared rough draft of the manuscript. Both authors read and approved the final manuscript.

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INTRODUCTION

Wetlands are dynamic ecosystems that link aquatic and terrestrial habitats (Zedler & Kercher 2005; Bassi et al. 2014; Panda et al. 2021; Rajpar et al. 2022; Yashmita-Ulman & Singh 2022). They have specific ecological characteristics, functions, and values. Approximately 5–8% of the Earth's surface is occupied by wetlands (Anand et al. 2023). In addition to providing various ecosystem services, wetlands are reservoirs of incredible biodiversity, including invertebrates, fish, amphibians, reptiles, birds, mammals, and plants. Therefore, wetlands are often viewed as treasuries of biodiversity within a region or landscape (Bhat et al. 2009; Singh & Brraich 2022; Anand et al. 2023; Byju et al. 2023).

Birds occupy several trophic levels in the nutrient cycle of wetland ecosystems (Kumar & Sharma 2018; Chakraborty et al. 2021; Rai & Vanita 2021; Rajpar et al. 2022; Yashmita-Ulman & Singh 2022). Wetlands are important for both resident and migratory birds. They are used by birds for various purposes such as foraging, breeding, roosting, and nesting habitats, and sometimes also as stopover sites (Ganbold et al. 2018; Kumar & Sharma 2018; Panda et al. 2021; Yashmita-Ulman & Singh 2022; Anand et al. 2023; Muralikrishnan et al. 2023). Birds are extremely sensitive to changes in their habitats such as human disturbance, poisoning, pollution, eutrophication, and siltation; therefore, they can be used as an excellent ecological indicator for assessing the quality, productivity, and stability of wetlands (Mistry et al. 2008; Amat & Green 2010).

In India, 4.6% of the geographical area is under wetlands (Bassi et al. 2014; Anand et al. 2023). Of the 1,353 bird species reported from various habitats within the geographical limits of India (Praveen & Jayapal 2023), 310 are recognised to be dependent on wetlands (Kumar et al. 2005). However, wetlands in India, as elsewhere, are facing anthropogenic pressures like conversion of wetlands into agricultural lands or for commercial fishing purposes, industrial pollution, fertilisers run-off from surrounding agricultural fields, hunting, unsustainable harvest of wetland resources, invasion of alien species, eutrophication, and draining of water for agricultural purposes (Ganbold et al. 2018; Kumar & Sharma 2018; Mandal et al. 2021; Panda et al. 2021; Rashiba et al. 2022; Yashmita-Ulman & Singh 2022). This threatens the existing avifaunal diversity of wetlands. Information on species composition and seasonal assemblages of birds in a particular wetland habitat is very helpful in understanding the habitat condition and designing suitable conservation and management strategies for sustainable biodiversity conservation (Kumar et al. 2016; Ganbold et al. 2018; Mandal et al. 2021; Muralikrishnan et al. 2023).

Dighal wetlands have been identified as an Important Bird and Biodiversity Area (IBA) of India with the IBA code of IN-HR-06 by the ENVIS Centre on Wildlife and Protected Areas (Rahmani et al. 2016). Spread over an area of about 131.5 ha, Dighal wetlands in the Jhajjar District of Haryana are a complex of many small and large ponds along with vast areas of wet fields left for several years because of high water table and water logging conditions in the Dighal Village. These wetlands serve as important wintering sites for large congregations of migratory birds. To understand the anthropogenic impacts on wetland birds and their habitat in the future, it is essential to have information on the species composition, seasonality, and conservation status of bird assemblages. Such information will help in the long-term monitoring of the wetlands and preparing conservation and management strategies for the avifauna as well as their habitat. In this context, the present study was designed to document the community composition and status of avifauna in the Dighal wetlands of Haryana, India.

MATERIAL AND METHODS

Study area

The present study was conducted in the Dighal wetlands, located in the southeastern region of Haryana state, India, with coordinates at 28.222°N and 76.187°E. These wetlands, positioned within Beri Tehsil of Jhajjar District, encompass an area of approximately 131.5 ha , comprising a complex of ponds, both small and large, as well as extensive wet fields that have remained unused for several years due to consistent high water tables and recurrent waterlogging in the village of Dighal. As a critical part of the Central Asian Flyway, the Dighal wetlands serve as crucial wintering grounds for numerous migratory bird species. This research involved selecting eight specific wetland sites for bird surveys, with their key characteristics detailed in Table 1. The study area experiences a subtropical climate featuring three distinct seasons: a rainy period from July to September, a cool dry season from October to February, and a hot dry season from March to June. Temperatures range from a scorching 45°C in summer to a chilly 6°C in winter, with an average annual rainfall of 444 mm recorded in the district.

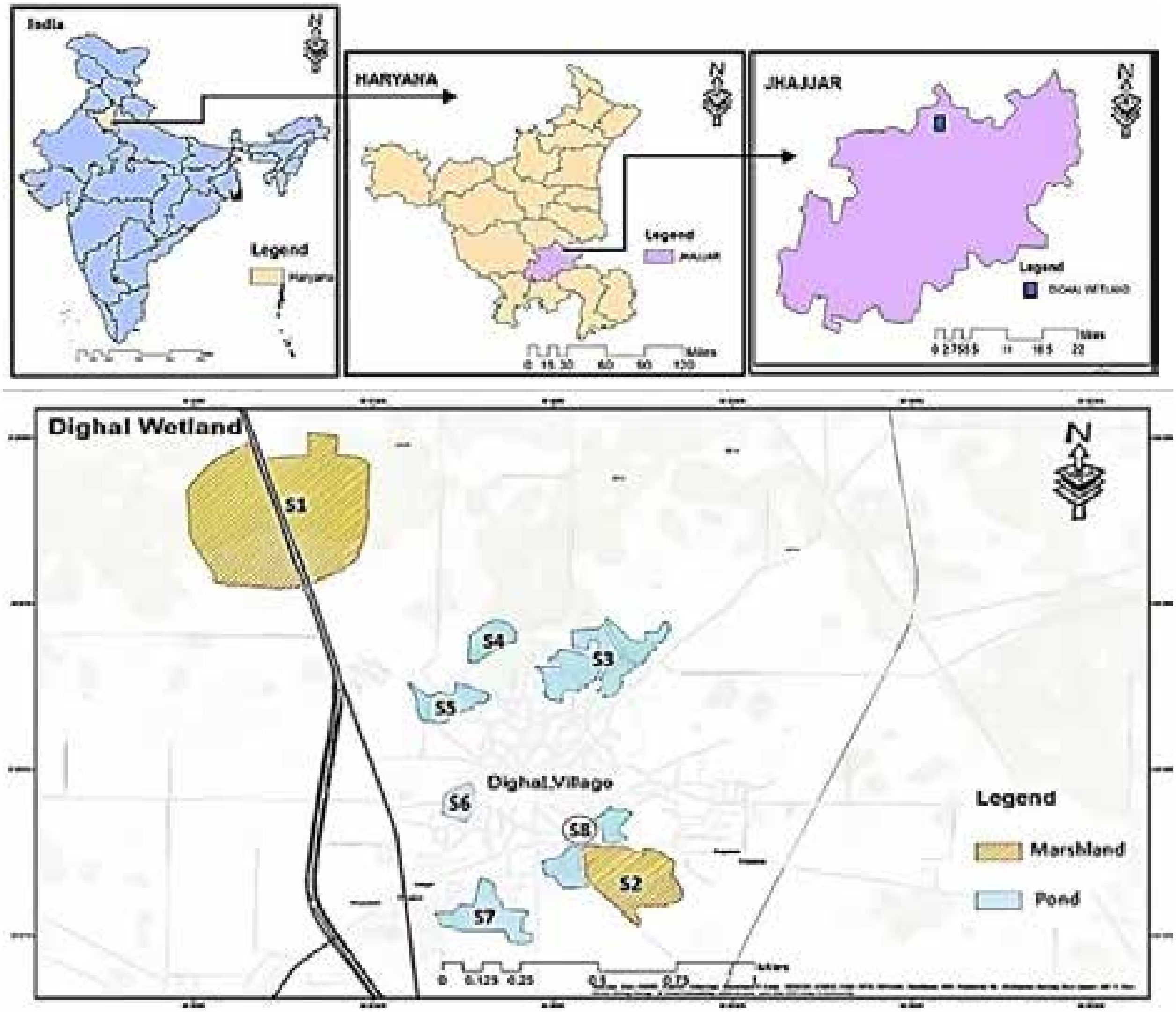

Figure 1. Map of study area showing eight different sites (S1 to S8) of Dighal Wetland in district Jhajjar, Haryana.

Data collection

Avian surveys were conducted using the point count method, following a bi-weekly schedule from October 2020 to September 2022. Four to six counting points, spaced at least 250 m apart, were strategically positioned along the perimeter of the wetlands, totaling 48 surveys at each location over the study period. A five-minute settling period preceded ten minutes of active bird observation, employing Nikon 10x50 field binoculars during peak activity hours (0600–1000 h or 1600–1800 h). Additional opportunistic observations were carried out by scanning the wetlands' peripheries and banks to compile a comprehensive avifauna checklist. Bird identification followed Grimmett et al. (2011), with Praveen & Jayapal (2023) for taxonomic references. Abundance status relies on sighting frequency, which can be categorised as common (CO), fairly common (FC), uncommon (UC), or rare (RA) based on Mackinnon & Phillipps (1993). The residential status (resident, summer migrant, or winter migrant) was determined with the presence-absence method (Kumar & Sharma 2018). Conservation status aligned with IWPA (1972) and CITES (2012), while the Red List of the IUCN (2022) guided assessment for conservation status and global population trend.

Relative diversity (RDi) of bird families was computed as

$$\text{RDi} = \frac{\text{Number of Bird Species in a Family}}{\text{Total Number of Bird Species}} \times 100$$

following the Torre-Cuadros et al. (2007) formula.

Table 1. General features of the selected Dighal wetland sites in Jhajjar District of Haryana, India.

Wetland site	Co-ordinates	General features
S1	28.780°N, 76.620°E	This wetland habitat is comprised of waterlogged agricultural fields spread on both sides of the Jhajjar-Rohtak toll plaza expressway. The roadside plantation was dominated by Eucalyptus trees.
S2	28.762°N, 76.636°E	A water-logged barren land on one side of one of the water houses of Dighal Village. It is comprised of small shrubs and bushy plantations all over the area.
S3	28.772°N, 76.635°E	This site is a complex of four adjoining ponds fragmented by road. It is surrounded by a gaushala, a graveyard, and a water house.
S4	28.772°N, 76.630°E	This pond is located on the back side of the sports stadium building and comprises comparatively less plantation.
S5	28.769°N, 76.627°E	This site is comprised of four ponds fragmented by road in the Dighal Village. An old historical monument is present on the bank of the pond and surrounded by human habitations.
S6	28.764°N, 76.626°E	This site is a single large pond located in front of the central co-operative bank of the Dighal Village and has a community health centre nearby.
S7	28.759°N, 76.629°E	This pond is present in the locality of residential areas and an old well is present on one corner of the pond.
S8	28.761°N, 76.634°E	This is a fragmented large pond in front of a water tank area, a natural small pond, and waterlogged agricultural land nearby.

RESULTS

A total of 154 species of birds belonging to 108 genera, 47 families, and 18 orders were recorded from the study area (Table 2). Passeriformes was the most dominant order with 54 species, followed by Charadriiformes (22), Anseriformes (17), and the rest 15 orders (Figure 2). Analysis of the relative diversity index revealed that Anatidae was the most diverse family (17 species, RDi = 11.03), followed by Scolopacidae (13 species, RDi = 8.44), Muscicapidae (10 species, RDi = 7.46), and Accipitridae (9 species, RDi = 5.84). Whereas 16 families, namely, Phoenicopteridae, Podicipedidae, Anhingidae, Jacanidae, Burhinidae, Strigidae, Bucerotidae, Upupidae, Megalaimidae, Picidae, Coraciidae, Dicruridae, Nectariniidae, Phylloscopidae, Zosteropidae, and Rhipiduridae were least represented (one species each, RDi = 0.64) (Table 3).

Of the total bird species recorded, 75 species (49%) were resident, 69 species (45%) were winter migrants, and only 10 species (6%) were summer visitors (Figure 3). Based on the IUCN Red List of Threatened Species, one species, Egyptian Vulture *Neophron percnopterus* is 'Endangered', and three species—Common Pochard *Aythya farina*, Sarus Crane *Antigone antigone,* and River Tern *Sterna aurantia*—are 'Vulnerable'; eight species—

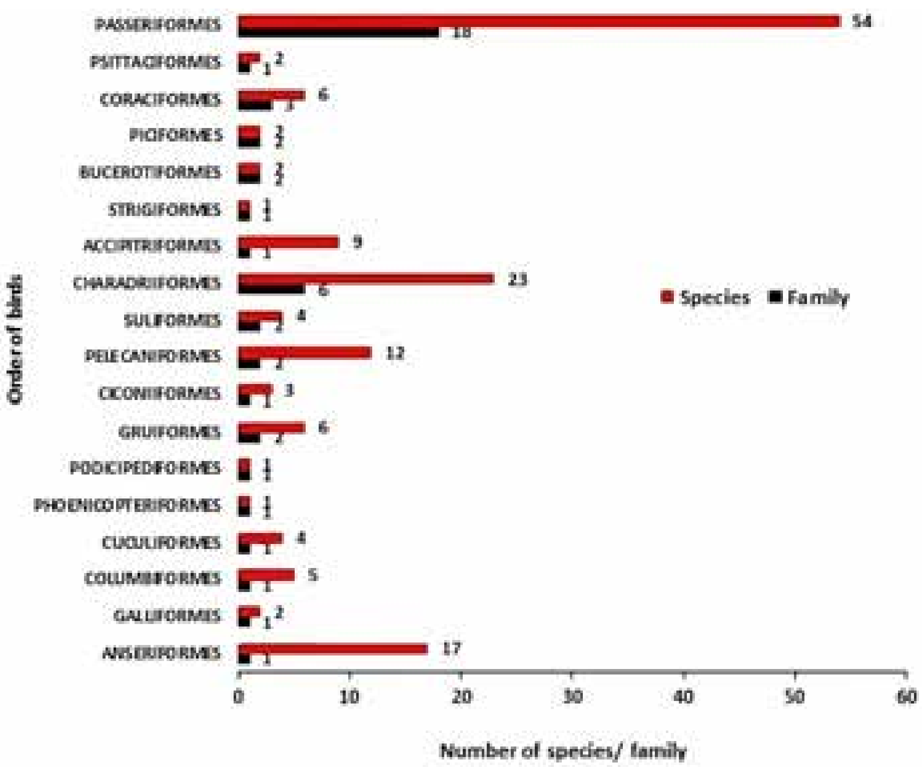

Figure 2. Composition of avian community in Dighal wetlands of Jhajjar District of Haryana, India.

Table 2. List of avian species recorded from Dighal wetlands of Jhajjar District, Haryana, India together with their respective taxonomic position, residential status, local abundance status, conservation status, and global population trend.

	Common name/ Order	Scientific name	Family	Residential status	Local status	Conservation status			Global population trend
						IUCN Red List	IWPA	CITES	
Order ANSERIFORMES									
1	Lesser-whistling Duck	*Dendrocygna javanica*	Anatidae (17)	S	UC	LC	IV	-	↓
2	Bar-headed Goose	*Anser indicus*		W	FC	LC	IV	-	↓
3	Greylag Goose	*Anser anser*		W	UC	LC	IV	-	↑
4	Ruddy Shelduck	*Tadorna ferruginea*		W	UC	LC	IV	-	?
5	Red-crested Pochard	*Netta rufina*		W	RA	LC	IV	-	?
6	Common Pochard	*Aythya farina*		W	UC	VU	IV	-	↓
7	Ferruginous Duck	*Aythya nyroca*		W	RA	NT	IV	-	↓
8	Tufted Duck	*Aythya fuligula*		W	UC	LC	IV	-	→
9	Garganey	*Spatula querquedula*		W	UC	LC	IV	-	↓
10	Northern Shoveler	*Spatula clypeata*		W	FC	LC	IV	-	↓
11	Gadwall	*Mareca strepera*		W	UC	LC	IV	-	↑
12	Eurasian Wigeon	*Mareca penelope*		W	UC	LC	IV	-	↓
13	Indian Spot-billed Duck	*Anas poecilorhyncha*		R	CO	LC	IV	-	↓
14	Northern Pintail	*Anas acuta*		W	UC	LC	IV	-	↓
15	Common Teal	*Anas crecca*		W	UC	LC	IV	-	?
16	Knob-billed Duck	*Sarkidiornis melanotos*		W*	UC	LC	IV	II	↓
17	Mallard	*Anas platyrhynchos*		W	RA	LC	IV	-	↑
Order: GALLIFORMES									
18	Indian Peafowl	*Pavo cristatus*	Phasianidae (2)	R	UC	LC	I	-	→
19	Grey Francolin	*Francolinus pondicerianus*		R	UC	LC	IV	-	→
Order: COLUMBIFORMES									
20	Rock Pigeon	*Columba livia*	Columbidae (5)	R	CO	LC	IV	-	↓
21	Eurasian Collared Dove	*Streptopelia decaocto*		R	FC	LC	IV	-	↑
22	Spotted Dove	*Streptopelia chinensis*		R	UC	LC	IV	-	↑
23	Laughing Dove	*Streptopelia senegalensis*		R	FC	LC	IV	-	→
24	Yellow-footed Green Pigeon	*Treron phoenicopterus*		R	RA	LC	IV	-	↑
Order: CUCULIFORMES									
25	Greater Coucal	*Centropus sinensis*	Cuculidae (4)	R	FC	LC	IV	-	→
26	Asian Koel	*Eudynamys scolopaceus*		S	UC	LC	IV	-	→
27	Grey-bellied Cuckoo	*Cacomantis passerinus*		S	RA	LC	IV	-	→
28	Pied Cuckoo	*Clamator jacobinus*		S	RA	LC	IV	-	→
Order: PHOENICOPTERIFORMES									
29	Greater Flamingo	*Phoenicopterus roseus*	Phoenicopteridae (1)	W*	RA	LC	IV	-	↑
Order: PODICIPEDIFORMES									
30	Little Grebe	*Tachybaptus ruficollis*	Podicipedidae (1)	R	CO	LC	IV	-	↓
Order: GRUIFORMES									
31	White-breasted Waterhen	*Amaurornis phoenicurus*	Rallidae (4)	R	UC	LC	IV	-	?
32	Grey-headed Swamphen	*Porphyrio poliocephalus*		R	FC	LC	IV	-	?

	Common name/ Order	Scientific name	Family	Residential status	Local status	Conservation status			Global population trend
						IUCN Red List	IWPA	CITES	
33	Common Moorhen	*Gallinula chloropus*		R	CO	LC	IV	-	→
34	Eurasian Coot	*Fulica atra*		W	FC	LC	IV	-	↑
35	Sarus Crane	*Antigone antigone*	Gruidae (2)	R	UC	VU	IV	II	↓
36	Demoiselle Crane	*Grus virgo*		W	RA	LC	IV	II	↑
Order: CICONIIFORMES									
37	Painted Stork	*Mycteria leucocephala*	Ciconiidae (3)	R	FC	NT	IV	I	↓
38	Asian Openbill	*Anastomus oscitans*		R	UC	LC	IV	-	?
39	Woolly-necked Stork	*Ciconia episcopus*		R	FC	NT	IV	-	↓
40	Black-crowned Night Heron	*Nycticorax nycticorax*	Ardeidae (8)	R	UC	LC	IV	-	↓
41	Indian Pond Heron	*Ardeola grayii*		R	CO	LC	IV	-	?
42	Cattle Egret	*Bubulcus ibis*		R	CO	LC	IV	-	↑
43	Grey Heron	*Ardea cinerea*		R	UC	LC	IV	-	?
44	Purple Heron	*Ardea purpurea*		R	UC	LC	IV	-	↓
45	Great Egret	*Ardea alba*		R	UC	LC	IV	-	?
46	Intermediate Egret	*Ardea intermedia*		R	UC	LC	IV	-	↓
47	Little Egret	*Egretta garzetta*		R	UC	LC	IV	-	↑
48	Black-headed Ibis	*Threskiornis melanocephalus*	Threskiornithidae (4)	R	FC	NT	IV	-	↓
49	Red-naped Ibis	*Pseudibis papillosa*		R	UC	LC	IV	-	↓
50	Glossy Ibis	*Plegadis falcinellus*		W*	UC	LC	IV	-	↓
51	Eurasian Spoonbill	*Platalea leucorodia*		W*	UC	LC	I	II	?
52	Little Cormorant	*Microcarbo niger*	Phalacrocoracidae (3)	R	FC	LC	IV	-	?
53	Great Cormorant	*Phalacrocorax carbo*		W	UC	LC	IV	-	↑
54	Indian Cormorant	*Phalacrocorax fuscicollis*		R	FC	LC	IV	-	?
55	Oriental Darter	*Anhinga melanogaster*	Anhingidae (1)	W*	UC	NT	IV	-	↓
56	Pied Avocet	*Recurvirostra avosetta*	Recurvirostridae (2)	W	UC	LC	IV	-	?
57	Black-winged Stilt	*Himantopus himantopus*		R	CO	LC	IV	-	→
58	Little-ringed Plover	*Charadrius dubius*	Charadriidae (4)	W*	RA	LC	IV	-	→
59	Red-wattled Lapwing	*Vanellus indicus*		R	CO	LC	IV	-	?
60	Yellow-wattled Lapwing	*Vanellus malabaricus*		W*	RA	LC	IV	-	→
61	White-tailed Lapwing	*Vanellus leucurus*		W	UC	LC	IV	-	?
62	Black-tailed Godwit	*Limosa limosa*	Scolopacidae (13)	W	UC	NT	IV	-	↓
63	Marsh Sandpiper	*Tringa stagnatilis*		W	UC	LC	IV	-	↓
64	Green Sandpiper	*Tringa ochropus*		W	UC	LC	IV	-	↑
65	Spotted Redshank	*Tringa erythropus*		W	RA	LC	IV	-	→
66	Little Stint	*Calidris minuta*		W	UC	LC	IV	-	↑
67	Temminck's Stint	*Calidris temminckii*		W	UC	LC	IV	-	?
68	Common Snipe	*Gallinago gallinago*		W	RA	LC	IV	-	→
69	Common Sandpiper	*Actitis hypoleucos*		W	UC	LC	IV	-	↓

	Common name/ Order	Scientific name	Family	Residential status	Local status	Conservation status			Global population trend
						IUCN Red List	IWPA	CITES	
70	Common Redshank	*Tringa totanus*		W	UC	LC	IV	-	?
71	Common Greenshank	*Tringa nebularia*		W	RA	LC	IV	-	→
72	Ruff	*Calidris pugnax*		W	UC	LC	IV	-	↓
73	Wood Sandpiper	*Tringa glareola*		W	UC	LC	IV	-	→
74	Eurasian Curlew	*Numenius arquata*		W	RA	NT	IV	-	↓
75	Black-headed Gull	*Chroicocephalus ridibundus*	Laridae (2)	W	RA	LC	IV	-	?
76	River Tern	*Sterna aurantia*		W*	UC	VU	IV	-	↓
77	Pheasant-tailed Jacana	*Hydrophasianus chirurgus*	Jacanidae (1)	S	RA	LC	IV	-	↓
78	Indian Thick-knee	*Burhinus indicus*	Burhinidae (1)	W*	RA	LC	IV	-	↓
79	Osprey	*Pandion haliaetus*	Accipitridae (9)	W	RA	LC	I	II	↑
80	Oriental Honey Buzzard	*Pernis ptilorhynchus*		W*	RA	LC	I	II	↓
81	Black-winged Kite	*Elanus caeruleus*		R	UC	LC	I	II	→
82	Egyptian Vulture	*Neophron percnopterus*		W*	UC	EN	I	II	↓
83	Shikra	*Accipiter badius*		R	UC	LC	I	II	→
84	Brahminy Kite	*Haliastur indus*		W	RA	LC	I	II	↓
85	Black Kite	*Milvus migrans*		R	FC	LC	I	II	→
86	Eurasian Sparrowhawk	*Accipiter nisus*		W	RA	LC	I	II	→
87	Short-toed Snake Eagle	*Circaetus gallicus*		W	RA	LC	I	II	→
88	Spotted Owlet	*Athene brama*	Strigidae (1)	R	UC	LC	IV	II	→
89	Indian Grey Hornbill	*Ocyceros birostris*	Bucerotidae (1)	R	UC	LC	IV	-	→
90	Common Hoopoe	*Upupa epops*	Upupidae (1)	R	UC	LC	IV	-	↓
91	Brown-headed Barbet	*Psilopogon zeylanicus*	Megalaimidae (1)	R	RA	LC	IV	-	→
92	Black-rumped Flameback	*Dinopium benghalense*	Picidae (1)	R	RA	LC	IV	-	→
93	Pied Kingfisher	*Ceryle rudis*	Alcedinidae(3)	R	UC	LC	IV	-	?
94	White-throated Kingfisher	*Halcyon smyrnensis*		R	FC	LC	IV	-	?
95	Common Kingfisher	*Alcedo atthis*		W*	RA	LC	IV	-	?
96	Indian Roller	*Coracias benghalensis*	Coraciidae (1)	R	UC	LC	IV	-	↑
97	Green Bee-eater	*Merops orientalis*	Meropidae (2)	S	UC	LC	IV	-	↑
98	Blue-cheeked Bee-eater	*Merops persicus*		S	RA	LC	IV	-	→
99	Alexandrine Parakeet	*Psittacula eupatria*	Psittaculidae (2)	R	UC	NT	IV	II	↓
100	Rose-ringed Parakeet	*Psittacula krameri*		R	FC	LC	IV	-	↑
101	Black Drongo	*Dicrurus macrocercus*	Dicruridae (1)	R	CO	LC	IV	-	?
102	Isabelline Shrike	*Lanius isabellinus*	Laniidae (2)	W	RA	LC	IV	-	→
103	Long-tailed Shrike	*Lanius schach*		R	UC	LC	IV	-	?

	Common name/ Order	Scientific name	Family	Residential status	Local status	Conservation status			Global population trend
						IUCN Red List	IWPA	CITES	
104	Rufous Treepie	*Dendrocitta vagabunda*	Corvidae (3)	R	UC	LC	IV	-	↓
105	House Crow	*Corvus splendens*		R	CO	LC	V	-	→
106	Large-billed Crow	*Corvus macrorhynchos*		R	UC	LC	IV	-	→
107	Purple Sunbird	*Cinnyris asiaticus*	Nectariniidae (1)	R	UC	LC	IV	-	→
108	Indian Silverbill	*Euodice malabarica*	Estrildidae (3)	R	UC	LC	IV	-	→
109	Scaly-breasted Munia	*Lonchura punctulata*		R	UC	LC	IV	-	→
110	Red Munia	*Amandava amandava*		W*	RA	LC	IV	-	→
111	House Sparrow	*Passer domesticus*	Passeridae (2)	R	FC	LC	IV	-	↓
112	Sind Sparrow	*Passer pyrrhonotus*		W*	RA	LC	IV	-	→
113	Crested Lark	*Galerida cristata*	Alaudidae (2)	R	UC	LC	IV	-	↓
114	Bengal Bushlark	*Mirafra assamica*		S	RA	LC	IV	-	→
115	Plain Prinia	*Prinia inornata*	Cisticolidae (5)	R	UC	LC	IV	-	→
116	Graceful Prinia	*Prinia gracilis*		S*	RA	LC	IV	-	→
117	Zitting Cisticola	*Cisticola juncidis*		R	UC	LC	IV	-	↑
118	Ashy Prinia	*Prinia socialis*		R	FC	LC	IV	-	→
119	Common Tailorbird	*Orthotomus sutorius*		R	UC	LC	IV	-	→
120	White-eared Bulbul	*Pycnonotus leucotis*	Pycnonotidae (2)	W*	RA	LC	IV	-	↓
121	Red-vented Bulbul	*Pycnonotus cafer*		R	FC	LC	IV	-	↑
122	Common Chiffchaff	*Phylloscopus collybita*	Phylloscopidae (1)	W	UC	LC	IV	-	↑
123	Brahminy Starling	*Sturnia pagodarum*	Sturnidae (3)	S*	RA	LC	IV	-	?
124	Common Starling	*Sturnus vulgaris*		W	RA	LC	IV	-	↓
125	Asian Pied Starling	*Gracupica contra*		R	FC	LC	IV	-	↑
126	Red-breasted Flycatcher	*Ficedula parva*	Muscicapidae (10)	W	RA	LC	IV	-	↑
127	Black Redstart	*Phoenicurus ochruros*		W	RA	LC	IV	-	↑
128	Common Myna	*Acridotheres tristis*		R	FC	LC	IV	-	↑
129	Bank Myna	*Acridotheres ginginianus*		R	FC	LC	IV	-	↑
130	Indian Robin	*Copsychus fulicatus*		R	UC	LC	IV	-	→
131	Oriental Magpie Robin	*Copsychus saularis*		R	UC	LC	IV	-	→
132	Bluethroat	*Luscinia svecica*		W	RA	LC	IV	-	→
133	Brown Rock Chat	*Oenanthe fusca*		R	UC	LC	IV	-	→
134	Siberian Stonechat	*Saxicola maurus*		W	UC	LC	IV	-	→
135	Pied Bushchat	*Saxicola caprata*		R	UC	LC	IV	-	→
136	Rosy Pipit	*Anthus roseatus*	Motacillidae (8)	W	UC	LC	IV	-	→
137	Tawny Pipit	*Anthus campestris*		W	UC	LC	IV	-	→
138	Long-billed Pipit	*Anthus similis*		W	UC	LC	IV	-	→
139	Paddyfield Pipit	*Anthus rufulus*		R	UC	LC	IV	-	→
140	Western Yellow Wagtail	*Motacilla flava*		W	UC	LC	IV	-	→
141	Citrine Wagtail	*Motacilla citreola*		W	UC	LC	IV	-	↑
142	White-browed Wagtail	*Motacilla maderaspatensis*		W*	FC	LC	IV	-	→
143	White Wagtail	*Motacilla alba*		W	UC	LC	IV	-	→
144	Streak-throated Swallow	*Petrochelidon fluvicola*	Hirundinidae (4)	R	UC	LC	IV	-	↑

	Common name/ Order	Scientific name	Family	Residential status	Local status	Conservation status			Global population trend
						IUCN Red List	IWPA	CITES	
145	Wire-tailed Swallow	*Hirundo smithii*		R	FC	LC	IV	-	↑
146	Barn Swallow	*Hirundo rustica*		W	UC	LC	IV	-	↓
147	Grey-throated Martin	*Riparia chinensis*		W*	UC	LC	IV	-	↓
148	Indian White-eye	*Zosterops palpebrosus*	Zosteropidae (1)	R	UC	LC	IV	-	↓
149	Jungle Babbler	*Argya striata*	Leiothrichidae (2)	R	UC	LC	IV	-	→
150	Large grey Babbler	*Argya malcolmi*		R	UC	LC	IV	-	→
151	Streaked Weaver	*Ploceus manyar*	Ploceidae (3)	R	UC	LC	IV	-	→
152	Baya Weaver	*Ploceus philippinus*		R	UC	LC	IV	-	→
153	Black-breasted Weaver	*Ploceus benghalensis*		R	UC	LC	IV	-	→
154	White-browed Fantail	*Rhipidura aureola*	Rhipiduridae (1)	W*	RA	LC	IV	-	→

Residential Status: R—Resident | S—Summer Migrant | W—Winter Migrant.
Local Abundance Status: CO—Common | FC—Fairly Common | UC—Uncommon | RA—Rare.
IUCN Red List: LC—Least Concern | NT—Near Threatened | VU—Vulnerable | EN—Endangered.
IWPA (Indian Wildlife Protection Act, 1972): I— Schedule I | IV—Schedule IV,
Global Population Trend: ↑—Increasing | ↓—Decreasing | →—Stable | ?—Unknown.
*—Species that are resident in Haryana but recorded only in winter or summer in the study area.

Table 3. Relative diversity index (RDi) of various bird families in Dighal wetlands of Jhajjar District of Haryana.

Bird families	Number of species	RDi value
Anatidae	17	11.03
Scolopacidae	13	8.44
Muscicapidae	10	7.46
Accipitridae	9	5.84
Ardeidae, Motacillidae	8	5.19
Columbidae, Cisticolidae	5	3.24
Cuculidae, Rallidae, Threskiornithidae, Charadriidae, Hirundinidae	4	2.59
Ciconiidae, Phalacrocoracidae, Alcedinidae, Corvidae, Estrildidae, Sturnidae, Ploceidae	3	1.94
Phasianidae, Gruidae, Recurvirostridae, Laridae, Meropidae, Psittaculidae, Laniidae, Passeridae, Alaudidae, Pycnonotidae, Leiothrichidae	2	1.29
Phoenicopteridae, Podicipedidae, Anhingidae, Jacanidae, Burhinidae, Strigidae, Bucerotidae, Upupidae, Megalaimidae, Picidae, Coraciidae, Dicruridae, Nectariniidae, Phylloscopidae, Zosteropidae, Rhipiduridae	1	0.64

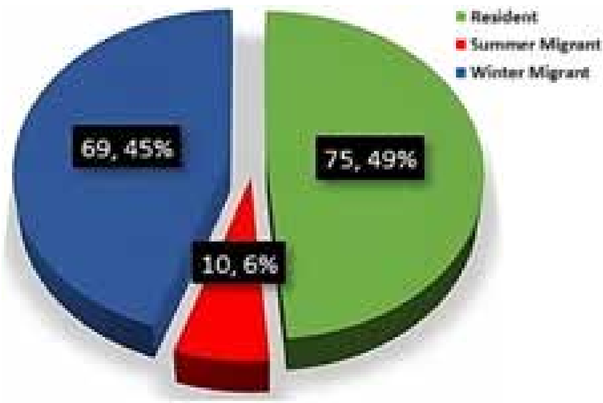

Figure 3. Residential status of avian species recorded from Dighal wetlands of Haryana, India.

Ferruginous Duck *Aythya nyroca*, Painted Stork *Mycteria leucocephala*, Woolly-necked Stork *Ciconia episcopus*, Black-headed Ibis *Threskiornis melanocephalus*, Oriental Darter *Anhinga melanogaster*, Black-tailed Godwit *Limosa limosa*, Eurasian Curlew *Numenius arquata,* and Alexandrine Parakeet *Psittacula eupatria*—are 'Near Threatened'; and the rest 142 species are Least Concern (Table 2). About the global population trend, the wetlands supported 62 globally stable species, 40 globally decreasing species, 29 globally increasing species, and 23 species whose global trend was unknown (Table 2). Data on local abundance revealed that 10 species were common, 23 species were fairly common, 83 species were uncommon, and 38 species were rare in the study area. It is pertinent here to mention that among the 38 species recorded rarely in the study area, 12 species—Ferruginous Duck *Aythya nyroca*, Mallard *Anas platyrhynchos*, Greater Flamingo *Phoenicopterus roseus*, Demoiselle Crane *Grus virgo*, Common Snipe *Gallinago gallinago*, Common Kingfisher *Alcedo atthis*, Isabelline Shrike *Lanius isabellinus*, White-eared Bulbul *Pycnonotus leucotis*, Red-breasted Flycatcher *Ficedula parva*, Osprey *Pandion haliaetus*, Oriental Honey Buzzard *Pernis ptilorhynchus,* and White-browed Fantail *Rhipidura aureola*—were spotted only once or

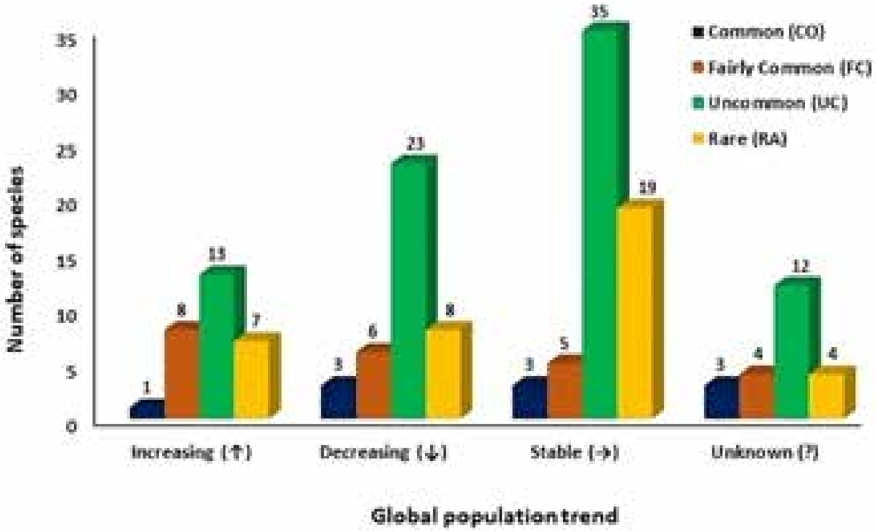

Figure 4. Comparison of local abundance status and global population trend of avian species recorded from Dighal wetlands of Jhajjar District, Haryana, India.

twice during the study period. Comparison of the local abundance status of recorded avian species with their global population trend revealed that three species—Indian Spot-billed Duck *Anas poecilorhyncha*, Rock Pigeon *Columba livia,* and Little Grebe *Tachybaptus ruficollis*—had a globally declining population trend and were found to be common in the study area (Figure 4). In addition to this, one species recorded from these wetlands was listed in Appendix I, and 16 species were listed in Appendix II of CITES (Table 1). According to the IWPA (1972), out of 154 recorded species, 11 were under Schedule I, one was in Schedule V, and the rest (n = 142) were in Schedule IV (Table 1).

DISCUSSION

The study area boasts an impressive avian diversity, contributing to approximately 29% of the bird species recorded in Haryana (Kalsi et al. 2020) and 11% of India's avifauna (Praveen & Jayapal 2023). The findings suggest that the avifaunal richness observed in the studied wetlands aligns with previous research conducted in various regions of Haryana (Table 4). Nevertheless, it is important to note that Alfred et al. (2001) documented 216 wetland bird species in the more expansive Sub-Himalayan Terai and Indo-Gangetic Plains of northern India. In the survey, Passeriformes were identified as the dominant order, with 54 species representing 18 families. These results corroborate earlier records highlighting Passeriformes as the primary avian taxa in India (Praveen & Jayapal 2023).

Table 4. A comparison of avifauna recorded from different study areas of Haryana, India.

	Study area	Recorded avifauna			Reference
		Species	Family	Order	
1	Khaparwas Bird Sanctuary, district Jhajjar	164	44	16	Gupta et al. 2012
2	Sultanpur National Park, district Gurugram	161	47	16	Kaushik & Gupta 2016
3	Kalesar National Park, district Yamunanagar	126	51	14	Rai et al. 2017
4	Basai Wetland, district Gurugram	171	51	17	Rai et al. 2019
5	Bhindawas Bird Sanctuary, district Jhajjar	119	43	17	Singh & Malik 2019
6	Sultanpur National Park, district Gurugram	111	42	17	Singh et al. 2021
7	Ottu Reservoir, district Sirsa	114	47	18	Rai & Vanita 2021

Among the documented bird families in the selected wetlands of Jhajjar District, Anatidae emerged as the most diverse. This observation aligns with prior studies demonstrating Anatidae as a prevalent bird family in various freshwater wetlands across India (Tak et al. 2010; Kumar & Sharma 2018; Rai et al. 2019; Kaur & Brraich 2021; Singh & Brraich 2022; Yashmita-Ulman & Singh 2022). The findings indicate that the majority of recorded species are residents, followed by winter and summer visitors, consistent with earlier reports on freshwater wetlands in Haryana (Kumar & Gupta 2013; Kumar et al. 2016; Rai et al. 2019). It is worth noting that 20 bird species recorded as migrants in the study area are considered residents of Haryana (Kalsi et al. 2020),

Image 1–12. Threatened Birds in Dighal wetlands of district Jhajjar, Haryana.
1—Black Tailed Godwit *Limosa limosa* | 2—Egyptian Vulture *Neophron percnopterus* | 3—Black-headed Ibis *Threskiornis melanocephalus* | 4—Wooly Necked Stork *Ciconia episcopus* | 5—Oriental Darter *Anhinga melanogaster* | 6—Painted Stork *Mycteria leucocephala* | 7—River Tern *Sterna aurantia* | 8—Common Pochard *Aythya farina* | 9—Alexandrine Parakeet *Psittacula eupatria* | 10—Ferruginous Duck *Aythya nyroca* | 11—Sarus Crane *Antigone antigone* | 12—Eurasian Curlew *Numenius arquata*. © Parul.

as outlined in Table 2. Given Haryana's location within the Central Asian Flyway, it serves as a crucial wintering ground for migratory birds travelling from northern Asia and parts of Europe (Kumar et al. 2016; Kumar & Sharma 2018; Rai & Vanita 2021).

The suitability of the Dighal wetlands, surrounded by irrigated agricultural fields featuring wheat and paddy crops, as well as tree species like Safeda (*Eucalyptus* spp.), Kikar (*Acacia* spp.), and Ber (*Ziziphus* spp.) along the wetland edges, provides ample resources for migratory birds during the winter months. This resource availability contributes to the rich avian diversity, particularly during the winter season.

The survey identified 12 bird species of global conservation significance, including one Endangered species, three Vulnerable species, and eight Near Threatened species. Furthermore, 17 species listed in the CITES appendices inhabit these wetlands. All the recorded bird species are also protected under various Schedules of the Indian Wildlife (Protection) Act, 1972. Notably, the study identified three species with declining global populations as common in the study area, indicating the continued availability of suitable resources for these species in the wetlands.

The presence of a substantial number of winter migrants and species of global conservation concern underscores the importance of these wetlands for avian conservation in Haryana.

However, wetland habitats across India face significant threats, including habitat loss, fragmentation, and degradation; water quality deterioration due to contamination; recreational pressures; and developmental activities (Kumar & Sharma 2018; Chakraborty et al. 2021; Kaur & Brraich 2021; Mandal et al. 2021; Yashmita-Ulman & Singh 2022; Anand et al. 2023; Muralikrishnan et al. 2023). The Dighal wetlands are no exception, as they support a diverse community of winter migrants and species of global conservation concern, all of which are vulnerable to various anthropogenic pressures. These threats include extensive fishing activities, electrocution, construction near ponds, domestic waste dumping, water drainage during the winter, and plastic pollution in ponds.

The study serves as a valuable baseline for future research on bird population monitoring and the effective management of the Dighal wetlands in the Jhajjar District of Haryana.

CONCLUSION

The documentation of 12 bird species of global conservation importance and 40 species of birds with a declining population trend globally emphasises the importance of studied wetlands from a global bird conservation perspective. These wetlands, along with surrounding agricultural fields and plantations, provide a congenial habitat for both resident and migratory avian species. Therefore, these wetlands should be given conservation and research priorities and regularly assessed for their existing bird diversity. This study provides valuable information on the ecological health and status of these wetlands and will be useful for increasing awareness regarding their conservation value.

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COMMUNICATION

Studies on the response of House Sparrow *Passer domesticus* to artificial nest-boxes in rural Arakkonam and Nemili taluks, Vellore District, Tamil Nadu, India

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Abstract: This study evaluated the response of House Sparrows *Passer domesticus* to artificial nest-boxes installed in human dwellings in 30 villages in Arakkonam and Nemili taluks, Vellore District, Tamil Nadu between February and July 2019, with help of school students who installed 245 artificial nest-boxes in their houses. House Sparrows attempted to build nests in 32 nest-boxes by frequent visits, built partial nests in 51, and built active nests followed by successful breeding in 32 nest-boxes; there was no response to the remaining 130. A significant relationship was detected between the type of house and the adoption of boxes by the birds. The maximum response was seen in tiled houses, followed by concrete and thatched houses. House Sparrows preferred nest-boxes placed at heights between 3 and 4 m. At the end of the breeding season, a total of 80 chicks successfully emerged from 32 active nests. Some mortality in adult birds due to ceiling fans and predatory animals such as House Crows and Domestic Cats was reported. Active nests in nest-boxes and birds were found in villages where mobile phone towers were installed. Of 32 active nests enumerated in nest-boxes, 22 were found within a 500 m radius of mobile phone towers, two from 500–1,000 m and eight from 1,000–2,000 m. Further study is planned to examine the relationship between mobile towers and nest site selection by sparrows. A survey done through a questionnaire reveals that 95% of residents were aware of and concerned about the declining populations of House Sparrow.

Keywords: Active nests, Electromagnetic radiations, Mobile-phone towers, Nesting sites, Predatory animals.

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INTRODUCTION

The House Sparrow *Passer domesticus,* a native of Eurasia, is the most widespread bird in the world (Anderson 2006) and its geographical range extends over Europe, North Africa, and parts of Asia including the Indian subcontinent (Ali & Ripley 1987). These sparrows prefer holes or crevices near roofs of human residences as nesting sites (Ali & Ripley 1987). They have been declining since the 1980s in several parts of the world, including Europe (Kelcey & Rheinwald 2005; Kekkonen et al. 2011), where the decline may be due to changes in farming practices, use of pesticides/herbicides, and predation. The species was red-listed in the U.K. in 2002 as a result of population decline (Summers-Smith 1988, 2005). Loss of suitable nesting sites and foraging habitats are the reasons for declining populations of House Sparrow in urban and suburban landscapes (Robinson et al. 2005).

In India, sparrow populations are reported to have decreased considerably in Bengaluru, Delhi, Mumbai, and Hyderabad (Rajashekar & Venkatesha 2008; Daniels 2008; Khera et al. 2010; Bhattacharya et al. 2010; Ghosh et al. 2010). Rahmani et al. (2013) have stated that House Sparrows and their nests were found in fewer places in India during 2005–2012 when compared to the time before 2005. This is because the bird prefers holes or crevices near roofs of human residences as nesting sites (Ali & Ripley 1987). The declining trend is consistent in all the regions and major cities except Coimbatore (Rahmani et al. 2013). The IUCN Red List has evaluated the House Sparrow's conservation status as 'Least Concern' (BirdLife International 2018).

House Sparrows are flexible in selection of nesting sites, and will build nests in places such as artificial nest-boxes when modern buildings lack suitable nesting sites (Shaw et al. 2008). Availability of nesting sites is a major factor that determines House Sparrow populations in urban areas (Anderson 2006). In India, the response of House Sparrow towards artificial nest-boxes has been poorly studied. In India, maximum numbers of active nests were found in wall cavities followed by artificial/man-made nest boxes (Rahmani et al. 2013). In view of urbanization and lack of nesting sites in the modern buildings the House Sparrow populations had preferred artificial nest-boxes in Udhagamandalam urban areas in Nilgiris District (Jayaraman et al. 2017).

In view of the growing concern over the decline of House Sparrow population in India, in this paper I sought answers to the questions considering their habitats, with specific reference to Arakkonam and Nemili taluks in Vellore District, Tamil Nadu, India. The following objectives were kept in mind: 1. How do House Sparrows respond towards artificial nest-boxes? 2. What types of houses are preferred by the bird and heights preferred to build nests? 3. What are the impacts of electrical appliances and predatory animals on House Sparrows?, and 4. What is the correlation between mobile towers and site selection by House Sparrows?

MATERIALS AND METHODS

Study Area

Arakkonam (13.07 N & 79.67 E) and Nemili (12.58 N & 78.50 E) taluks, Vellore District occur in the northeastern part of Tamil Nadu, 70 km from Vellore Town (12.25–13.25 N and 78.25–79.83 E) and 71 km west from Chennai (13.08–80.28 E) covering 828 km^2 with a human population of c. 500,000 (2011 census). The present study was undertaken in 30 villages in Arakkonam and Nemili taluks (Figure 1). The principal occupation of residents here is agriculture followed by weaving. The average elevation from the sea level is about 81 m. The maximum and minimum annual temperatures in the district are 34.1 ^{o}C and 22.4 ^{o}C, respectively. The average annual rainfall is 1,000 mm (www.tn.gov.in).

Methods

With help of four school teachers and five informants/field assistants, I identified 30 villages having House Sparrow populations. Artificial nest-boxes (245) made of hard cardboard (12 X 12 X 12 cm size) were distributed to 245 students (6th to 12th standard) of three higher secondary schools (one in Nemili Taluk and two in Arakkonam Taluk) during the first week of December 2018. Supplementary cushion materials like fibres, leaves, twigs or any other plant materials were not placed inside the nest boxes because birds would place nest materials once it selects the nest-boxes for nesting. The students were briefed about the life cycle of House Sparrows including breeding period and were instructed to hang the nest-boxes in their houses at reasonable heights (above 3 m) beyond the reach of human beings and predatory animals. Out of 300 nest-boxes distributed, students had placed 245 nest-boxes in their houses at the end of the third week of December 2018. Students had placed 80% of nest-boxes (195) facing outwards/exteriors from the houses and in the remaining 20% nest-boxes (50) the entrances were facing inwards to the houses. The remaining 55 students had not responded and did not place nest-boxes in

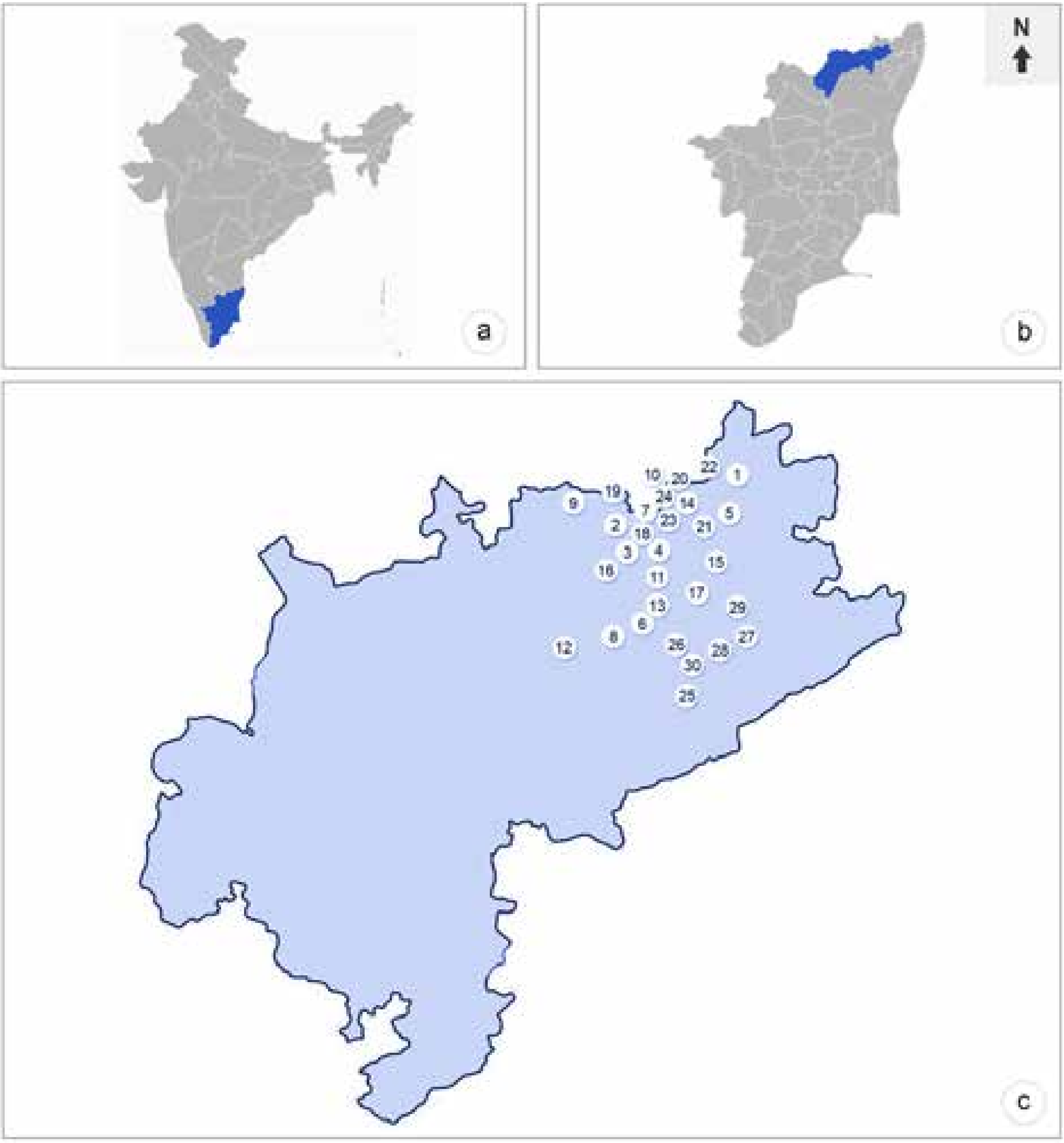

Figure 1. Study area: a—India map showing Tamil Nadu (blue colour) | b—Tamil Nadu map showing Vellore District (blue colour) | c—Eastern part of Vellore District map showing villages having nests of House Sparrows. List of villages: 1—Ayyanthangal Kandigai | 2—Chinna sembedu | 3—Gandhinagar | 4—Guruvarajapetai | 5—Ichiputhur | 6—Kailasapuram | 7—Kannigapuram | 8—Minnal | 9—Nandhiveduthangal | 10—Periyakadambur | 11—Perumalrajapet | 12—Ramapuram | 13—Salai | 14—Sembedu | 15—Chithambadi | 16—Soganur | 17—Vedal | 18—Viswanathapuram | 19—Chinna kadambur mottur | 20—Periya kadambur mottur | 21—Gadavari kandigai | 22—Karthikeyapuram | 23—Kesavarajapettai | 24—Chinna kadambur | 25—Arumbakkam | 26—Arumbakkam | 27 —Melandurai | 28—Nagavedu | 29—Paruthiputhur | 30—Ochalam.

their houses. The students placed nest-boxes at various heights ranges from 3–8 m depending on their type of houses. Nest boxes placed at 7–8 m height was in the upper floor of concrete houses. Hence, the study was carried out only on those 245 houses where nest-boxes were installed. Between February and July 2019, all the houses (245) were visited and the response of House Sparrows towards nest-boxes was studied.

The nest-boxes and birds were surveyed between 0600 h & 0900 h and 1500 h & 1800 h over the mentioned six months. Students who received and placed nest-boxes had spent time to observe the nest boxes in the morning (0600–0830 h) and in the evening (1600–1800 h) and during holidays they spent more time (0600–1800 h) to monitor the activity of birds. Then they were interviewed within the age group (between 11–17 years) at the end of the breeding season, i.e., during July 2019 and concluded during 20 August 2019. Elderly persons (age 60–70 y) were interviewed using a questionnaire in Tamil language.

The breeding of this species occurred between February and July 2019. Details such as types of houses, responses of birds toward nest-boxes such as number of attempts to visit nest-boxes, number of partially built nests, number of active nests and successful breeding, number of chicks grown and flown from each successful nest, impact of electrical ceiling fans, accidental fall of eggs/chicks, extent of increase or decrease in the populations of House Sparrow and impact of predatory animals. The heights of nest-boxes from the floor of the houses were measured using measuring tape. The study on the breeding biology of House Sparrows such as number of eggs laid, incubation, and hatching in the active nests was not done, as it would cause harm to the breeding of this species. The numbers of mobile phone towers in the villages were verified and listed. Photograph was made using a digital camera without disturbing the nests and birds.

Chi-Square test was applied to determine whether any significant differences exist between the types of houses (namely concrete flat-terraced houses and tile-rooftop houses) and the selection of nesting sites by House Sparrows. For analysis, SPSS (Statistical Package for Social Sciences) software was used. The test of significance was assessed at $p<0.05$. Since the number of thatched houses (6) and shops (2) were few in number, they were ignored and not taken for analysis. Collected data were tabulated, analysed, and given as graphical representations.

RESULTS

Of 245 nest-boxes placed in 30 villages, House Sparrows responded to 47% (115) and no response was found towards the remaining 53% (130). The House Sparrows visited 32 nest boxes but did not nest, in 51 nest-boxes birds built partial nests, and 32 pairs built complete and active nests (Table 1). Maximum response of birds to nest-boxes was reported in tiled houses (64 nest-boxes), followed by concrete houses (47). Similarly, maximum numbers of attempts occurred in concrete houses (21), followed by tiled houses (10). Successful breeding occurred in 21 nest-boxes installed in tiled houses followed by 10 in concrete house and a solitary case reported in thatched house.

House Sparrows preferred to nest (65%) in nest-boxes which were placed between 3 m and 4 m height. Sparrows did not inhabit or lay eggs in nest-boxes which were installed above 7 m height. In these limited observations, the birds preferred to select artificial nest-boxes in the ground floor for the construction of nests (Figure 2).

Out of 245 nest-boxes, the entrance of 80% (195) were found facing outwards/exteriors from the houses and in the remaining 20% (50) the entrances were facing inwards. At the end of the breeding season, the response of House Sparrows towards nest-boxes which were facing outward from the houses were found higher (50%; 97) than the nest-boxes facing interior of the houses (36%; 18). Though the nest-boxes installation was skewed, proportionately the birds preferred a greater number of nest-boxes facing exterior from the houses than the nest-boxes facing interior of the houses in the study area (Table 2).

A significant relationship exists between the type of house and attempts to use nest-boxes ($X^2 = 7.069$; $p <0.008$) and partially built nests ($X^2 = 4.155$; $p <0.042$). But no significant relationship exists between the types of houses and construction of active nests ($X^2 = 2.548$; $p <0.11$) (Table 3). Study revealed that the birds had shown more preference towards artificial nest-boxes placed in tiled houses than concrete houses in the studied villages. The existence of many entry/exit spaces between roof & wall, wall cavities, and scaffold holes in the walls in tiled houses might have been the probable reasons for the preference of tiled houses and these entry/exit spaces seldom found in concrete houses. However, House Sparrow's preference of houses need further studies in larger areas covering urban and rural habitats. In the present study the observation of fewer chicks, i.e., one to two chicks per active nest (18 out of 32 nests) could be considered a matter of great concern (Table 4).

Incidents of adult House Sparrows suffering mortality by collision with the blades of ceiling fans occurred in 10 houses, i.e., two birds in concrete houses and eight in tiled houses in the study area. Three incidents of accidental fall of chicks in concrete houses and two incidents of fall of eggs in tiled houses were also reported. In the present study, incidents of predators visiting

Table 1. Type of houses and response of House Sparrow in the construction of nests in artificial nest-boxes in the study area.

Type of Building	Total no. of nest-boxes placed	% of nest boxes placed	No. of nest-boxes in which birds attempted	No. of nest-boxes where partially built nests	No. of active nests built	Total no. of positive response	% of positive response
Tiled houses	129	52.65 %	10	33	21	64	26.12 %
Concrete houses	108	44.08 %	21	16	10	47	19.18 %
Thatched houses	6	2.45 %	1	1	1	3	1.22 %
Grocery shops	2	0.82 %	0	1	0	1	0.41 %
Total	245	100%	32	51	32	115	46.94%

Table 2. Relationships between orientation of entrance of nests boxes and response of House Sparrows.

Orientation of nest entrance	Total no. of nest-boxes placed	%	Total no. of responses of House Sparrows	%	No. of mere attempts	%	No. of partially built nests	%	No. of active nests	%
Entrance of nest-boxes facing out wards from houses	195	79.6	97	39.59	28	11.43	42	17.14	27	11.02
Entrance of nest-boxes facing in wards to houses	50	20.4	18	7.35	4	1.63	9	3.67	5	2.04
Total	245	100	115	46.94	32	13.06	51	20.82	32	13.06

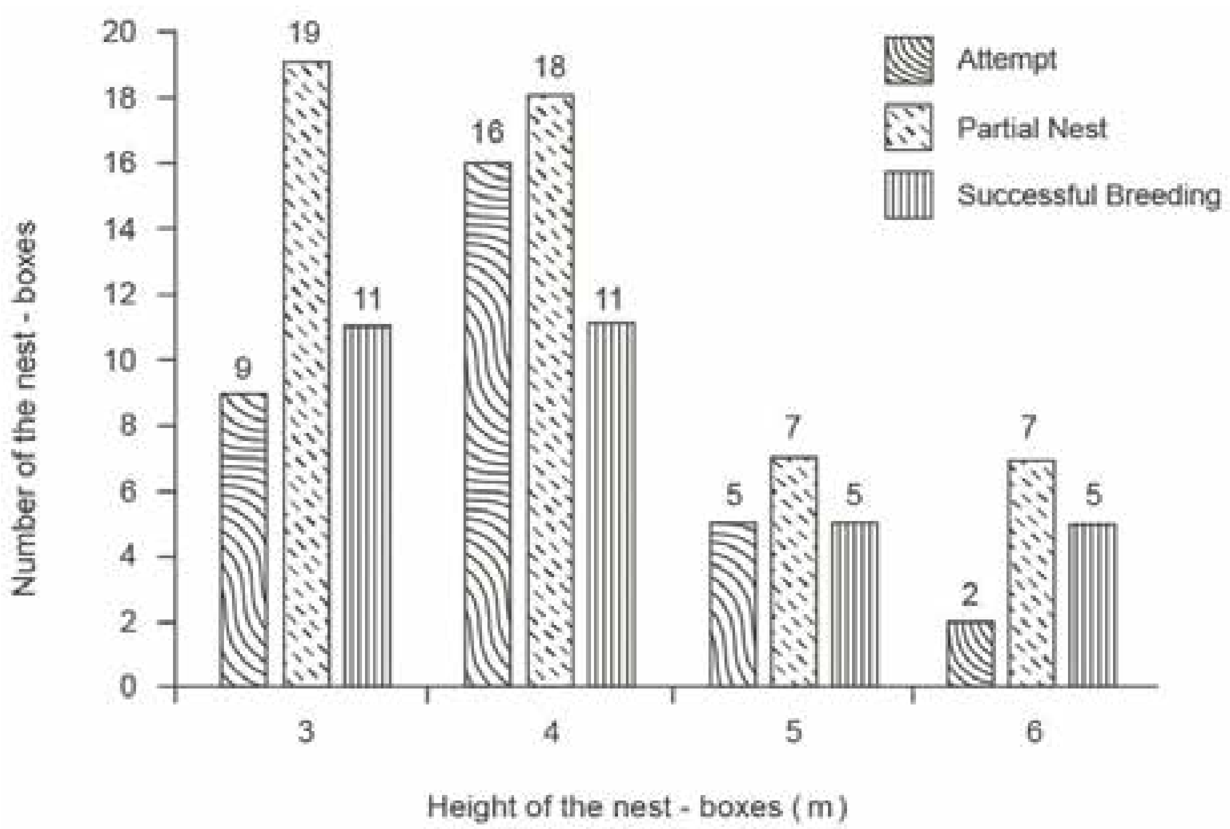

Figure 2. Relationship between heights of nest-boxes and House Sparrows' mere attempts, built partial nests, and built complete nests by successful breeding in the nest-boxes.

Table 3. Chi-square test between type of houses and response of House Sparrow to artificial nest-boxes.

Types of houses	House Sparrows attempted to adopt nest-boxes				House Sparrows partially built nests				House Sparrows built active nests and bred successfully			
	Merely attempted		Nil attempts		No. of houses where partially built nest		Nil partial nests		Active nests Successful breeding occurred		Nil breeding occurred	
	Count	%	Count	%	Count	%	Count	%	Count	%	Count	%
Tiled house	10	7.8	119	92.2	33	25.6	96	74.4	21	16.3	108	83.7
Concrete House	21	19.4	87	80.6	16	14.8	92	85.2	10	09.3	98	90.7
	X^2 = 7.069 ; p <0.008				X^2 = 4.155 ; p <0.042				X^2 = 2.548 ; p <0.11			

Table 4. Details of number of chicks fledge after successful breeding from nests built in artificial nest-boxes of House Sparrow.

	No. of active nests	No. of chicks that came out from active nests
1.	6	1
2.	12	2
3.	8	3
4.	4	4
5.	2	5
Total	32	80

nest-boxes and causing disturbance to adult birds were found in 39 houses (concrete house 21; tiled house 18). Residents reported that House Crows *Corvus splendens* and Domestic Cats *Felis catus* had caused disturbances to House Sparrows. These predatory animals disturbed adult House Sparrows during nest-building and delivery of food to chicks. Residents stated that House Crows in 30 houses and Domestic Cats in nine houses had caused disturbances to House Sparrows by chasing the latter.

In the present study active nests and birds were found in villages where mobile-phone towers were installed. The analysis on the locations of active nests found in nest-boxes and their proximity to mobile-phone towers in the villages revealed that 22 active nests were found within 500 m radius from mobile-phone towers, two active nests occurred between 500 m and 1,000 m radius and another two nests beyond this up to 2,000 m radius from mobile-phone towers. In case of remaining six nests, no mobile towers were found within 2,000 m radius from the nesting sites.

A closed type questionnaire survey revealed that 95% of the adult residents were aware of and concerned about the declining populations of House Sparrow in general, and particularly in their villages.

DISCUSSION

The response of House Sparrow towards artificial nest boxes was greater in Udhagamandalam, Tamil Nadu (Jayaraman 2017). The number of House Sparrow breeding pairs in the nest boxes was increased to 50% over a period of five years in Poland (Dulisz et al. 2022). In India, next to wall cavities, maximum numbers of nests were found in the artificial/man-made nest boxes (Rahmani 2013). In the present study, the response of *P. domesticus* to 47% of the total artificial nest-boxes matches with the views of Jayaraman (2017), Rahmani (2013), and Dulisz et al. (2022). The British Trust for Ornithology has suggested that the heights of nest-boxes should be 3 m above the ground. In the present study also, responses of *P. domesticus* were found maximum in the nest-boxes which were placed between 3 m and 4 m heights.

House Crows predate nests of House Sparrow in Delhi (Khera et al. 2010) and Domestic Cats in Bandel region of West Bengal and Chennai in Tamil Nadu (Daniels 2008; Ghosh et al. 2010). Similarly, in the present study, incidents of nest predation by House Crows and Domestic Cats were recorded and hence, it corroborates with the earlier mentioned findings.

Clutch size is determined by various environmental factors, age of the female, breeding density, and the usual clutch size is composed of 4–5 eggs (Summers-Smith 1988; Anderson 2006). In all the active nests (32), 1–5 nestlings came out at the end of their breeding. Cases of eggs not hatched and mortality of chicks within the nest-boxes were not studied in the present investigation. Detailed study alone will throw more light on the causes for such reduced number of nestlings, i.e., one or two per active nest in the study area.

Electromagnetic radiations from mobile-phone towers are linked to population declines of House

Sparrow in Europe (Crick et al. 2002; Balmori & Hallberg 2007; Everaert & Bauwens 2007). Although equal numbers of nest-boxes were not installed at equal distance from mobile-phone towers, considerable number of active nests occurred within 500 m radius from mobile-phone towers. However, in the event of existence of mobile-phone towers in almost all villages, the exact impact of mobile-phone towers on the breeding of House Sparrows in the larger geographical areas need further study.

CONCLUSION

The present study reveals that the rural Arakkonam and Nemili taluks in Vellore District are potential breeding grounds of the House Sparrow. The birds show a considerable response to artificial nest-boxes. Efforts need to be taken to create further awareness among the general public, including students, about the need to save House Sparrows and create more nesting sites in newly constructed houses, government buildings, schools, and colleges, besides placing nest-boxes. Predatory animals and accidental fall of eggs and broods, and ceiling fans in human dwellings pose threats to the House Sparrow populations. The impact of ceiling fans on the House Sparrow needs further study as ceiling fans have become ubiquitous in rural areas. In order to mitigate such mortality, installation nest-boxes near ceiling fans or halls having ceiling fans may be avoided. A special management plan for Vellore district must be established and it is essential to conduct sustained surveys and monitor the nesting sites during the subsequent breeding seasons and efforts should be taken to create suitable nesting habitats by installing more artificial nest-boxes in the villages for successful breeding. The present study was a model study of conservation of such a semi domesticated natural avian population. Community participation to ensure installation of sufficient number cavities in the newly constructed modern buildings and also participation of like school/college students to place more number of nest-boxes in the government buildings should be encouraged.

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COMMUNICATION

Threat assessment and conservation challenges for the herpetofaunal diversity of Dampa Tiger Reserve, Mizoram, India

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Abstract: Herpetofauna is an important group of vertebrates with key functions in ecosystem sustenance. Nonetheless, with ever-increasing anthropogenic activities and lack of evidence-based studies, about 80% of the herpetofauna diversity of southern Asian region is threatened. Our study reports 80 herpetofauna species distributed across different habitat types in Dampa Tiger Reserve (DTR), Mizoram. We revise the amphibian list of DTR throughthe addition of seven species and establish the identity of cryptic species such as *Microhyla ornata* which is actually two distinct species,i.e., *M. mukhlesuri* and *M. mymensinghensis*. Through the questionnaire survey, it was found that 90% of the respondents depended on varied forms of forest resources. Herpetofaunal species account for 30% of the faunal resources with *Varanus bengalensis, Ophiophagus hannah*, and *Python bivittatus* being the most consumed reptile species. All chelonians and some amphibians like *Duttaphrynus melanostictus*, *Pterorana khare*, *Hoplobatrachus tigerinus*, *Hoplobatrachus litoralis*, *Hydrophylax leptoglossa*, *Minervarya asmati*, *Polypedates teraiensis*, and *Sylvirana lacrima* were also found to be consumed and used for their presumed medicinal values. In addition to hunting, road-kills, use of chemical pesticides, and habitat alteration were recorded to be the prominent threats in the region. The land use and land cover (LULC) data shows a steady recovery of dense forest and a better forest fire scenario as over 90% of the DTR core region falls in the 'low severity' and 'unburned' category. With continuous alteration in forested areas, the present study will not only provide a fundamental baseline for the conservation of herpetofauna and better management of protected areas but also stimulate future herpetological-based research.

Keywords: Anthropogenic, habitat, land use land cover, northeastern India, resource management, sustainable.

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Author details & **Mizo abstract:** See end of this article.

Author contributions: SG and HTD carried-out all the field surveys and prepared the initial draft manuscript. FF, LB has performed the molecular analysis of cryptic species and ascertains the identity of the collected specimens. Mr. Z has analysis the LULC and other GIS related works. HTL has supervised the entire survey period and approved the final draft upon suggesting the necessary corrections to the initial draft

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INTRODUCTION

Amphibians and reptiles are amongst the most diverse and unique group of vertebrates. They play varied roles in natural systems as predators, prey, seed dispersers, or as commensal species (da Silva & de Britto-Pereira 2006). Several herpetofauna species are known to serve as bio-indicators of environmental health providing ideal models for biological and evolutionary studies (Böhm et al. 2013; Hernández- Ordóñezet al. 2015; Erawan et al. 2021). Although available in different land forms, the herpetofauna are sensitive to habitat modification and face global extinction crises because of habitat loss and climate change (Lesbarrères et al. 2014; Trimble &van Aarde 2014; Musah et al. 2019).

Globally, around 41% of amphibians and 21% of reptiles are categorized as 'threatened' by the IUCN Red List (Cox et al. 2022), due to habitat destruction, hunting, poaching, and pet trade (Böhm et al. 2013; Measey et al. 2019; Hughes et al. 2021; Cox et al. 2022). While large body-sized vertebrates are comparatively well studied, little is being documented about the responses of amphibians and reptiles to such changing landscapes and anthropogenic activities (Fulgence et al. 2021).

Nestled in the eastern Himalayan region, the northeastern states of India are located at the crossroads of two biodiversity hotspots of the world, i.e., the Eastern Himalaya and the Indo-Burma biodiversity hotspot (Saikia & Kharkongor 2017). The forests of Mizoram are mainly tropical forests with high species richness and endemicity (Lalremsanga 2018). Recent studies from Mizoram have reported several new species to the state that include *Amolopsindo burmanensis*, *Limnonectes khasianus*, *Microhyla mukhlesuri*, and *M. mymensinghensis*. New country records like *Raorchestes rezakhani* and *Sylvirana lacrima* were also described from the state (Decemson et al. 2021a). Reptiles like *Cyrtodactylus montanus* (Muansanga et al. 2020), and *Gekko lionotum* (now *Gekko mizoramensis*) are some other records. However, with rising incidences of forest fire, shifting cultivation, change in land use, land cover pattern, local consumption, and ethnomedicinal usage, the herpetofauna diversity of the state is in peril (Pawar et al. 2004; Lalremsanga 2018; Gouda et al. 2021). As the pressure on herpetofauna in the region continues to mount, threat assessments and inventory studies are of great importance to document and conserve the rich herpetological diversity of the state. Through this study, we aim to: (a) assess the herpetofaunal diversity in and around Dampa Tiger Reserve (DTR), which has been unrepresented or poorly documented, (b) determine the conservation status and existing threats, and (c) identify and address the research gaps that seek urgent attention from the stakeholders and concerned authorities.

MATERIALS AND METHODS

Study Site

The study was undertaken in and around Dampa Tiger Reserve located in the Mamit District of Mizoram. DTR is part of both the Himalaya and the Indo-Burma biodiversity hotspot. The reserve also forms the international border with Bangladesh. It stretches over an area of 500 km^2 of the core area (23.3486–23.7972 N, 92.2688–92.5275 E) and a buffer zone of 488 km^2 (23.8005–23.3533 N, 92.3175–92.5288 E) (Mandal & Raman 2016) (Figure 1). Vegetation type in the area comprises tropical wet evergreen, semi-evergreen and bamboo forests. The terrain in the reserve is steep and rugged ranges run in an altitudinal range of ~230–1100 m. Several perennial streams and small water bodies flow across the reserve. The habitat types in the peripheral areas of DTR are secondary forests of medium-sized, abandoned jhum fields, and small forest patches mixed with shrubs and bamboo plants at varied elevations. The peripheral areas of DTR consist of 14 villages where the main source of livelihood is agriculture and Non-timber forest products (NTFP's) collected from the reserve (Gouda et al. 2021).

Herpetological Sampling

Sampling was carried out in both the core and buffer areas of DTR between July 2020 and November 2022. Extensive surveys were carried out in different seasons and habitat types using multiple approaches such as trail walks, line transect surveys and road surveys by a group of two–four individual researchers (Prasad et al. 2018). Different gradients of fallow lands and secondary forested areas (community forest) were also surveyed. Surveys were carried out in the early morning (0500–0800 h) and evening (1800–2300h). For the chelonian diversity, surveys were carried out along the water bodies, drainage, and river beds that flow along DTR and the surrounding village areas. Common species were photographed and released back to the wild after examination and measurement, while unidentified species including road kills were collected and preserved in 70% ethanol for future evaluation.

Genetic analysis

Liver and other suitable tissue samples were used for the DNA extraction process through DNeasy

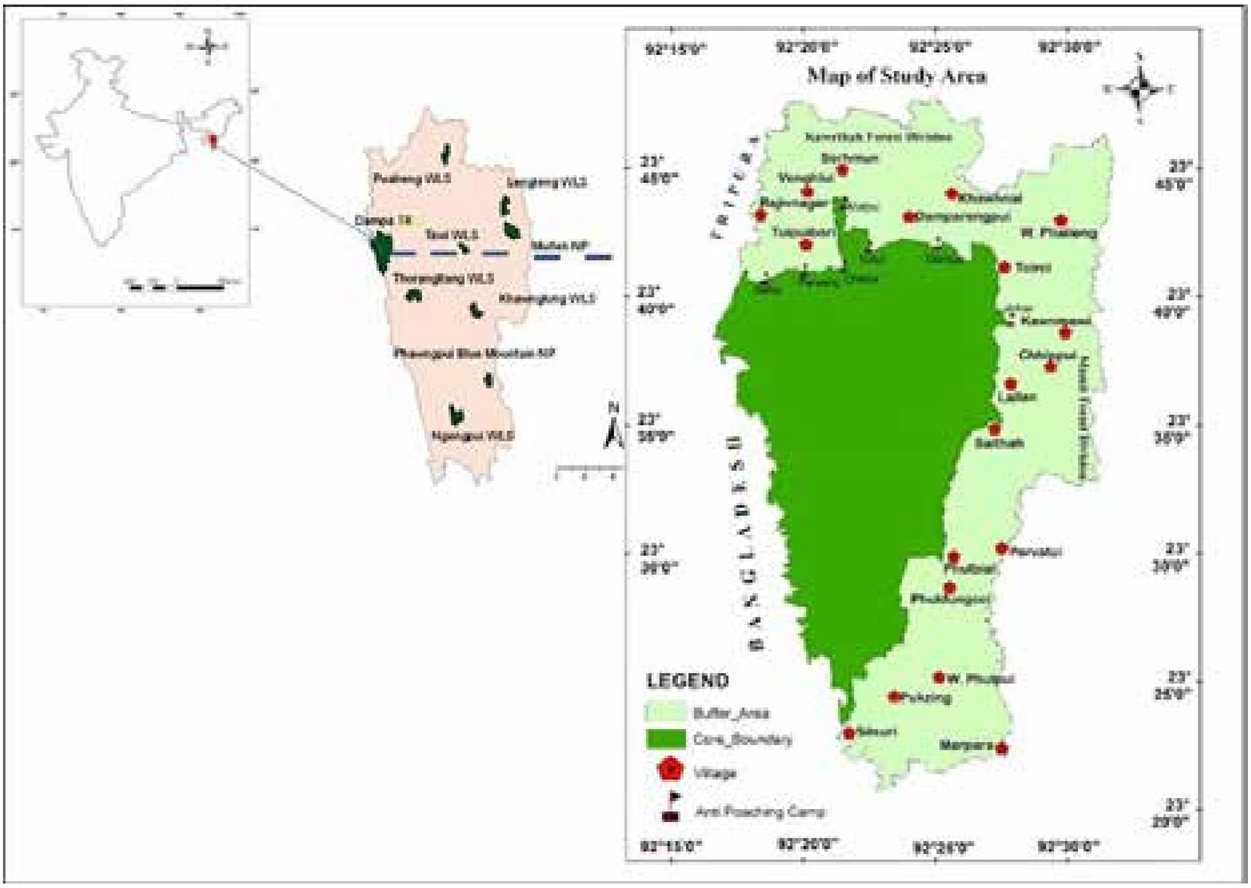


Figure 1. Map representing the study sites.

(Qiagen™) blood and tissue kits. Standard polymerase chain reactions (PCR) were run for amplification using forward primer L02510 and reverse primer H3056. The amplified samples were then sequenced using a sequencer (Agrigenome Labs Pvt. Ltd.) in both directions following Sanger's dideoxy method (Sanger et al. 1977). The obtained chromatograms of 16S rRNA sequences were screened through nucleotide BLAST (https://blast.ncbi.nlm.nih.gov/) and ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/), the generated sequences were deposited in the GenBank repository and accession numbers were acquired for the same. Voucher specimens for all species were also deposited at the Department Museum of Zoology, Mizoram University (MZMU) for future reference.

Threat assessment

Socio-economic survey

For the assessment of anthropogenic pressure, a semi-structured questionnaire, informal interviews, and interactions with local communities were used to gather information relating to the livelihood options, agricultural practices, knowledge on herpetofaunal diversity, and usages of the herpetofaunal species (Gouda et al. 2021; Adil et al. 2022). The survey was conducted among all ethnic communities across the fringe villages of DTR, i.e., Mizo, Bru, and Chakma tribes. All discussions and interactions were conducted with the consent of the respondents through the local dialect, which is 'Mizo'.

Land use and land cover (LULC) survey

The presence of humans including settlements, forest cover, forest fire, and agricultural land in fringe villages of DTR was acquired through the Indian Remote Sensing satellite data (LISS-III and Cartosat-I) and digitized using QGIS software for the preparation of LULC maps. LULC classification and NBR mapping were primarily relayed on Landsat 8 data, Level2 products developed and distributed by United States Geological (USGS) Earth Explorer (https://earthexplorer.usgs.gov/). Understanding the pattern of LULC change, the study utilized three separate satellite images with different years, months, and dates, like 19 February 2014, 29

January 2018, and 08 January 2023. The study area is covered by path136 and row44. The selection of the data set was influenced by the image quality, especially for those days with limited and low cloud cover. All the selected images had less than 5% cloud cover.

For assessing the forest fire severity, the study utilized multi-date images from Landsat 8 data, firstly, the image representing the pre-fire scenario, acquired on 08 January 2023, and second, the post-fire scenario acquired on 21 March 2022. Specifically, the near-infrared band with 0.845–0.885 µm and short-wave-infrared band with 2.10–2.30 µm forest fire severity were utilized for forest fire severity calculation.

RESULTS

Herpetofaunal diversity of DTR

The study reports 80 herpetofaunal species consisting of ophidians (20 species), saurians (20 species), chelonians (six species) and amphibians (34 species) (Tables 1&2). Among the reptilian fauna, all chelonian species except *Cyclemys gemeli* are categorized as threatened as per the IUCN Red List while among the ophidians, two species—*Python bivittatus* and *Ophiophagus hannah* are 'Vulnerable', 15 are in 'Least Concern' and three are in 'Not Assessed' category. Of the 20 saurian species, *Cyrtodactylus montanus*, is considered 'Critically Endangered', *Tropidophorus assamensis* as 'Vulnerable', *Varanus bengalensis* as 'Near Threatened', and *Sphenomorphus maculatus*, *Gekko mizoramensis* as 'Not Assessed', while the remaining 15 species are of 'Least Concern' status (Table 1). Among the amphibians, one species—*Bufoides meghalayanus* is categorized as 'Critically Endangered', while 16 species are of 'Least Concern', four species are 'Data Deficient', and 11 are 'Not Assessed' (Table 2).

Through this study, we have also updated the amphibian checklist of DTR to 34 species by the addition of seven new species namely *Raorchestes manipurensis* (Departmental Museum of Zoology, Mizoram University MZMU2326–2328 and MZMU2350), *Polypedates braueri* (MZMU2261), *Theloderma baibungense* (MZMU2108), *Kurixalus yangi* (MZMU2273 and MZMU2274), *Ichthyophis multicolor* (MZMU2494A–G), *Bufoides meghalayanus* (MZMU2078 and MZMU2091), and *Ichthyophis benji* (MZMU2809) (Table 3) (Image 1).

The diversity of amphibians was profoundly distributed in small perennial streams, roadside water holes, moist temperate bamboo forests, and secondary forests within the core and also along the buffer areas. Man-made water bodies like fish ponds in the buffer areas accounted for species such as *Euphlyctis adolfi*, *Fejervarya multistriata*, *Microhyla berdmorei*, *and Sylvirana lacrima* while species like *Amolops indoburmanensis* and *Odorrana chloronota* were more prominent in the cascade flowing of the lotic ecosystem. Agricultural crop fields/ jhum fields were found to be inhabited by species like *Duttaphrynus melanostictus*, *E. adolfi*, *Hydrophylax leptoglossa, Hoplobatrachus litoralis*, *Hoplobatrachus tigerinus*, *Kaloula pulchra* and *Minervarya asmati.* Small seasonal drains along roadside were found to harbour species such as *D. melanostictus, Ichthyophis multicolor* and *R. manipurensis.* Species like *Rhacophorus bipunctatus*, and *Theloderma baibungense* were more prevalent in the primary forests, while, *Ingerana borealis*, *Limnonectes khasianus, Leptobrachium smithi*, *Leptobrachella tamdil* and *Pterorana khare* were found in the slow-flowing streams in the core areas of DTR.

Although we observed saurian and other reptilian species both from primary as well as secondary forests along DTR, activities such as encroachment by humans, increase in number of agricultural crop fields, over-harvesting, and use of chemical pesticides appears to influence the distribution pattern of reptiles in DTR and its peripheral areas. Several species such as *Bungarus fasciatus*, *Bungarus niger, Naja kaouthia*, *Ophiophagus hannah*, *Trimeresurus popeiorum*, *Trimeresurus erythrurus*, *Python bivittatus*, *Varanus bengalensis*, *Varanus salvator*, *Cyrtodactylus montanus*, *Calotes irawadi*, *Draco maculatus*, *Ptyctolaemus gularis* and *Tropidophorus assamensis* were frequently encountered in secondary forests, thereby highlighting the role of these mosaic forest patches in the conservation of the herpetofaunal diversity of DTR. All six chelonian species in the study were observed from the streams flowing in the primary forest. However, human activities including illegal hunting and excessive release of pesticides to the water bodies that connect the streams appear to be a challenge for their conservation.

We ascertain the identity of several cryptic species previously misidentified from the region by sequencing mitochondrial genes, 16S ribosomal RNA, and bioinformatics tools The tissue sample earlier assigned to *Microhyla ornata* was sequenced and the obtained genetic data revealed that it is two distinct species, i.e., *M. mukhlesuri* and *M. mymensinghensis*. Another amphibian species *Xenophrys parva* was established as *X. serchhipii*, which is endemic to the northeastern region of India. *Hoplobatrachus* sp. which was reported as a single species from DTR was sequenced and

Table 1. Chelonian, saurian, and ophidian diversity in and around Dampa Tiger Reserve, Mizoram.

	Common name	Scientific name	Family	Red List status
Chelonia				
1	Assam Leaf Turtle	*Cyclemys gemeli*	Emydidae	NT
2	Keeled Box Turtle	*Cuora mouhotii*	Emydidae	EN
3	Asian Giant Tortoise	*Manouria emys*	Testudinidae	CE
4	Yellow Tortoise	*Indotestudo elongata*	Testudinidae	CE
5	Black Softshell Turtle	*Nilssonia nigricans*	Trionychidae	CE
6	Asiatic Softshell Turtle	*Amyda ornata jongli*	Trionychidae	VU
Sauria				
1	Forest Garden Lizard	*Calotes emma*	Agamidae	LC
2	Indian Garden Lizard	*Calotes irawadi*	Agamidae	LC
3	Smooth-scaled Mountain Lizard	*Cristidorsa planidorsata*	Agamidae	LC
4	Blanford's Flying Lizard	*Draco maculatus*	Agamidae	LC
5	Green Fan-throated Lizard	*Ptyctolaemus gularis*	Agamidae	LC
6	Burmese Glass Snake	*Dopasia gracilis*	Anguidae	LC
7	Jampui Bent-toed Gecko	*Cyrtodactylus montanus*	Gekkonidae	CE
8	Common House Gecko	*Hemidactylus frenatus*	Gekkonidae	LC
9	Fox Gecko	*Hemidactylus garnotii*	Gekkonidae	LC
10	Flat-tailed House Gecko	*Hemidactylus platyurus*	Gekkonidae	LC
11	Mizoram Parachute Gecko	*Gekko mizoramensis*	Gekkonidae	NA
12	Tokay Gecko	*Gekko gecko*	Gekkonidae	LC
13	Khasi Hill Long-tailed Lizard	*Takydromus khasiensis*	Lacertidae	LC
14	Bronze Grass Skink	*Eutropis macularia*	Scincidae	LC
15	Common Mabuya	*Eutropis multifasciata*	Scincidae	LC
16	Indian Forest Skink	*Sphenomorphus indicus*	Scincidae	LC
17	Spotted Forest Skink	*Sphenomorphus maculatus*	Scincidae	NA
18	North-eastern Water Skink	*Tropidophorus assamensis*	Scincidae	VU
19	Bengal Monitor Lizard	*Varanus bengalensis*	Varanidae	NT
20	Common Water Monitor	*Varanus salvator*	Varanidae	LC
Ophidia				
1	Yellow Whipsnake	*Ahaetulla flavescens*	Colubridae	LC
2	Tawny Cat Snake	*Boiga ochracea*	Colubridae	LC
3	Golden/Indian Flying Snake	*Chrysopelea ornata*	Colubridae	LC
4	Common/Painted Bronzeback	*Dendrelaphis proarchos*	Colubridae	LC
5	Asiatic Water Snakes/ Checkered Keelback	*Fowlea piscator*	Colubridae	LC
6	Common Ringneck	*Gongylosoma scriptum*	Colubridae	NA
7	Chin Hills Keelback	*Hebius venningi*	Colubridae	LC
8	Wall's Keelback	*Herpetoreas xenura*	Colubridae	NA
9	Zaw's Wolf Snake	*Lycodon zawi*	Colubridae	LC
10	Light-barred Kukri Snake	*Oligodon albocinctus*	Colubridae	LC
11	Heller's Red-necked Keelback	*Rhabdophis helleri*	Colubridae	NA
12	Banded Krait	*Bungarus fasciatus*	Elapidae	LC
13	Greater Black Krait	*Bungarus niger*	Elapidae	LC
14	Monocled Cobra	*Naja kaouthia*	Elapidae	LC
15	King Cobra	*Ophiophagus hannah*	Elapidae	VU
16	Common Slug Snake	*Pareas monticola*	Pareidae	LC
17	Common Mock Viper	*Psammodynastes pulverulentus*	Pseudaspididae	LC
18	Burmese Python	*Python bivittatus*	Pythonidae	VU
19	Pope's Bamboo/ Green Pit Viper	*Trimeresurus popeiorum*	Viperidae	LC
20	Redtail (Bamboo) Pit Viper	*Trimeresurus erythrurus*	Viperidae	LC

CE—Critically Endangered | EN—Endangered | LC—Least Concern | NT—Near Threatened | NA—Not Assessed | VU—Vulnerable.

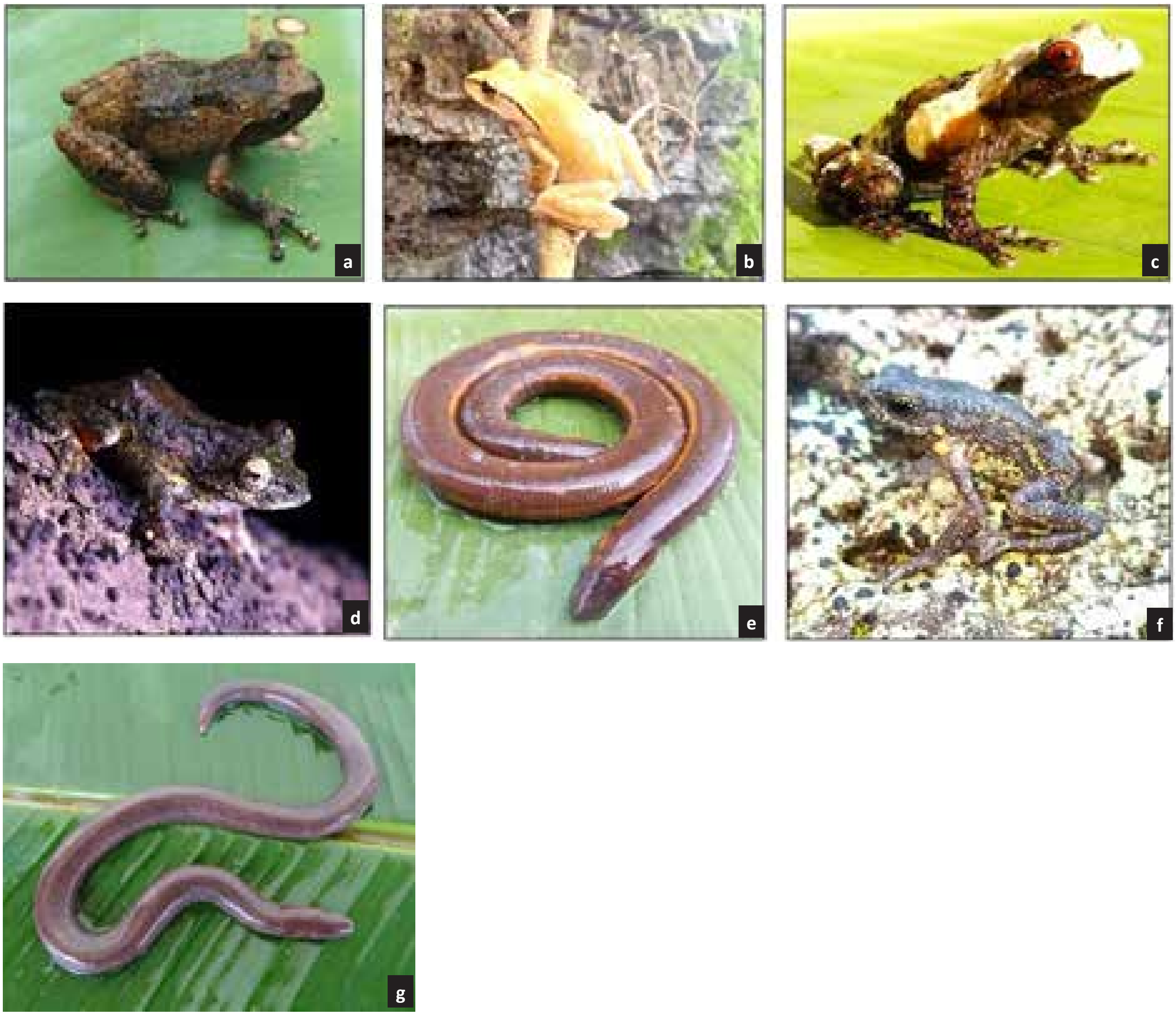


Image 1. Some amphibian species reported through the study: a—*Raorchestes manipurensis* | b—*Polypedates braueri* | c—*Theloderma baibungense* | d—*Kurixalus yangi* | e—*Ichthyophis multicolor* | f—*Bufoides meghalayanus*| g—*Ichthyophis benjii*. © HT Lalremsanga.

confirmed to be two separate species, i.e., *H. tigerinus* and *H. Litoralis.*

Threats and conservation challenges

The threats for the herpetofaunal diversity were assessed through the socio-economic survey and understanding of the land cover change in and around the DTR region. Some of the major conservation challenges for the herpetofauna of DTR include:

Dependency on forest resources

During the questionnaire survey, it was found that more than 90% of the respondents collected NTFPs and other forms of forest resources such as bamboo shoots, wild berries, different frog, snails, and crab species. from the surrounding forested areas of DTR. About 19% of the locals interviewed stated that they collect bamboo—*Dendrocalamus longispathus*, *D. asper*, *Melocanna baccifera*, and *Bambusa tulda* for construction, consumption, and fuelwood, while 2% collect timber—*Gmelina arborea* and *Derris robusta*, 24.5% collect fuel wood, and 12.5% collect edible food/medicines. About 39% of the respondents stated to collect all the above forest items, while 3% of the surveyed locals do not collect any form of forest products. Several faunal resources such as fish, tadpoles, crabs and snails are also regularly collected by the locals and sold in the market (Image 2). Large mammals hunted for bushmeat include *Sus scrofa*, *Muntiacus muntjak*, *Rusa unicolor*, *Capricornis sumatraensis*, and *Macaca assamensis.* Encouragement to adopt alternative livelihoods and expand their income sources will be crucial for reducing the pressure on the biodiversity of DTR and improving the socio-economic status of the tribal communities.

Over-harvesting of herpetofauna

Herpetofaunal species account for 30% of the faunal resources used by locals around DTR. *Varanus bengalensis*, *Ophiophagus hannah*, and *Python bivittatus* were the most consumed reptile species. Several snake species although not consumed, were many a time killed out of fear of snake bite. Among amphibians *Duttaphrynus melanostictus* and *Pterorana khare* were consumed as a delicacy and also used for the treatment of ailments like common cold and cough. Species like *Hoplobatrachus tigerinus*, *H. litoralis*, *Hydrophylax leptoglossa*, *M. asmati*, *P. teraiensis*, *Duttaphrynus* sp., and *S. lacrima* were found to be sold alive or dried at ₹100/ package weighing about 500g in the local market (Pers. obs. of HTL and SG during field visits to the study sites) (Image 3). Tadpoles of *Clinotarsus alticola* and *L. smithi* caught from the streams along the buffer region were also reported to be consumed regularly by locals. Ethnomedicinal or traditional medicines play an important role in the exploitation of herpetofauna species in and around DTR where health facilities are lacking or poor. The questionnaire survey revealed that many locals use reptiles and amphibian's species for treating several health ailments such as skin infections, stomach problems, and burns. The fats of *Python bivittatus* are applied to burns and inflammation, and the glands are sometimes used as a sedative. The fatty oil obtained from *Trimeresurus erythrurus* is used for treating warts. Soup of *D. melanostictus* is administered for common cold and cough. Different species of chelonians including *I. elongata*, *Cyclemys gemeli*, and *Cuora mouhotii* are regularly consumed by the locals and also traded to neighbouring states like Assam and Tripura for their medicinal values (Image 4).

Road kills and lack of awareness

During our survey period, we encountered several species of amphibians and reptiles that were killed on the road that stretches from W. Phaileng to Marpara (84.9 km) and between W. Phaileng to Rajiv Nagar (64 km) (Image 5). Some of the commonly observed roadkill species included *I. multicolor*, *Calotes irawadi*, *Coelognathus radiatus*, *D. melanostictus*, *Pareas monticola*, *M. berdmorei*, *S. lacrima*, *F. multistriata*, and *M. asmati*. (Image 5a-i). Ignorance or lack of awareness of the role of amphibians in biodiversity sustainability, as biological indicators of climate change, pollution, and the benefits of amphibians as pest control agents have led

Table 2. Amphibian diversity of Dampa Tiger Reserve region, Mizoram.

	Common name	Scientific name	Red List status
I	**Anurans**		
1	Smith's Litter Frog	*Leptobrachium smithi*	LC
2	Tamdil Leaf-litter Frog	*Leptobrachella tamdil*	NA
3	Serchhip Horned Frog	*Xenophrys serchhipii*	DD
4	White-lipped Horned Toad	*Xenophrys major*	LC
5	Painted Kaloula/ Painted Bullfrog	*Kaloula pulchra*	LC
6	Pegu Rice Frog	*Microhyla berdmorei*	LC
7	Mukhlesur's Narrow-mouthed Frog	*Microhyla mukhlesuri*	NA
8	Mymensingh Narrow-mouthed Frog	*Microhyla mymensinghensis*	NA
9	Adolf's Speckled / Bangladesh Skittering Frog	*Euphlyctis adolfi*	LC
10	Indian Bullfrog	*Hoplobatrachus tigerinus*	LC
11	Bangladesh Coastal Bull Frog	*Hoplobatrachus litoralis*	LC
12	Khasi Wart Frog	*Limnonectes khasianus*	LC
13	Indo-Burma Torrent Frog	*Amolops indoburmanensis*	NA
14	Malay Pointed-snout Frog/ Assam Hill Frog	*Clinotarsus alticola*	LC
15	Chin WoodFrog	*Sylvirana lacrima*	NA
16	Copper-cheeked Stinky Frog/ ChloronateHuia Frog	*Odorrana chloronota*	LC
17	Khare's Gliding Frog	*Pterorana khare*	LC
18	Cope's Assam Frog	*Hydrophylax leptoglossa*	LC
19	Terai Tree Frog	*Polypedates teraiensis*	NA
20	Himalaya Flying Frog	*Rhacophorus bipunctatus*	LC
21	Leimatak'sBush Frog	*Raorchestes manipurensis*	NA
22	White-lipped Tree Frog	*Polypedates braueri*	NA
23	Baibung Small Tree Frog	*Theloderma baibungense*	LC
24	Yang's Frill-limbed Tree Frog	*Kurixalus yangi*	NA
25	Paddy Frog	*Fejervarya multistriata*	DD
26	Bangladeshi Cricket Frog	*Minervarya asmati*	NA
27	Rotung Oriental Frog	*Ingerana borealis*	LC
28	Asian Toad	*Duttaphrynus melanostictus*	LC
29	Khasi Hill Toad/ Mawblang Toad	*Bufoides meghalayanus*	CR
II	**Ichthyophiidae – Caecilians**		
1	Manipur Moustached Ichthyophis	*Ichthyophis moustakius*	DD
2	Colourful Ichthyophis	*Ichthyophis multicolor*	DD
3	Benji's Caecillian	*Ichthyophis benjii*	NA

CR—Critically Endangered| DD—Data Deficient| LC—Least Concern| NA—Not Assessed.

Image 2. a—Tadpoles collected by locals | b—Snails collected from the peripheral areas of DTR | c—Shrimps | d—Bamboo shoots | e— Dried fish and frogs sold in the local market. © HT Lalremsanga.

Table 3. New record of amphibian species from Dampa Tiger Reserve, Mizoram.

	Species	Common name	Family	Voucher/ GenBank accession no.	Red List status	Distribution
1	*Raorchestes manipurensis* (Mathew & Sen 2009)	Leimatak'sBush Frog	Rhacophoridae	MZMU2326, 2327 & 2328 (GBA no. MZ148621, MZ148620 & MZ148619, respectively)	NA	India (Manipur, Mizoram)
2	*Polypedates braueri* (Vogt, 1911)	White-Lipped Tree Frog	Rhacophoridae	MZMU2261 (GBA no. MH938688.1)	DD	Tropical and Sub tropical China, Taiwan, Vietnam, Thailand, Myanmar and India (Mizoram)
3	*Theloderma baibungense* (Jiang, Fei & Huang, 2009)	Baibung Small Tree Frog	Rhacophoridae	MZMU2108 (GBA no. OK474164)	DD	Tibet, China, Bangladesh and India (Arunachal Pradesh, Assam, Nagaland)
4	*Kurixalus yangi* Yu, Rao &Yang, 2018	Yang's Frill-limbed Tree Frog	Rhacophoridae	MZMU2273 & MZMU2274 (GBA no. MT808303.1)	NA	Western Yuannan, China, Northern Myanmar and India (Nagaland, Mizoram)
5	*Ichthyophis multicolor* Wilkinson, Presswell, Sherratt, Papadopoulou & Gower, 2014	Colourful Ichthyophis	Ichthyophiidae	MZMU1758 (GBA no. MZ098158	DD	Ayeyarwady region of Myanmar and India (Mizoram)
6	*Ichthyophis benjii* (Lalremsanga, Purkayastha, Biakzuala, vabeiryureilai, Muansanga and Hmar, 2021)	Benji's Caecillian	Ichthyophidaae	MZMU2809 (GBA No. OR689358)	NA	Mizoram, India
7	*Bufoides meghalayanus* (Yazdani & Chanda, 1971)	Khasi Hill Toad/ MawblangToad	Bufonidae	MZMU2078 & MZMU2091 (GBA no. MW741545 & MW741544)	EN	Meghalaya, Assam, Mizoram (India)

DD—Data Deficient| E—Endangered| LC—Least Concern| NA—Not Assessed.

to their random killing and consumption. Uncontrolled use of chemical pesticides in agricultural crop fields that ultimately get deposited in the nearby water bodies as a result of water runoff was another factor that led to

Image 3. Different amphibian species skilled, dried and sold at the local market of Dampa Tiger Reserve region, Mizoram. © HT Lalremsanga.

a decline in the local population of certain amphibian species. Hindlimb malformation in Adolf's Speckled Frog *E. adolfi*, Tamenglong Horned Frog *X. numhbumaeng*, Mawphlang Odorous Frog *O. mawphlangensis*, Nagaland Montane Torrent Toad *D. chandai*; anophthalmia in *X. major* are some of the cases reported from the state of Mizoram. From DTR region abnormalities in skinks such as Indian Forest Skink *Sphenomorphus indicus*, and Spotted Forest Skink *S. maculatus* are also reported (Decemson et al. 2021b; Lalremsanga 2022; Siammawii et al. 2022).

Land use pattern

The forest is a crucial component for the prosperity and sustenance of wildlife in any given area. Data on LULC generated for the years 2014, 2018, and 2022 shows a recovery of densely forested areas in DTR. Nevertheless, substantial alteration in forest cover along the peripheral areas of DTR also cannot be denied (Image 6). Another interesting finding from the LULC is the reduction in areas under 'jhum cultivation' also known as slush and burnt form of cultivation, thereby resulting in an increased area of open forest. The areas under shifting cultivation recorded during the last five years showed a decrease from 306 km^2 to 180.49 km^2 throughout Mizoram. Adaptation of mixed farming over the traditional Jhum cultivation by local farmers around DTR has also helped increase the fallow period between successional Jhum fields allowing the forest vegetation to recover. An increase in plantation areas was also recorded for DTR. Many of the local farmers now grow various forms of cash crops like Betel Nut *Areca catechu*, Cavendish Banana *Musa acuminata*, Plantain *Musa paradisiaca*, Tree Bean/ Stink Bean *Parkia timoriana* apart from their traditionally grown paddy in the crop fields. Such adaptation in agriculture can be attributed to better management practices by the concerned department and the implementation of the New Land Use Policy (NLUP).

Forest fire

Forest fires in the region were categorized based on the frequency of detected forest fires in an area over period of time and the probabilities of occurrence (proneness) shortly as suggested by Kumar et al. (2019). Analysis of satellite imageries of forest fire data shows that more than 90% of DTR's core areas fall under the category of 'low severity' and 'unburned' (Image 7). Some areas namely Tuichar, Charte, and Saithah composed mainly of bamboo forest that were previously cleared for developing grasslands, represent a 'moderate–high severity' zone. The buffer areas close to villages like Rajiv Nagar, Tuipuibari, Damparengpui, and Silsuri pose a greater threat of fire crossover into the core regions and hence require proper monitoring while jhum fields are burnt by the local farmers. Although forest fire prevalence and severity in DTR are relatively low, with the growing incidences of forest fires and increase in temperature (Pers. Obs of HTL and Pramanick et al.

Image 4. Chelonian species from Dampa Tiger Reserve that are locally consumed: a—*Manouria emys* | b—*Nilssonia nigricans* | c—*Indotestudo elongata* | d—*Cuora mouhotii* | e—*Cyclemys gemelli* | f—*Amyda ornata jongli*. © HT Lalremsanga.

2023), timely vigilance by the concerned department and proper awareness among locals will be crucial for the prevention of forest fire in the near future.

DISCUSSION

Herpetofauna is one of the most threatened groups of vertebrates on the planet. The synergistic effect of habitat loss, fragmentation, over-harvesting, pet trade, traditional medicine, and climate change has threatened the global herpetological population with extinction in the next 50 years, especially in southern Asia (Stuart et al. 2008; Rowley et al. 2010; Nori et al. 2015; Hughes 2017; Choquette et al. 2020; Montgomery et al. 2022). Being the largest protected area in the state of Mizoram, the DTR and its surrounding areas hold a rich and diverse group of herpetofauna of the state. Although *Bufoides bhupathyi* was recently described as new species by Naveen et al. (2023) from the similar study area in DTR, we recommend taxonomic reassessment of the Bufoides population from this area using a more holistic data with implementing integrated approach (e.g., multilocus phylogeny, natural history, robust morphological distinctness, etc.) because recognizing cryptic species through a short fragment of 16S rRNA (~413 bp) and few morphological attributes, particularly webbing formulae and shape of parotoid is ambiguous. Thus, we consider *B. bhupathyi* as a subjective junior synonym of *B. meghalayanus* for the time being. Furthermore, the shallow genetic divergence (0-0.6%) across the sequences of *R. manipurensis* from Mizoram (DTR: GBA MZ148617-21; Sailam: GBA MZ148616, MW938629-30; Lunglei: GBA MZ148622) and the type locality in Manipur (GBA MW680944-47), *R. cangyuanensis* from China (Yunnan: GBA MN475866-7), and *R. longchuanensis* from India (West Bengal: GBA MH423740) and Bangladesh (Habigonj: GBA MH699074) suggested that these samples are conspecific and warrant the treatment of *R. cangyuanensis* as a subjective junior synonym of *R. manipurensis* with the subsequent amendment on the taxonomic status of *R. longchuanensis* from India (West Bengal) and Bangladesh into *R. manipurensis*. We report several reptilian species from the reserve such as *R. manipurensis*, *Leptobrachella tamdil, R. senapatiensis, I. moustakius, I. benjii, C. montanus, G. mizoramensis, T. assamensis, H. xenura,* and *B. meghalayanus* that are endemic to the northeastern states of India. Other

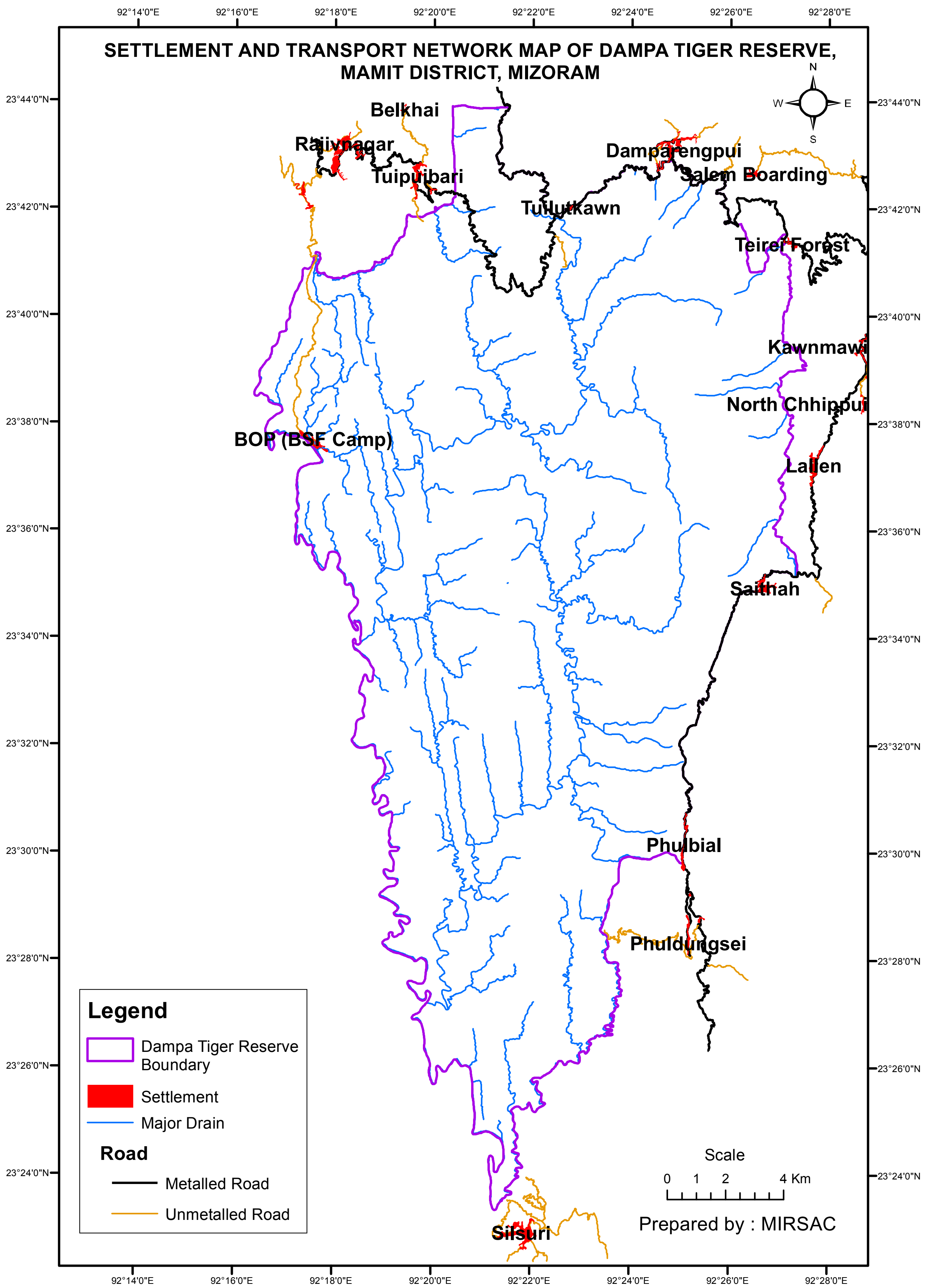


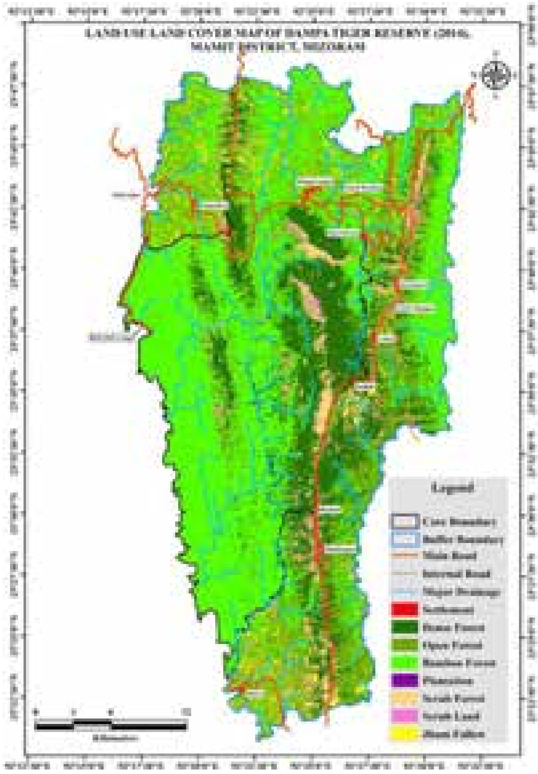
Figure 2. Map representing the road network around Dampa Tiger Reserve.

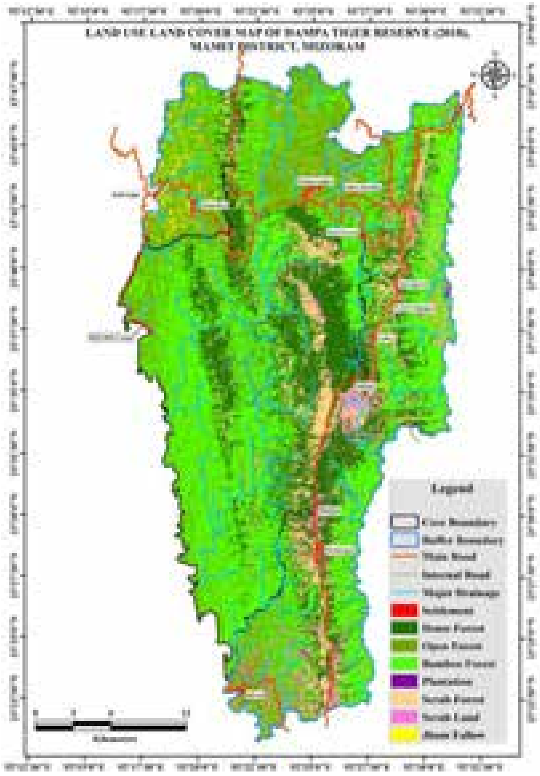
Image 5. Some road kills recorded from adjacent road to Dampa Tiger Reserve: a—*Coelognathus radiatus* | b—*Pareas monticola* | c—*Boiga ochracea* | d—*Ichthyophis multicolor* | e—*Ovophis monticola* | f&g—*Calotes irawadi* | h—*Sylvirana lacrima* | i—*Duttaphrynus melanostictus*. © Ht. Decemson.

important species reported include *C. montanus*, *T. assamensis*, and *N. nigricans* which are highly cryptic. Several cases of misidentification and misclassification in some of the previous studies from DTR (Pawar et al 2004; Decemson et al. 2021a) such as that of *X. serchhipii* as *X. parva*; *Amolops indoburmanensis* as *A. marmoratus*, *E. adolfi* as *E. cyanophlyctis*, *L. khasianus* as *L. laticeps* are also resolved in the study However, similar to other tropical forests of southern Asia, DTR too faces the challenge of habitat alteration, increasing human population in the vicinity of villages, hunting, frequent forest fires, monoculture plantation, and high harvesting pressures. A study on the impact of climate change on amphibians by Lalremsanga (2018) showed several distinct changes like altered reproductive activity, phenology, and altitudinal migration from 100–500m to over 824m in Microhylids, and among *Clinotarsus alticola* and *Ingerana borealis* respectively. Biodiversity in protected areas across southern Asia is facing a huge threat mainly due to human population growth (as 25% of the world's population inhabits the region) and the extension of agricultural lands (Ghosh-Harihar et al. 2019; Chowdhury et al. 2022). Although pet trade and poaching of herpetofauna are yet to be reported from the DTR region, their local consumption and ethno-zoological usage pose a serious threat (Gouda et al. 2021). Mardiastuti et al. (2021) and Montgomery et al. (2022) have also categorized humans as exceptional wildlife consumers pursuing prey species from 34 taxonomic orders of body size ranging from 27 g to 4,400 kg. All of the listed chelonian species in the study are categorized as threatened species and require immediate conservation interventions.

Frequent forest fire is another factor that greatly hampers the herpetofaunal diversity of DTR. As highlighted by Wang et al. (2021), the northeastern region of India alone contributes for about 550,086 ha of the 658,778.4 ha of forested land that is lost every year in India due to various reasons.

A significant proportion of the herpetofaunal diversity in the DTR region is likely to be hidden within morphologically cryptic species groups or genera which are mostly treated as a single species or as many species. Misidentifications between closely resembling species





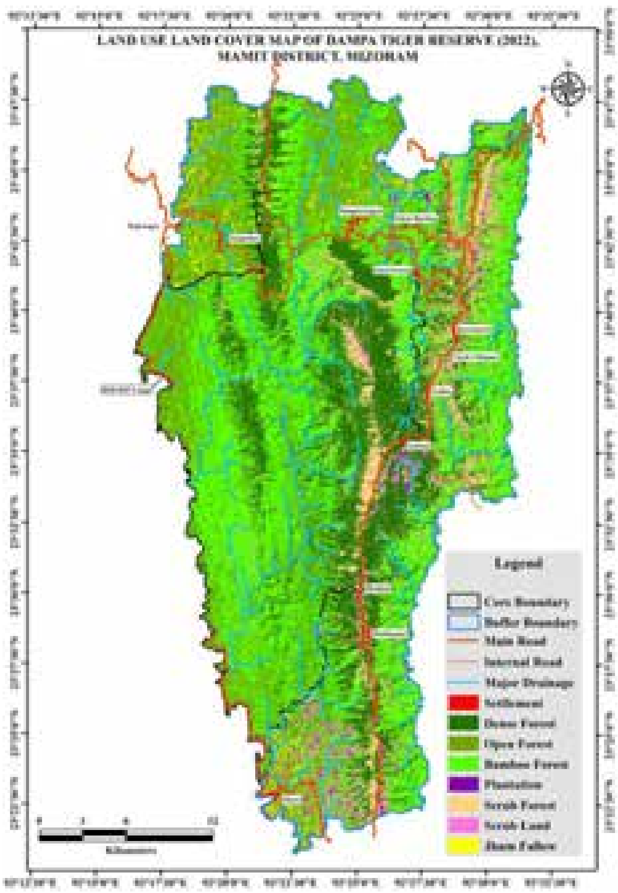


Image 6. Land Use Land Cover pattern in Dampa Tiger Reserve and its surrounding areas from 2014–2022.

Image 7. Forest fire in peripheral areas of Dampa Tiger Reserve. © Sushanto Gouda.

like *Euphlyctis cyanohlyctis* and *E. adolfi*, *Raorchestes manipurensis* and *R. rezakhani*, *Hoplobactrachus tigerinus* and *H. litoralis*, *Microhyla mukhlesuri* and *M. mymensinghensis*, *F. multistriata* and *M. asmati* are quite common in this region and hence require more concerted approaches (Stuart et al. 2006; Kundu et al. 2020; Neves et al. 2020).

While the crucial role of indigenous people and local communities in biodiversity conservation has been greatly valued by organisations such as the United Nations Convention on Biological Diversity, we believe that region-based conservation measures are more likely to have a better impact. As observed in our study, the locals reported the declining trend in amphibian fauna especially the bullfrogs (*Kaloula pulchra, Hoplobatrachus litoralis*, and *Hoplobatrachus tigerinus*) which are an important component of their diets. Similar to the findings of Mandal & Raman (2016) and Gouda et al. (2021), the locals during the questionnaire surveys also cited different factors like usage of chemical pesticides, low rainfall, monoculture plantations, habitat alteration and the use of modern machinery as causes for the decline in the population of such species. Since human-dominated landscapes are known for the distribution of over 65% of gap species (Acevedo-Charry & Aide 2019), with the continuous conversion of forested areas into agricultural fields and human habitats in the DTR region, the surrounding areas of DTR can serve as a valuable site for survival and recovery of herpetofauna communities. Considering the paucity of research in the region and cryptic nature of herpetofauna species, more research initiatives and the knowledge of the local communities are necessary for detailing the diversity and upgradation of the current Red List status. Acknowledging the importance of inventory studies and the role of herpetofauna in a balanced ecosystem will also be necessary for its management and conservation across the eastern Himalayan range and the Indo-Burma biodiversity hotspot. If done regularly, these studies will not only provide a fundamental baseline for the conservation of herpetofauna and better management of protected areas, but also stimulate future herpetological-based research.

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Mizo abstract: Rul, laiking leh uchang te hi nungcha hnungzangruh nei zingah chuan an chenna chhehvel inrelbawlna kawngah an pawimawh hle a. Chutih laiin, Asia chhimchhaklam a heng nungcha chi hrang hrang 80% vel chu mihring hnuhma lo pungchho zel leh behchhan nei a zirchianna mumal awm loh avangin dinhmun derthawng an ni tawh a. Dampa Tiger Reserve (DTR)-a heng nungchate leh an chenna hmun chi hrang hrang kan zirchiannaah hian chi hrang 80 chhinchhiah a ni a. Hengte bakah hian chi hrang pasarih dang hmuh belh niin tunhma a uchang chikhat *Microhyla ornata* ang hriat thin chu chi hrang pahnih, *M. mukhlesuri* leh *M. mymensinghensis* inphumru an lo ni reng zawk tih hmuhchhuah a ni. Zirchianna atan a zawhna siam chhangtu zinga 90% te chu ramngaw hausakna chi hrang hrangah an innghat tih a hriat a. Heng ramsa zing a mihringte ei thin zingah hian 30% chu *Varanus bengalensis, Ophiophagus hannah*, leh *Python bivittatus* te an ni. Satel leh sumsi te leh uchang thenkhat, *Duttaphrynus melanostictus, Pterorana khare, Hoplobatrachus tigerinus, Hoplobatrachus litoralis, Hydrophylax leptoglossa, Minervarya asmati, Polypedates teraiensis*, leh *Sylvirana lacrima* te hi ei thin niin tualchher damdawi ang a hman thin an ni bawk. Helai hmun a nungchate dinhmun tiderthawng nasa ber zingah hian ei atan a tihhlum bakah lirthei chilhlumte, hlotur hman nasat leh an chenna hmun tih chereu nasat vang te a ni. Land use leh land cover (LULC) data atanga a landan chuan helai ramngaw chhehvel 90% hi insiamthar leh chak tak leh kangmei pawhin a tihchhiat loh a ni a. Kan ramngaw te tihdanglam zel an nih lai hian, tun a kan zirna hi heng nungchate leh an chenna ramngaw humhalhna atan chauh niloin, hetiang lam zirbing mi te tan ala tangkai dawn chauh a ni.

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COMMUNICATION

Taxonomy and conservation status of swamp eels (Synbranchiformes: Synbranchidae) of West Bengal, India

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Abstract: In a comprehensive study spanning January 2019 to April 2023 within the state of West Bengal, the research focused on elucidating the taxonomy and conservation status of swamp eels in the state. Swamp eels were harvested using traditional fishing techniques, and sampling sites were randomly selected across nine districts: Cooch Behar, Alipurduar, Jalpaiguri, Uttar Dinajpur, Purba Bardhaman, Nadia, Purba Medinipore, North 24 Parganas, and South 24 Parganas, accounting for variations in climatic zones and topography. Through meticulous examination involving X-ray radiographs and morphometric measurements, two distinct swamp eel species, *Ophichthys cuchia* (Hamilton, 1822) and *Ophisternon bengalense* McClelland, 1844, were identified, both falling under the 'Least Concern' category according to the IUCN Red List of Threatened Species. Rapid population decline of swamp eels in West Bengal is primarily attributed to habitat degradation and the indiscriminate use of pesticides and chemical fertilisers.

Keywords: Bengal Mud Eel, diagnosis and description, Gangetic Mud Eel, *Ophichthys cuchia*, *Ophisternon bengalense*, Synbranchiform fishes.

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INTRODUCTION

The swamp eel, a distinctive eel-like percomorph fish, belongs to the Synbranchidae family. These eels are found across tropical and sub-tropical regions globally, excluding Antarctica (Rosen & Greenwood 1976; Bera 2007; Britz et al. 2021a,b). According to Praveenraj et al. (2021), the Synbranchidae family comprises four genera and approximately 26 species, with 12 residing in India (Gopi et al. 2017; Praveenraj et al. 2021), including *Monopterus eapeni* Talwar, 1991, *M. albus* (Zuiew, 1793), *Ophichthys cuchia* (Hamilton, 1822), *O. fossorius* (Nayar, 1951), *O. hodgarti* (Chaudhuri, 1913), *O. ichthyophoides* (Britz, Lalremsanga, Lalrotluanga & Lalramliana, 2011), *Rakthamichthys digressus* (Gopi, 2002), *R. indicus* (Silas & Dawson, 1961*), R. roseni* (Bailey & Gans, 1998), *R. rongsaw* (Britz, Sykes, Gower & Kamei, 2018), *R. mumba* Jayasimhan, Thackeray, Mohapatra & Kumar, 2021, and *Ophisternon bengalense* McClelland, 1844 (Gopi et al. 2017; Britz et al. 2020, 2021a,b)

The state of West Bengal contains diverse habitats ranging from the eastern Himalaya in the north to the Bay of Bengal in the south (Das et al. 2020; Bera et al. 2018). This geographic diversity underscores the state's significance in terms of biodiversity. Surprisingly, no comprehensive study on the taxonomy and conservation status of swamp eels in West Bengal, India, has been conducted by any previous author. These fishes inhabit a variety of environments, including subterranean waters and mud holes in swamps and caves, making their harvesting challenging with conventional fishing methods. Additionally, their superficial anatomy lacks distinctive features, and the limited external characteristics are highly variable, posing identification challenges. Consequently, this study seeks to investigate the taxonomy and conservation status of swamp eels in West Bengal, India, including the identification of major threats to facilitate effective conservation strategies.

MATERIALS AND METHODS

The research spanned January 2019 to April 2023 in West Bengal (Figure 1), involving the collection of swamp eel specimens from nine districts of West Bengal (Cooch Behar, Alipurduar, Jalpaiguri, Uttar Dinajpur, Purba Bardhaman, Nadia, Purba Medinipore, North 24 Parganas and South 24 Parganas; Table 1). Sampling sites were systematically chosen to ensure representation across diverse climatic and topographical conditions. Traditional fishing techniques such as Shuli, kodal-assisted digging, and handpicking were used in rice fields and marginal water areas, while drag nets and mosquito nets were employed in weed-infested wetlands like oxbow lakes. Additionally, baited hooks on hand lines were used to capture species in ditches and fish ponds. Post-harvest, specimens were photographed and preserved in 10% formalin solution, each assigned a museum voucher/accession number for documentation. Detailed morphometric measurements were conducted using a digital caliper to measure various morphometric measurements like total length (TL), head length (HL), snout length (SL), pre-anal length, gape length, the distance between anterior-posterior naris, the width of the body at vent and depth of the body at the vent. The meristic character, like vertebral count, was also undertaken. The vertebrae counts were determined through X-ray radiography and examined various anatomical features, such as fins, scales, gill cleft, soft tissue around the upper jaw, branchiostegal membrane, holobranchs, suprapharyngeal pouches, and afferent and efferent blood vessels of 4th gill arch. Fishes were identified as per the standard taxonomic keys (Rosen & Greenwood 1976; Jayaram 2010).

The assessment of species threat status adhered to the criteria outlined by the IUCN Red List of threatened species (Dahanukar 2010; Dahanukar et al. 2019). Additionally, the frequency of each species' occurrence was determined by calculating the number of times it was collected during the sampling process. This determination was facilitated by employing a standard catch frequency chart, as presented by Tamang et al. (2007), where catch frequencies were categorized as follows: 91–100% (common), 81–90% (abundant), 61–80% (frequent), 31–60% (occasional), 15–30% (sporadic), 05–14% (rare), and less than 5% (extremely rare).

RESULTS

The study revealed the occurrence of two species of swamp eel, *Ophichthys cuchia* (Hamilton, 1822) and *Ophisternon bengalense* McClelland, 1844 in the study area (Table 2).

Taxonomic account

The recorded swamp eels belong to the family Synbranchidae, which is characterized by anterior and posterior nostrils widely separated; gill openings united to form a single pore or slit under the head or throat,

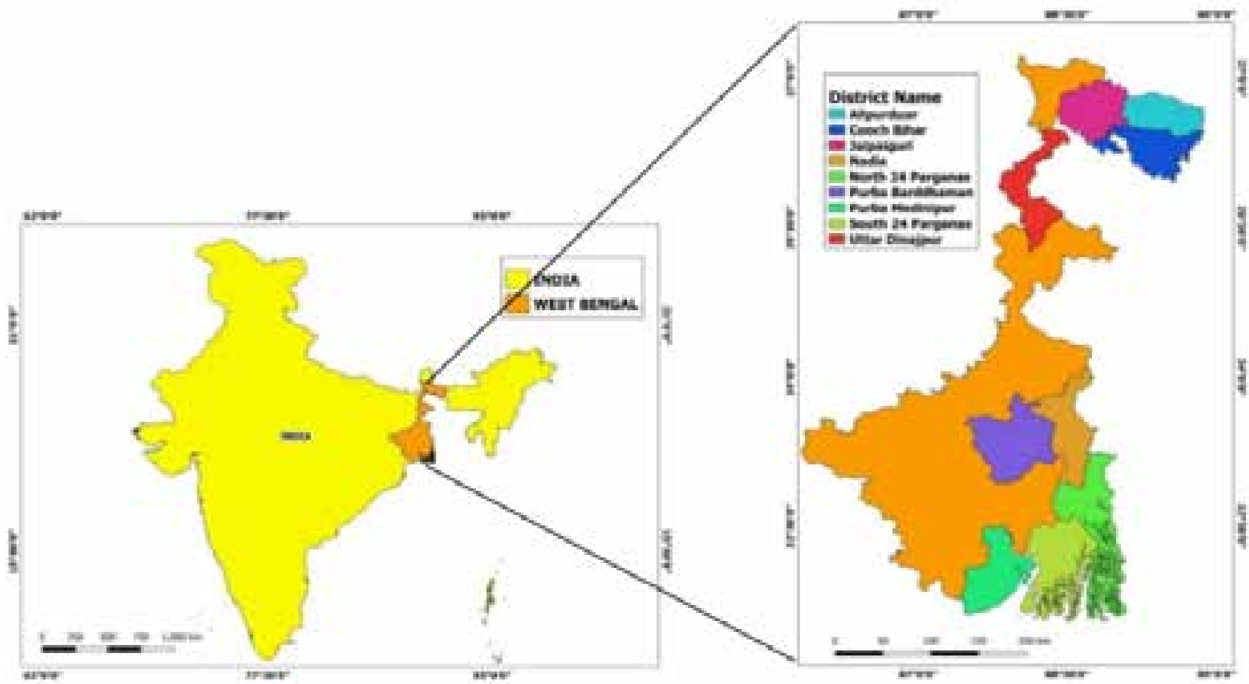

Figure 1. Map of West Bengal in the India map showing the study area.

Table 1. Details of the sampling sites.

District	Block	Village	GPS Readings	Type of water bodies
Cooch Behar	Tufanganj II	Bochamari	26.2500°N, 89.4400°E	Bochamari Beel (ox-bow lake)
	Cooch Behar II	Kharija Kakribari	26.2292°N, 89.2557°E	Beel (ox-bow lake)
		Baneswar	26.2400°N, 89.2993°E	Ditches
	Sitalkuchi	Gosaierhat	26.2048°N, 89.2876°E	Beel (ox-bow lake)
Alipurduar	Kumargram	Hindupara	17.4291°N, 83.1805°E	Rice Field
		Madhuvasa	26.2822°N, 89.4345°E	Beel (ox-bow lake)
Uttar Dinajpur	Hemtabad	Balliadighi	25.4410°N, 88.1260°E	Beel (ox-bow lake)
Purba Bardhaman	Jamalpur	Balarampur	23.5500°N, 87.5932°E	Beel (ox-bow lake)
Nadia	Chapra	Padmamala	23.3319°N, 88.3510°E	Fish Pond
North 24 Parganas	Habra II	Ashokenagar	22.5070°N, 88.3652°E	Fish Pond
South 24 Parganas	Canning II	Canning	22.3007°N, 88.6671°E	Tidal Creek, Matla River estuary
Purba Medinipore	Bhagbankhali	Chandipur	22.1406° N, 87.8715° E	Paddy field
Jalpaiguri	Ambari	Rajgunge	26.6388° N, 88.4971° E	Wetland

so they are named Synbranchidae, which means fused gills; the dorsal and anal fins are reduced to ray less skin folds, and the caudal fin is reduced, pectoral and pelvic fins absent; scales may be present or absent; eyes small or vestigial; 4th aortic arch is complete and swim bladder is absent.

Class: Actinopterygii
Order: Synbranchiformes
Family: Synbranchidae

Ophichthys cuchia **(Hamilton, 1822) (Image 1)**

Unibranchapertura cuchia (Hamilton, 1822)

Amphipnous cuchia (Hamilton, 1822) Muller 1839

Monopterus cuchia (Hamilton, 1822) Rosen & Greenwood 1976

Monopterus (Amphipnous) cuchia (Hamilton, 1822) Talwar & Jhingran 1991

Ophichthys cuchia (Hamilton, 1822) Britz et al. 2020

Materials examined

2 exs., 03.iii.2019, 750–860 mm TL, Rice field (17.4291 N, 83.1805 E), Paschim Nararthali, Hindupara, Alipurduar, West Bengal, India, Coll. R.K.Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae/1-2; 1 ex., 20.vi.2019, 380 mm TL, an oxbow lake (Beel) (26.2822N, 89.4345E), Madhuvasa, Alipurduar, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae/3; 1 ex., 13.i.2019, 710 mm TL, small ditches (26.2400N, 89.2993E),Kaljani, Baneswar, Cooch Behar, West Bengal, India, Coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae/4; 1 ex., 04.xi.2020, 460 mm TL, Bochamari beel (26.2500N, 89.4400E), Tufangunge, Cooch Behar, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae/5; 2 exs., 25.xi.2020, 680–700 mm TL, ditches (26.2048N,89.2876E), Gosaierhat, Sitalkuchi, Cooch Behar, West Bengal, India, Coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /6–7; 1 ex., 06.xi.2020, 630 mm TL, Beel (ox-bow lake) (26.2292N, 89.2557E), Kharija Kakribari, Cooch Behar, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /8; 2 exs., 8.xii.2020, Beel (25.4410N, 88.1260E), Balliadighi, Hemtabad, Uttar Dinajpur, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /9–10; 2 exs., 22.ii.2021, 520–550 mm TL, Beel (23.5500N, 87.5932E), Jamalpur, Balarampur, Purba Bardhaman, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /11–12; 2 exs., 10.iii.2022, 715-770 mm TL, seasonal fish pond (23.3319N, 88.3510E), Chapra, Padmamala, Nadia, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /13–14; 2 exs., 25.xii.2022, 600-630 mm TL, fish pond (22.5070N, 88.3652E), Habra, Ashokenagar, North 24 Parganas, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /15-16; 2 exs., 20.iv.2023, 465–565 mm TL, fish pond (22.3007 N, 88.6671 E), Canning, South 24 Parganas, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /17–18.)

Common name Gangetic mud eel.

Local name Kuchia, Kuichha, Kuche.

Description

The body is elongated, eel-shaped with a rounded/oval abdomen but laterally compressed at the caudal peduncle; upper lip with a characteristic overhanging or jowl-like structure (Image 2); fins absent; very minute cycloid scales at the posterior part of the body; pre-maxilla with a single row of teeth with 2–3 rows of teeth at symphysis, the maxilla is equal to pre-maxilla in length, without any teeth, palatine/ectopterygoid with a row of teeth, each half of mandible is with more than

Image 1. *Ophichthys cuchia* (Hamilton, 1822). Scale bar: 3.2 cm. © R.K. Das.

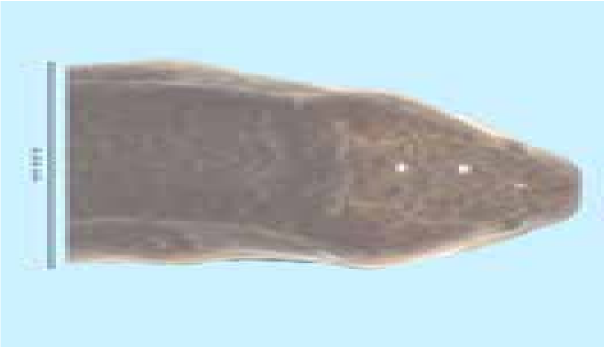

Image 2. Dorsal view of the head of *Ophichthys cuchia* showing sense organs. Scale bar: 5.23 cm. © R.K. Das.

Image 3. Lateral view of the head of *Ophichthys cuchia* showing eyes, gape of mouth, and gill slit. Scale bar: 2.59 cm. © R.K. Das.

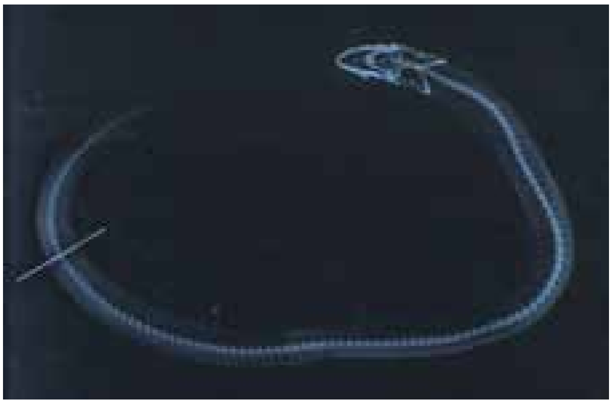

Image 4. X-ray radiograph of *Ophichthys cuchia* showing vertebrae.

Table 2. The list of swamp eels recorded from West Bengal.

Swamp eel species	English name / Common name	Local name	Catch frequency	IUCN category
O. cuchia (Hamilton, 1822)	Gangetic Mud Eel	Kuchia, Kuichha, Kunche	Abundant (85.71%)	LC
O. bengalense McClelland, 1844	Bengal Mud Eel	Nona Kuchia, Kuchia	Rare (14.28%)	LC

Image 5(a). Detection of swamp eels in a rice field in Alipurduar District. © R.K. Das.

Image 5(b). Harvesting of swamp eels from a rice field in Alipurduar District. © R.K. Das.

Image 5(c). Harvesting of swamp eel from a rice field in Alipurduar District. © R.K. Das.

one row of teeth anteriorly but a single row of teeth in posterior part; branchiostegal rays six in number; the triangular gill opening which is internally attached

Image 6(a). Mud holes in ditches in Cooch Behar District. © R.K. Das.

Image 6(b). Harvesting of swamp eel from ditches in Cooch Behar District. © R.K. Das.

Image 6(c). Harvesting of swamp eels in Cooch Behar District. © R.K. Das.

Image 6(d). Harvesting of a *Ophichthys cuchia* from Cooch Behar District. © R.K. Das.

Image 6(e). Detection of swamp eels in ditches in Cooch Behar District. © R.K. Das.

Image 6(f). Harvesting of swamp eels from ditches in Cooch Behar district. © R.K. Das.

with isthmus (Image 3); a single layer of gill filaments are present in 2nd and 3rd gill arches; 1st, 4th, and 5th gill arches are without any gill filaments, 5th ceratobranchial with several teeth-like structures. In the morphometric measurement, the head length 7.35–9.16 % of the total length, pre-anal length is 70.83–78.87 % of the total length, depth of the body at vent 2.08–4.65 % of the total length, the width of the body at vent 1.25–2.20 % of the total length, snout length 13–20 % of head length, the distance between anterior and posterior naris 15.71–22.50 % of head length, snout length 40–50 % of the gape length. The total vertebral count was 165 to 167 in which abdominal vertebrae was 97–98 and caudal vertebrae was 68–69 (Image 4). Morphometric measurements of the species are presented in Table 3.

Colour

In living conditions, the dorsal side of the *O. cuchia* was yellowish/greenish/brownish with black spots. Whereas the ventral side was yellowish or whitish in colouration. However, the formalin preserved specimens were dark/blackish in colour.

Remarks

O. *cuchia* (Hamilton, 1822) differs from all the species of synbranchidae except *O. hodgarti* (Chaudhuri, 1913), *O. ichthyopthoides* (Britz, Lalremsanga, Lalrotluanga & Lalramliana, 2011), O*. fossorius* (Nayar 1951), *O. indicus* (Silas & Dawson, 1961) and *O. desilvai* (Bailey & Gans, 1998) by the presence of scales. *O. cuchia* differs from the latter five species having scales in the posterior part of the body. It also differs by having the highest vertebral count (165–167) compared to the remaining species of the genus *Ophichthys*.

Habitat

The study revealed the occurrence of *O. cuchia* in diverse aquatic habitats in the study area. They were found to live in the mud holes of rice fields in the Alipurduar and Purba Medinipore districts (Image 5a–c). However, most of the specimens were found to inhabit the marginal areas of ditches in Cooch Behar and Uttar Dinajpur districts (Images 6a–f). Some individuals were also found to live in the weed-infested wetlands in Alipurduar and North 24 Parganas districts (Oxbow lake) (Image 7), and fish ponds in Nadia and South 24 Parganas district (Image 8). Higher density of the individual was observed in the clayey soils and weed-infested water bodies. During drying seasons, they move to a greater depth of soil, having a very small amount of water. Some specimens were also observed to live in subterranean water. They create a characteristic canal located in deep soils in the marginal areas of water bodies (Image 9). All of the *O. cuchia* were collected from the freshwater region of the study area.

Distribution

India (West Bengal, Assam); Bangladesh; Nepal; Myanmar; Pakistan; and USA.

Image 7. Harvesting of swamp eel from a weed-infested wetland (oxbow lake) in Alipurduar District. © R.K. Das.

Image 8. Harvesting of swamp eel from a fish pond in Nadia District. © R.K. Das.

Image 9. Canal of swamp eels below the soil in the marginal area. © R.K. Das.

Status

According to catch frequency, the species can be categorised as an abundant species (Table 2). On the other hand, the species is recognized as a 'Least Concern' (LC) category as per the IUCN Red List of Threatened Species (Dahanukar 2010).

Ophisternon bengalense McClelland, 1844 (Image 10)

Materials examined

1 ex., 20.iv.2023, 330 mm TL, Tidal creek of Matla river estuary (22.3007 N, 88.6671 E), Canning, South 24 Parganas, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /19; 2 exs., 22.iv.2023, 381–487 mm TL, Tidal creek of Matla river estuary (22.3007 N, 88.6671 E), Canning, South 24 Parganas, West Bengal, India, coll. R.K. Das, Reg. No. AABM/IFF/AC/Fish/Synbranchidae /20–21.

Common name: Bengal Mud Eel.

Local name: Nona kuchia, Kuchia.

Description

Body elongated, eel-like, abdomen rounded; eyes visible through skin (Image 11), vent in the posterior part of the body; head short, compressed; mouth wide, terminal, the gap of mouth extending to some distance behind the posterior border of the eye (Image 11); both the jaws equal with villiform teeth; four branchial arches with well-developed gills; scales absent; pectoral and pelvic fins absent; dorsal, anal, and caudal, fins are rudimentary; dorsal originates ahead of anal; dorsal, caudal and anal fins are confluent with each other. The snout length (SL) is 40% of the gape length; the head length is 9.65–9.71 % of the total length; the pre-anal length is 74.01–75.75 % of the total length, the depth of the body at vent 2.87–3.67 % of total length, the width of the body at vent 1.43–2.36 % of total length, snout length 9.4–10.8 % of head length, the distance between anterior and posterior naris 12.50–16.21 % of head length, snout length 44.40–45.45 % of the gape length. The total vertebral count was 128–132 in which abdominal vertebrae was 73–75 and caudal vertebrae was 55–57 (Image 12). The morphometric measurements of the species are presented in Table 3.

Colour

In living conditions, the dorsal side of the fish was deep brownish, and the ventral side was light brownish with small spots. Whereas the formalin preserved specimens were reddish/brownish in colour.

Table 3. Selected morphometric data for *Ophichthys cuchia* and *Ophisternon bengalense*.

	***O. cuchia* (n = 18)**		***O. bengalense* (n = 3)**	
	Range	Mean±SD	Range	Mean±SD
Total Length (TL) in mm	340–860	600.27±141.60	330–487	399.33±80.08
In percent of total length				
Head length (HL)	7.35–9.16	8.49±0.48	9.65–9.71	9.68±0.03
Pre-anal length	70.83–78.87	74.82±2.26	74.01–75.75	75.03±0.91
Body depth at the vent	2.08–4.65	3.39±0.57	2.87–3.67	3.19±0.42
Body width at the vent	1.25–2.20	1.60±0.25	1.43–2.36	1.76±0.51
In percent head length				
Snout length	13.33–20.00	16.77±1.52	9.37–10.81	10.27±0.78
Distance anterior-posterior naris	15.71–22.50	19.20±1.82	12.50–16.21	13.82±2.07
In percent gape length				
Snout length(SL)	40–50	44.17±4.61	44.40–45.45	44.93±0.50
Ratio				
Depth/Width of body	1–1.66	1.23±0.22	1.00–1.06	1.02±0.03
Total length/Head length	10.90–13.60	11.80±0.71	10.29–10.36	10.32±0.03
Total length/Body depth	20–28.88	23.19±2.41	30.43–33.00	31.72±1.28
Vertebrae				
Abdominal	97–98	97.5±0.70	73–75	74±1.41
Caudal	68–69	68.5±0.70	55–57	56±1.41

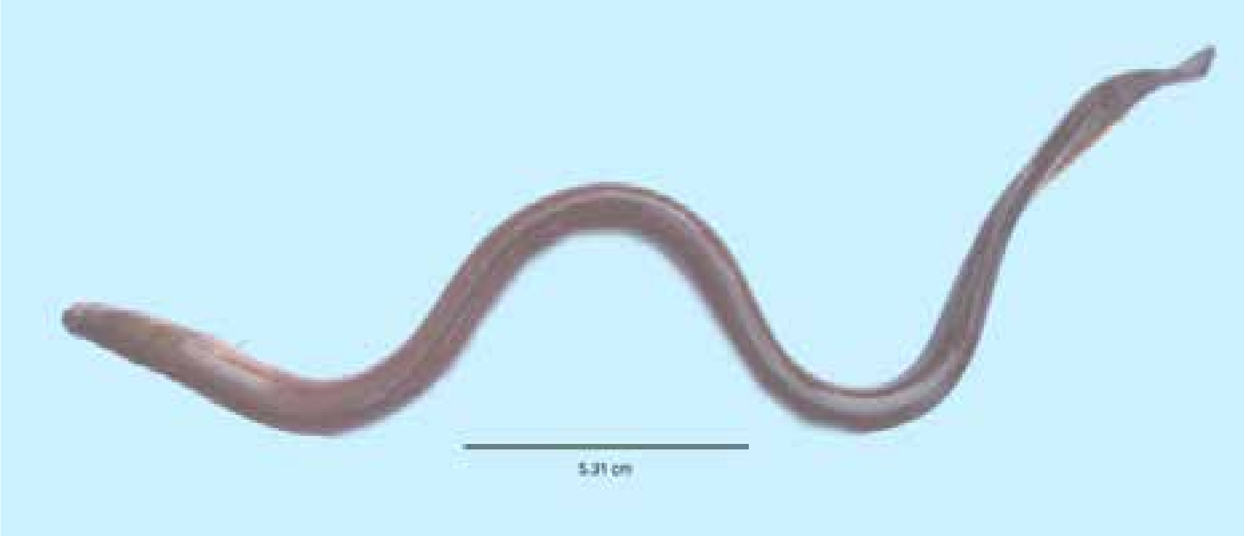

Image 10. *Ophisteron bengalense* McClelland, 1844. Scale bar: 5.31 cm. © R.K. Das.

Image 11. Ventral view of the head of *Ophisteron bengalese*. Scale bar: 2.46 cm. © R.K. Das.

Remarks

O. *bengalense* McClelland differs from all the species of the genus *Ophisternon*, having caudal vertebrae of 55–57, and a snout length of 40% of the gape length. In *O. bengalense*, pectoral and pelvic fins are absent; dorsal, anal, and caudal fins are rudimentary; dorsal originates ahead of anal; dorsal, caudal and anal fins are confluent with each other.

Habitat

The specimens of *O. bengalense* were collected from the mud holes located in the marginal areas of the tidal creek of the Matla River estuary (Image 13), Canning, South 24 Parganas, West Bengal, India. Thus, the results of the present study reveal that *O. bengalense* is a brackish water swamp eel.

Image 12. X-ray radiograph of *Ophisteron bengalense* showing vertebrae.

Distribution

India (West Bengal); Sri Lanka; Bangladesh; Indo-Malayan region; Philippines**.**

Status

According to catch frequency, the species can be categorized as a rare species (Table 2). On the other hand, the species is recognized as a 'Least Concern' (LC) category as per the IUCN Red List of Threatened Species (Dahanukar et al. 2019).

Image 13. Harvesting of swamp eel, *Ophisteron bengalense*, from the Tidal Creek of Matla River estuary, South 24 Parganas. © R.K. Das.

DISCUSSION

The swamp eel species, *O. cuchia,* was originally described as *Unibranchapertura cuchia* by Hamilton in 1822 using the type locality of the Ganges river in southeastern Bengal. Later, Muller (1839) transferred the species *U. cuchia* to the genus *Amphipnous*. Rosen & Greenwood (1976), in their well-known revisionary work on the taxonomy of swamp eels, placed the species in the genus *Monopterus.* Recently, Britz et al. (2020) again revised the taxonomy of this fish, placing the species in the genus *Ophichthys.* On the other hand, *O*. *bengalense* was described by McClelland in 1844 with the type locality of Hooghly River, West Bengal, India. The swamp eels identified in the present study are in accordance with the holotype of the species. In an earlier study, Mishra et al. (2003) recorded the *O. cuchia* in the rivers Kansai, Subarnarekha, and Shilabati of West Bengal. Recently, Mishra & Gopi (2017) recorded both species in the Sundarban Biosphere region of West Bengal, India.

The results of the present study showed that *O. cuchia* is widely distributed in the state of West Bengal. The adaptive features like air breathing organs, the ability to tolerate a wide range of salinity, the capacity to withstand extreme draught and cold by living in burrows, the ability to survive without feeding for considerable periods, and the crawling type of movement are evident in the swamp eel species *O. cuchia*, which possibly caused the wide distribution of the species in the state of West Bengal (Nico et al. 2019). However, *O. bengalense* has been recorded only in the tidal creek of the Matla River estuary, demonstrating brackish water of living habit, thus showing a very restrictive distribution of the species in the state compared to the *O. cuchia*.

Swamp eels occur in a variety of habitats, both freshwater and brackish water. Although some species live in clear flowing streams, most inhabit sluggish or standing waters, often with low oxygen content, like swamps or marshy areas, ponds, and lakes, where borrowing and amphibious habits are commonly displayed. They are admirably adapted for cave life, and some species from both the New World and Old World are cavernicolous (Rosen & Greenwood 1976; Bailey & Gans 1998). In addition, some species, like *M*. *eapeni* Talwar and *R*. *roseni* (Bailey & Gans 1998), are blind cavernicoles living in subterranean waters (Gopi 2002).

Overall, swamp eels are not usually considered to be heavily threatened or endangered. So, that is the reason IUCN is listed as the 'Least Concern' category. However, the populations of both species are declining rapidly in the state. Habitat loss due to urbanisation, agricultural expansion, and pollution of freshwater systems is probably responsible for the reduction of the population of swamp eels in West Bengal. Barman (2007) also considered habitat loss to be the chief threat to the fish in West Bengal. Overexploitation for food and trade can also impact local populations of these fish. As agricultural land or rice fields are considered one of the habitats for swamp eel, the indiscriminate use of pesticides and chemical fertilisers also causes the decline of the swamp eel population in West Bengal. Thus, localised threats such as habitat loss, water pollution, and overexploitation can affect the populations of swamp eels, especially in areas where human activities significantly impact their habitats.

CONCLUSION

The present study documented two species of swamp eels, *O*. *cuchia* (Hamilton, 1822) and *O. bengalense* McClellands, 1844 within the region of West Bengal, India. These swamp eels inhabit diverse environments, including mud holes of the rice field, ditches, fish ponds, wetland or oxbow lakes, and tidal creeks. Based on the catch statistics, *O. cuchia* is notably abundant, whereas *O. bengalense* is relatively rare species. Nevertheless, it is noteworthy that both species are categorized as ‘Least Concern’ category as classified by the IUCN Red List of Threatened Species. The decline in swamp eel populations in West Bengal can be attributed to habitat loss due to urbanisation, and indiscriminate use of pesticides and other agro-chemicals. Further studies endeavors are warranted to delve into the biology of the swamp eels for the development of commercial swamp eel fisheries in West Bengal.

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COMMUNICATION

Sacred river of Pune: boon or bane for the diversity of aquatic beetles (Insecta: Coleoptera)

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Abstract: Aquatic beetles are potential indicators of freshwater ecosystem and play an important role in food web and nutrient cycling. Parameters like pH, temperature, conductivity, total dissolved solids, salinity, and dissolved oxygen, are important water quality parameters. The present study is focused on the diversity of aquatic beetles and assessing water quality parameters of the sacred Indrayani River from various sites namely Valvan, Kamshet, Warangwadi, Begadewadi, Moshigaon, Alandi, Dhanore, and Tulapur. A total of 94 examples of aquatic beetles belonging to 31 species under 19 genera and four families from Indrayani River were recorded along with water quality parameters.

Keywords: Abiotic factors, checklist, Dytiscidae, fauna, Gyrinidae, habitat, Hydrophilidae, Maharashtra, Noteridae.

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PDEA

INTRODUCTION

Aquatic insects are integral part of aquatic ecosystems, representing essential components of biodiversity and play a profoundly significant role in recycling nutrients and form a crucial part of the natural food web in these environments (Subramanian & Shivaramakrishnan 2007). Among aquatic insects, there are 13,000 described species of aquatic beetles in the world (Short 2017), of which 776 species are known from India (Chandraf et al. 2017). The Indrayani River originates in the scenic northern Western Ghats of India, specifically at Kurwande Village (18.7310°N & 73.3820°E) near Lonavala, Pune, Maharashtra. It flows eastwards from Pune, passing through the revered Hindu pilgrimage centres of Dehu and Alandi, before eventually merging with the Bhima River at Tulapur, Pune. The Indrayani River is a significant tributary of the Bhima River and it is also called as sacred river. The Valvan dam, situated on the river, serves the dual purpose of irrigation and generating hydroelectric power. Flowing along the northern border of Pune City, the river's catchment area encompasses numerous villages, housing complexes, several cities, educational institutes, and various industrial areas, including Maharashtra Industrial Development Corporation (MIDC) and over the past three decades, industrialization has been rapidly expanding in this area. The deterioration of river water quality of rivers Pawana, Mula, Mutha, and Indrayani of Pune Metropolitan area resulted from the growth of industrial activities and associated unplanned concentration of people in the suburban areas (Hui & Wescoat 2019; Bhagwat et al. 2021). There is no proper sewage collection and treatment provided for thousands of people who assemble twice a year and for the local residents. These activities are taking toll on river's health thereby affecting its faunal status as well as human health (Dahanukar et al. 2012). There are also many news reports on formation of toxic foam on the banks of the Indrayani River from factories and sewage. Therefore, the present study is focused on the aquatic beetle's diversity and to assess water quality, including water temperature, dissolved oxygen, pH, electrical conductivity, total dissolved solids and salinity from Indrayani River.

MATERIAL AND METHODS

Collections from the Indrayani River were conducted monthly throughout the year 2022, encompassing all seasons. The beetle samples were collected from eight different sites (Image 1) of Indrayani River (Image 2) such as Valvan (site A), Kamshet (site B), Warangwadi (site C), Begadewadi (site D), Moshigaon (site E), Alandi (site F), Dhanore (site G), and Tulapur (site H). The beetles were collected using the line transects method, using a pond net with a square frame (mesh size 0.5 mm). The net was systematically swept back and forth at 100 m intervals in the water bodies. Once collected, the beetles were preserved in 70% ethanol and appropriately labelled with corresponding information about the sample sites, date, and time of collection. Collected beetles were studied and photographed under Leica EZ4 HD microscope. Identification was done using standard literature mainly by Sharp (1882), Vazirani (1968, 1984), Pederzani (1995), Toledo (2008), Nasserzadeh & Komarek (2017), Sheth et al. (2018, 2021), and Girón & Short (2021). All the identified specimens are deposited in the Zoological Survey of India, Pune with registration numbers from ENT-1/4220 to ENT-1/4267.

During the beetle collection, water samples were also collected from each sampling site. At the location of sampling, three replicates of selected physicochemical water quality parameters were recorded. The water quality parameters, such as pH, salinity, conductivity, total dissolved solids, and temperature, were measured directly on-site using a multiparameter probe Eutech PCS Tester 35. However, dissolved oxygen measurements were recorded in the laboratory, utilizing the digital bench top DO meter (Aquasol AB-DO-01). The geographic coordinates were obtained using Google Earth. Analysis of variance (ANOVA) was utilized to assess the statistical differences between the means of the water quality parameters of the Indrayani River using R-Software-version R 4.3.1 [Package R studio - (1) library (dplyr), (2) library (gplots)].

RESULT AND DISCUSSION

A total of 94 individuals of aquatic beetles were collected from six eight sites in 36 sampling efforts during the year. There were all belonging to 31 species (Image 3–6) under 19 genera and four families from Indrayani River (Table 1). The family Dytiscidae was the most abundant with 15 species followed by Hydrophilidae with 11 species, Gyrinidae with three species, and Noteridae with two species (Figure 1). Among the family Dytiscidae, the genus *Laccophilus* was found in five out of eight sites which makes it more prevalent.

Family Hydrophilidae was found to be the second

Image 1 . Collection sites and habitats of aquatic beetles from Indrayani River: A—Valvan | B—Kamshet | C—Warangwadi | D—Begdewadi | E—Moshigaon | F—Alandi | G—Dhanore | H—Tulapur. © P. Takawane.

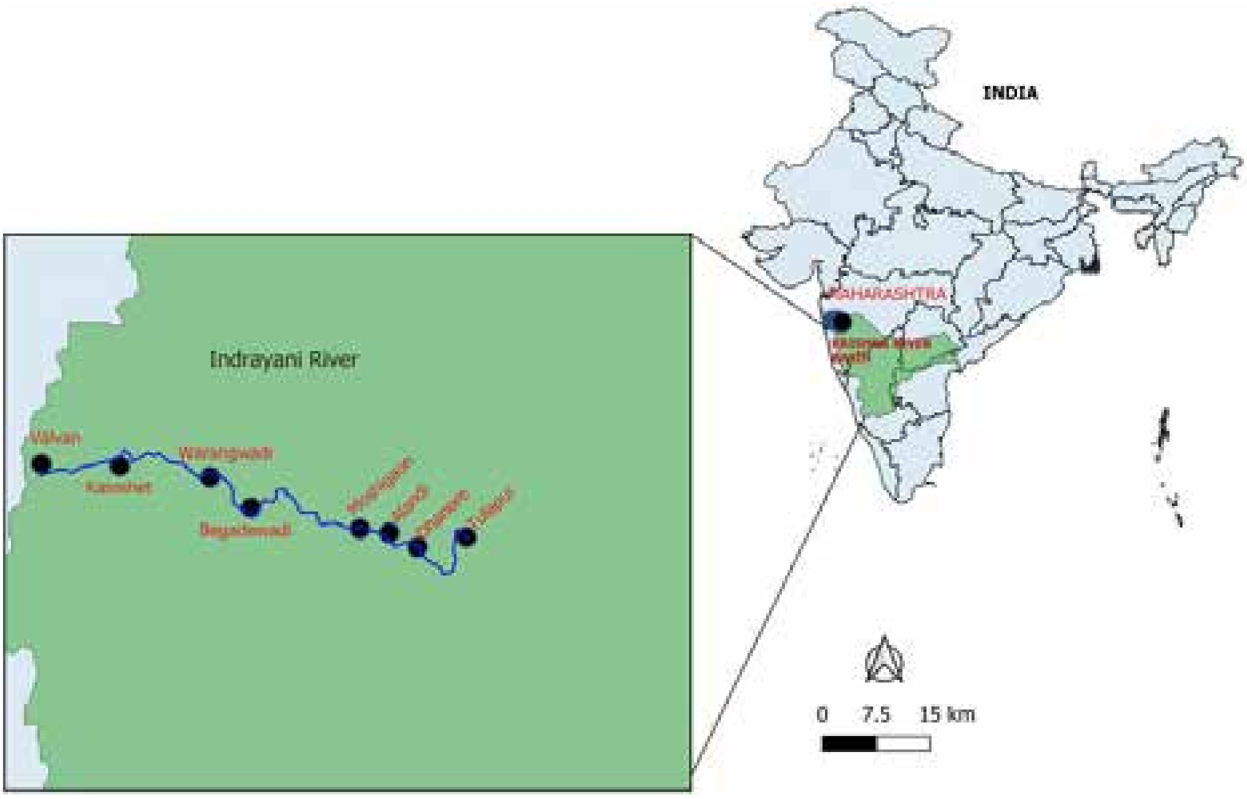

Image 2. Collection sites on the bank of Indrayani River.

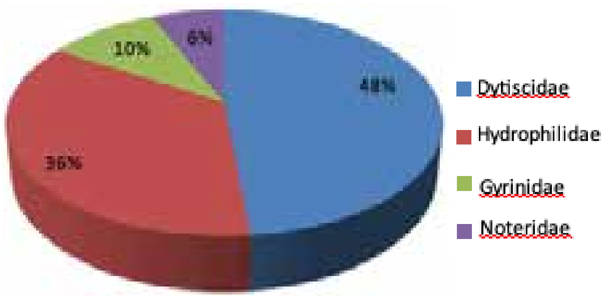

Figure 1. Graph showing species richness with respect to families in Indrayani River.

most abundant and the genus *Sternolophus* was collected more than any other hydrophilid genus from all the six localities. Two sites namely Alandi and Tulapur did not show any aquatic beetles.

The collected data of water quality for the Indrayani River from January to December 2022 is presented in Table 2. The investigation of physicochemical parameters in this study revealed that the minimum pH value was recorded at Begadewadi (pH 6.8 ± 0.14), while the maximum was observed at Alandi (pH 7.72 ± 0.46). Furthermore, the minimum water temperature was measured at Begadewadi (27.61 ± 1.90°C), and maximum temperature recorded at Alandi (28.4 ± 1.93°C). The dissolved oxygen concentration exhibited higher values at Tulapur (5.72 ± 0.30 mg/l) and the lowest values at Moshigaon (3.69 ± 0.45 mg/l). The minimum salinity recorded was 43.41 ± 16.25 ppt at Valvan, whereas the maximum salinity was observed at Dhonore, with a value of 397.41 ± 24.25 ppt. The total dissolved solids were found to be least at Warangwadi, with a measurement of 177.16 ± 32.17 ppm, and highest at Dhanore, reaching 575 ± 40.53 ppm. Furthermore, the lowest conductivity was observed at Kamshet, with a reading of 196 ± 11.15 µS/cm, while the highest conductivity value was recorded at Dhanore, measuring 784.16 ± 37.01 µS/cm. The pH, dissolved oxygen, salinity, total dissolved solids, and conductivity exhibited significant variations ($p < 0.05$) among the different sampling sites, as determined by the analysis of variance (ANOVA) (Figure 2). However, temperature did not show any significant difference. The odour of the water was unpleasant at site 5–8 and the colour of the water was slightly greenish-black to brownish-black with enormous growth of

aquatic plants like water hyacinth *Pontederia*

Table 1. Distribution of aquatic beetles in collection sites of Indrayani River.

	Family	Species	Valvan	Kamshet	Warangwadi	Begadewadi	Moshigaon	Alandi	Dhanore	Tulapur
1	Gyrinidae	*Dineutus (Cyclous) indicus* Aube, 1838	+	-	-	-	-	-	-	-
2	Gyrinidae	*Patrus punctulatus* (Regimbart, 1886)	+	-	-	-	-	-	-	-
3	Gyrinidae	*Patrus limbatus* (Regimbart, 1883)	+	-	-	-	-	-	-	-
4	Dytiscidae	*Laccophilus ceylonicus* Zimmermann, 1919	+	-	-	-	-	-	-	-
5	Dytiscidae	*Laccophilus flexuosus* Aube, 1938	+	-	-	-	+	-	+	-
6	Dytiscidae	*Laccophilus inefficiens* Walker, 1859	-	-	+	-	-	-	-	-
7	Dytiscidae	*Laccophilus parvulus* Aube, 1838	-	-	-	+	-	-	-	-
8	Dytiscidae	*Hydaticus fabricii* M'Leay,1833	+	-	-	+	-	-	-	-
9	Dytiscidae	*Hydaticus incertus* Regimbart, 1888	+	-	-	-	-	-	-	-
10	Dytiscidae	*Hydaticus luczonicus* Aube, 1838	-	-	-	+	-	-	-	-
11	Dytiscidae	*Copelatus neelumae* Vazirani, 1973	-	-	-	-	+	-	-	-
12	Dytiscidae	*Copelatus schuhi* Hendrich & Balke,1998	+	-	-	-	-	-	-	-
13	Dytiscidae	*Copelatus deccanensis* Sheth, Ghate & Hajek, 2018	+	-	-	-	-	-	-	-
14	Dytiscidae	*Cybister sugillatus* Erichson, 1834	-	-	-	+	-	-	-	-
15	Dytiscidae	*Hydroglyphus inconstans* (Regimbart, 1892)	-	-	-	-	+	-	-	-
16	Dytiscidae	*Hyphydrus renardi* Severin, 1890	-	-	-	+	-	-	-	-
17	Dytiscidae	*Peschetius nilssoni* Sheth, Ghate, Dahanukar & Hajek, 2021	-	-	+	+	-	-	-	-
18	Dytiscidae	*Peschetius toxophorus* Guignot, 1942	+	-	-	-	-	-	-	-
19	Hydrophilidae	*Sternolophus rufipes* (Fabricius, 1792)	+	-	-	+	-	-	+	-
20	Hydrophilidae	*Regimbartia attenuata* (Fabricius, 1801)	-	-	-	+	-	-	-	-
21	Hydrophilidae	*Hydrophilus olivaceous* (Fabricius,1781)	-	-	-	+	-	-	-	-
22	Hydrophilidae	*Helochares anchoralis* Sharp, 1890	-	-	-	+	+	-	-	-
23	Hydrophilidae	*Helochares crenatus* Regimbart, 1903	-	-	-	+	-	-	-	-
24	Hydrophilidae	*Enochrus esuriens* Walker, 1858	-	-	+	-	-	-	-	-
25	Hydrophilidae	*Coelostoma vitalisi* Orchymont, 1936	-	-	-	-	+	-	-	-
26	Hydrophilidae	*Coelostoma fallaciosum* Orchymont, 1936	+	-	-	-	-	-	-	-
27	Hydrophilidae	*Berosus (Berosus) pulchellus* M'Leay, 1825	+	-	-	+	-	-	-	-
28	Hydrophilidae	*Amphiops mater* Sharp, 1873	-	-	-	+	-	-	-	-
29	Hydrophilidae	*Agraphydrus obscuratus* Komerak, 2018	+	-	+	+	-	-	-	-
30	Noteridae	*Canthydrus laetabilis* Walker, 1858	-	-	-	+	-	-	-	-
31	Noteridae	*Canthydrus angularis* Sharp, 1882	+	-	-	-	-	-	-	-

+—Presence of species in the site | -—absence of species in the site .

crassipes, *Hydrilla* sp., *Pistia* sp. and algal blooms of Chlorophyta and Bacillariophyta on surface. Suspended sediments were also observed.

Water beetles are an important part of the biotic component of any aquatic habitat or wetlands and they are considered as indicators of ecological diversity and habitat characteristics (Foster 1987; Eyre & Foster 1989; Sánchez-Fernández et al. 2004) as they meet most of the criteria usually accepted in the selection of indicator taxa (Holt & Miller 2011). The distribution of aquatic beetles in upper basin namely Valvan, Kamshet, Warangwadi, and Begdewadi was seen more due to the quality of water and less anthropogenic disturbance as compared to the aquatic beetle's availability in lower basin namely Moshigaon, Alandi, Dhanore, and Tulapur. The river is polluted due to industrial effluents, sewage,

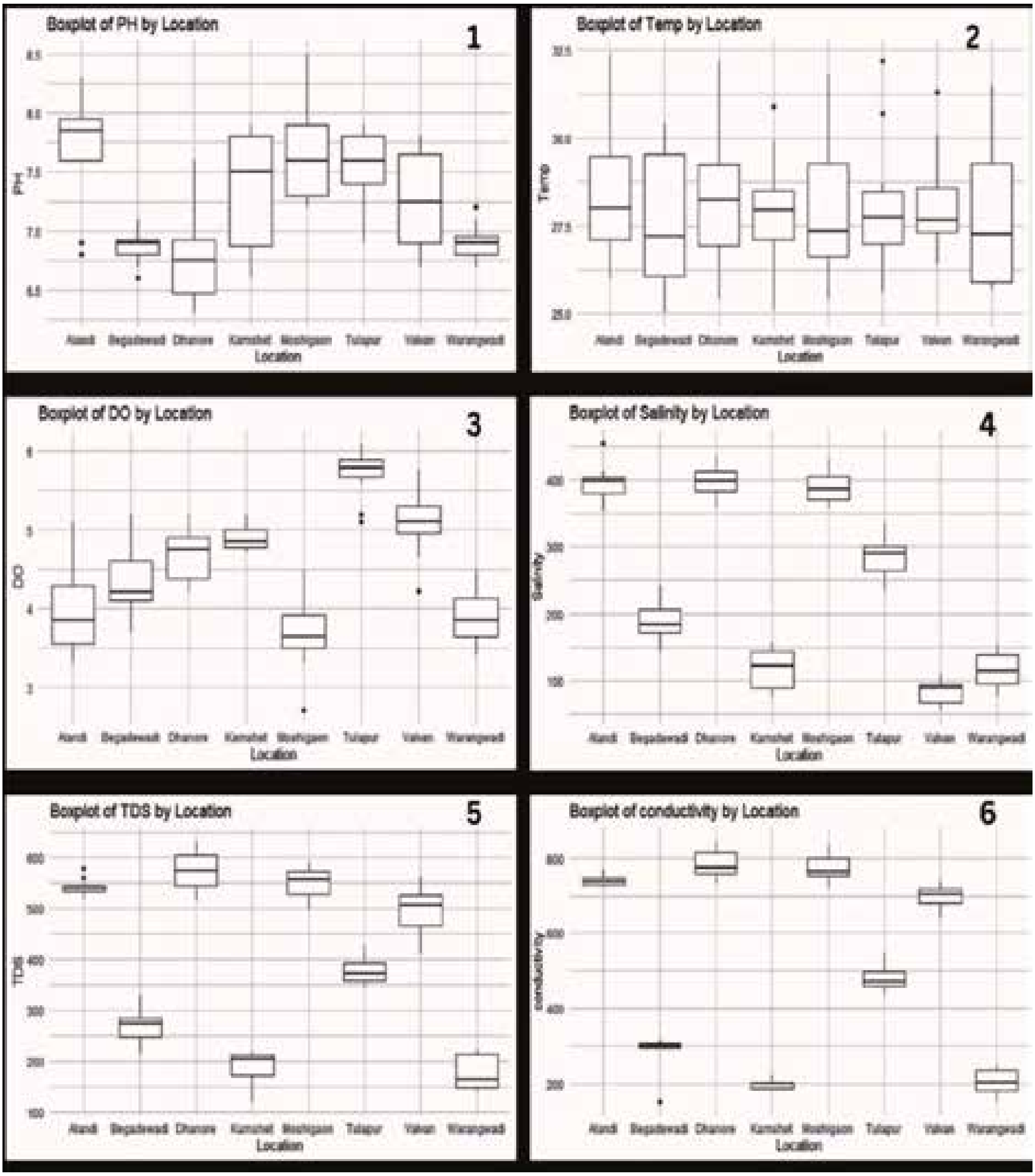

Figure 2. Graphs of Analysis of Variance (ANOVA) of Indrayani River water: 1—pH | 2— Temperature | 3—Dissolved oxygen | 4—Salinity | 5—Total dissolved solids | 6— Conductivity.

constructions and various recreational activities in the river basin (Dahanukar 2011). If the present activities continue, the harmful effect may lead to loss of aquatic fauna in Indrayani River. This study can be a baseline data for future research on aquatic beetles from the river as it is the first data on aquatic beetles throughout the stretch of Indrayani River.

Table 2. Physicochemical parameters of Indrayani River (January–December 2022).

Locations	Physicochemical parameters of Indrayani River (January–December 2022)					
	PH	Temp (°C)	DO (mg/l)	Salinity(ppt)	TDS (ppm)	Conductivity (µS/cm)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Valvan	7.27 ± 0.4	28.15 ± 1.42	5.08 ± 0.39	43.41 ± 16.25	500 ± 46.66	696 ± 30.37
Kamshet	7.33 ± 0.51	27.9 ± 1.71	4.89 ± 0.16	119.4 ± 28.94	193 ± 30.14	196 ± 11.15
Warangwadi	6.9 ± 0.14	27.83 ± 2.10	3.9 ± 0.36	114.83 ± 26.62	177.16 ± 32.17	202.25 ± 33.39
Begadewadi	6.8 ± 0.14	27.61 ± 1.90	4.39 ± 0.45	190.08 ± 29.30	267.33 ±32.20	289 ± 44.36
Moshigaon	7.65 ± 0.433	28.02 ± 2.12	3.69 ± 0.45	389.10 ± 24.28	547.91 ± 30.83	773 ± 35.36
Alandi	7.72 ± 0.46	28.4 ± 1.93	3.98 ± 0.58	394.25 ± 26.38	541 ± 16.23	739 ± 15.16
Dhanore	6.82 ± 0.44	28.27 ± 2.03	4.67 ± 0.33	397.41 ± 24.25	575 ± 40.53	784.16 ± 37.01
Tulapur	7.52 ± 0.34	28 ± 1.83	5.72 ± 0.30	282.75± 29.83	378.5 ± 27.10	481.41 ± 35.45

SD—Standard deviation | Temp—Temperature | DO—Dissolved oxygen | TDS—Total dissolved solids.

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Image 3. Dorsal and ventral images of aquatic beetles collected from Indrayani River, Pune: 1 a & b—*Dineutus* (*Cyclous*) *indicus* Aube, 1838 | 2 a & b—*Patrus punctulatus* Regimbart, 1886 | 3 a & b—*Patrus limbatus* Regimbart, 1883 | 4 a & b—*Laccophilus ceylonicus* Zimmermann, 1919 | 5 a & b—*Laccophilus flexousus* Aube, 1938 | 6 a & b—*Laccophilus inefficiens* Walker, 1859 | 7 a & b—*Laccophilus parvulus* Aube, 1838 | 8 a & b— *Hydaticus fabricii* M'Leay, 1833.

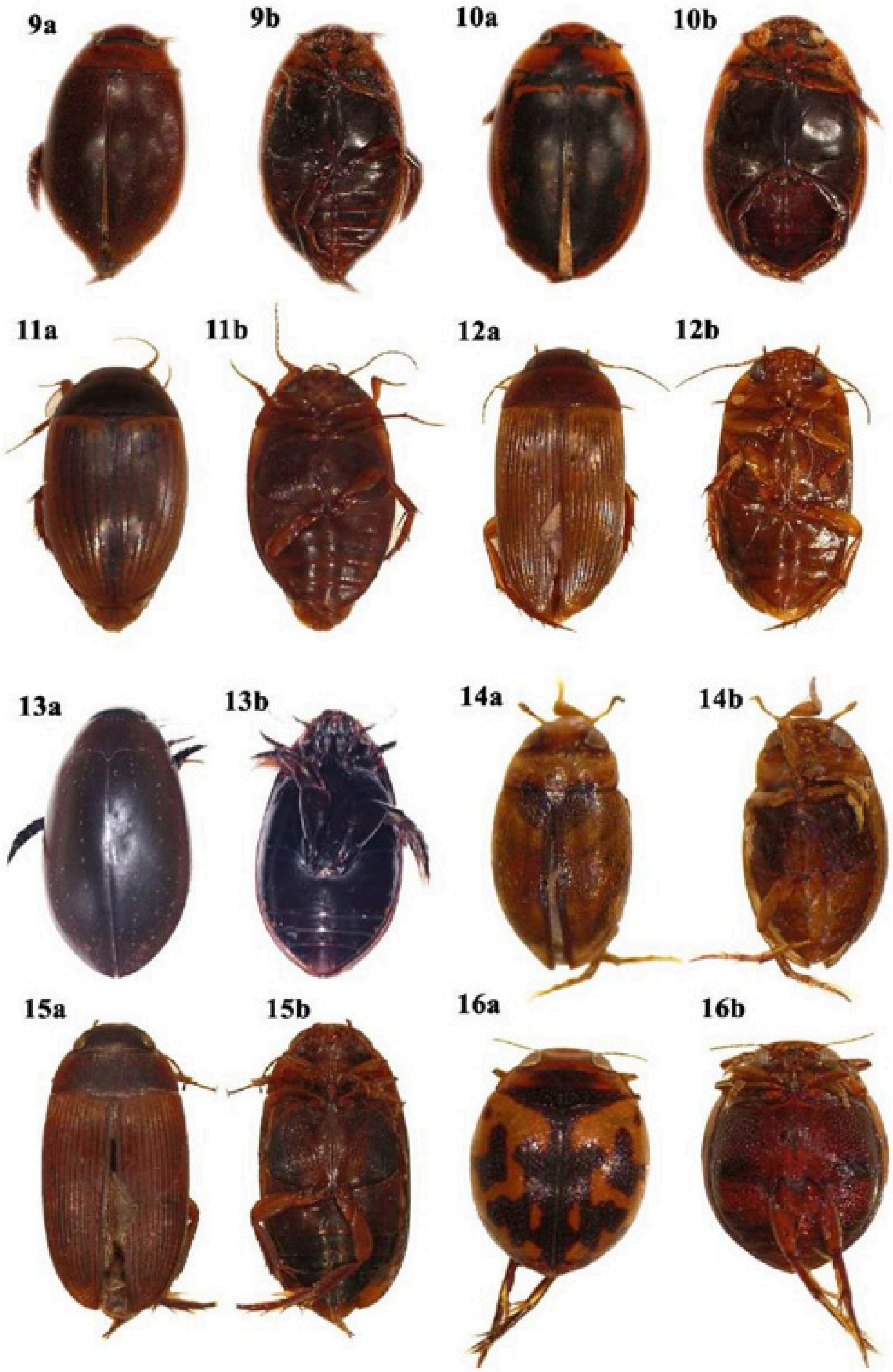

Image 4. Dorsal and ventral images of aquatic beetles collected from Indrayani River, Pune: 9 a & b—*Hydaticus incertus* Regimbart, 1888 | 10 a & b—*Hydaticus luczonicus* Aube, 1838 | 11 a & b—*Copelatus neelumae* Vazirani, 1973 | 12 a & b—*Copelatus schuhi* Hendrich & Balke, 1998 | 13 a & b—*Cybister sugillatus* Erichson, 1834 | 14 a & b—*Hydroglyphus inconstans* Regimbart, 1892 | 15 a & b—*Copelatus deccanensis* Sheth, Ghate & Hajek, 2018 | 16 a & b— *Hyphydrus renardi* Severin, 1890.

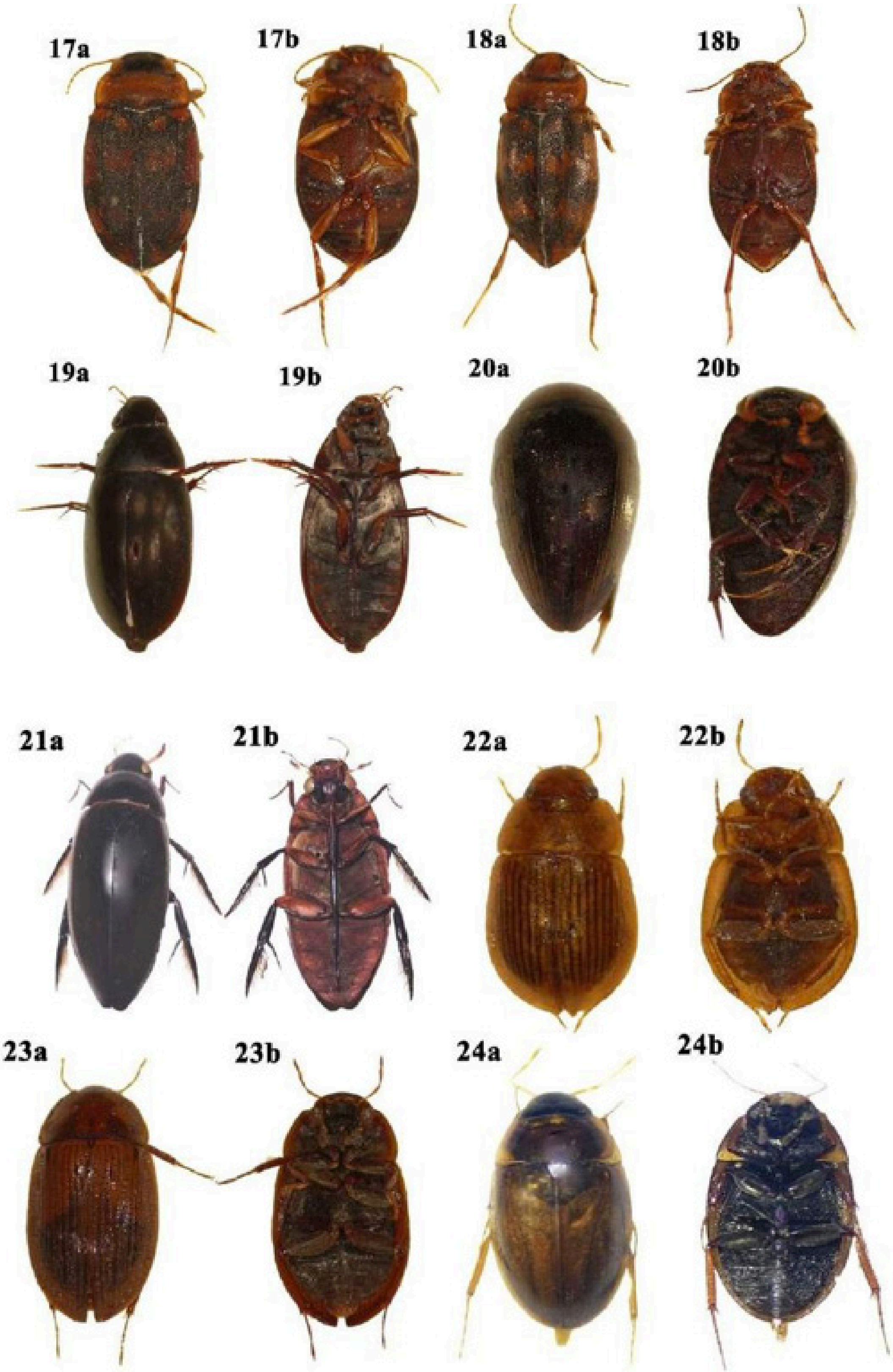

Image 5. Dorsal and Ventral images of aquatic beetles collected from Indrayani River, Pune: 17 a & b—*Peschetius nilssoni* Sheth et al. 2021| 18 a & b—*Peschetius toxophorus* Guignot, 1942 | 19 a & b—*Sternolophus rufipes* Fabricius, 1792 | 20 a & b—*Regimbartia attenuata* Fabricius, 1801 | 21 a & b—*Hydrophilus olivaceous* Fabricius, 1781 | 22 a & b—*Helochares anchoralis* Sharp, 1890 | 23 a & b—*Helochares crenatus* Regimbart, 1903 | 24 a & b— *Enochrus esuriens* Walker, 1858.

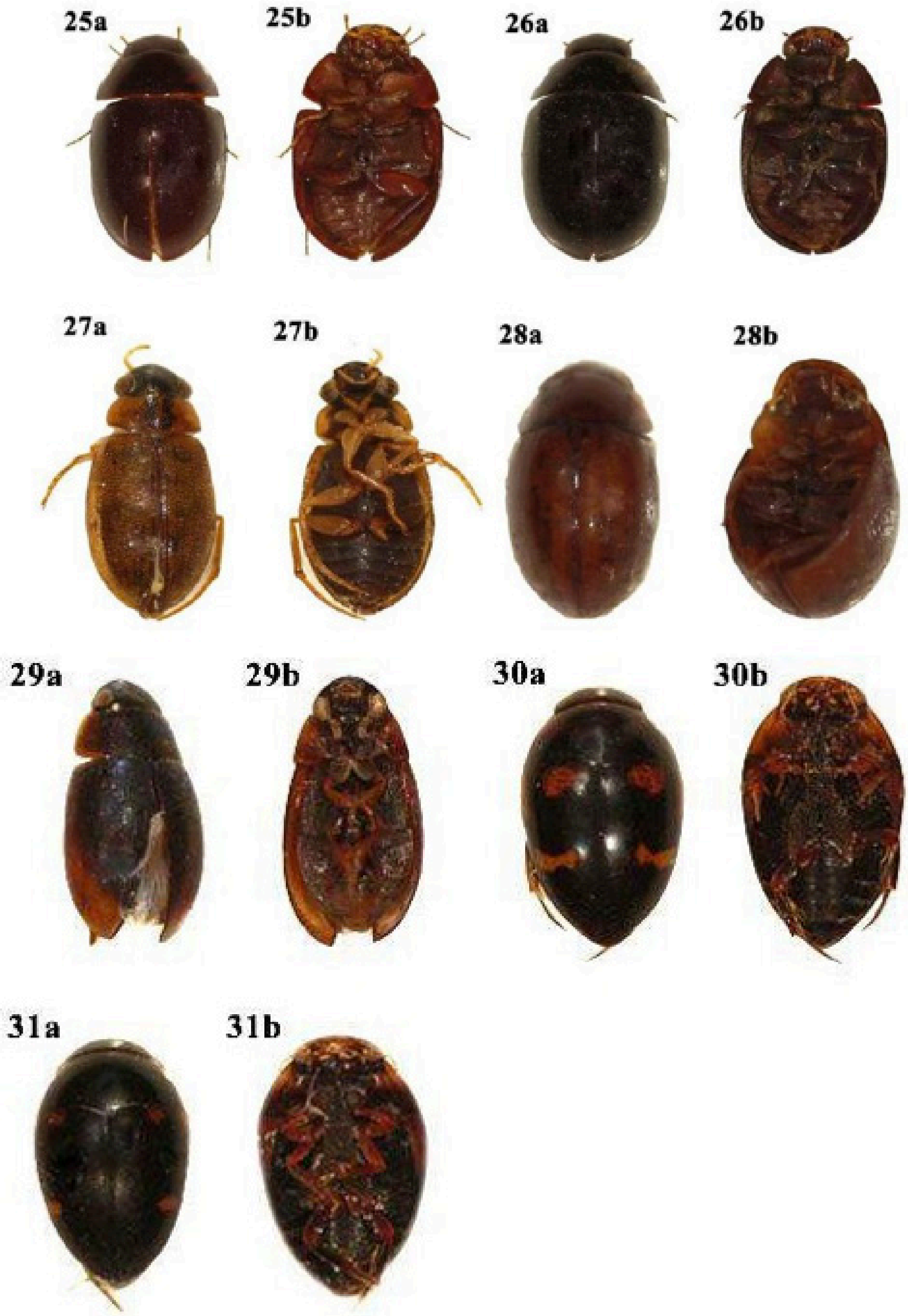

Image 6. Dorsal and ventral images of aquatic beetles collected from Indrayani River, Pune. 25 a & b—*Coelostoma vitalisi* Orchymont, 1936 | 26 a & b—*Coelostoma fallaciosum* Orchymont, 1936 | 27 a & b—*Berosus (Berosus) pulchellus* M'Leay, 1825 | 28 a & b—*Amphiops mater* Sharp, 1873 | 29 a & b—*Agraphydrus obscuratus* Komerak, 2018 | 30 a & b—*Canthydrus laetabilis* Walker, 1858 | 31 a & b—*Canthydrus angularis* Sharp, 1882.

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COMMUNICATION

Fine structure of sensilla on the proboscis of the Indian Honey Bee *Apis cerana indica* Fabricius (Insecta: Hymenoptera: Apidae)

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Abstract: Honey bees feed on flowers from which they collect nectar and pollen and their mouth parts are designed for fluid-feeding from flowers. The proboscis consists of a 'tongue' that includes a long glossa and ends in a spoon-shaped labellum, labial palp, galea and mandibles. The sensilla on the proboscis assists in nectar feeding. A study of the chemosensory hairs on the proboscis was carried out in *Apis cerana indica* collected from apiaries at the foot of Western Ghats, India. Light- and scanning electron microscopy were employed. In addition, silver staining was carried out to distinguish different types of chemosensilla. The glossa has 60 sensilla chaetica that stain by silver nitrate technique. The length (110 µ), width (2 µ) and spacing of microtrichia on glossa and forked hairs on the labellum are suited for the collection of nectar due to viscosity and to reduce leakiness while feeding. The length of the glossa being short suggests that *A. cerana indica* feeds on small-sized flowers that are not tubular. The labial palp has sensilla chaetica A and sensilla chaetica B distinguished by their length and sensilla basiconica, all of which are silver nitrate-positive and thus chemosensory in nature. Distal galea has sensilla basiconica, sensilla chaetica A and B and sensilla coeloconica. The maxillary palp is a mechanosensory structure. The bulge on the galea near the maxillary palp has chemosensory sensilla chaetica. Mandibular hairs did not stain with silver and are hence mechanosensory. The sensilla on proboscis in *A. cerana indica* is comparable to mouth part sensilla in *Apis mellifera* and *Apis florea*. The position of the chemosensilla at different regions suggests their role in tasting nectar, detecting the flow of nectar, and the dimensions of the flower and pollen.

Keywords: Basiconica, chaetica, coeloconica, epipharynx, hotspots, olfactory, sensory, silver-staining, taste, Western Ghats.

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Author contributions: SK performed all the experiments; SVR and RKP analyzed the data and wrote the paper.

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INTRODUCTION

In the course of evolution, flowering plants have developed form, colouration, and nectar to entice bees for pollination. Visit to flowers is the major point of interaction between plants and bees, with the bee getting nectar and pollen and the flower getting pollinated. Palynological studies to deduce host plants' sources of nectar and/or pollen are a favored approach to identify the host plants (Lau et al. 2019) and to reveal the plants preferred by honey bees. The choice of food plants by honeybees is a deliberate process and involves learning, memory and nutritional requirements. The mouth part of honey bees can be expected to assist feeding and their structure can be related to flower morphology and location of nectar and pollen.

The Asiatic Honeybee *Apis cerana indica* F. is distributed in southeastern Asia and the Indian subcontinent (Jaffe et al. 2010). It is suitable for apiculture along the Western Ghats, one of the biodiversity hotspots. To protect honeybee production and conserve bees, there is a need to gain insights into the biology of *Apis cerana indica* F. and its principal sources of nectar and pollen. The distribution of *Apis cerana indica* and its pollen sources in the vicinity of Mangalore near the Western Ghats region has been surveyed by a palynological analysis (Krishna & Patil 2019). The principal pollens found in honey samples in the study were *Areca catechu*, *Cocos nucifera*, *Hopea* sp., *Ixora coccinea*, *Mimosa pudica*, and *Psidium guajava*. *A. cerana indica* is a short-range forager and its foraging is restricted to a radius of ~500 m from the hive (Punchihewa et al. 1985). While sensilla on the galea of honeybees respond to salts, sugars (Whitehead & Larsen 1976a) and umami (taste of amino acids) as reported by Lim et al. (2019), they have a limited number of taste receptors (Sanchez et al. 2007). It is believed that honeybees have limited gustatory abilities (Monchanin et al. 2022). They may, therefore, be unable to detect and avoid pesticides and hence vulnerable to exposure to pesticides. It is essential to understand the gustatory abilities of honey bees and there is a need to survey the chemosensilla on their mouth parts, which is the focus of the present study.

Drinking nectar and gathering pollen is a specialized mode of feeding in insects. They need structural specialization of mouth parts (Krenn et al. 2005) and their operation. Secondly, chemoreception is crucial for foraging and feeding. The broad types of sensilla in insects and in honey bees are described by (Esslen & Kaissling 1976). Preliminary studies on the olfactory capabilities of honeybee *A. cerana indica* have been carried out wherein the ultrastructure of the antenna and electroantennogram has been studied (Bhowmik et al. 2016). The proteome of antennae of *A. mellifera linguistica* and *A. cerana* drones and workers suggest differences in the olfactory capabilities of the two species (Woltedji et al. 2012) suggesting differences in olfactory senses. Such mechanisms may also affect exclusive food preferences. Work on contact chemoreceptors on the mouthparts of *Apis mellifera* has been carried out by (Galic 1971) and (Whitehead & Larsen 1976a,b) and on *Apis florea* (Kumar & Kumar 2016). While studies on *A. florea* focused on sensilla types only, Whitehead & Larsen (1976b) studied the number and innervations of sensilla using both transmission and scanning electron microscopic studies. The response properties have been studied by electrophysiology by Whitehead & Larsen (1976a) and shown to sense salts and sugars but not water. Galic (1971) studied the chemoreceptors in the epipharynx and hypopharynx. Study of insect feeding behavior requires knowledge of its taste repertoire and distribution of taste hairs on the mouth parts is necessary for this purpose. The form of mouthparts in honey bees is a determinant of the type of flower that the honey bee feeds on. The present study focuses on the distribution and organization of sensilla of the mouthparts of *A. cerana indica* F. We used scanning electron microscopic and silver nitrate staining approaches (Babu et al. 2011) to characterize the sensilla and its distribution. The current study reliably differentiates chemoreceptor sensilla from mechanoreceptor sensilla and describes their functional role in nectar feeding.

MATERIAL AND METHODS

Microscopic Observations

Asian worker honey bees *Apis cerana indica* were collected from apiaries in Puttur (12.7687° N, 75.2071° E, 87 m elevation), Karnataka, India and the region is located at the base of the Western Ghats. Fifty honey bees were collected from colonies and seven bees were randomly selected for SEM analysis. The bees were dissected with the help of fine forceps, the mouthparts were carefully excised under a dissecting microscope and later fixed in 4% glutaraldehyde (in 1 M phosphate buffer) for four hours. The dirt on the bees was cleaned by ultra-sonication (Sidilu Ultrasonic, Bangalore) for 5 s. The mouth parts were washed further in distilled water followed by serial dehydration. The whole mounts were prepared and mounted using DPX. The number of

Table 1. Chemosensory hairs were found on different regions of mouthparts of *A. cerana indica* (present study). The numbers in *A. mellifera* were reported by (Whitehead & Larsen 1976b). The numbers are comparable, except in the region of glossa where *A. cerana indica* has less sensilla chaetica A. Sensilla coeloconica occurs at the distal end of galea and these sensilla on galea have not been reported so far in honey bees. Sensilla chetica A (SC A), Sensilla chetica (SC B), Sensilla basiconica (SB) and Sensilla coeloconica (S CO) have not been reported earlier in honeybees (*Apis mellifera* and *Apis florea*)

	A. Mellifera		*A. cerana indica*				Sample size
	SC (A)	SC (B)	SC (A)	SC (B)	S. Ba	S.CO	
Region							
Glossal tip	12 (6 + 6)		12 (6 + 6)				7
Distal glossa	66–78	0	21 ± 3	4			7
Labial palp segment 1	0	0	0	0			7
Labial palp segment 2	4–6	8–13	3	6			7
Labial palp segment 3	10	11–15	10	4			7
Labial palp segment 4	7–9	9–12	8 ± 1	7 ± 1			7
Distal galea	12–16	10–16	11 ± 1	22 + 3	4	12 ± 2	7
Adjacent to maxillary palp	0	43–47	0	33 ± 2			7

samples observed is provided in Table 1.

Scanning Electron Microscopy

The samples were fixed in absolute alcohol or Karnovsky's fixative (Karnovsky 1964). Specimens were dehydrated by incubating through a series of alcohol grades. After a final wash in acetone, the specimens were gold-coated at 5 nm thickness using a Class I gold sputtering system and observed using a field emission scanning electron microscope (FESEM, Carl Zeiss Ltd., Germany). The images were processed using Adobe Photoshop. The sensilla are named based on the description provided by Callahan (1975) and that of Whitehead & Larsen (1976b) for *Apis mellifera*.

Silver Nitrate Staining

Porous chemosensilla were stained with silver nitrate following the method described by (Babu et al. 2011) and observed using an Olympus BX51 microscope. Anesthetized bees were washed with 1% acetone and incubated in 1% silver nitrate ($AgNO_3$) for five minutes. Subsequently, they were washed thrice for two minutes each to remove $AgNO_3$. The specimens were treated by soaking the samples in photo/film developer for five–seven minutes. Then the specimens were immediately rinsed in 3% acetic acid for one minute. The specimens were dehydrated using 70%, 80%, 90%, and 100% alcohol (10 minutes each). The dehydrated samples were washed in methyl salicylate. The antenna/maxillary palp was mounted on a slide using DPX.

Nomenclature of Sensilla

Sensilla is named based on the descriptions by Callahan (1975) and of Whitehead & Larsen (1976b) for *Apis mellifera*. The naming of sensilla by (Kumar & Kumar 2016) in *A. florea* is ambiguous.

RESULTS

Sensilla on the segments of the mouth parts, viz., glossa, labellum, labial palp, galea including maxillary palp are described here. Glossa and labellum together measure 1.8 ± 0.1 mm in length and 120 ± 8 µm in width. The labellum is oval measuring 120 ± 7µ and 80 ± 6µ. The glossa bears hairs to collect nectar by surface tension and capillarity. The labellum has forked hairs on the dorsal side whereas the ventral surface is bald (Image 1). The grooved labellum assists in sucking the nectar and also helps draw in nectar due to capillarity. The glossa is sheathed by labial palp and galea. The various types of sensilla that were observed are:

1. Sensilla chaetica, type A and type B, based on their length.
2. Sensilla basiconica, and
3. Sensilla coeloconica.

The base of the labellum has six sensilla chaetica on either side, slightly curved, measuring 40 ± 4 µm in length. They are stained by the silver staining technique and are therefore chemosensory and possibly gustatory in function (Image 1). Along the distal two-thirds of the glossa, there are sensilla, measuring 30 µm, thus shorter in length. They are silver-positive. A large number of thin

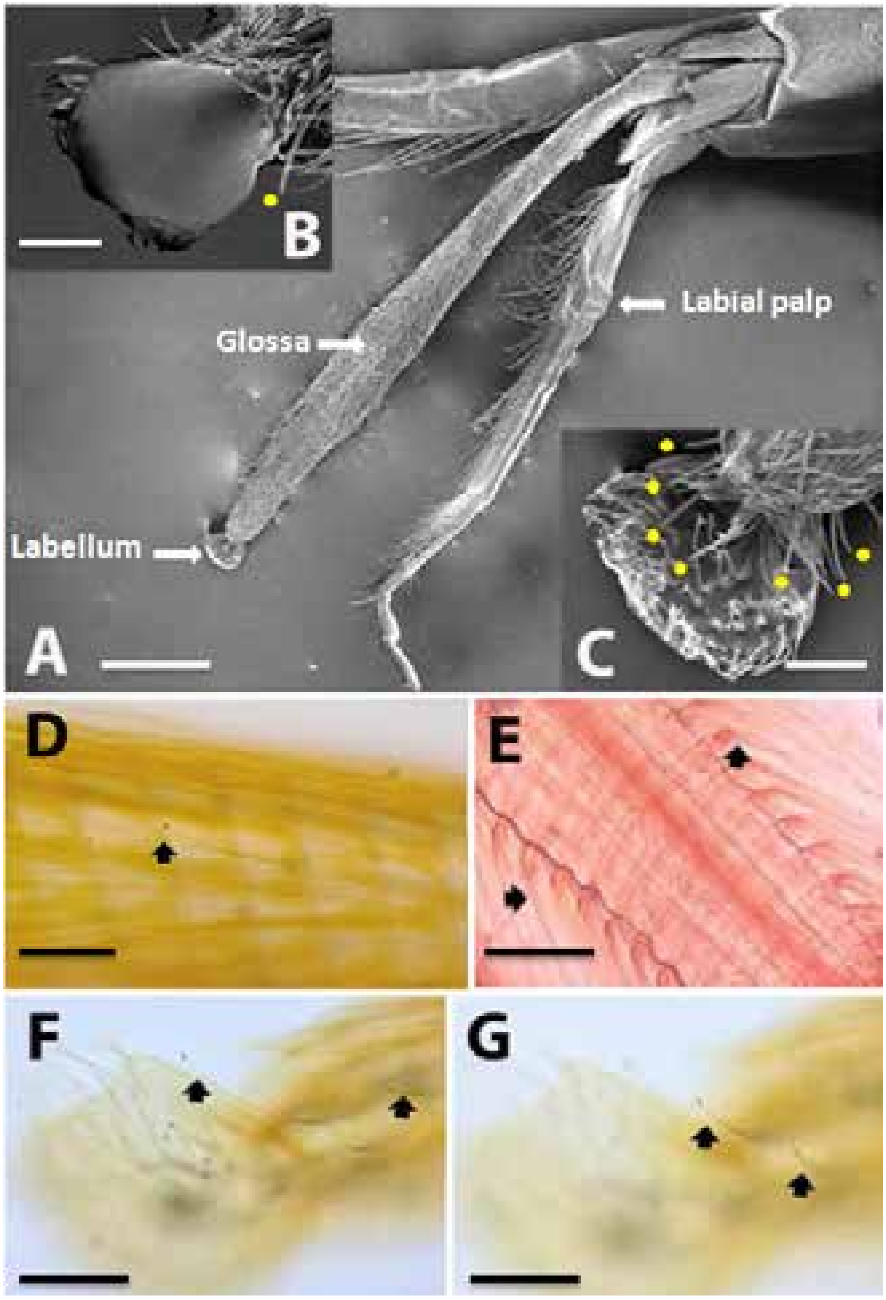

Image 1. Glossa and labial palp of *A. cerana indica*. Galea is removed for clarity and shown in Image 3: A—the elongated glossa measures 1.9 mm long with an oval labellum measuring (100 µ) | B—dorsal side of the labellum has forked hairs (arrowhead) | C—the ventral side of the labellum, taste hairs are indicated by asterisk | D—glossa has annular arrangement with microtrichia and silver stained trichoid sensilla (arrow) | E—there are about 90 annuli on the rim of which microtrichia are arranged with interspersed trichoid sensilla (arrow) | F & G—taste hairs at the distal region at the base of the labellum are stained with silver nitrate and are indicated by arrow. Scale Bar: A—100 µm | B–G—20 µm. © Rajashekhar Patil.

microtrichia (120 ± 9 µm long and 2 ± 0.12 µm wide) cover the glossa facilitating the collection of nectar. They are placed at a distance of 20 ± 1.1 µ from each other on the annulus.

The labial palp has four segments with segments 3 and 4 protruding out of the galea that en-sheath them (Image 2). The length of segments 1 and 2 helps the segments protrude out of the galea and segments distal regions segment 2, segment 3, and segment 4 have chemosensilla. There are three broad types of sensilla – sensilla chaetica A, sensilla chaetica B, and sensilla bascionica. Their absolute numbers are provided in Table 1. These are clearly stained by silver staining, predominantly at their tips.

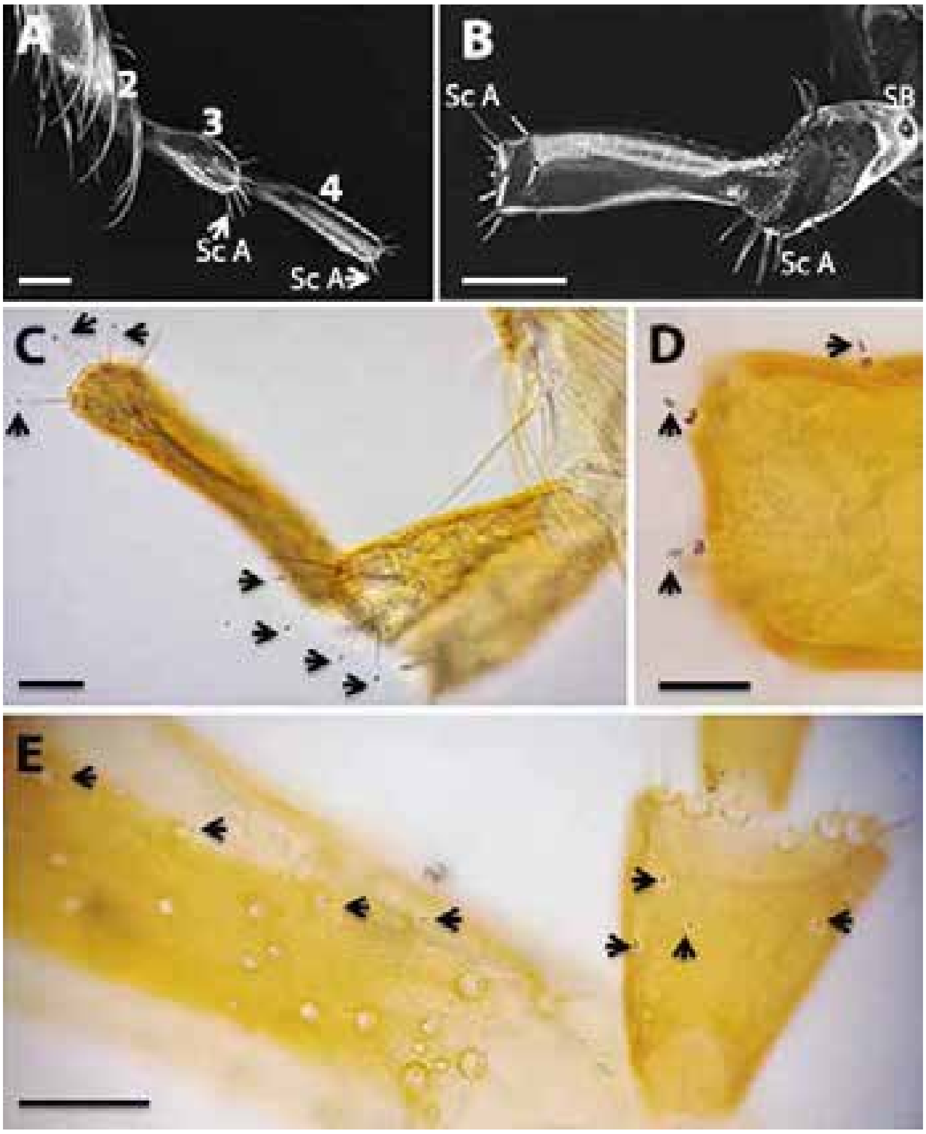

Image 2. Labial palp of *A. cerana indica*: A—dorsal view of the 2nd, 3rd, & 4th segments | B—ventral view of segments 2 & 3, showing sensilla chaetica A (ScA), and sensilla basiconica (SB). Their actual numbers are given in Table 1. A cobble-stone-like cuticle is seen at the joints in which is supposed to provide lubrication while folding of joints due to dipping of labial palp in nectar. The arrangement reduces drag in a high-viscosity fluid. Silver-stained chemosensilla are seen in C, D, & E | C—sensilla chaetica (ScA) (arrow) | D—Sensilla basiconica are marked with arrow | E—Silver-stained sensilla chaetica B on segments 2 & 3. Scale Bar: A, B & C—40µm | D—10 µm | E—20 µm. © Rajashekhar Patil.

The galea is tapered and en-sheaths the labial palp and the glossa. It bears 12 sensilla coeloconica on the proximal region and sensilla chaetica A measuring 12 ± 1µm and sensilla chaetica B measuring 20 ± 1.8 µm (Image 3). The ventral surface has four sensilla basiconica which have been described hitherto in *A. mellifera* or *A. florea*. Sensilla chaetica are identified to be chemosensory by being permeable to silver nitrate. The second segment of the maxillary palp bears nine mechanosensory hairs measuring 12 µm to 24 µm that are not porous. The bulged surface of the galea at the base of the maxillary palp bears a group 34 ± 2 sensilla basiconica measuring 2 ± 0.2 µm that is permeable to silver (Image 4). The mandibles bear numerous microtrichia measuring 10 µm and are possibly mechanosensory as they are not stained by silver.

DISCUSSION

Feeding in honeybees is well-studied by several authors as summarized by (Düster et al. 2018). A recent study shows that honeybees can switch from lapping to sucking mode when needed (Wei et al. 2023). While

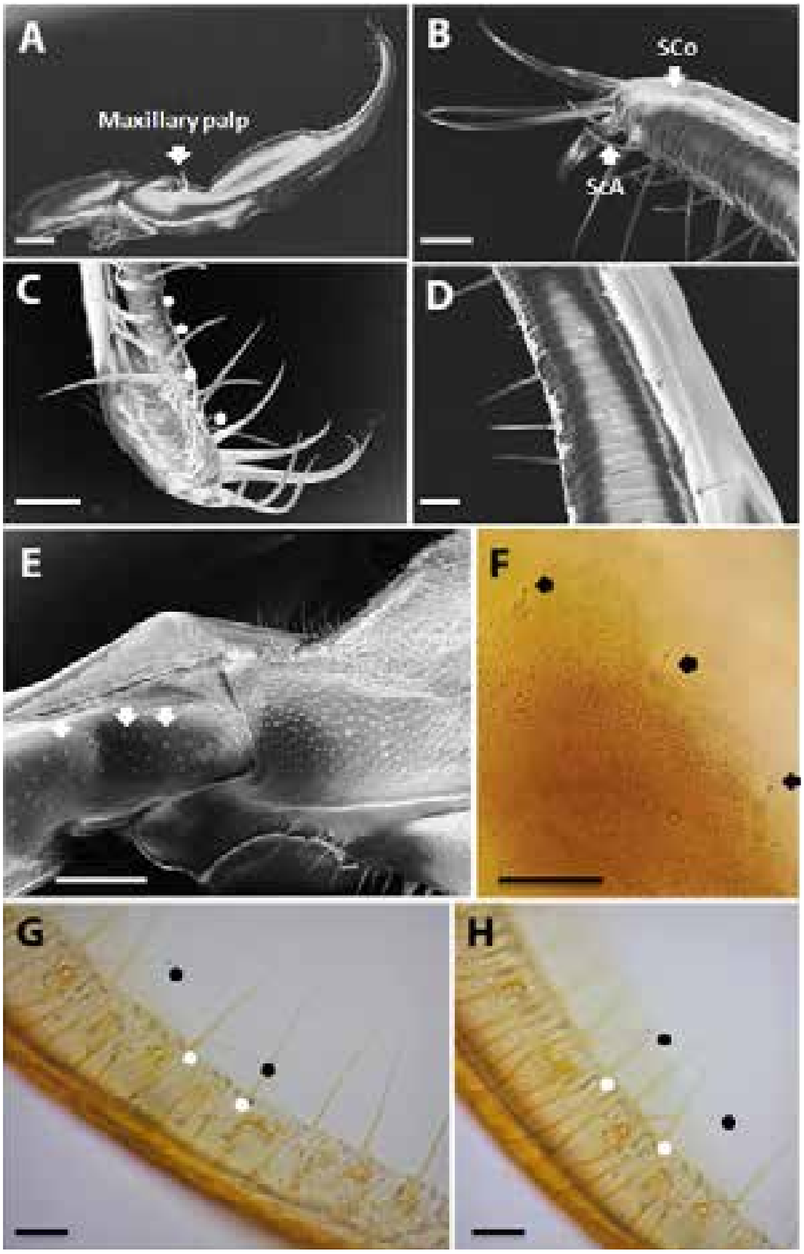

Image 3. Scanning electron micrographs of galea: A—entire galea in lower magnification. The arrangement of sensilla on the distal region is shown in B, C, & D | B—arrangement of Sensilla chaetica A, Sensilla chaetica B, Sensilla coeloconica on the dorsal side of distal region | C—ventral side of distal region showing Sensilla basiconica (asterisk and inset). D—Medial region | E—the bulge adjacent to maxillary palp (Sc A—Sensilla chetica A | Sc B—Sensilla chetica B | SCo—Sensilla coeloconica) | F—Silver stained (asterisk) corresponding to sensilla shown in E suggesting them to be chemosensory which number about 33. Scale bar: A—100 µm | B–D—20 µm |E—200µm | F–H—20µm. © Rajashekhar Patil.

feeding nectar, the erectable microtrichia gets extended due to the viscosity of the nectar. This event helps to collect nectar by surface tension. Nectar is held between microtrichia due to the length of the microtrichia and is influenced by the viscosity of nectar (He et al. 2020). The taste hairs observed in the present study are shorter than microtrichia and may provide information about nectar contents. The taste hairs are largely hidden among the microtrichia and cannot be observed by scanning electron microscopy. Silver staining and transmitted light microscopy help reveal taste hairs (Image 1). The glossa is a long structure with an oval labellum. It is made up of annular segments which number about 90. The hairs are arranged on the margins of the annuli. Sensilla chaetica, possibly taste hairs and stained at the tip by silver are found along with setae. There are taste hairs for providing chemosensory input during feeding. The present work excludes the sensilla

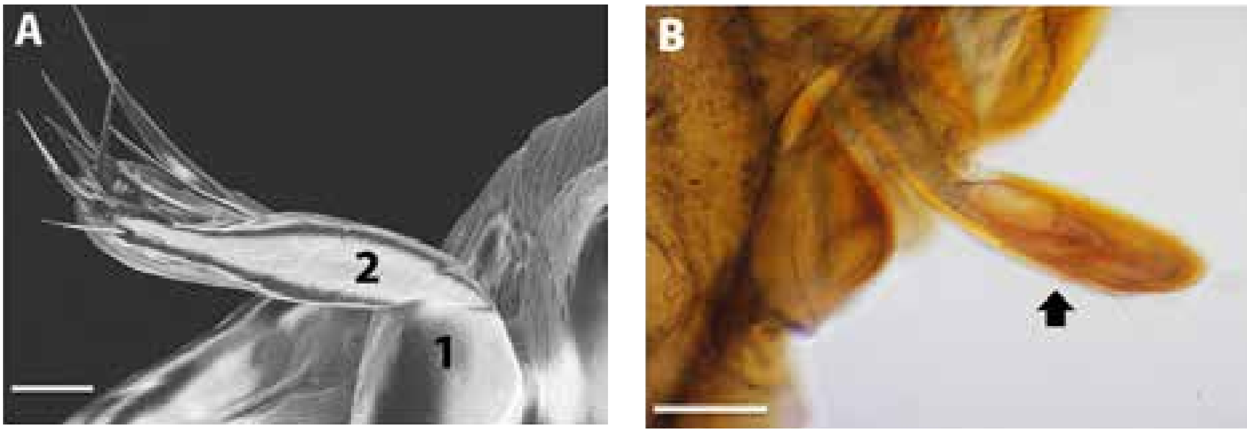

Image 4. Maxillary palp on the galea of *A. cerana indica*: A—SEM image of maxillary palp showing mechanosensory hairs (segments 1 & 2) | B—these hairs are silver-negative suggesting them to be mechanosensory. Scale bar: A & B—10 µm. © Rajashekhar Patil.

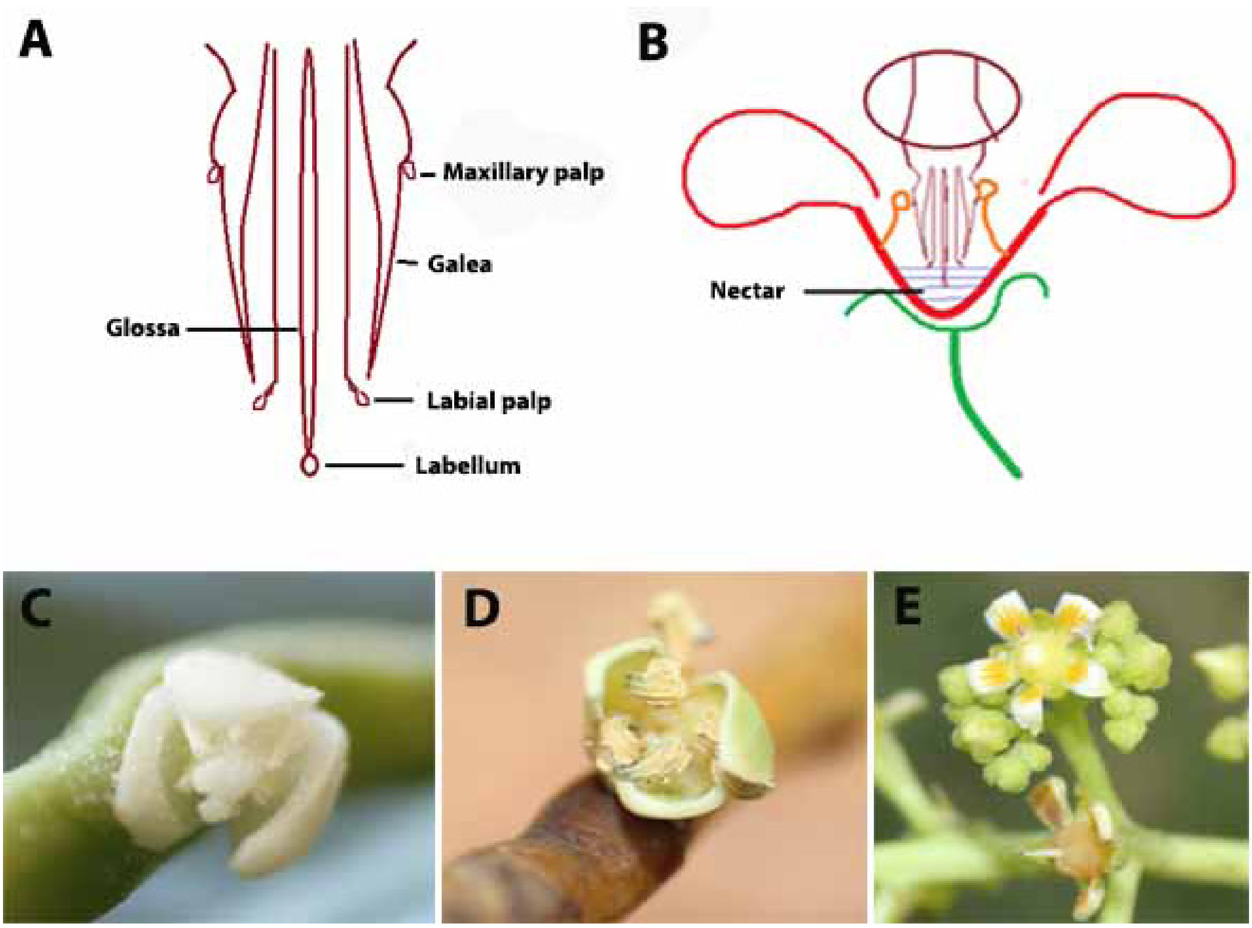

Image 5. A—schematic diagram of the proboscis of *A. cerana indica* | B—the position of different appendages of mouth parts in relation to parts of a flower indicate the possible role played by sensilla in feeding. The distal half of glossa dips into nectar. The taste hairs on glossa, labial palp and galea may help sense nutrients and flow of nectar. The labial palp is also designed to reduce drag encountered in viscous nectar. Maxillary palp is mechanosensory and may provide information on dimensions of flower (Duster et al. 2017). A patch of chemosensory hairs posterior to maxillary palp are suitably placed to come in contact with the anther. Length of the glossa suggests that the proboscis is designed for non-tubular polypetalous flowers (Style µ and stigma not shown). Flowers of: B—*Cocos* | C—*Areca* | D—*Mangifera*, frequently visited by *Apis cerana indica*. © Rajashekhar Patil.

in the groove identified by (Whitehead & Larsen 1976b). The taste hairs at the distal regions protruding out from among the microtrichia are comparable to the distal taste hairs of *A. mellifera* (Table 1) and *A. florea*. The number of taste hairs along the length of the glossa is lesser in *A. cerana indica* than in *A. mellifera*. A Sensilla number is suggested to enhance sensitivity. However, the difference between the two species (*A. mellifera* and *A. cerana indica*) is not large. The forked hairs of the labellum may increase the ability to take up and retain nectar.

Taste hairs occur on the distal region of segments 3 and 4 of the labial palp. They are positioned to come in contact with nectar/food while feeding. Their number and types are comparable to labial palp hairs found to occur in *A. mellifera* and *A. florea*. Segments 1 and 2 are long to ensure that they protrude beyond the envelope of galea. The arrangements of micro-protuberances seen in the articulations of segments 3 and 4 of the labial palps help reduce resistance during feeding in *A. mellifera* (Ji et al. 2017). This region has been wrongly attributed as plate sensilla by Kumar & Kumar (2016) in *A. florea*. The sensilla basiconica occur on the surface of segments 2, 3, and 4 and may also act as olfactory hairs.

The sensilla chaetica A of the galea are long and are less in number than sensilla chaetica B. The shorter sensilla chaetica B are 12 in number and the two types are well positioned to be contact chemosensory hairs (Image 3). These hairs may also help sense the occurrence of pollen grains and the stage of floral development. The maxillary palp appears to be mechanosensory in function as none of the hairs are stained with silver (Image 4). Their function as mechanosensory is suggested by (Whitehead & Larsen 1976a). Similarly, the mandibles have hairs that do not stain with silver and one sensillum was found to innervate them by TEM studies (Whitehead & Larsen 1976a), suggesting them to be mechanosensory. The observations in the present study using silver nitrate and the previous TEM study corroborate each other. Honeybees have adapted to exploit different types of nectar/pollen sources. The length of glossa in *A. mellifera* (3.3 mm measured from illustrations of Zhu et al. (2016) and *A. cerana indica* (1.8 mm, present study) render them suitable for foraging small-sized flowers. Bees such as *Euglossa championi* (glossa length 11.25 mm) and *Euglossa imperialis* (glossa length 22.25 mm) forage on long, tubular orchids. The length of an orchid bee proboscis is three–five times more than that of honey bees. Despite the differences in lengths, the types of sensilla in the three species of *Apis* (Whitehead & Larsen 1976b; Kumar & Kumar 2016; present study) and in Euglossini (Düster et al. 2018) are comparable. The observations that the number of sensilla does not differ much and their positions are comparable has prompted (Düster et al. 2018) to make interesting suggestions summarized below: (1) The sensilla of galea and labial palp provide information on nectar availability and quality, (2) The flexed segments 3 and 4 of labial palp helps detect whether flowers are open and (3) The hairs on inner galea, labial palp and between microtrichia of labellum detect the flow of the nectar. The mouth parts of honey bees are thus crafted and endowed with strategically placed sensilla to probe the morphology, nutrient quality and the stage of blooming of flowers for feeding.

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COMMUNICATION

A compendium of *Aphelenchoides* (Fischer, 1894) (Nematoda: Tylenchina: Aphelenchoidea) nematodes with the description of a new species from Manipur, India

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Abstract: The present compendium is based on the findings of a research work on the survey of nematodes belonging to the family Aphelenchoidea in the northeastern states of India and the literature available on this particular species, mainly from Manipur. During the study, a total of 12 *Aphelenchoides* spp. were found, among which six species were reported for the first time from Manipur. A new species, *Aphelenchoides oryzae* is also described in the present article. The present study will help in making us understand the biodiversity status of *Aphelenchoides* nematodes in the region. Diagnosis of the species and illustrations along with dichotomous keys are provided in the manuscript.

Keywords: Aphelenchid, Coconut tree, fungivore, food-web, soil fertility, soil dwelling, *Pinus* sp., *Morus* sp., Orange plant and species richness.

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Author contributions: LBC conducted the survey, collected samples, processed the samples, extracted nematodes, identified the nematodes, did statistical calculations and wrote the manuscript. NM helped in designing the survey and collection, in the identification of the nematodes, and in final proofing of the manuscript.

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INTRODUCTION

Forest conservation improves ecosystem functions and will help to protect natural biodiversity. Plant communities are the critical indicators for forest restoration. Below-ground diversity relates very closely with above-ground biodiversity. Plant parasitic nematodes are found in every soil of varied ecosystems. Nematodes are often sensitive to habitat disturbance, showing the characteristic sequence of recolonization after disturbance. Furthermore, they are represented in a wide array of trophic groups as herbivores (Tylenchid), bacterivores & fungivores (Aphelenchid), omnivores (Dorylaimid), and predators (Mononchid) reflecting resource availability and changes of environmental conditions in the soil providing information on succession and changes in decomposition pathways in the soil food – web, nutrient status & soil fertility, acidity, and the effects of soil contaminants (Yeates & Bongers 1999).

Bacterial as well as fungal feeding nematodes like aphelenchids have a high carbon: nitrogen (C:N) ratio (±5.9) than their substrate (±4.1), consuming bacteria, they take in more N than necessary for their body structure. The excess nitrogen is excreted as ammonia. The bacterial and fungal feeding nematodes' community in the top 15 cm of the field soil mineralizes N at rates increasing to 1.01µg - N g – soil -1d - 1 in the rhizosphere (Ferris et al. 1995; 1996; 1997).

Aphelenchid nematodes have diverse habitats. Several aphelenchids are associated with insects, some spending a part of their life - cycle in insects besides being phytophagous, while others are mycetophagous. Some forms of aphelenchids are true plant parasites and are, therefore, economically significant. Of the available aphelenchid nematodes, three species are major pests of agricultural and horticultural crops, i.e., white-tip nematode *Aphelenchoides besseyi*; Red-ring nematode of coconut *Rhadinaphelenchus cocophilus,* and pine-wilt nematode *Bursaphelenchus xylophilus* respectively in the world. So far, 138 species of *Aphelenchoides* (Fischer, 1894) have been identified, of which India contributed more than 12 species.

Recognizing the importance of bacterial and fungal decomposition in forest ecosystems, survey for Aphelenchid nematodes is very important in every region of the Earth. Recently, a survey was conducted on the *Aphelenchoides* nematodes in the different ecosystems of the north-eastern region of India. During the work, 12 *Aphelenchoides* were encountered from Manipur, among which six species were recorded for the first time from this particular region. The species encountered were *Aphelenchoides aligarhiensis* (Siddiqi et al., 1967); *A. baquei* (Maslen, 1978); *A. confusus* (Thorne & Malek, 1968); *A. minor* (Seth & Sharma, 1986); *A. swarupi* (Seth & Sharma, 1986); *A. vigor* (Thorne & Malek, 1968); *A. dhanachnadi* (Chanu et al., 2012) and *A. neoechinocaudatus* (Chanu et al., 2012); *A. aerialis* (Chanu et al., 2015); *A. longistylus* (Chanu & Mohilal, 2014); *A. neominoris* (Chanu & Mohilal, 2014), and *A. manipurensis* (Chanu & Mohilal, 2018). These species are presented along with their dichotomous keys.

METHODS

Study site

Manipur has a geographical area of 22,327 km^2, situated at an altitude of 790 m with a longitude of 24.733333333333334 and a latitude of 93.9666666666667 (Figure 1). Soil samples were collected from different localities of Manipur.

Methodology

For collection of soil samples around the rhizospheric region of a particular host, 500 g of soil around the plant from 8 different sides were taken. The soil was mixed together thoroughly. From the thoroughly mixed soil, again 500 g were taken, serving as the sample soil for a particular host plant or tree. The samples were processed for extraction of nematodes through the Cobb's (1918) sieving and decanting method and Baermann's funnel technique. Collected nematodes through the process were fixed with warm formalin alcohol (F.A) (4:1) for 24 hours and afterward, dehydrated under the Seinhorst (1952) dehydration techniques. Dehydrated nematodes were mounted on clean non-greased slides with dehydrated glycerin as mountants. The specimens were studied, measurements taken and diagrams were drawn using a drawing tube attached to a microscope.

RESULTS

SYSTEMATICS

***Aphelenchoides aeralis* Chanu et al., 2015**
(Figure 2, Table 1 & 3)

Diagnosis

Female: Body contour S-shaped with fine cuticular striations having a lateral field with two incisures. The cephalic region set off with weak sclerotization, spear 15.3 µm long with small basal thickenings. The median

Figure 1. Expanded map of Manipur.

oesophageal bulb is oblong with centered valvular apparatus. Oesophagous overlap intestine. Nerve ring behind the esophagus-intestinal junction. Excretory pore above nerve ring. Vagina with sphincter and raised vulval lips. Gonads monoprodelphic and oocytes are arranged in single rows. Spermatheca is filled with sperms and with uterine sac. Tail 42.5–48.2 µm long,conical, and with small single mucro.

Male: Body more curved at tail region. Testis long and outstretched. Spicule is typical of the genus. Dorsal limb without knob. The capitulum and rostrum very well developed. Post anal genital papillae, one pair situated above the tail terminus. Tail terminating into a long spine-like mucro.

Remark: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

Aphelenchoides aligarheinsis Siddiqi et al., 1967
(Table 1)

Diagnosis

Female: Body contour slightly curved. Cuticle fine, striations about 1.7 µm at mid-body. Lip region set-off round. Lateral fields with four incisures. Stylet slender 10.2 µm long with weakly developed basal knobs. Oesophagous typical of the genus. Excretory pore at level of the nerve ring. Ovary outstretched and oocytes arranged in single row. Spermatheca oblong with

Table 1. Morphometric data of female species of *Aphelenchoides* spp. from Manipur.

Character	*A. aeralis* Chanu et al., 2013	*A. aligar-hiensis* Siddiqi et al., 1967	*A. ba-guie* Maslen, 1973	*A. confusus* Thorne & Malek, 1968	*A. dhanach-adi* Chanu et al., 2012	*A.longistyl-us* Chanu & Mohilal, 2014
L	0.46–0.51	0.42–0.61	0.58–0.74	0.48–0.79	0.37–0.50	0.59–0.66
a	32.1–33.8	25.1–30.2	35.2–38.2	28.6–46.6	25.2–36.1	34.4–35.85
b	6.15–7.2	6.1–8.1	5.2–7.2	2.5–4.4	6.6–7.7	8.53–9.81
b´	3.66–4.1	-	3.6–4.2	-	3.3–7.4	6.03–6.49
c	9.8–10.1	11.4–16.1	14.2–16.2	23.8–38.8	5.3–7.4	13.67–14.58
c´	5–6.4	2.5–3	3.8–4.4	2–2.4	6.6–9.2	5.6–6.14
V	66.7–68.2	64.2–71.8	62.8–68	76.8–78.6	57.9–62.9	67.78–69.76
G_1	26.9–28.2	30.6–34.8	42.4–48.6	29–42.6	13.6–15.3	24.2
G_2	-	-	14–16.2	4.3–18.2	-	39.39–43.08
Spear	15.3	10.2	10.2–12.8	10.2–17	-	-
Oesophago-us	125.8	73.1–81.6	146.4–152	134.7–241.4	95.2–129.0	98.61–102.07
Nerve ring	68			90.1–119	56.1–73.1	-
Excretory pore	62.9	52.7	82.4–94.2	86.7–115.6	61.2–85.0	-
Tail	42.5	35.7–39.1	38.4–46.2	8.5–10.2	62.9–85.0	41.52–48.44
ABD	8.5	11.9–15.3			8.5–10.2	6.92–8.65
PUS	-	35.7–37.1	92.4–96.4	20.4	-	-

discoidal spermatozoa. The Uterine sac is five times body width in length. Tail 35.7–39.1 µm long, elongate-conoid, ventrally arcuate, rounded with spine like mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al., (2015).

Aphelenchoides baguei Maslen, 1978
(Table 1)

Diagnosis

Female: Body contour slightly ventrally curved. Fine cuticularisation with four incisures throughout body length. Cephalic region set off flattened. Spear 10.2–12.8 µm long with a small basal knob. Oesophagous typical of aphelenchoid. Mono-prodelphic reproductive system and ovary outstretched. Vulva is a transverse slit with slightly protruding lips. Oocytes in single row with a uterine sac. Tail 38.4–46.2 µm long, about 4–5 times anal body width long, terminus with a small ventral mucro which is multi-papillate almost to its tips.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

Aphelenchoides confusus Thorne & Malek, 1968
(Table 1)

Diagnosis

Female: Body contour gradually tapering near extremities. Cuticle with fine striations and lateral fields marked by four fine lines. Cephalic region set-off. Spear 10.2–17 µm long. Oesophagous typical aphelenchoid with massive valvular apparatus. Nerve ring is behind the oesophageal bulb. Excretory pore at level of the nerve ring. Hemizonid posterior to excretory pore.

Vulva with protuberant labia and vagina directed forward. Uterus spheroid-shaped, filled with sperm. Ovary outstretched and post uterine sac collapsed. Tail 20.4–25.3 µm long without a mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

Aphelenchoides dhanachandi Chanu et al., 2012
(Figure 3, Table 1)

Diagnosis

Female: Body contour slender ventrally curved. Lateral filed marked by three incisures. The body cuticle is fine. The cephalic framework is high. Spear 13.6–15.3 µm long, slender with indistinct basal knobs. Oesophagous typical with tamarind seed-shaped median bulb. Nerve ring behind the median bulb, 59.5–69.0

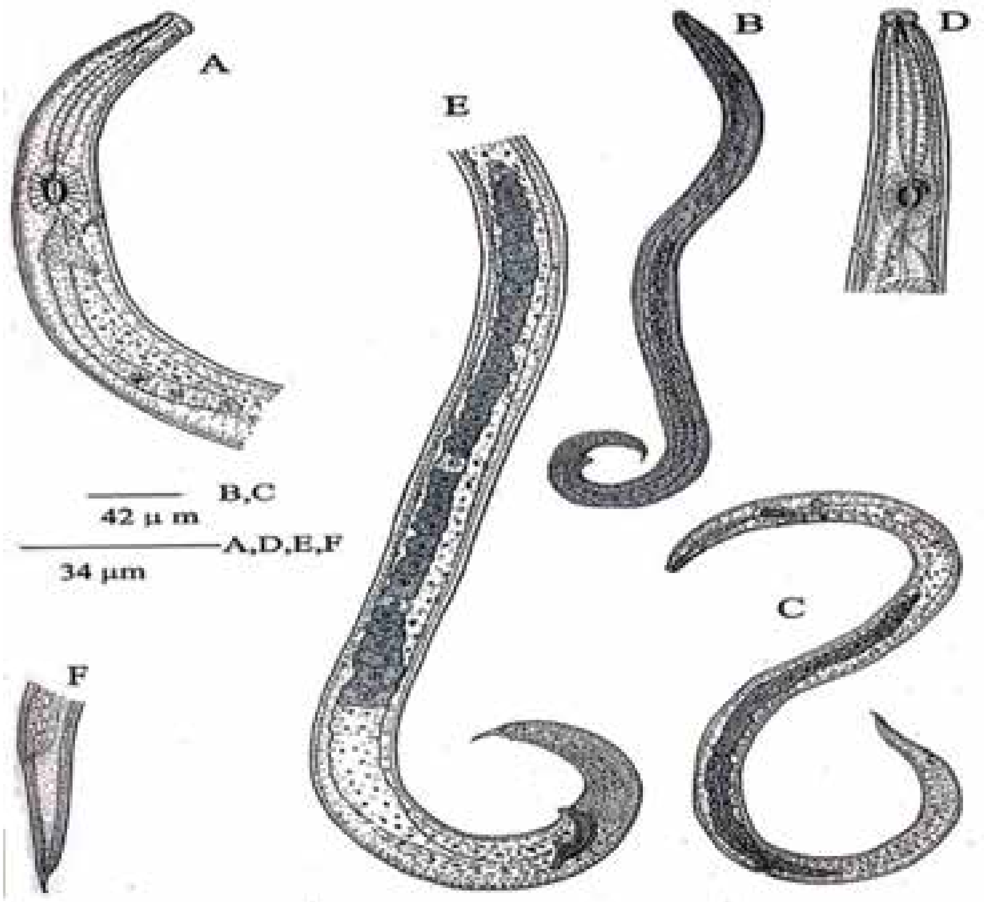

Figure 2. *Aphelenchoides aerialis* Chanu et al., 2013: A—Anterior part of Female body | B—Entire body of Male | C—Entire body of Female | D—Anterior body part of male | E—Posterior end of body (enlarged) | F—Tail region of Female.

µm long. Excretory pore at the level of the nerve ring. Oesophageal gland lying dorsally along the intestine.

Monoprodelphic reproductive system and oocytes arranged in a single row with uterine sac. Vulva protrudes in some species. Tail 62.9–98.0 µm long, highly curved ventrally tapering into a pointed terminus.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2012).

***Aphelenchoides longistylus* Chanu & Mohilal, 2014**
(Figure 4, Table 1 & 3)

Diagnosis

Female: Body contour slightly curved with fine annulation. Lateral fields with four longitudinal lines merge into two lines at around the tail region. The cephalic region indistinctly set off with six equal lips. Spear 24.22 µm long with indistinct basal knobs. Procorpus straight, median bulb spherical to pyriform in shape. Excretory pore at the base of the median bulb. The vulva is a transverse slit. The monoprodelphic reproductive system and oocytes are arranged in a single row. Uterine sac well developed. The tail gradually tapers into a cylindrical tube, terminating in a ventral prong tip.

Male: Slightly smaller than female. Tail slender with single terminal mucro. Spicules absent about 24.22 µm long. Testis single, 335.62–342.45 µm long.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2014).

***Aphelenchoides manipurensis* Chanu & Mohilal, 2018**
(Figure 5 & 6, Table 2 & 3)

Diagnosis

Female: Body contour cylindrical, ventrally arcuate, the cephalic framework set off and flat. Cuticle marked by fine annulus. Lateral fields with two incisures. Spear short, 10.33–13.84 µm long with small rounded basal knobs. The median bulb is spherical and basal bulb bifurcated. Excretory pore at 51.9–76.12 µm from anterior end of body. Nerve ring behind median bulb,

Table 2. Morphometric data of female species of *Aphelenchoides* spp. from Manipur.

Characters	*A. manipure-nsis* Chanu & Mohilal, 2018	*A. minor* Seth & Sharma. 1986	*A. neoechino-caudatus* Chanu et al., 2012	*A. neominoris* Chanu & Mohilal, 2014	*A. swarupi* Seth & Sharma, 1986	*A. vigor* Thorne & Malek, 1968
Length	0.294–0.461	0.28–0.36	0.53–0.60	0.35–0/43	0.52–0.68	0.44–0.49
a	24.28–38.14	18.2–26.2	25.5–28.6	36.29–40.6	34.4–42.4	24.8–29.3
b	6.3–8.65	4.8–7.2	8.3–9.0	4.23–6.51	5.8–8.2	2.7–4.8
b´	3.61–8.15	2.4–4.4	4.5–5.0	6.03–6.49	-	5.6–8.6
c	12.47–16.46	12.2–16.2	11.0–11.7	13.53–36.29	12.2–16.2	12.4–30.3
c´	3–4.25	2.2–2.6	5.3–5.4	1.75–3.75	2.2–2.6	1.5–3.5
V	68.88–71.69	66.4–72.2	64.4–64.9	69.95–71.65	66.4–72.2	69.2–88.6
Stylet	10.38–13.84	48.2–56.2	11.9	8.65	8.2–10.2	11.9–13.6
G_1	24.34–33.01	4.2–5.2	-	41.87–50.0	32.4–36.4	33.5–45.9
G_2	-	4.8–8.2	-	-	-	10.5–13.3
Oesophagous	-		119.0122.4	67.47–83.04	-	102–164.9
Nerve ring	-	-	66.3–68.0	-	-	74.8–88.4
Exc. Pore	-	47.6–54.6	62.9–64.6	-	-	61.2–78.2
Tail	20.76–31.14	22.4–30.8	45.9–54.4	12.11–25.95	32.3–37.4	15.3–35.7
ABD	6.92–8.65		8.5–10.2	6.92	8.5–12.4	10.2

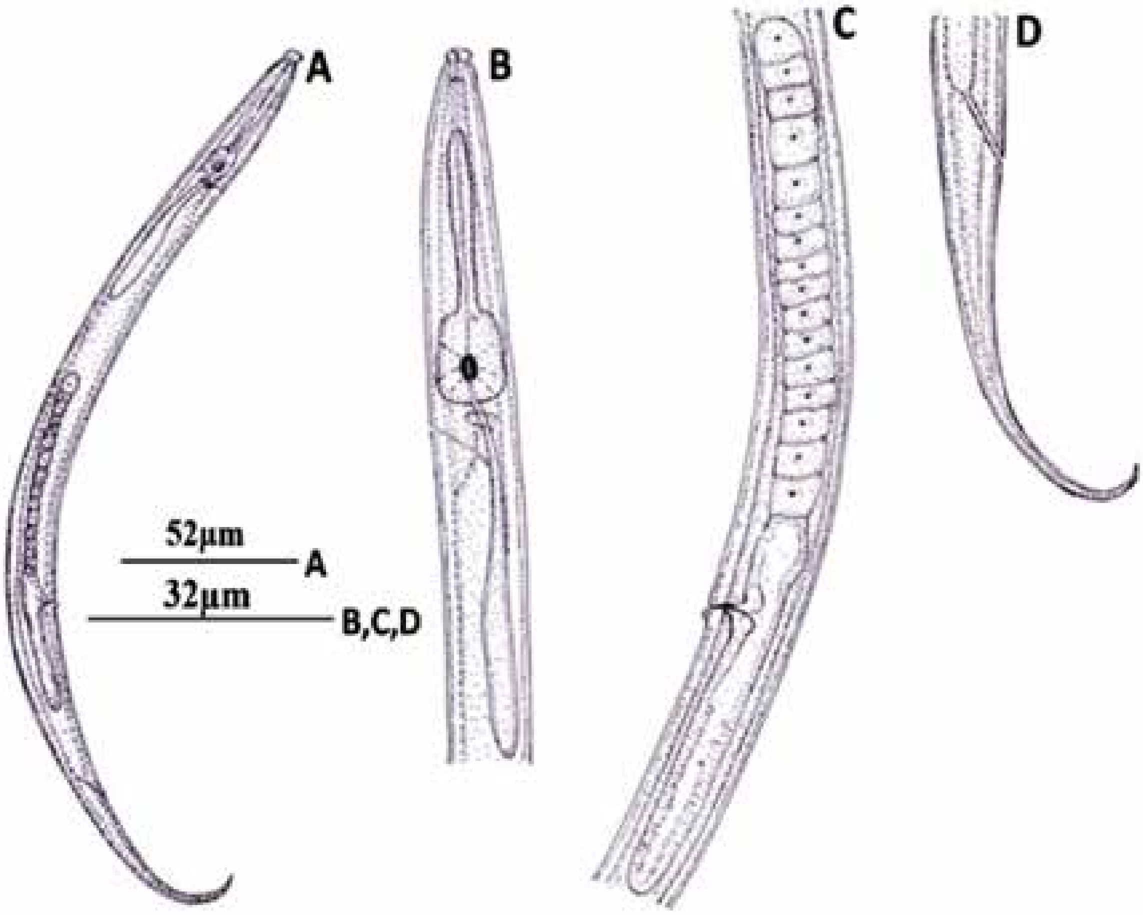

Figure 3. *Aphelenchoides dhanachandi* Chanu et al., 2012: Female A—Entire body | B—Anterior region | C—Reproductive system | D—Tail region.

ovary single, outstretched, and oocytes arranged in single row reaching the basal bulb. Spermatheca elongated oval with sperms. Uterine sac filled with sperms and ventral rounded tip. Vulva protuberant and vagina at right angle to the body. Tail curved ventrally with rounded tip with a small mucro at tip.

Male: Body ventrally curved. Tail conoid with mucronated lip. Testis 138.4–190.3 µm. spermatocytes in single row, spicules simply arcuate, rostrum rounded, and prominent apex. Three pairs of sub ventral papillae present towards tip of spicule. Bursa and gubernaculum absent.

Remarks: The morphometric details of the present species conform well with those described by Chanu & Mohilal (2018).

Table 3. Morphometric data of male species of *Aphelenchoides* spp. from Manipur.

Characters	*A. aerialis* Chanu et al., 2013	*A. longistylus* Chanu & Mohilal, 2014	*A. manipurensis* Chanu & Mohilal, 2018
L	0.46–0.51	0.562–0.62	264.69–320.05
a	29–29.41	38.2–42.0	25.5–33.39
b	5.9–6.92	7.24–8.33	25.59–41.52
b´	3.4	3.25–5.60	3.47–4.02
c	8.7	13.54–17.45	12.0–13.91
c´	4.2	4–6.2	2.4–6
T	46.71–47.8	92.61–102.84	50.89–61.09
Testis	46.71–47.8	335.62–342.45	138.4–190.3
Spicule	23.8–25.95	24.22	10.38–17.3
Tail	51.0–55.36	41.52–46.23	20.76–25.95
ABD	11.9–13.84	10.32	6.92–8.65
Stylet	15	24.22	10.38–13.84

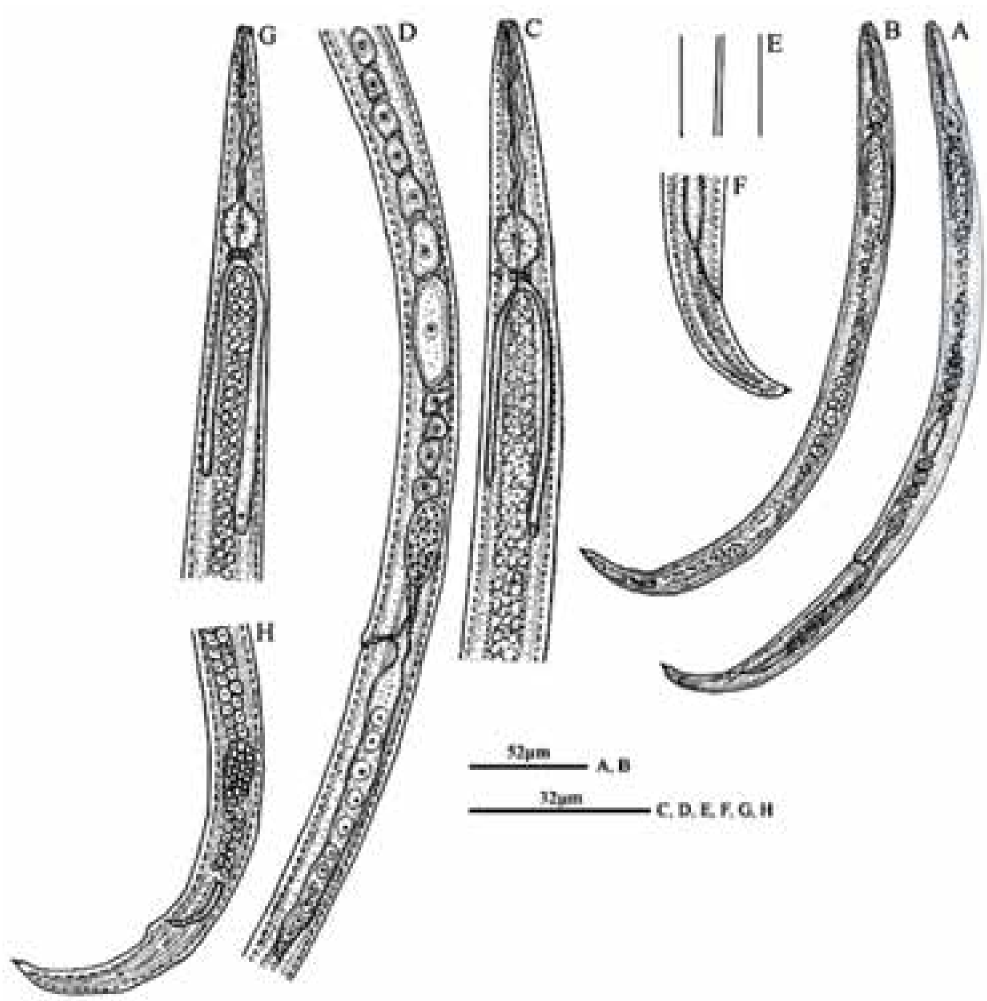

Figure 4. *Aphelenchoides longistylus* Chanu & Mohilal, 2014: Female. A—Entire body | C—Anterior body | D—Reproductive system | E—Lateral lines | F—Tail region. Male. | B—Entire body | G—Anterior body | H—Tail region.

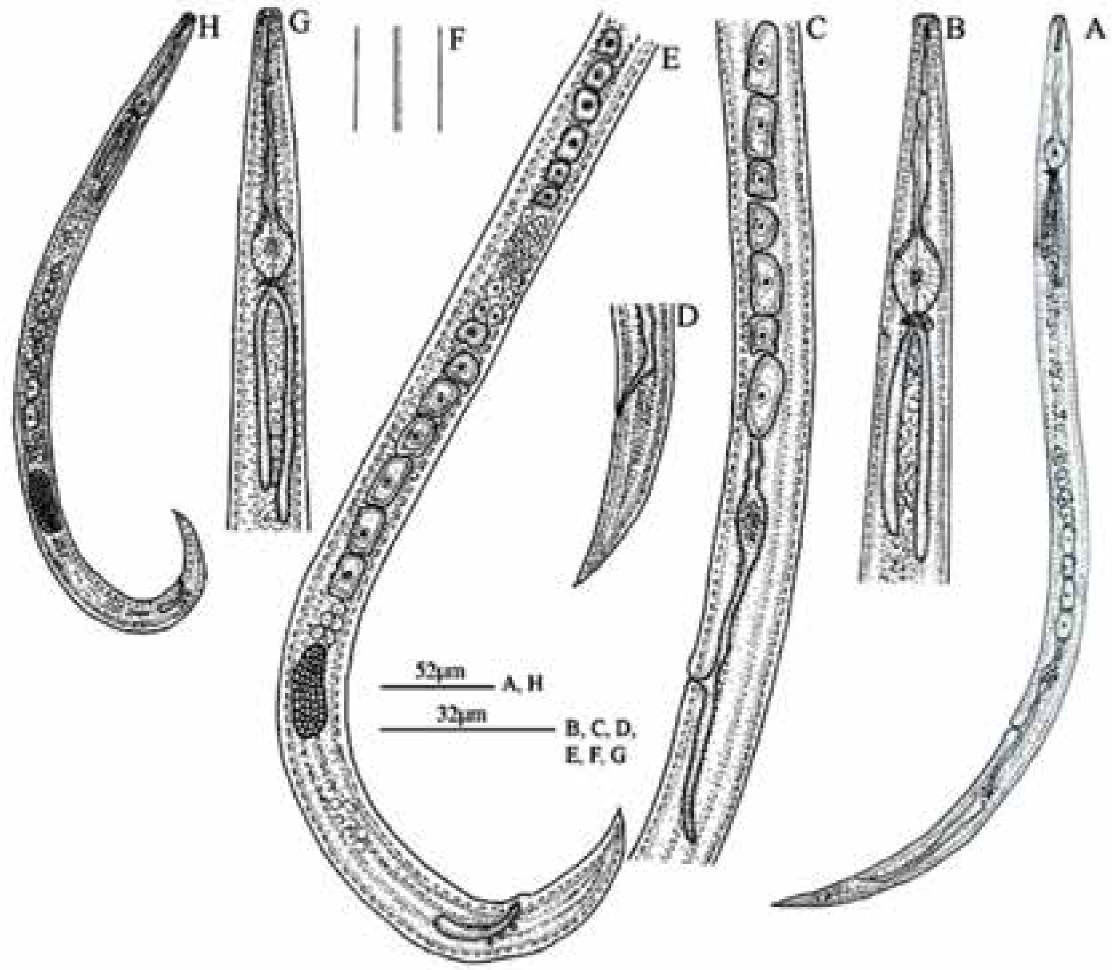

Figure 5. *Aphelenchoides manipurensis* Bina & Mohilal, 2018 from Manipur University Campus: Female. A—Entire body | C—Anterior body | D & I—Reproductive system (variation) | E—Body incisures | F—Anal region. Male. B—Male | Entire body | G—Anterior region | H—Posterior body.

Table 4. Species of *Aphelenchoides* spp. with their hosts and localities.

Species	Host	Locality
A. aerialis Chanu et al., 2015	Pine tree, *Pinus roxburghii* Sarg	Nongpok Sekmai, Thounal District
A. aligarhiensis Siddiqi et al., 1967	Pine tree, *Pinus kesiya* Royle	Khuga Dam, Churchandpur District
A. baguei Maslen, 1973	*Morus alba* Lin, *Pinus kesiya* Royle	Kakwa Naorem Leikai, Imphal West Distrcit; Keibul Lamjao, Bishnupur District
A. confusus Thorne & Malek, 1968	*Morus alba* Linn	Matai garden, Imphal East District
A. minor Seth & Sharma, 1986	*Morus alba* Linn	Kalika Village, Irilbung, Kyamgei, Imphal East District; C. I. College, Bishnupur district; Govt. Silkfarm Wangbal , Thoubal district; Regional Tasar Research Station, Chingmeirong, Imphal West district.
A. swarupi Seth & Sharma, 1986	*Morus alba* Linn, *Morus indica* Linn, *Pinus roxburghii* Sarg	Regional Tasar Research Station, Chingmeirong, Imphal West District; Bishnupur ward no. 4, Bishnupur district; Nongpok Sekmai, Thoubal District
A. vigor Thorne & Malek, 1968	*Morus indica* Linn	Bishnupur ward no. 4, Bishnupur District
A. dhanachandi Chanu et al., 2012	Mulberry plant, *Morus alba* L.	Yurembam Rose Garden, Imphal West District
A. neoechinocaudatus Chanu et al., 2012	Mulberry plant, *Morus alba* L.	Yurembam Rose Garden, Imphal West District
A. neominoris Chanu & Mohilal, 2014	Orange plants	Sibilong, Chandel district
A. longistylus Chanu & Mohilal, 2014	Coconut tree (*Cacos nucifera*)	Ninghsing Khul, Jiri, Imphal West District
A. manipurensis Chanu & Mohilal, 2018	Rooten wood lock	Manipur University campus, Canchipur.

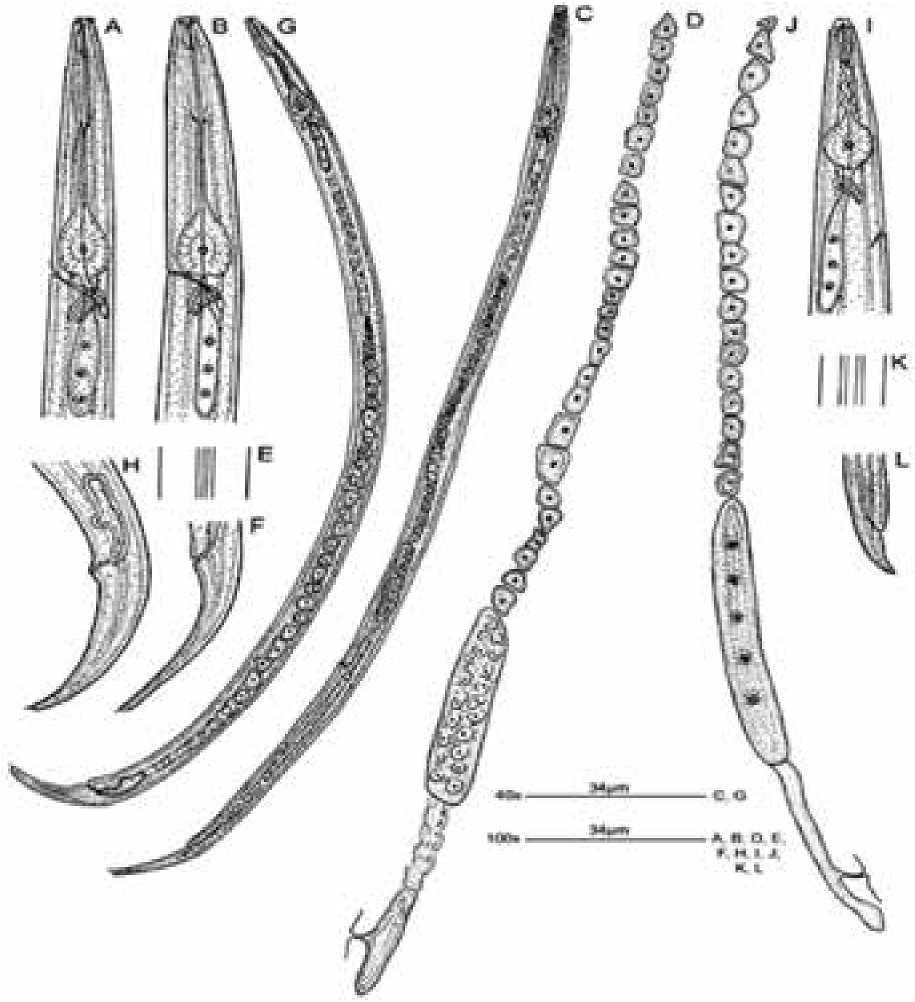

Figure 6. ***Aphelenchoides manipurensis*** **Bina & Mohilal, 2018 from Nongpok Sekmai Pine Reserve Forest. Female: A—Entire body | B—Anterior body | C—Reproductive system | D—Anal region | F—Body incisures. Male: E—Reproductive system | G—Anterior region | H—Entire body.**

Aphelenchoides minor Seth & Sharma, 1986
(Table 2)

Diagnosis

Female: Body contour straight to slightly curved ventrally. Lateral fields with three incisures. Cephalic framework set off without annulation. Spear 4.8–8.2 µm long with indistinct basal thickenings. Oesophagous is aphelenchoid type with squarish muscular median bulb with a flat base and crescentric wave. Excretory pore at level of nerve ring. Vulva, a transverse slit with prominent lips. Oocytes are arranged in single row with a uterus. Tail 22.4–30.8 µm long, rounded with ventral mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2018).

Aphelenchoides neoechinocaudatus Chanu et al., 2012
(Figure 7, Table 2)

Diagnosis

Female: Body contour is slender with three lateral incisures. The cephalic region is slightly set off. Stylet slender 11.9 µm long without basal swellings. Oesophagous typical, median bulb elongated pear-shaped. Nerve ring 66.3–68.0 µm long. Excretory pore at 62.9–64.4 µm, at the level of the nerve ring. The oesophageal gland was dorsal to the intestine. Reproductive system monoprodelphic and oocytes arranged in a single row, and uterine sac well developed. Tail 45.9–54.4 µm long, short, and pointed with a ventral mucro.

Male: not found

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2012).

Aphelenchoides neominoris **Chanu & Mohilal, 2014**
(Figure 8, Table 2)

Diagnosis

Female: Body contour straight, tapering towards both extremities with four incisures in lateral field. Cephalic framework smooth and set-off. Spear 8.65 µm long with distinct stylet knobs. Procorpus zig-zag, coiled, strongly rounded corpus with sclerotized plates and elongated gland lobe, dorsal to the intestine. The excretory pore is close to the nerve ring. Vulval lips protrude with an inclined vagina. Monoprodelphic reproductive system, oocytes are arranged in a single row reaching up to the oesophageal bulb. Spermatheca large elongated and uterine sac empty. The anterior lip of the anus protrudes, the tail bluntly rounded, 12.11–25.95 µm in length, with a small hair-like mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu & Mohilal (2014).

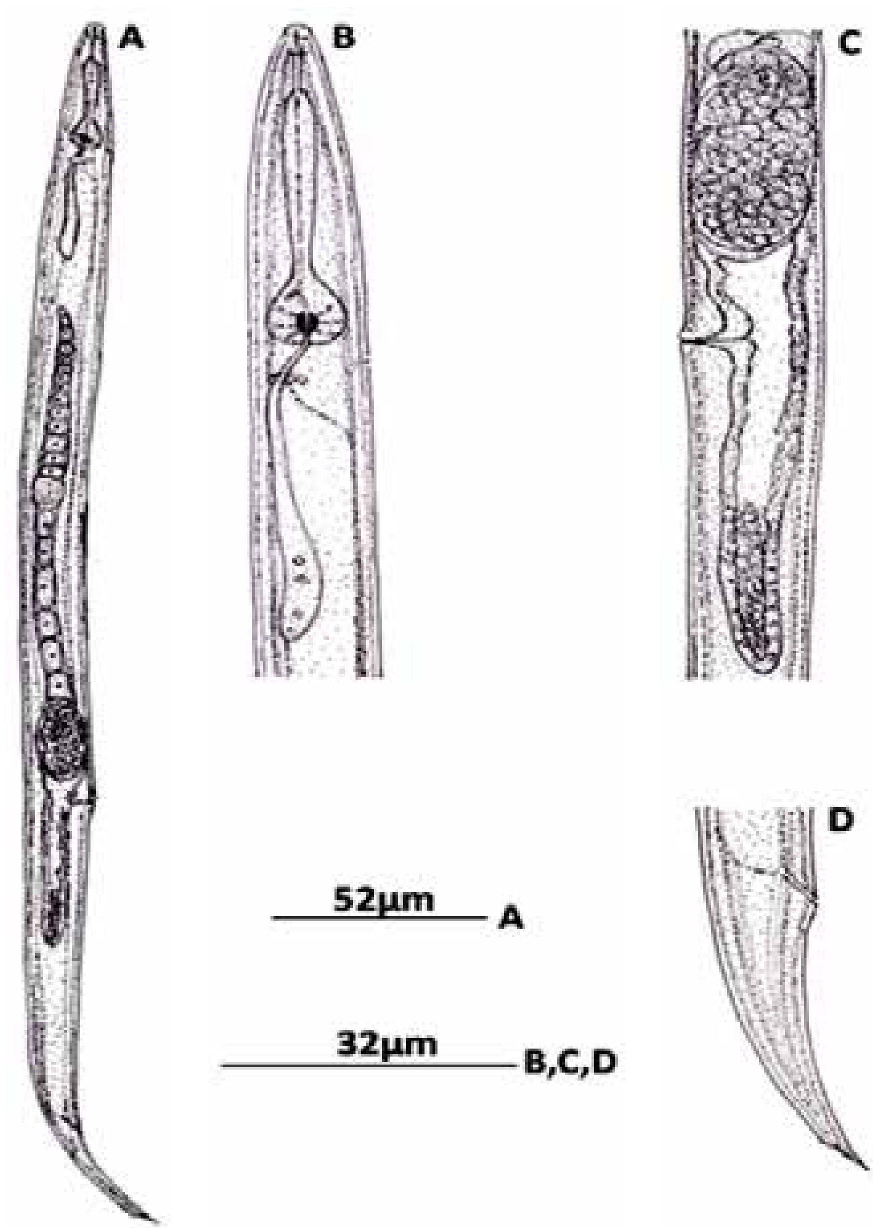

Figure 7. *Aphelenchoides neoechinocaudatus* Chanu et al., 2012. Female: A—Entire body | B—Anterior region | C—Reproductive system | D—Tail region. Photomicrographs: E—Anterior region | F—End bulb | G—Reproductive system | H—Tail region.

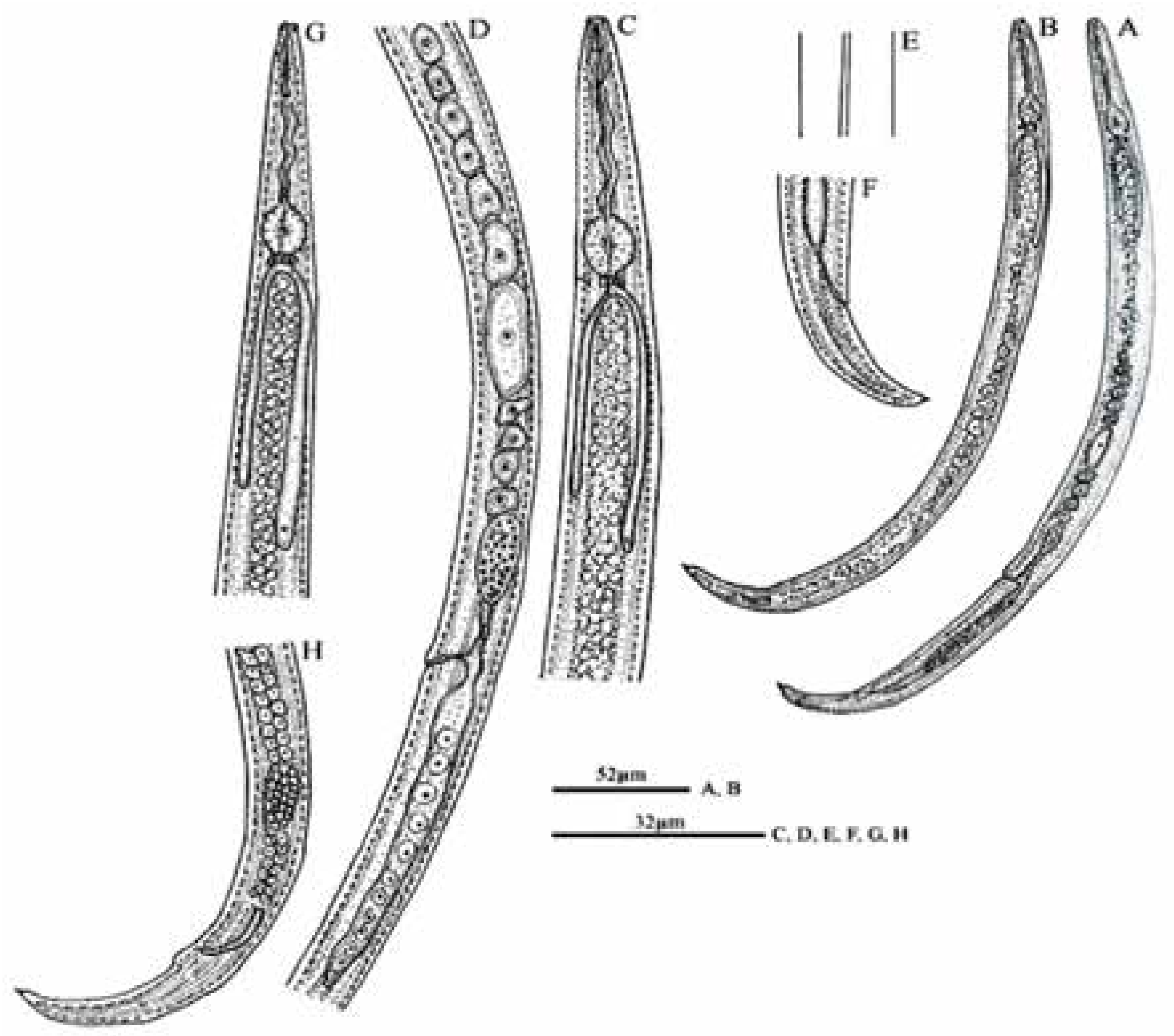

Figure 8. ***Aphelenchoides neominoris*** **Chanu & Mohilal, 2014; A—Female whole body |B—Male whole body | C—Anterior portion of female | D—Body portion showing reproductive organs of female | E—Lateral lines | F—Female tail region | G—Anterior body region of male | H—Posterior body region of male showing testes.**

Aphelenchoides swarupi Seth & Sharma, 1986
(Table 2)

Diagnosis

Female: Body contour cylindrical to slightly curved, with three incisures in the lateral lines. Cephalic region set off without annules. Spear 8.2–10.2 µm long with basal thickenings, procorpus muscular. Nerve ring at 72.8 µm from anterior body. Excretory pore ventral at level of nerve ring. Tail 32.8–37.4 µm long, bluntly rounded with a ventral mucro. Vulva a transverse slit with vulval lips. Post vulval uterine sac well developed.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

Aphelenchoides vigor Thorne & Malek,1968
(Table 2)

Diagnosis

Female: Body cylindrical with coarse annulations. Lateral filed with two incisures. The cephalic framework is set off by constriction. Spear 11.9–13.6 µm long with distinct knobs. Nerve ring at 74.8–88.4 µm long and excretory pore at 78.2 µm from the anterior body region. Vulva sclerotized, overlapping with jointed flap. Ovary outstretched, tubular uterine sac filled with sperms. Tail 15.3–35.7 µm long, arcuate, blunt tip without mucro.

Male: Not found.

Remarks: The morphometric details of the present species conform well with those described by Chanu et al. (2015).

The hosts and localities of all the species are mentioned in Table 4.

***Aphelenchoides oryzae* sp. nov.**

(Figure 9, Table 5)

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Material examined

Holotype: Collected on August, 2015 from paddy fields (*Oryza sativa* L. growing field) by L. Bina chanu, from Thoubal Khekman, Thoubal District, Manipur with a longitude of 24.5036 and latitude of 93.9116. The specimen is deposited on nematode collection of Parasitology Section, Centre for Advanced Study in Life Sciences, Manipur University, Canchipur, Manipur under the Voucher no. ZoDMU_MN02 with holotype female on slide FTY_4 ♀1.

Paratype: Females on slides FTY_4 ♀2-12 and males on the slides FTY_4 ♂1-7, same data as holotype.

Descriptions

Holotype female: Body straight, cylindrical, and robust upon fixation, 685.08 µm long. Lip region offset with rounded sides & flattened anteriorly, 5.19 µm wide and 1.7 µm high, and smooth in appearance. Body elongate with fine transverse annulations, 0.8 µm at mid body region. Lateral lines extend almost to tail tip with two ridges having four evenly spaced lines in the middle of the body.

Stylet is slender, 17.3 µm long, the conus slightly shorter than the shaft with indistinct swellings. Median oesophageal bulb rounded to slightly oval with the refractive thickenings usually placed centrally, 13.84µm

Table 5. Morphometric data of species of *Aphelenchoides oryzae* sp. nov. All measurements in µm except L in mm.

Characters	Holotype female	Paratype females	Paratype males
n	1	18	7
Length	685.08	569.17–750.82 (675.045±58.74)	536.3–615.88 (576.09±39.79)
a	44	36.55–44 (42.39±2.93)	34.44–39. 55 (36.99±2.65)
b	7.33	6.18–7.33 (6.72±0.51)	5.74–5.83 (5.78±0.04)
b´			-
b_1	9.65	9.65–11.12(10.26±0.55)	7.75–9.12 (8.43±0.68)
c	36	16.45–36 (25.70± 8.59)	15.5–19.77 (17.63±2.13)
c´	1.83	1.83–4 (3.144±0.94)	2.57–4 (3.28±0.71)
T	-	-	81.86–93.54 (94.24±0.69)
V	69.44	68.20–70.7 (69.65±0.89)	-
G_1	49.24	29.95–49.24 (43.55±7.66)	-
Post. Uterine sac	46.71	46.71–86.5 (62.97± 15.61)	-
PVS/ V-A%	24.54	24.54–51.02 (34.65±14.15)	-
Oesophagous	93.42	91.69–114.18 (101.2±8.91)	91.69–114.18 (101.2±8.91)
Stylet	17.3	17.3	17.3
Lip width	5.19	5.19	5.19
Lip height	1.73	1.73	1.73
Median bulb length	13.84	10.38–13.84 (12.68±1.28)	10.38–13.84 (12.68±1.28)
Median bulb diam.	8.65	6.92–8.65 (7.49± 0.81)	-
Spicule	-	-	15.57–17.3(16.43±0.86)
Ovary	337.35	224.9–337.35 (293.05±54.2)	-
PUS/ VBD	3	3–6.25 (4.27±1.2)	-
Nerve ring	74.39	69.2–76.12 (72.94±2.72)	69.2–76.12 (72.94±2.72)
Excretory pore	72.66	72.66–77.85 (744.04±2.01)	72.66–77.85 (744.04±2.01)
Spermatheca	29.41	29.41	-
Rectum	8.65	5.19–8.65 (7.26± 1.69)	-
Tail	19.03	19.03–41.52 (29.41± 8.59)	31.14–34.6 (32.87±1.73)
ABD	10.38	8.65–10. 38 (9.51± 0.94)	8.65–12.11 (10.38± 1.73)

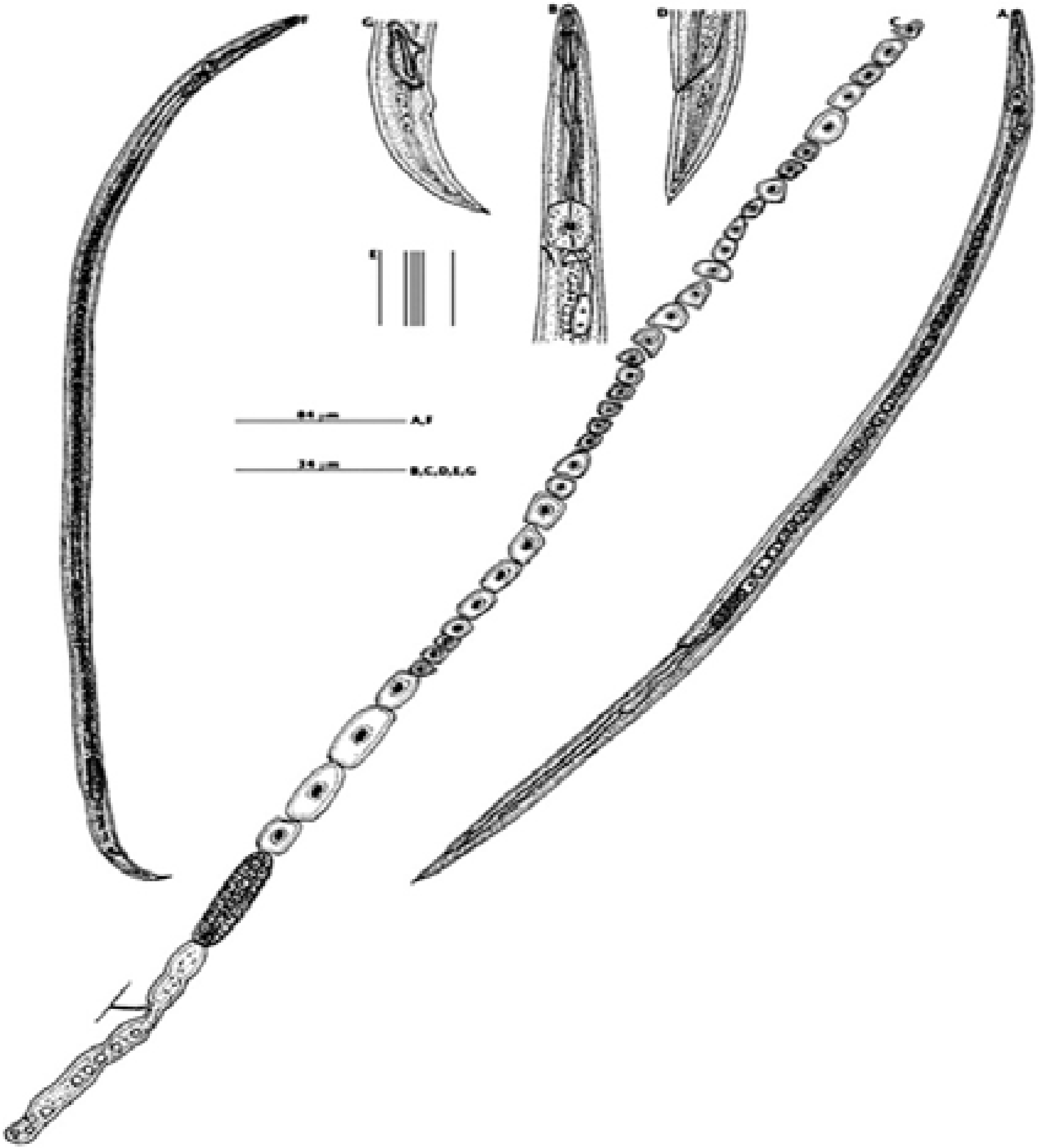

Figure 9. *Aphelenchoides oryzae* sp. nov.: Female, A—Entire body length | B—Anterior region | C—Female reproductive system | D—Tail region | E—Lateral lines |Male, F—Entire body length | G—Tail region.

high and 8.65µm across in length. Excretory pore and nerve ring, one to two body widths posterior to median bulb, excretory pore at 72.66µm from the anterior end and nerve ring at 74.39µm from the anterior end of the body. Oesophageal glands overlap the intestine.

Vulva is at about two thirds of the body length from the anterior end. Reproductive system with a single anterior ovary, oocytes arranged in single rows, with spermatheca, 29.41µm long filled with sperms and a prominent post-vulval uterine sac which usually extends just half the distance from the vulva to the anus. Post uterine sac is about 46.71µm in length.

Tail convex-conoid, 19.03µm long and straight, usually 10.35 µm anal body widths long with a rounded tail tip bearing a small terminal mucro.

Paratype males: Lip region, stylet and oesophagous similar to female. Tail ends curls ventrally through 45–90° when killed by heat and usually with simple terminal mucro. Spicules well developed; 15.57–17.3 (16.43 ± 0.86) µm long, the dorsal limb smoothly curved in its proximal half but flattened to concave tip; the ventral limb appears much weaker than the dorsal limb. The

Table 6. Characters differentiating *Aphelenchoides oryzae* sp. nov. from other related Aphelenchoides species.

***Aphelenchoides* spp.**	**Character differentiation**
A. blastophthorus Franklin, 1952	Shorter stylet (15–16 µm) with prominent knobs
A. brassicae Edward & Misra, 1969	Excretory pore opposite median bulb base, female tail, shorter (c´=3) with longer mucro, spicules more smoothly curved.
A. baguei Maslen, 1979	The female tail is ventrally concave with a longer mucro, and spicules more smoothly curved with a more prominent apex.
A. hamatus Thorne & Malek, 1968 [After Vovlas, 1982]	Shorter stylet (12–13 µm), female tail ventrally curved with a ventral mucro, spicule larger
A. helophilus (de Man, 1880) Goodey, 1933	Female body length over 1mm, stylet 14–16 µm with prominent knobs, spicules smoothly curved
A. lanceolatus Tandon & Singh, 1974	Lip region continuous, stylet shorter (12.5–13 µm), female body thinner (a=33), shorter post–vulval sac, spicules smoothly curved
A. lichenicola Siddiqi & Hawksworth, 1982	Female body was shorter (L=610 µm), female tail longer (c´=3.5), spicules were characteristically swollen near the distal end of the dorsal limb
A. lilium Yokoo, 1964	Excretory pore a body width posterior to nerve ring, shorter stylet (12.5 µm), shorter post–vulval sac, female tail ventrally curved, spicules smoothly curved
A. saprophilus Franklin, 1957	Shorter female body (L= 546 µm), ventrally curved female tail and larger spicules
A. sexlineatus Eroskenko ,1967	Shorter stylet (9 µm), shorter female body (L = 605–645 µm), longer post vulval sac, female tail with a longer mucro.
A. submerses Truskova, 1973	Lip region narrower than the adjacent body; female tail more curved ventrally; excretory pore anterior to median bulb
A. suipingensis Feng & Li, 1986	Female with the thinner body (a=32); excretory pore opposite median bulb base; female tail ventrally curved with hair-like mucro
A. Tumulicaudatus Truskova, 1973	Lip region not offset, post vulval sac shorter; female tail with characteristic terminal swelling.
A. nechaleos Hooper & Ibrahim, 1994	Female body longer (L=10.5–11.5 mm); shorter stylet (10.5–11.5 µm) and shorter tail (3.9–4.6 µm)
A. paranechaleos Hooper & Ibrahim, 1994	Longer body (631–860 mm); shorter stylet (9.5–10.5µm); thinner body (a=37–46) and shorter tail (c´=2.6–3.6)

rostrum and apex are moderately developed; a tangent drawn from the apex to the spicule tips is separated from the tangent from the apex through rostrum.

Etymology: The species name is derived from the host plant.

Diagnosis and Relationships

Aphelenchoides oryzae sp. nov. is characterized by narrow cylindrical body, adults being 569.17–750.82 (675.04±53.74)µm long with a stylet about 17.3 µm long with indistinct basal swellings, a prominent median bulb and short end bulb, 4 lateral lines throughout the body, and tail straight, convex-conoid with a simple terminal mucro.

Males are common and functional with prominal spicules with dorsal limb flattened to indent in its distal half and the tip curled ventrally. Lateral fields of adults usually with four lines.

In view of its association with paddy plants, *Aphelenchoides oryzae* sp. nov. might be confused with *Aphelenchoides besseyi* Christie, 1942 the rice nematode. However, *Aphelenchoides oryzae* sp. nov. is separated from *A. besseyi* Christie, 1942 in having a single, simple, tail mucro instead of three–four processes as in *A. besseyi*. The present species also has longer oesophagus and stylet than *A. besseyi* Christie, 1942 whereas oesophagus ranges from 64–68 µm and stylet 10.0–12.5 µm in *A. besseyi* Christie, 1942.

Aphelenchoides oryzae sp. nov. differed from other species of *Aphelenchoides* in having a female body length of 569.17–750.82 (675.04 ± 58.74) µm, with a slender stylet length of 17.3 µm and a convex conoid tail with a simple terminal mucro with four lateral lines along the whole body length.

The differences of characters between closely related species of *Aphelenchoides* is provided in Table 6. Based on these morphometric differences the present species is reported as new to science.

The Shannon - Wiener species diversity index and Evenness for all the mentioned species are given in the table 7.

CONCLUSIONS

The richness of the species in the region may be due to warm climatic conditions, suitable habitats, and hosts as well as due to the absence of drastic changes in the climatic conditions during the past few years. But the present work could not cover all the varied ecosystems of Manipur. Since, nematodes are soil dwelling living around the roots of plants as well as plant parasitic

Table 7. Shannon-Wiener species diversity index and evenness of the species of *Aphelenchoides*. Shannon-Wiener index is denoted by *H* and evenness by *E*. Total number of species is 255 and the Shannon-Wiener species index is 3.783.

Species	No. of individuals (*n*)	Proportion, *pi=n/N*	*ln(pi)*	*pi ×ln(pi)*	*H =* -∑[(*pi*)×ln(*pi*)]	*lnk*	*E=H / ln(k)*
A. aeralis Chanu et al., 2015	20	20/40=0.5	-0.6931	-0.346	0.346	3.688	0.093
A. aligarheinsis Siddiqi et al., 1967	5	5/35=0.1428	-1.9463	-0.277	0.277	3.555	0.077
A. baguei Maslen, 1973	18	18/90=0.2	-1.609	-0.321	0.321	4.499	0.071
A. confusus Thorne & Malek, 1968	10	10/32=-0.3125	-1.1631	-0.321	0.321	3.465	0.092
A. dhanachandi Chanu et al., 2012	12	12/53=0.2264	-1.485	-0.336	0.336	2.484	0.135
A. longistylus Chanu & Mohilal, 2014	17	17/46=0.3695	-0.995	-0.367	0.367	3.850	0.095
A. manipurensis Chanu & Mohilal, 2018	38	38/58=0.6551	0.422	-0.277	0.277	4.060	0.068
A. minor Seth & Sharma, 1986	51	51/200=0.255	-1.366	-0.348	0.348	5.298	0.065
A.neoechinocaudatus Chanu et al., 2012	4	4/53=0.0754	-2.5849	-0.194	0.194	3.970	0.048
A. neominoris Chanu & Mohilal, 2014	11	11/47=0.2340	-1.452	-0.339	0.339	3.850	0.088
A. swarupi Seth & Sharma, 1986	36	36/143=0.251	-1.382	-0.346	0.346	4.962	0.069
A. vigor Thorne & Malek, 1968	7	7/38=0.1842	-1.6917	-0.311	0.311	3.687	0.084
A. oryzae sp. nov.	26	26/43=0.4651	-0.7655	-0.356	0.356	5.366	0.074

Key to the species of *Aphelenchoides* spp. from Manipur

1. Cephalic region set-off from body 2
   Cephalic region not set-off from body .... *A. longistylus* Chanu & Mohilal, 2014
2. Lateral fields with 2 incisures 3
   Lateral fields with 3–4 incisures 6
3. Basal bulb elongated 4
   Basal bulb bifurcated .... *A. manipurensis* Chanu & Mohilal, 2018
4. Vagina with lips .... *A. aerialis* Chanu et al., 2015
   Vagina without lips 5
5. Distinct spear knob, spear 11.9–13.6 µm long, arcuated blunt tail .... *A. vigor* Thorne & Malek, 1968
   Indistinct spear knob, spear 17.3 µm long, tail convex-conoid with blunt tip .... *Aphelenchoides oryzae* sp. nov.
6. Lateral fields with 3 incisures 7
   Lateral fields with 4 incisures 10
7. Tail tip pointed 8
   Tail tip bluntly rounded 9
8. Spear 4.8–8.2 µm, indistinct knob, tail 22.4–30.8 µm .... *A. minor* Seth & Sharma, 1986
   Spear 8.2–10.2 µm, distinct knob, tail 32.8–37.0 µm long .... *A. swarupi* Seth & Sharma, 1986
9. Tail with a mucro .... *A. neoechinocaudatus* Chanu et al., 2012
   Tail without a mucro .... *A. dhanachandi* Chanu et al., 2012
10. With simple single tail mucro 11
    With multi-papillated tail mucro .... *A. baguei* Maslen, 1978
11. Uterine sac collapsed/absent .... *A. confusus* Thorne & Malek, 1968
    Uterine sac well-developed 12
12. Body contour curved, weak knob, tail elongated conoid with spine like mucro ....
    .... *A.aligarhiensis* Siddiqi et al., 1967
    Body contour straight, tapering at extremities, knob distinct, tail blunt conoid with hair like mucro ....
    .... *A. neominoris* Chanu & Mohilal, 2014

forms, there is potential for availability of the organism in various other plant and tree varieties in different ecosystems of Manipur. There are various forest ecosystems found in Manipur with varied tree species along the Himalayan range. Further survey and in-depth taxonomic works incorporating molecular taxonomic techniques can reveal the rich diversity of the nematode group in Manipur.

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COMMUNICATION

Efficacy of levamisole and oxyclozanide treatment on gastrointestinal nematodes of ungulates at the Central Zoo, Nepal

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Abstract: The efficacy evaluation of levamisole and oxyclozanide treatment on the gastrointestinal nematodes of ungulates at the central zoo, Nepal was carried out from June—August 2021. A total of 40 fecal samples were collected from 10 species of ungulates from the central zoo for determining the efficacy of the anthelmintic given at day 0 of pretreatment and post-treatment analysis on day 07 and day 14. The concentration method (floatation concentration) was used for the microscopic examination of eggs, and quantitative examination (EPG) of nematode eggs was carried out with the help of modified McMaster slides. The identification was done using an optic micrometer and fecal egg culture. Anthelmintic resistance status was evaluated by the Fecal Egg Count Reduction Technique (FECRT) based on the method described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines and with the Bayesian hierarchical model. Out of 40 samples, nematode prevalence was found to be 68%, in which single infection was detected in 48% and double infection in 52%. The efficacy of Zanide L forte (levamisole-0.75 g and oxyclozanide-1.00 g) was found to be 85% (UI 80-89) at day 07 and 89% (UI 85-92) at day 14 by using Hierarchical Modelling of Fecal Egg count based on 'eggCounts-2.3 on R version 3.6.1 and 86% (CI 61.51–95%) at day 07 and 90% (CI 74.18–95%) at day 14 by WAAVP guidelines. This study represents the first documented case of ineffectiveness of anthelmintic treatment resulting in anthelmintic resistance in the central zoo. Thus, there is a requirement for a suitable and efficacious anthelmintic program.

Keywords: Anthelminthic, captive wild ungulates, efficacy, FECRT %, nematodes.

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Author contributions: PK—conceptualization, lab works, manuscript writing, editing, data compilation and analysis. AS, PJT & CPP—conceptualization, field methodology, review and editing.

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INTRODUCTION

Zoos are centers in which wild animals are kept for aesthetic, educational and conservation purposes (Thawait et al. 2014). The Central Zoo of Nepal was established in 1932. Ungulates cover the major population of the zoo animals in the Central Zoo, which includes Spotted Deer, Sambar Deer, Four-horned Antelopes, Himalayan Goral, Blue Bull, Barking Deer, One-horned Rhinoceros, Wild Boar, and Wild Water Buffalo.

Parasitic infection is one of the causes of morbidity and mortality in captivity, along with improper diet and poor husbandry practice (Singh et al. 2006; Mir et al. 2016; Kolapo & Jegede 2017). In the wild, animals generally have a natural resistance to parasites due to their diverse habitat and food, but due to the confinement and change in living conditions, captive wild animals might be more susceptible to many diseases caused by viruses, bacteria, rickettsia and parasites (Goossens et al. 2005; Thawait et al. 2014).

Nematodes are generalist parasites of a wide range of hosts (Walker & Morgan 2014). Generally, ungulates are infected by nematodes by ingesting infective larvae from the pasture, and in some species, larvae also penetrate through the skin (Walker & Morgan 2014). Zoo ruminants are particularly vulnerable to gastrointestinal nematodes due to high stocking density without the possibility of pasture rotation, leading the pasture to heavy exposure to infective nematode larvae or eggs (Goossens et al. 2006).

The epidemiology of nematodosis in domestic ruminants is well studied, but there are limited studies and reports that directly address parasite control programs in captive wild ruminants (Isaza et al. 1990; Goossens et al. 2006). Regular parasite load examination, anthelmintic efficacy, and resistance evaluation are not frequently done in many zoological gardens and parks. Furthermore, there is no published data on the efficacy of anthelmintics in captive wild ungulates in Nepal. Fecal egg count reduction (FECR) is the simplest, most effective, and most widely used method to evaluate the efficacy of anthelmintics (Coles et al. 1992; Cabaret & Berrag 2004) and has been used in captive wild animals (Nalubamba & Mudenda 2012; Pawar et al. 2020). Anthelmintic resistance is becoming a threatening issue in every livestock class and in every anthelmintic class globally (Kaplan 2004). Idiosyncrasies are also one of the major factors that contribute to the efficacy of anthelmintics on different wild animals on certain occasions (Ortiz et al. 2001). Thus, this present study will aim to contribute to establishing the prevalence of gastrointestinal nematode parasites and the anthelminthic efficacy of oxyclozanide and levamisole administration in the ungulates in Central Zoo, Nepal.

MATERIALS AND METHODS

Time and place of research

The research was carried out at the Central Zoo from 19 June 2021 and ended on 19 August 2021.

Sample collection

Pooled fecal samples were collected from the fresh feces of the ungulates early in the morning from different spots of the enclosure with the help of zoo keepers. The fresh sample was randomly taken on the basis of the number of ungulates in each enclosure. The sample was labelled accordingly and the method was followed as per Soulsby (2005). The sample of around 15 gm was kept in an airtight plastic zipper bag and transported in a cool box to the laboratory at the Department of Animal Science, Institute of Agriculture and Animal Science (IAAS), Tribhuvan University. Macroscopic examination of the helminths, if present, was done from the feces. The concentration method (floatation concentration) was used for the microscopic examination of eggs, and quantitative examination of eggs was carried out with the help of modified McMaster slides. The size of the eggs was measured using an optic micrometer. The sample containing more than one species of nematodes were kept as mixed infection sample and while samples with only one species were labelled as single infection.

For further confirmation, the fecal culture method using the 'Falcon tube method of fecal culture' for nematode larva was also carried out in accordance with the method provided by Soulsby (2005) and Zajac & Conboy (2012). One gram of feces was wrapped up in a blotting paper making a pouch. In a falcon tube, water was placed up to the circular rim at the distal end of the tube. The pouch was attached to the distal interior end of the Falcon tube using a long piece of the same blotting paper. The tube was now made airtight and left in a dark place for up to 7–8 days for incubation of nematodes larvae. After about 7–8 days, the blotting paper and the sample were removed. Twenty microliters of water was transferred to a glass slide with the help of a micropipette, which was then examined for the larvae of nematode under a microscope.

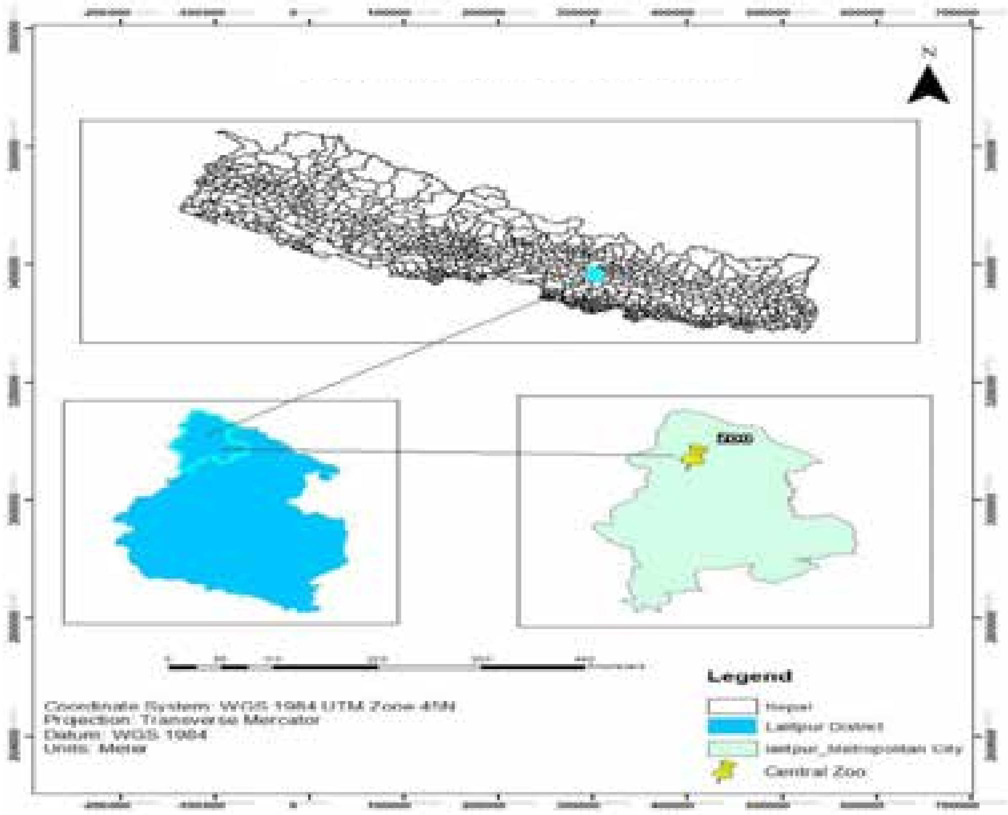

Figure 1. The location of the Central Zoo, Nepal.

Assessment of drug efficacy and anthelmintic resistance

Anthelmintic resistance status was evaluated by FECRT based on the method described by the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles et al. 1992; McKenna 1994; Storey 2015). The FECRT has been the most recommended method so far being broadly utilized for field or research studies (Coles et al. 1992). FECRT assesses the anthelmintic resistance of a given compound by comparing worm egg counts from animals before and after treatment.

All the individuals who were positive for nematode eggs were subjected to EPG on day 0 before treatment, day 07 and day 14 after treatment.

$$FECR\ (\%) = 100\ \%*(1-[T2/T1])$$

Here, T1 is the pre-treatment EPG

T2 is post-treatment EPG.

(Coles et al. 1992; McKenna 1994; Cabaret & Berrag 2004; Pawar et al. 2020)

Resistance is present when two criteria are met:

I. The percentage reduction in egg count is less than 95%.

II. The lower limit of its 95% confidence interval is equal or below 90%.

Treatment

At 10 mg per kg, ZANIDE- L Forte Bolus (Levamisole Hydrochloride BP 0.75 g and Oxyclozanide BP (Vet) 1 g) was given to the ungulates. There was a specific deworming practice at the zoo of changing the anthelminthic drug types regularly at the interval of 4-months. Ivermectin was used 4-months prior to this research and four months before Ivermectin, albendazole was used. So, this time it was the turn of ZANIDE- L Forte Bolus (Levamisole Hydrochloride BP 0.75 g and Oxyclozanide BP (Vet) 1 g). So, in accordance with that schedule, ZANIDE-L Forte was used. This research showed the deworming status of levamisole and oxyclozanide in the nematodes. After the determination of pre-anthelmintic EPG at Day 0, the post-anthelmintic EPG at Day 07 and Day 14 were also determined using the same procedure as mentioned earlier. The mean EPG of Day 7 and Day 14 is used to determine the efficacy of the respective days.

Data analysis

Fecal egg count in EPG is determined from a sample taken on day 0 prior to treatment with an anthelmintic drug, as well as on days 07 and 14 following treatment. The data were entered into a spreadsheet and imported into IBM SPSS version 25 to test for statistical significance.

Egg count data on FECRT was analyzed for fecal egg count reduction (%FECR) using 'eggCounts-2.3' on R version 3.6.1. (Young et al. 2000; Torgerson et al. 2014; Wang et al. 2018)

For analysis of drug efficacies, a 'z'-test (Sample size > 30) was done to analyze the significance of the pretest and the posttest group on different days. Similarly, to determine the association within different groups, a chi-square test was done.

A p-value of less than 0.05 at 95% CI was considered statistically significant. Finally, tables and charts were used to present the results generated from SPSS and the graphical presentation was completed in MS Excel 2016.

RESULTS

During the study, out of 40 samples examined by the floatation concentration method, 27 samples were positive for the presence of nematode eggs as given in Table 1. Thus, the prevalence was found to be 67.5%. Single parasitic infection was detected in 13 (48.15%) and mixed parasitic infection in 14 (51.85%) samples. The intensity of eggs belonging to eight different types of nematodes, i.e., *Bunostomum* spp., *Strongyloides* spp., *Trichuris* spp., *Ostertagia* spp., *Haemonchus* spp., *Capillaria* spp., *Ascaris* spp., and *Oesophagostomum* spp., varied from + to +++ in the study. The eggs were identified on the basis of their sizes using the calibrated optic micrometer (Soulsby 2005). Further confirmation was done by the fecal culture method with reference to Soulsby (2005) and Zajac & Conboy (2012).

The *Strongyloides* spp. were major nematode eggs seen during the study with 44.44% prevalence, followed by *Bunostomum* spp. 22.22% and *Trichuris* spp., *Ostertagia* spp., *Haemonchus* spp., *Capillaria* spp., *Ascaris* spp., *Oesophagostomum* spp., with 5.56% each as shown in Figure 2.

The efficacy of Zanide L forte (levamisole-0.75 g and oxyclozanide-1.00 g) was found to be 85.3% (CI 80.4–89) at day 07 and 89.2% (CI 85–92.3) at day 14 by using hierarchical modelling of fecal egg count based on 'eggcounts-2.3 on R version 3.6.1 and 85.47% (CI 61.51–94.48%) at day 07 and 89.67% (CI 74.18–95.61%) at day 14 by WAAVP guidelines.

Since, the P value is less than 0.05 in both the days, the pretest at day 0 and post test data at day 07 and day 14 are statistically significant respectively. So, we reject the null hypothesis, i.e., there is a statistical difference between the mean of the two data sets. The anthelminthic treatment at day 0 has a significant effect on the EPG count of day 7 and day 14.

DISCUSSION

This study shows the overall prevalence of 67.5% of nematode infection in the total of 40 samples taken,

Table 1. Prevalence of gastro intestinal nematode infection in captive ungulates of the Central Zoo.

	Ungulate species	No. of sample collected	No of sample positive for nematodes	Sample demonstrating single infection	Sample demonstrating mixed infection	Types of infection
1	Spotted Deer *Axis axis*	12	6(50%)	3	3	*Bunostomum* spp., *Strongyloides* spp., *Trichuris* spp.
2	Blue Bull *Boselaphus tragacamelus*	4	2(50%)	0	2	*Ostertagia* spp., *Strongyoides* spp.
3	Black Buck *Antelope cervicapra*	2	2(100%)	0	2	*Bunostomum* spp., *Haemonchus* spp., *Strongyloides* spp.
4	Barking Deer *Muntiacus muntjak*	9	7(77.78%)	4	3	*Bunostomum* spp., *Trichuris* spp., *Strongyloides* spp.
5	Sambar Deer *Rusa unicolor*	1	0	0	0	-
6	Himalayan Goral *Naemorhedus goral*	2	2(100%)	1	1	*Trichuris* spp., *Strongyloides* spp.
7	Four-horned Antelop *Tetracerus quadricornis*	1	1(100%)	1	0	*Strongyloides* spp.
8	Wild Boar *Sus scrofa*	2	2(100%)	0	2	*Ascaris* spp., *Oesophagostomum* spp.
9	Wild Water Buffalo *Bubalus arnee*	4	3(75%)	2	1	*Bunostomum* spp., *Strongyloides* spp., *Capillaria* spp.
10	One-horned Rhino *Rhinocerous unicornis*	3	2(66.67%)	3	0	*Strongyloides* spp.
	Total	40	27(67.50%)	13(48.15%)	14(51.85%)	

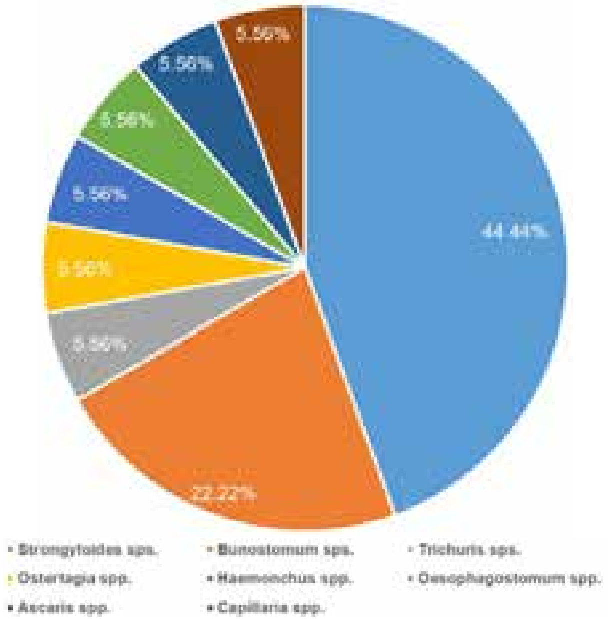

Figure 2. Overall prevalence of nematode species found in ungulates of the Central Zoo.

in which single infection was detected in 48.15% and double in 51.85%. The findings of Pun (2014) were similar to the research conducted, i.e., prevalence of 59% of parasite infection in the central zoo, Kathmandu. The present findings in respect to overall prevalence in captive herbivores agreed with Bir Moti Bagh Mini Zoo, India (68%) (Mir et al. 2016), Dehiwala National Zoological Gardens, Sri Lanka (62.9%) (Aviruppola et al. 2016), Ljubljana Zoo, Slovenia (61%) (Kvapil et al. 2017), Rangpur Recreational Garden and Zoo, Bangladesh (60%) (Khatun et al. 2014) but disagreed with research at the Zoological gardens of Malaysia (45.7% of ungulates) (Lim et al. 2008), Maharajbag Zoo, Nagpur (50%) (Borghare et al. 2009), the Antwerp Zoo and the Animal Park Planckendael, Belgium (36.5%) (Goossens et al. 2005), and Mahendra Choudhury Zoological Park, Chhatbir, Punjab (25.17%) (Singh et al. 2006).

Strongyloides spp. (44.44%) were the major nematodes detected in the study, followed by *Bunostomum* spp. (22.22%) and *Capillaria* spp., *Haemonchus* spp., *Trichuris* spp., *Oesophagostomum* spp., *Ascaris* spp., and *Oestertagia* spp., were found at 5.65% each. Nematodes were the group of concern in this research because the majority of studies reported a null prevalence of parasitic infection with trematode and cestode (Atanaskova et al. 2011; Pun 2014; Mir et al. 2016; Pawar et al. 2020;). Cestodes and trematodes need intermediate hosts and are less likely to accumulate in captive and enclosed ecosystems (Atanaskova et al. 2011). On the contrary, nematodes are one of the most important veterinary helminths that have a negative impact on wildlife health as well as conservation ecology (Goossens et al. 2006; Singh et al. 2006).

Table 2. Nematode species identified from the size of their eggs.

	Nematode Species	Size of Egg	Reference value Soulsby (2005).
1	*Strongyloides* spp.	47 by 20 µm	40–60 by 20–25 µm
2	*Bunostomum* spp.	84 by 49 µm	79–97 by 47–50 µm
3	*Trichuris* spp.	67 by 34 µm	68–75 by 36–40 µm
4	*Haemonchus* spp.	78 by 36 µm	70–85 by 41–48 µm
5	*Ascaris* spp.	59 by 41 µm	50–75 by 40–50 µm
6	*Oestartagia* spp.	65 by 35 µm	60–85 by 40–45 µm
7	*Capillaria*.spp.	42 by 25 µm	45–50 by 22–25 µm
8	*Oesophagostomum* spp.	35 by 63 µm	35-45 by 60–80 µm

Many nematode parasites of veterinary importance have a huge genetic diversity and features that favor the development of anthelmintic resistance (Kaplan 2004). Similarly, the author has also stated that anthelmintic resistance has been reported in every anthelmintic class.

The present study reports the baseline study of the effectiveness of the levamisole and oxyclozanide treatment on the nematodes of the captive ungulates of the Central Zoo. The present study agrees with WAAVP guidelines (Coles et al. 1992) for the diagnosis of anthelmintic resistance without using a control group (pretreatment mean was used for comparison) and similar studies were also conducted by Young et al. (2000), Goossens et al. (2006), and Pawar et al. (2020).

The result (<90% FECR and lower CI <95%) indicated presence of anthelmintic resistance (Coles et al. 1992; McKenna 1994). Similar results were obtained from captive wild impala in Zambia, in which the efficacy using FECR % was around 90% showing low efficacy and suggesting anthelmintic failure (Nalubamba & Mudenda 2012).

The failure of an anthelmintic could be the result of resistance, either from the survival of existing nematodes or the establishment of a new infection. The unavailability of the correct dose for the specific wild captive animals with an improper route of anthelmintic (causing more wastage and low dosage) (Nalubamba & Mudenda 2012) and idiosyncrasies (Ortiz et al. 2001) may have contributed to the development of the anthelmintic resistance in the current study. Additionally, ZANIDE-L Forte Bolus (Levamisole Hydrochloride BP 0.75 gm and Oxyclozanide BP (Vet) 1 gm is not a specific drug for nematodes, especially the oxyclozanide may not be

Table 3. Egg per gram (EPG) counts and FECR% (Bayesian hierarchical model) of captive wild ungulates from Nepal's central zoo treated with Zanide-L forte* at 10mg/kg body weight.

	Ungulate species	No. of sample taken	EPG Day 0	EPG Day 7	EPG Day 14	FECR Day 7 UI	FECR Day 14 UI
1	Spotted Deer *Axis axis*	12	1050	150	150	82.1% (51%.9–95%)	82.1% (51%.9–95%)
2	Blue Bull *Boselaphus tragacamelus*	4	7950	1250	650	83.4% (75.2–89.3)	91.2% (85.3–95.1)
3	Black Buck *Antilope cervicapra*	2	4150	350	250	90.5% (81.5–95.7)	93% (85.3–97.3)
4	Barking Deer *Muntiacus muntjak*	9	1500	200	300	84.3% (62.1–94.9)	77.5 (51.4–91.1)
5	Sambar Deer *Rusa unicolor*	1	-	-	-	-	-
6	Himalayan Ghoral *Naemorhedus goral*	2	1400	250	200	78.9% (52.1–92.1)	82.6 (57.5–94.2)
7	Four-horned Antelop *Tetracerus quadricornis*	1	500	50	0	81.6% (24.7–97.77)	92.1% (48.6–99.7)
8	Wild Boar *Sus scrofa*	2	700	200	200	64.9% (16.3–88.7)	65% (16.6%–89.2%)
9	Wild Water Buffalo *Bubalus arnee*	4	450	100	50	69.7% (11.1–93.8)	80.07% (26.9–97.5)
10	One-horned Rhino *Rhinocerous unicornis*	3	200	50	50	56.3% (41.6–94.3)	56.3% (41.6–94.3)
	Total	40	17900 ± 2527.81	2600 ± 373.14	1850 ± 181.04	85.3% (80.4–89)	89.2% (85–92.3)

Table 4. 'Z' test two sample means for day 0 and day 7.

	Sample size	Treatment	Pre-treatment Day 0 (Mean EPG ± S.E)	Post- treatment Day 07 (Mean EPG ± S.E)	'z' value	p value
1	40	Levamisole and Oxyclozanide	17900± 2527.81	2600 ± 373.14	2.520991	0.012

Table 5. 'Z' test two sample for means of Day 0 and Day 14.

	Sample size	Treatment	Pre-treatment Day 0 (Mean EPG ± S.E)	Post- treatment Day 14 (Mean EPG ± S.E)	'z' value	p value
1	40	Levamisole and Oxyclozanide	17900± 2527.81	1850 ± 194.36	2.654587	0.007

effective to the extent required. So, the necessity in this case is to change the drugs used for the rotation, based on the infection that is prevalent.

CONCLUSION

Infection with nematodes is of major veterinary importance. Frequent, unnecessary, and under-dosing of anthelmintics has given rise to a major problem of anthelmintic resistance in animals. The serious problem of anthelmintic resistance is based on the fact that levels of resistance can increase rapidly and the development of new classes of drugs is less. The efficacy of levamisole and oxyclozanide is found to be less than 90% with a lower confidence limit of 95% confidence level less than 90% suggesting the presence of resistance of gastrointestinal nematodes against the anthelmintic at the Central Zoo. This study is the first documentation of the efficacy of the anthelmintic used in the captive wild animal setting, in the Central Zoo, Kathmandu. The low efficacy of the anthelmintic is a concerning factor that requires proper nutrition, sanitation, and periodic deworming strictly based on advanced scientific strategies with periodic checks on anthelmintic resistance, which will aid in combating the serious issue of anthelmintic resistance.

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COMMUNICATION

Ocimum gratissimum L. ssp. *gratissimum* var. *macrophyllum* Briq. (Lamiaceae: Nepetoideae: Ocimeae) a new record from northeastern India

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Abstract: The genus *Ocimum* means fragrant-lipped, characterized by the presence of the upper lobe of the calyx, which is large and decurrent. *Ocimum gratissimum* L. is conventionally known as Clove Basil due to its foliage which smells like cloves. The present study reports the extant distribution of *O. gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq. across northeastern India. It is a new distribution record for the flora of Assam and northeastern India. A comprehensive description along with photographs, taxonomic notes, and diagnostic keys has been provided to aid identification.

Keywords: Assam, distribution, flora, keys, lipped, taxonomy.

Abbreviations: L./LINN—Linnaeus | APG—Angiosperm Phylogeny Group | GPS—Global Positioning System | ARUN—Arunachal Pradesh Regional Centre, Itanagar, Arunachal Pradesh | ASSAM—Eastern Regional Centre, Shillong, Meghalaya | CAL—Central National Herbarium, Howrah, West Bengal | GUBH—Gauhati University Botanical Herbarium | BSI—Botanical Survey of India | IVH—Indian Virtual Herbarium | JSTOR—Journal Storage | G—Conservatoire et Jardin botaniques de la Ville de Genève | K/KEW—Royal Botanic Garden, Kew | MNHN—Muséum national d'Histoire naturelle | MO—Missouri Botanical Garden's Herbarium | NY—New York Botanical Garden Herbarium | BSID—Deccan Regional Centre, Hyderabad.

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Author contributions: ND and DN conceptualized and supervised the research work; MK did the field and laboratory works, and drafted the manuscript. ND and DN finalized the manuscript, and MK communicated to the Journal.

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INTRODUCTION

Commonly known as 'Tulsi' in Hindi and 'Toolakhi' in Assamese, 'Basil' (Empress of all herbs) descended from the greek word 'Basileus' which means royal, and '*Ocimum*' from 'okimon' which purports an aromatic herb. *Ocimum* L. is chiefly an 'East Indian' genus (Bentham 1832). The primary centre of origin is Africa, Tropical Asia, and Central and South America, while India is the secondary centre (Pushpangadan & Sobti 1982). According to APG IV, *Ocimum gratissimum* is a member of the tribe Ocimeae Dumort., subfamily Nepetoideae Burnett in the mint family Lamiaceae Martinov (Stevens 2001 onwards). The specific epithet '*gratissimum*' explains an exaggerated expression of pleasantness due to the aroma of the species. The species have a more substantial degree of fragrance than other *Ocimum* L. species (Roxburgh 1832). *O. gratissimum* has two accepted sub-specific taxa, *O. gratissimum* ssp. *gratissimum* and *O. gratissimum* ssp. *iringense* Ayob. ex Paton. The latter subspecies is confined to Tanzania, while ssp. *gratissimum* is native to the tropical and sub-tropical old world. The variety *macrophyllum* was first acknowledged by Briquet (1894) affirming the distribution of var. *macrophyllum* in India Orientalis. According to Ryding (2000), var. *macrophyllum* is widespread in the tropics from India to western Africa. The var. *macrophyllum* got introduced from or to India and later disseminated through African cultivation. The variety was acknowledged by Paton (1992) while investigating *Ocimum* L. in Africa. He found a few forms of *O. gratissimum* in Uganda and Tanzania, corresponding to var. *macrophyllum* in having lax inflorescence, calyx, and leaf indumentum. The distinction of the varieties based on morphological characteristics conceals the facts acquired from genetic and secondary product variation. Such high degree variation is found in var. *gratissimum* against var. *macrophyllum* (Vieira et al. 2001). The var. *macrophyllum* is recognized by glabrous or pubescent leaves (hairs scattered over lower nerves) and hairy inflorescence (Albuquerque & Andrade 1998). While revising the tribe *Ocimeae* Dumort., Suddee et al. (2005) distinguished both varieties of *O. gratissimum* ssp. *gratissimum* (var. *gratissimum* and var. *macrophyllum*) and their distribution from India. The var. *macrophyllum* might have arisen from var. *gratissimum* in response to environmental constrain (Paton et al. 2009).

The Indian subcontinent is acknowledged by ssp. *gratissimum.* The var. *macrophyllum* is treated within ssp. *gratissimum* and reported earlier from different states of India, except Himachal Pradesh, Jammu & Kashmir, Uttarakhand, and northeastern India. In the present study, the variety *macrophyllum* is being reported for the first time from Assam.

MATERIALS AND METHODS

The specimens of the var. *macrophyllum* were collected from Jorhat district of Assam during our field survey conducted in 2019–22. Field photographs and GPS locations were recorded using a digital camera. The micro-morphological features were investigated on living specimens using a Labomed CZM4 stereo zoom binocular microscope. Further, photo plates were prepared using Adobe Photoshop 7.0. The variety was identified by consulting regional and national herbaria, such as ARUN, ASSAM, CAL, and GUBH, and through relevant literature (Floras, Journals, Revisions, and Synopsis). The microfilms of herbarium specimens from online databases BSI-IVH, G, JSTOR, KEW, LINN, MNHN, MO, and NY were also consulted for identification. The new distributional record of the variety was confirmed through research articles and literary works such as checklist, flora, and floristic records of northeastern India, along with physical verification of herbarium records held by ARUN, ASSAM, CAL, and GUBH. The morphological affirmations were correlated with lectotype G00018935 and photographs acquired from MNHN (Image 1). The common and vernacular names are given in English (E), Hindi (H), and Assamese (A).

Taxonomic treatment

***Ocimum gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq.**

Bull. Herb. Boissier 2: 120.1894; Paton in Kew Bulletin. 47: 417.1992; Paton, Harley & Harley in Holm & Hiltunen, Basil: *Ocimum*. 25.1999; Ryding in Fl. Somalia 3: 341.2006.

Type: Lectotype (LT): G00018935, Madagascar, Bourbon, Boivin L.H. LT present in Conservatoire et Jardin botaniques de la Ville de Genève (G) and photo of type in K!

Description: Perennial shrubs, 1.5–2 m tall; Stem erect, much branched, woody at the base, rounded quadrangular, glabrous; Leaves 6–12 × 4.5–7 cm in size, serrate, surface smooth, hairs restricted to veins beneath, apex acuminate, multicostate divergent reticulate venation; Petiole 1.5–4 cm long, slender; Inflorescence 15–22 cm long, lax, axis glabrescent, verticils 0.8–1.2 cm apart; Bracts 3–4 × 1.8–2.5 mm, green, ovate with broad base, caducous, apex acute, base cordate, sub sessile or

Image 1. *O. gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq: A–C—Lectotype specimen (G00018935) | B—Magnified view of inflorescence | C—Leaves | D—PDIG00000651 | E—PDIG00001680 | F—PDIG00000652 | G—PDIG00001681 | H—Magnified sight of verticils and flowers | I—Habit | J—Leaves with Inflorescence. Source: (A–C) Reproduced with permission from Catalogue des herbiers de Genève (CHG)| (D–G) with permission from Fabien Barthelat / Muséum national d'Histoire naturelle (MNHN–Paris)| (H–J) © Mamita Kalita.

sessile, margin ciliate, pubescent on both sides; Pedicels 3–3.5 mm long, pubescent, spreading, recurved; Calyx 2–3.2 × 2–3.5 mm, slightly downwards pointing against the inflorescence axis, green, slightly purplish at tips, posterior lip rounded, wider at tip, acute apex, decurrent on tube, anterior lip shorter than posterior, two hooked lateral curved teeth (uncinate lip) slightly lower than the two median teeth, median lobes of anterior lip pressed against posterior one in fruiting calyx, throat closed, tube with patent hairs or without; Corolla 4–5.5 × 2–3 mm, light pastel yellow, barely exceeding the calyx, lobes obscurely crenate, minute hairs at back, posterior lip oblong and comparatively shorter than anterior lip, lobes are equal in length, anterior lip boat shaped, tube straight, puberulous outside, glabrous inside; Stamens 4.5–5 mm, occasionally equal in length with anterior stamens, posterior pair having tufts of hairs at base (barbate filament base); Gynoecium 6.5–8 mm, two equal lobes, curled bifid stigma, ovary more or less globose; Nutlets 1.8–2.2 × 1.5–2 mm, ivory in colour, brown at maturity, sub globose, minutely tuberculate, producing mucilage when wet (Image 2).

Flowering and Fruiting: It was observed in July.

Common names: African Basil, Clove Basil, East Indian Basil, Russian Basil, Shrubby Basil, Tree Basil, Wild Basil, Tea Bush (E), Ban Tulsi, Jangli Tulsi, Vriadha Tulsi, Mali Tulsi, Ram Toolsee (H) and Ram toolakhi (A).

Key to the Infra-specifics of *Ocimum gratissimum* L.

1. Flower verticils 1–1.5 cm apart, not strongly reflexed towards the inflorescence axis; flowering and fruiting calyces recurved .... *O. gratissimum* ssp. *gratissimum*
2. Inflorescence 10–13 cm long, dense, axis softly pubescent; leaves pubescent on both sides; calyx horizontal or slightly upward pointing .... *O. gratissimum* var. *gratissimum*
2. Inflorescence 15–22 cm long, lax, axis glabrescent; hairs restricted to veins beneath; calyx downward pointing .... ***O. gratissimum* var. *macrophyllum***
1. Flower verticils 0.7–1.0 cm apart, strongly reflexed towards the inflorescence axis; flowering and fruiting calyces decurved .... *O. gratissimum* ssp. *iringense*

Specimen examined

Africa: Réunion. Boivin, L.H. -21.1216E , 55.5380S. *O. gratissimum* L. var. *macrophyllum* Briq. Herbarium Genavense (G), Lectotype (G00018935!). **Madagascar:** L.J. Dorr, 24.ii.1985. Original material? of *O. gratissimum* var. *macrophyllum* Briq. MO-694055! Coll.No.3779. Verified by Paton, 1998. **Bangladesh:** Flora of Chittagong hill tracts, Dr. King's Collector, viii.1886, Herb. Hort. Bot. Calcuttensis, CAL 351774! CAL 351775! **India, Andhra Pradesh:** Godavari, M.S. Ramaswami, 13.viii.1914, CAL 351792! Coll.No.1682; East Godavari, Daragatta, M. Mohanan, 17.xii.1993, altitude 550m, BSID0005839! Coll.No.100749. **Assam:** Flora of North Cachar, Haflong, William Craib, 17.viii.1908, Herb. Hort. Bot. Calcuttensis, CAL 351776! Koliapani, N. Kalita & S. Haque, 20.v.2001, ARUN000012603! and 16.xii.2008, ARUN000012604! **Karnataka:** Flora of North Kanara, W.A Talbot, 1889, CAL 351800! Coll.No.1935; Coorg, Mercara, B.C Banerjee, 31.x.1976, CAL 0000008663! Coll.No.11686. **Kerala:** Flora of Travancore, Quilon, M. Rama Rao, 13.viii.1913, CAL 351785! Coll.No.2252; Kozhikode, Kapad Shore, T.A. Rao, 09.xi.1972, CAL 11412! Coll.No.9839. **Lakshadeep:** Chetlat Island, B.M. Wadhwa, 28.ii.1959, CAL 6616! CAL 6617! Coll.No.49132. **Manipur:** Flora of Munneypore, Irang, C.B. Clarke, 27.xi.1885, CAL 351777! Flora of Manipur, Bishenpur, A. Meebold, xi.1907, No accession number. **Odisha:** Ganjam, Rocky hill Gopalpur, D. Prain, 1889, CAL 351793! Ramgiri, G.V. Subba Rao 19.xii.1962, ASSAM 36069! ASSAM 36070! **Tamil Nadu:** Coimbatore, Bolampatti Valley, C.E.C. Fisher, 22.ix.1900, altitude 1600m, CAL 351790! Coll. No.2205; AlagarKoil reserve forest, S. R. Srinivasan, 20.x.1988, altitude 300 m, BSID0012445! Coll. No.89407. **Telangana:** Khammam, Perantappally Forest, Pappikonda Hills, R. Chandrasekaran, 19.ii.1994, altitude 250 m, BSID0005841! Coll.No.98988; Borapuram (Mahabubnagar), B. Sadasivaiah & S. Khadar Basha, 04.xi.2008, altitude 615 m, BSID0005843! Coll.No.32360. **Tripura:** Rajnagar, B.K. Huidrom, 26.viii.1995, ASSAM 57229! **Assam:** Jorhat, Near Hoollongapar Gibbon sanctuary, 26.6785654N 94.3555723E, altitude 93 m, 21.vii.2019, Mamita Kalita, Coll.No. 63 (JHOG02).

Taxonomic note

The variety epithet *'macrophyllum'* is a Greek word which intent large-sized leaves of the specimen. Earlier, five varieties of ssp. *gratissimum,* have been recognized, one by Hooker (1885) and the rest four by Briquet (1894, 1898). Hooker (1885) reduced *O. suave* Willd. to a variety of *O. gratissimum* var. *suavis*, and distinct the variety from *O. gratissimum* in leaf pubescence. Briquet (1894) established three varieties, viz., *macrophyllum, mascarenarum, hildebrandtii,* and later *subdentatum* in 1898. However, only a single variety (*macrophyllum*) is acknowledged, and the others are accepted as synonyms. Morton (1962) found insufficient evidence for establishing intermediates of *O. gratissimum*. He considered *O. suave* and *O. gratissimum* as different species based on chromosome number, 2n = 64 and 2n = 40, 48, respectively. Similar chromosome numbers (2n = 64) were obtained by Darlington & Wylie (1955) from the Indian material of *O. gratissimum*. Also, differences based on leaf epidermal characteristics were analyzed by Olowokudejo & Pereira-Shateolu (1988). Khosla (1995) found *O. suave* contrasting from *O. viride* Willd. and *O. gratissimum*. Based on taxonomic and genetic relationships, he further concluded their origin from a common ancestor. Currently, both *O. suave* and *O. viride* exists as a synonym of *O. gratissimum* ssp. *gratissimum*. The var. *macrophyllum* is definite from var. *gratissimum* in having lax inflorescence and sparse indumentum. This incarceration is held up by referencing Indian material, where the discontinuity between the two varieties is also supported. The consulted herbarium specimens ARUN000012603, ARUN000012604, CAL 351776 pertaining to Assam and CAL 351777, ASSAM 57229 of Manipur and Tripura, respectively, are identified as *Ocimum gratissimum*. However, these specimens were found morphologically dissimilar from the variety described in the present study. Thus, it led to an establishment of new distribution record for the var. *macrophyllum* in northeastern India.

DISCUSSION

The species *O. gratissimum* popularly known as scent leaf, has potential bioactive compounds such as polyphenols and flavonoids. The var. *macrophyllum* is undoubtedly similar to clove basil, which may serve as an alternative to drugs. The variety can also make its appearance as a new medicinal plant. *O. gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq. is a new distributional record for northeastern India and Assam. The investigations of var. *macrophyllum* are similar to the description given by Paton (1992) while revising the tribe *Ocimeae* in Africa. The present study has provided comprehensive data on the odoriferous specimen's diagnosis, distribution, elucidation, and taxonomic status.

Image 2. *Ocimum gratissimum* L. ssp. *gratissimum* var. *macrophyllum* Briq: A—Habit of the plant | B—Leaf adaxial side showing multicostate reticulate (mr) venation | C—Leaf abaxial side | D—Inflorescence | E—Inflorescence tip showing flower bud (fb), inflorescence axis (ia) and bract (Br) | F—Closure view of inflorescence showing floral arrangement | G—Complete flower | H— Bract | I—Calyx dissected view displaying uncinate lip (ul) | J—Dissected corolla showing epipetalous stamen, barbate filament (bf) base | K— Gynoecium exhibiting gynobasic incurved style (is) and bifid stigma | L—Ovary revealing ovules | M—Nutlets. © Mamita Kalita.

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COMMUNICATION

The study of biogeographic patterns of the genus *Parmotrema* in Wayanad District, Kerala with a new record in India

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Abstract: This research focuses on Wayanad District within the Nilgiri Biosphere Reserve of the Western Ghats, a renowned hotspot for lichen diversity. A thorough investigation documented 10 distinct *Parmotrema* species, with one newly identified species (*Parmotrema clavuliferum*). Each species was comprehensively described, encompassing their morphological, chemical, and biogeographical characteristics. The core objective of this study revolves around conservation and sustainable utilization of this valuable bioresource. This research contributes to our understanding of lichen ecosystems, particularly in regions facing diverse threats, and underscores the importance of the Wayanad District within the broader context of biodiversity conservation.

Keywords: Antimicrobial agents, biogeographical characteristics, conservation, hotspot, lichen diversity, morphology, Nilgiri Biosphere Reserve, secondary metabolites.

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Author contributions: BJ has designed and conceptualized the present study, performed the field collection, and formal data analysis, and prepared the original draft. ETS has participated in the field collection and contributed to the review and editing of the draft. VTJ has participated in the field collection and contributed to the review and editing of the draft. HP has participated in the field collection, prepared QGIS maps and spatial data analysis, and contributed to the review and editing of the draft. NSP has provided proper guidelines for the research and supervised the field collection, data analysis and the preparation of the final manuscript. All authors read and approved the final manuscript.

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INTRODUCTION

Lichens are fascinating organisms, formed by the symbiotic association between algae and fungi, functioning together as a single organism (Honegger 1991). Lichens contribute to 8% of global biodiversity and approximately 19,387 species that comprise 995 genera, 115 families, 39 orders, and eight classes (Lücking et al. 2016). The Indian subcontinent has approximately 3,028 lichens (Awasthi 2000; Sinha 2021) and Kerala has 27% of this diversity (Purushothaman et al. 2021; Anilkumar et al. 2022; Sequeira et al. 2022). All the members of the genus *Parmotrema* A.Massal. are foliose lichens. The ventral of the thallus has green or pale green or ash color and the margins may or may not have cilia and pored epicortex. The dorsal side has brown, tan or black color and the lower margin is tan, brown or white in color. The margin of the dorsal side is generally devoid of rhizines. This erhizinate condition of the dorsal side is used as a key character to separate the lichens of the genus *Parmotrema* A.Massal. from other foliose lichens in the family Parmeliaceae. All the members in this genus have distinct cortex and medulla. The upper cortex is maculated due to the extension of medullar fungal hyphae to the cortex, which can be identified by the regions devoid of phycobiont (Spielmann & Marcelli 2009, 2020; Mishra & Upreti 2017). All the members of the genus *Parmotrema* are rich in pharmaceutically important secondary metabolites. Atranorin is a commonly occurring compound with anti–microbial properties against bacteria *Bacillus cereus, B. subtilis, Staphylococcus aureus, S. faecalis, Proteus vulgaris*, the fungi *C. albicans and C. glabrata* as well as the mycobacterium, *M. aurum*. In addition to this Atranorin has anticarcinogenic properties (Sroka et al. 2017). Salazinic acid is another secondary metabolite of the genus *Parmotrema* and has antimicrobial and cytotoxic properties. Lecanoric acid is a bioactive compound and has antiproliferative activity against HeLa cells (IC50 = 123.97 µg/ml). Lecanoric acid is a potential antioxidant and can be used as a molecular scavenger against free radicals **(**Zambare & Christopher 2012).

Lichens are generally sensitive to habitat, host (John 1992), environmental factors, latitude, climate (Fryday 2000) and environmental pollution (Larsen et al. 2007). The present study is focused on the assessment of biogeographic patterns of distribution of the genus *Parmotrema* in the rapidly urbanizing zones of Wayanad District within the Nilgiri Biosphere Reserve of the Western Ghats, for the lichen diversity status assessment and future reference. This is the first study that analyzes the ecology and population aspects of the genus *Parmotrema* in the Wayanad District of Kerala, in a scenario of lichens facing challenges of extinction due to endemism, ever increasing pollution, urbanization, & lack of studies and endeavors to protect them.

Study area

Wayanad (Figure 1) is a small hilly district in Kerala with an area of 2,131 km^2, located at 11.685^{0}N, 76.132^{0}E with the highest tribal population of about 1.25 Lakh, consisting of 17% of the total tribal population of the state. Wayanad has a salubrious climate with a mean rainfall of 2,786 mm and the elevation varies 700–2,061 m.

MATERIALS AND METHODS

The diversity assessment of lichens of the genus *Parmotrema* in Wayanad was based on 460 specimens of lichens collected from October 2021 to October 2022. The specimens were systematically identified using the keys of macro lichens of Awasthi (1976, 1991, 2007), Divakar & Upreti (2005), and Mishra & Upreti (2017). Out of the 460 samples, 258 samples belong to the genus *Parmotrema* and these specimens were further studied using a Leica MC170 stereo microscope for morphological studies and a Leica DM750 compound microscope for anatomical studies. The secondary metabolites in the thallus were also considered for species delimitation. The compounds were preliminarily determined through spot tests and thin-layer chromatography (Orange et al. 2001). Thin-layer chromatography was performed using solvent system A (toluene: dioxane: acetic acid = 180:45:5) (Nayaka 2014). Nomenclature was confirmed with the database Index Fungorum (http://www.indexfungorum.org). All the morphological characters noted were compared with the morpho-taxonomic accounts of Mishra & Upreti (2017) and Spielmann & Marcelli (2009). All the specimens were systematically processed and deposited at the herbarium of KSCSTE-Malabar Botanical Garden & Institute for Plant Sciences (MBGH).

The distribution maps were prepared using open source QGIS 3.16 software and the ecological studies were conducted using narrow frequency grid (sampling ladder) (Scheidegger et al. 2002). The study was carried out in 16 sample sites of Wayanad District and these sites were grouped into three zones based on elevation. The geographical parameters and zonal classification is given in Table 1. A total of 60 quadrats were laid randomly for

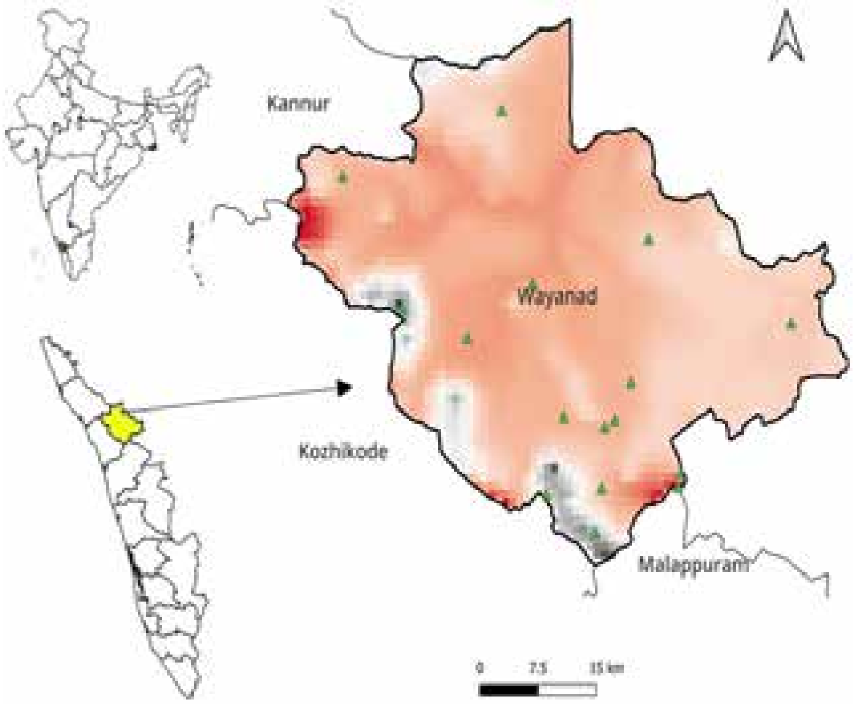

Figure 1. Study area - Wayand, southern Western Ghats showing sample sites.

the collection of ecological data (20 quadrats were used for each zone). The quadrat analysis of the study area is listed in Table 2. The alpha diversity of lichen habitats was assessed using the Shannon-Weiner index (Shannon & Weiner 1949), Simpson's index (Simpson 1949) and evenness. The following equations are used to calculate the alpha diversity of 16 lichen habitats of Wayanad.

Frequency and relative frequency were calculated as,

$$\text{Frequency} = \left(\frac{\text{Number of quadrats in which a species occurs}}{\text{Total number of quadrats sampled}}\right) * 100$$

$$\text{Relative Frequency} = \left(\frac{\text{Frequency of the individual species}}{\text{Total frequency of all the species}}\right) * 100$$

Density and relative density were calculated as,

$$\text{Density} = \left(\frac{\text{Total number of individuals in all the quadrats}}{\text{Total number of quadrats studied}}\right)$$

$$\text{Relative density} = \left(\frac{\text{Total number of individuals of the species in all quadrats}}{\text{Total number of individuals of all species in all quadrats}}\right) * 100$$

The importance value index (IVI) was used for the assessment of the ecological distribution of species in the ecosystem (Curtis & Mc Intosh 1950; Misra 1968) of lichens in different zones. IVI is the sum of relative frequency and relative density (Phillips 1959).

Shannon-Wiener Index (H') (1949) of species richness is based on the total number of species and the total number of individuals of each species in a sample. This index represents the average degree of uncertainty in predicting to which particular species, an individual randomly chooses from the sample.

$H' = -\sum [(ni/N) \ln (ni/N)]$

Where,

Ni = number of individuals of i^{th} species

N = total number of individuals of all species

H' = index value

Simpson's index (D) (1949) is used to measure the degree of concentration when individuals are classified into types.

$$D = \sum_{i=0}^{s} \left(\frac{ni(ni-1)}{N(N-1)}\right)$$

Where,

ni = number of individuals in the i^{th} species

N = total number of individuals of all species

D = index value

Evenness of the region expresses the Shannon-Weiner function (H'), relative to the maximum value that H' can be obtained. Evenness reaches a maximum when all the species in the sample have the same number of individuals.

$$E = \left(\frac{H'}{H'max}\right)$$

Table 1. Geographical factors of the study area.

	Location	Zone	Elevation (m)	Latitude (⁰ N)	Longitude (⁰ E)
1	Vaduvanchal	Zone 1 Altitude below 800 m	400	11.5210364	76.2393654
2	Chennalode		690	11.6631996	75.98487763
3	Arijermala		719	11.7146403	76.06218148
4	Neelimala		725	11.5327544	76.23645906
5	Kolagapara		753	11.61955001	76.17823095
6	Karappuzha		766	11.58407531	76.15903076
7	Puthoorvayal		766	11.58748825	76.09855323
8	Periya		769	11.81961661	75.83803087
9	Meppadi		785	11.57728355	76.14728895
10	ChooralMalai	Zone 2 Altitude between 800–1,200 m	818	11.51799489	76.14309851
11	Thirunelli		851	11.88324358	76.02617202
12	Wayanad Wildlife Sanctuary		856	11.67626877	76.36811484
13	Irulam		890	11.75776861	76.19951054
14	Chembra	Zone 3 Altitude above 1,200 m	1,252	11.51220149	76.07799674
15	Vellarimala		1,389	11.47523043	76.13555719
16	Banasuramalai		1,911	11.69295873	75.90831713)

Table 2. List of species and occurrence in the study area.

	Location	List of species and occurrence									
		*Au**	*Ce**	*Cl**	*Cr**	*Ha**	*Pl**	*Pr**	*Re**	*St**	*Ti**
1	Vaduvanchal	-	-	9	-	-	-	1	-	-	14
2	Chennalode	-	-	-	4	6	-	-	-	-	15
3	Arijermala	-	-	-	-	1	-	-	5	1	2
4	Neelimala	-	1	1	-	-	7	-	4	1	3
5	Kolagapara	1	-	-	-	-	1		-	1	6
6	Karappuzha	-	-	-	-	-	-	-	8	4	9
7	Puthoorvazhal	-	-	-	1	1	-	-	-	-	9
8	Periya	3	-	-	-	-	6	-	-	-	9
9	Meppadi	4	-	-	-	-	4	-	-	1	4
10	Chooral Malai	-	1	4	1	-	-	-	6	1	11
11	Thirunelli	-	-	1	1	-	-	-	16	6	9
12	Wayanad Wildlife Sanctuary	-	1	-	-	-	3	-	9	-	8
13	Irulam	1	-	-	-	-	2	-	-	1	4
14	Chembra	-	-	-	-	1	-	-	2	-	2
15	Vellarimala	-	-	-	-	1	-	-	2	-	4
16	Banasuramalai	4	-	-	-	3	-	-	-	-	6
	TOTAL	13	3	15	7	13	23	1	52	16	115

Au—P. austrosinense | Ce*—P. cetratum | Cl*—P. clavuliferum | Cr*—P. cristiferum | Ha*—P. hababianum | Pl*—P. planatilobatum | Pr*—P. praesorediosum | Re*—P. reticulatum | St*—P. stuppeum | Ti*—P. tinctorum.*

Where,
H' = Shannon-Wiener Index
H' max = Species Richness

RESULTS

The detailed description of various *Parmotrema* lichens collected from the study area are presented as 'Taxonomic studies' and detailed biogeographic features and distribution of species in the study area are described under 'Biogeography of the lichen genus *Parmotrema* in the study area'.

Taxonomic studies

The 10 species recorded from the study area are described here. *Parmotrema clavuliferum* (Räsänen) Streimann is reported here as a new record to India.

1) ***Parmotrema austrosinense*** (Zahlbr.) Hale, Phytologia 28(4): 335 (1974)

Index Fungorum Number: IF343014

Thallus foliose, corticolous, loosely attached to the substratum, 5–10 cm across; lobes rotund, each lobe 5 to 20 mm wide, margins ascending imbricate, sinuous; eciliate; upper surface pale green or grey colour, smooth, white-maculate, more or less rugose in the centre; soralia marginal, linear, soredia farinose to granular, sorediate margins are wavy and assenting imbricate, wide marginal zone ivory, tan or brown mottled; erhizinate shiny marginal zone; lower side centrally black; rhizines sparse in the centre part, simple; short; up to 1 mm long, medulla white. Apothecia rare, isidia absent (Image 3B).

Chemistry: Cortex K+ yellow; medulla K—, C+ rose red, KC+ red, P—

TLC: atranorin and lecanoric acid (Image 4(10)) Distribution: This taxon is found in elevations above 700m (Image 5e).

2) ***Parmotrema cetratum*** (Ach.) Hale, Phytologia 28(4): 335 (1974)

Index Fungorum Number: IF343018

Thallus foliose, corticolous or saxicolous, loosely adnate to the substratum, 7–20 cm across; lobes rotund, 5–10 mm wide, margin ciliate; cilia black with tapering end, simple to furcated, 1–3 mm long; upper side grey to darker green, densely white-maculate; maculae reticulate and fissured into a network (appearing as pseudocyphellae); isidia and soredia absent; lower side centrally black, marginal narrow zone ciliate; sparsely

Image 1. A—*Parmotrema reticulatum* (Taylor) M.Choisy | B—*Parmotrema clavuliferum* (Räsänen) Streimann | C—*Parmotrema cristiferum* (Taylor) Hale | D—*Parmotrema stuppeum* (Taylor) Hale. © Bibin Joseph.

rhizinate; rhizines restricted to the central part of the thallus, simple, black, 0.5–1 mm long; medulla white. Apothecia up to 10 mm in diameter, perforate; ascospores, colourless, simple, 13–17 × 6–10 µm. Pycnidia not seen (Image 3A).

Chemistry: Cortex K+ yellow; medulla K + yellow then red, C—, KC + red, P+ orange

TLC: atranorin, salazinic and consalazinic acids (Image 4(5)).

Distribution: In Kerala, this taxon is reported from Wayanad only (Image 5j).

3) *Parmotrema clavuliferum* (Räs.) Streimann, Bibliotheca Lichenologica 22: 93 (1986)

Index Fungorum Number: IF129346

Thallus foliose, corticolous, loosely attached with the substratum, 15–20 cm across; lobes dichotomously branched 5–15 mm wide, lobe margins ciliate rotund, margins entire ciliate, 0.3–1.5 mm long, black; upper side pale green or whitish-gray, dull to shiny, reticulatly maculate and cracked; soralia capitates and stalked, marginal, present in the laciniate lobes which appear as palmate, the lower side of the lacciniae white in colour; soredia found in a large cluster, granular, rotund; lower side centrally black narrow marginal zone, 2 mm wide, brown erhizinate; rhizines abundant, up to 1 mm; medulla white. Apothecia and pycnidia were not observed among the Wayanad specimens (Image 1B).

P.clavuliferum and *P. reticulatum* are similar in cortical and medullary chemistry in colour test and

Image 2. A—*Parmotrema planatilobatum* (Hale) Hale | B—*Parmotrema hababianum* (Gyeln.) Hale | C—*Parmotrema praesorediosum* (Nyl.) Hale | D—*Parmotrema tinctorum* (Despr. ex Nyl.) Ha. © Bibin Joseph.

reticulate cracked upper side and differs in having palmate and elongate laciniae with capitates and stalked soralia; typically erhizinate and white lower side, lacking pigmentation by the former.

Chemistry: Cortex K+ yellow, KC–, C–, P+ yellow; medulla K + yellow then soon turning blood-red, C—, KC + red, P+ orange or deep yellow.

TLC: atranorin, salazinic acid (Image 4(2)).

Distribution: In India, this taxon is reported from Wayanad only (New to India)

4) *Parmotrema cristiferum* (Taylor) Hale, Phytologia 28(4): 335 (1974)

Index Fungorum Number: IF34303

Thallus foliose, corticolous rarely saxicolous, loosely attached to the substratum, large, spreading, 10–25 cm across; lobes rotund, laterally ascending, sinuous, 10–15 mm wide, emaculate; axils incised; margins entire, eciliate; upper side grey to pale grey, centrally brownish, cracked, soralia marginal on lateral lobules in the central part, crescent-shaped or confluent; soredia marginal to submarginal, rounded to confluent, sinuous and revolute, granular; lower side centrally black, wide marginal zone, 3–5 mm wide, brown, nude; rhizines sparse in the central part, short, coarse, up to 1 mm long; medulla white. Apothecia rare and pycnidia are absent in Wayanad specimens (Image 1C).

Chemistry: cortex K+ yellow; medulla K + yellow turning red, KC—, C—, P+ orange-red.

TLC: atranorin, salazinic acid and consalazinic acids

Image 3. A—*Parmotrema cetratum* (Ach.) Hale | B—*Parmotrema austrosinense* (Zahlbr.) Hale. © Bibin Joseph.

Image 4. Chromatogram of acetone extracts of lichens using solvent system A:
C—Standard (*Parmelia wallichiana*) | 1—*Parmotrema reticulatum* | 2—*Parmotrema clavuliferum* | 3—*Parmotrema hababianum* | 4—*Parmotrema stuppeum* | 5—*Parmotrema cetratum* | 6—*Parmotrema praesorediosum* | 7—*Parmotrema tinctorum* | 8—*Parmotrema cristiferum* | 9—*Parmotrema planatilobatum* | 10—*Parmotrema austrosinense*.

(Image 4(8)).

Distribution: This taxon is found in elevations above 600 m (Image 5h).

5) *Parmotrema hababianum* (Gyeln.) Hale, Phytologia 28: 336 (1974)

Index Fungorum Number: IF343060

Thallus foliose, corticolous, loosely attached to the substratum, 8–10 cm across; lobes rotund, 5–15 mm wide, margin crenate, sparsely ciliate; cilia simple, 0.5–2 mm long; upper side grey to brownish grey, smooth, faintly white-maculate to emaculate, sorediate; soralia marginal or submarginal; sorediate lobes revolute; lower side centrally brown-black; wide marginal zone ivory to brownish mottled, nude; rhizines sparse, uneven, present in scattered groups, simple, 1–2 mm long; medulla white. Apothecia and pycnidia are not seen in Wayanad specimens (Image 2B).

Chemistry: cortex K+ yellow; medulla K—, C—, KC+ reddish or purple P—.

TLC: atranorin and protolichesterinic acids (Image 4(3)).

Distribution: This taxon is found in elevations above 750m (Image 5g).

6) *Parmotrema planatilobatum* (Hale) Hale, Phytologia 28(4): 338. (1974)

Index Fungorum Number: IF343105

Thallus foliose, corticolous or saxicolous, closely to loosely attached to the substratum, 5–10 cm across;

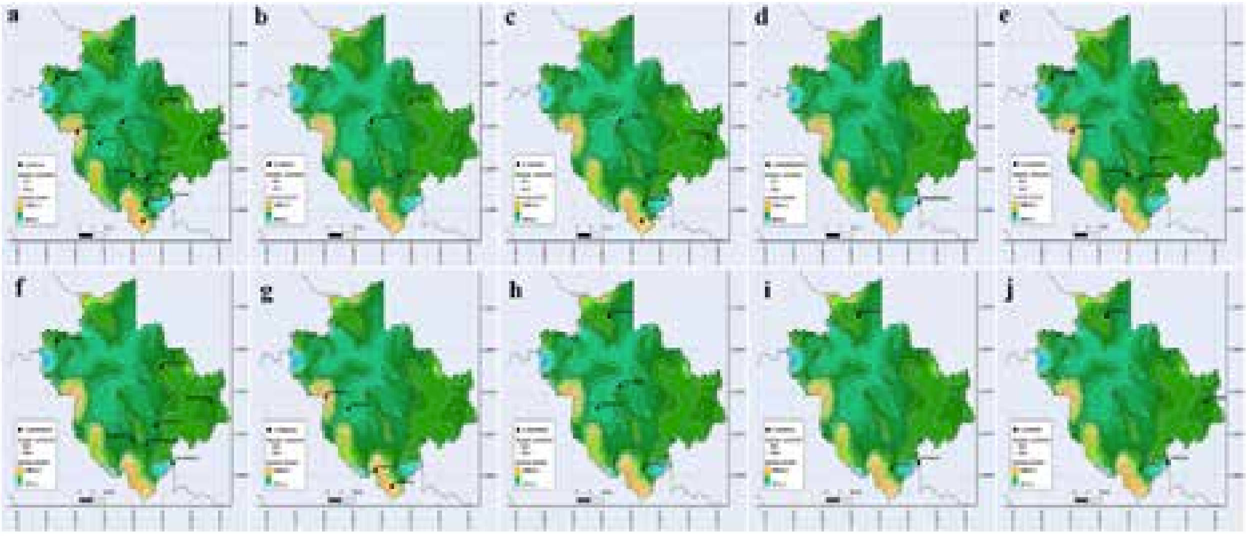

Image 5. QGIS distribution map of the genus *Parmotrema* in Wayanad: a—*Parmotrema tinctorum* | b—*Parmotrema stuppeum* | c—*Parmotrema reticulatum* | d—*Parmotrema praesorediosum* | e—*Parmotrema austrosinense* | f—*Parmotrema planatilobatum* | g—*Parmotrema hababianum* | h—*Parmotrema cristiferum* | i—*Parmotrema clavuliferum* | j—*Parmotrema cetratum*.

lobes rotund, 5–10 mm wide, apical margin entire or crenate, convolute, ciliate; cilia black, simple, 1–1.5 mm long; upper side grey, smooth, shiny, emaculate, with laminal to marginal isidia lacinulate thallus; lower side centrally black, marginal zone brown, nude; rhizines abundant, black, simple, 1–2 mm long; medulla white, with patches of K + purple pigment. Apothecia rare, up to 6 mm in diameter, disc imperforate, concave, pale brown; asci broad clavate, 38–42 × 20–25 µm, ascospores colourless, ellipsoid 15–18 × 7–9 µm. Pycnidia not seen (Image 2A).

Chemistry: cortex K+ yellow; medulla K—, C+ faint rose, KC+ red, P—

TLC: atranorin, gyrophoric acids and skyrin (Image 4(9)).

Distribution: This taxon is found in elevations above 750m (Image 5f).

7) ***Parmotrema praesorediosum*** (Nyl.) Hale, Phytologia 28(4): 338. (1974)

Index Fungorum Number: IF343106

Thallus saxicolous or corticolous, adnate, attached to the substratum, 3–10 cm across; lobes rotund, 5–10 mm wide, margins entire or crenate, sub erect and sorediate, eciliate; upper side grey to darker, emaculate, smooth, becoming slightly rugose and cracked in older parts, sorediate; soralia usually marginal, linear or crescent-shaped; soredia granular; lower side centrally black, narrow marginal zone lighter tan, nude; rhizines sparse, simple, short, 1–2 mm long; medulla white. Apothecia rare, short-stalked, 2–4 mm in diameter, disc imperforate, dark brown; asci clavate, 40–45 × 16–19 µm, ascospores simple, colourless, 15–21 × 7–10 µm. Pycnidia not seen (Image 2C).

Chemistry: cortex K+ yellow; medulla K—, C—, KC—, P—

TLC: atranorin, proto praesorediosic acid, praesorediosic and fatty acids (Image 4(6)).

Distribution: This taxon is found in elevations above 750 m (Image 5d).

8) ***Parmotrema reticulatum*** (Taylor) M. Choisy, Bull. Mens. Soc. Linn. Soc. Bot. Lyon. 21:175 (1952)

Index Fungorum Number: IF357464

Thallus foliose, corticolous or saxicolous, adnate loosely attached to the substratum, up to 10–20 cm across; lobes rotund, 5–15 mm wide, margin ciliate; cilia simple, black, 1–1.5 mm long; upper side grey to darker, smooth, densely white maculate; maculae eventually reticulately fissured, sorediate; soralia either capitate or marginal to submarginal on rounded or involute lobes; lower side centrally black, marginal zone white mottled or brown and nude or lower side black, rhizinate up to the margin; rhizines black, simple, 1–2 mm long; medulla white. Apothecia rare, up to 5 mm in diam., disc perforate or imperforate, brown; clavate asci; ascospores 8-spored, colorless, simple, 15–18 × 6–10 µm. Pycnidia not seen in Wayanad specimens (Image 1A).

Chemistry: Cortex K + yellow; medulla K + yellow then red, KC—, C—, P+ orange-red.

TLC: atranorin, salazinic and consalazinic acids

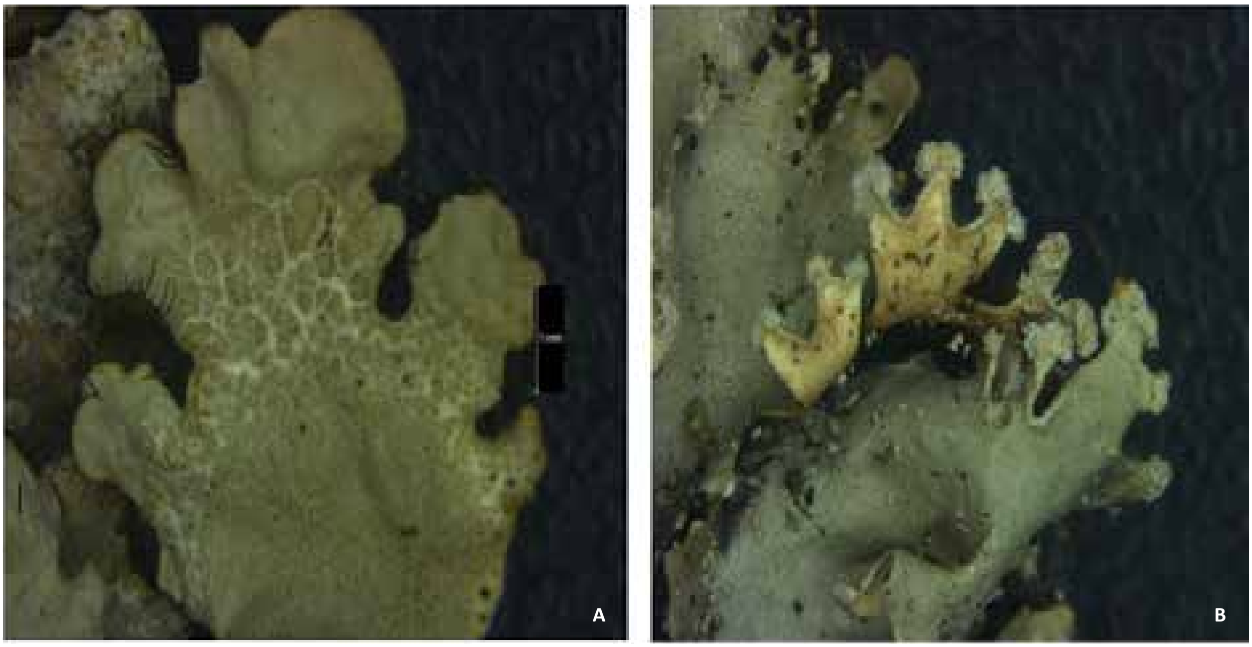

Image 6. *Parmotrema clavuliferum*: A—vegetative thallus with reticulate cracks and cilia | B—fertile thallus with variegated lacinules carrying soralia. © Bibin Joseph.

(Image 4(1)).

Distribution: This taxon is found in elevations above 600 m (Image 5c).

9) ***Parmotrema stuppeum*** (Taylor) Hale, Phytologia 28(4): 339 (1974)

Index Fungorum Number: IF343128

Thallus corticolous, rarely saxicolous, loosely adnate to the substratum, 10–15 cm across; lobes rotund, 10–20 mm wide, crenate-dentate, ciliate; cilia sparse to dense, simple, 1–3 mm long; upper side grey, dull, smooth, emaculate, cracked in older parts, sorediate; soralia marginal, on apices of dents in the central part, often confluent and submarginal; soraliate lobes involute; soredia farinose; lower side centrally black, wide marginal zone brown, nude or papillate; rhizines sparse, occur in patches in the central part, simple, 1–2 mm long; medulla white. Apothecia and pycnidia are not seen in Wayanad specimens (Image 1D).

Chemistry: Cortex K + yellow; medulla K + yellow turning red, C—, KC—, P+ orange-red.

TLC: atranorin, salazinic and consalazinic acids present in TLC (Image 4(4)).

Distribution: This taxon is found in above elevations 750 m (Image 5b).

10) ***Parmotrema tinctorum*** (Dèspr. *ex* Nyl.) Hale, Phytologia 28(4): 339 (1974)

Index Fungorum Number: IF343140

Thallus foliose, lobate, corticolous, saxicolous or terricolous, loosely attached to the substratum, membranaceous, 10–30 cm across; lobes irregular, 10–30 mm wide, apices rotund, margins entire to crenate, eciliate; upper side grey to pale green to mineral grey, emaculate; isidia granular to filiform becoming coralloid or rarely flattened; lower side centrally black, wide marginal zone, 3–6 mm, tan to brown, nude; rhizines sparse, dense at the centre, short 0.5–2.0 mm long; medulla white. Apothecia rare, not present in the specimens examined, up to 10 mm in diam., disc imperforate; asci clavate, 8 spored, ascospores simple, colourless, oval-ellipsoid 13–18 × 6–10 µm, epispore 1.5 µm thick (Hale 1965). Pycnidia not present (Image 2D).

Chemistry: Cortex K + yellow; medulla K—, C+ red, KC+ red, P—

TLC: Atranorin, lecanoric acid and traces of orsellinic acid (Image 4(7)).

Distribution: This taxon is abundantly present in all sample sites of the study area (Image 5a).

DISCUSSION

a) Study of species

In the comprehensive revisionary study of the lichen genus *Parmotrema* A.Massal. of India, Mishra & Upreti (2017) have provided a detailed morpho-taxonomic account of 53 species of *Parmotrma*. The

Table 3. Species diversity of the study area.

	Location	Shannon index (H)	Simpson's index (D)	Evenness (E)
1	Chembra	1.055	1.66	00.96
2	Karappuzha	1.05	0.31	00.95
3	Meppadi	1.285	00.21	00.927
4	Kolagapara	1.003	00.375	00.723
5	Vaduvanchal	00.815	00.441	00.742
6	Neelimala	1.512	00.208	00.844
7	Vellarimala	00.956	00.291	00.87
8	ChooralMalai	1.4	00.264	00.781
9	Thirunelli	1.227	00.314	00.763
10	Banasuramalai	1.058	00.285	00.963
11	Chennalode	00.942	00.443	00.74
12	Puthoorvayal	0.60	00.686	00.546
13	Irulam	1.213	00.22	00.875
14	Arijermala	1.149	00.275	00.829
15	Periya	1.011	00.33	00.920
16	Wayanad Wildlife Sanctuary	1.154	00.304	00.832

Table 4. Zone wise distribution of species.
The sixteen lichen habitats of the Wayanad District are divided into three zones based on elevation. The number of samples from each species collected from the study area is given in this table. The lichen habitats included in each zone were given in Table 1.

	Zone	*Au**	*Ce**	*Cl**	*Cr**	*Ha**	*Pl**	*Pr**	*Re**	*St**	*Ti**
1	Zone 1	8	1	10	*5*	*8*	18	1	17	8	71
2	Zone 2	1	2	5	2	0	5	0	31	8	32
3	Zone 3	4	0	0	0	5	0	0	4	0	12
	TOTAL	13	3	15	7	13	23	1	52	16	115

Au—P. austrosinense | Ce*—P. cetratum | Cl*—P. clavuliferum | Cr*—P. cristiferum | Ha*—P. hababianum | Pl*—P. planatilobatum | Pr*—P. praesorediosum | Re*—P. reticulatum | St*—P. stuppeum | Ti*—P. tinctorum.*

present study is adding *P. clavuliferum* (Räsänen) Streimann as the 54th species to the Indian lichen biota of the genus *Parmotrema*. The *P. clavuliferum* resembles *P. reticulatum* in its white to whitish-grey color, densely reticulate-maculate, often cracked upper surface abundantly sorediate margins, and simple cilia (Image 6A). They share the same chemistry (cortex with atranorin, medulla with salazinic acid). *P. clavuliferum* can be distinguished from *P. reticulatum*, the former having capitate soralia, sorediate lacinules at the lobe margins and the variegated lower side of the soredia (non–pigmented and white) (Image 6B). The distinctly stalked, capitate soralia protruding from long, slender, laciniate lobes is the characteristic feature of *P. clavuliferum*, whereas in *P. reticulatum* the soralia is in laminal to submarginal regions of the thallus (Moon et al.2001). This character can be used to segregate *P. clavuliferum* from *P. reticulatum*. Even though the erhizinate or nude and broad margins are the key characteristics of the genus *Parmotrema*, the broad lobes of *P. reticulatum* are typically densely rhizinate even close to the margin but the margins and clavulae of *P. clavuliferum* always devoid of rhizines (Bungartz & Spielmann 2019). The molecular studies of Ahn & Moon (2016) also provide evidence of the existence of *P. clavuliferum* as a separate species, not morphotypes. Spielmann & Marcelli (2009) reported *P. clavuliferum* from Brazil with filiform conidia and salazinic acid (K+ yellow turning blood red) as the secondary metabolite. Bungartz & Spielmann (2019) also recognised *P. clavuliferum* as separate species in their comprehensive inventory of all Galapagos lichens.

Table 4 shows that a total of 258 samples belonging to 10 different species is distributed through the three zones and among these zones; Zone 1 has a higher number of species and occurrences. The Zone 1, with nine lichen habitats has the maximum number of species in the genus *Parmotrema*, Zone 2 with four lichen habitats and Zone 3 has three lichen habitats. The Zone 1 has 10 species, Zone 2 has eight species and Zone 3 has four species of *Parmotrema*. From Table 5, *P. tinctorum* is the dominant species in all the zones, with the IVI value of 80.03, 71.346, and 88 respectively. *P. cetratum* is one species with a low IVI value and from Kerala, it is reported from Wayanad District only (Christy et al., 2022). Another species with a low IVI value is *P. praesorediosum* (IVI value 2.26), present only in zone 1, mainly due to its pollution–sensitive nature and hence can be used as a pollution indicator.

b) Biogeography and Ecological Studies

The diversity of species in the study area is termed alpha diversity. Simpson's Index of Diversity (D) is a measure of richness and relative abundance and also a measure of the dominance of the species in the population. Shannon-Wiener Index (H'), like the Simpson's Index, also measures the richness and abundance of the species. Evenness (E) gives us a picture of the relative abundances of the different species in the study area. Table 3 expresses the values of these indexes in the study area. Neelimala is the region with a high Shannon-Wiener Index (H'), Puthoorvayal is the region with a high Simpson's Index and Banasuramalai is the region with high species Evenness (E) with values of

Table 5. Ecological assessment of species occurrence in the three zones.

		Species	Number	Frequency	Density	Abundance	Relative density	Relative frequency	IVI
1	ZONE 1	*P. austrosinense*	6	30	0.4	1.33	5.4422	9.52	14.962
2		*P. cetratum*	1	5	0.05	1	0.7404	1.58	2.3204
3		*P. clavuliferum*	4	20	0.5	2.5	6.8027	6.34	13.14
4		*P. cristiferum*	4	20	0.25	1.25	3.40	6.34	9.74
5		*P. hababianum*	6	30	0.4	1.33	5.44	9.5	14.94
6		*P. planatilobatum*	8	40	0.9	2.25	12.24	12.69	24.93
7		*P. praesorediosum*	1	5	0.05	1	0.68	1.58	2.26
8		*P. reticulatum*	8	40	0.85	2.125	11.56	12.69	24.25
9		*P. stuppeum*	5	25	0.4	1.6	5.44	7.93	13.37
10		*P. tinctorum*	20	100	3.55	3.55	48.29	31.74	80.03
1	ZONE 2	*P. austrosinense*	1	5	0.05	1	1.16	2.43	3.59
2		*P. cetratum*	2	10	0.1	1	2.32	4.8	7.12
3		*P. clavuliferum*	3	15	0.25	1.66	5.81	7.31	13.12
4		*P. cristiferum*	2	10	0.1	1	2.32	4.8	7.12
5		*P. planatilobatum*	3	15	0.25	1.66	5.81	7.31	13.12
6		*P. reticulatum*	10	50	1.55	3.1	36.04	24.39	60.43
7		*P. stuppeum*	6	30	0.4	1.33	9.30	14.63	23.93
8		*P. tinctorum*	14	70	1.6	2.28	37.20	34.146	71.346
1	ZONE 3	*P. austrosinense*	2	10	0.2	2	16	13.33	29.33
2		*P. hababianum*	4	20	0.25	1.25	20	26.66	46.66
3		*P. reticulatum*	3	15	0.2	1.33	16	20	36
4		*P. tinctorum*	6	30	0.6	2	48	40	88

1.512, 0.60, and 0.963, respectively. All these areas show rich biodiversity. The higher values of alpha diversity indicate the wellbeing of the ecosystem. The data in Table 3 can be used as a baseline record of the ecological and population attribute of lichens of Wayanad. The increase in any of these values can be appreciated and negative change is the indication of depreciation of air quality and increasing pollution.

From the ecological perspective, lichens act as the first successors of barren rock, indicators of pollution, etc. The naturally healthy lichen biota of a region indicates the ecological well-being as well as lesser environmental pollution of the region. Information needs for biodiversity studies are many and varied. Any data that deals with biodiversity information has to be geographically based. The role of GIS is to integrate and analyze large varieties of spatial and attribute data for assessment and monitoring purposes of biodiversity. The QGIS data recorded in Image 5 can be used for the needs of today as well as tomorrow as a baseline date to understand the trends in changes of biodiversity of lichens due to pollution, urbanization and climate change.

CONCLUSION

Studying the lichen ecosystems in Wayanad, particularly those confronting multiple threats, holds the potential to contribute significantly to the conservation of the region's biodiversity. The diverse lichen species in this area are currently facing threats attributed to pollution stemming from automobile exhaust and the acidic residues of sulphides and nitrites, a consequence of various human activities. The current study underscores the significance of Wayanad district as an untarnished haven for lichens. Moreover, this study serves as a foundational dataset elucidating lichen diversity and bio-geographic patterns, specifically those of foliose lichens. It also offers a platform for evaluating the impacts of climate change and pollution on the biodiversity of Wayanad district and the broader Western Ghats region.

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REVIEW

Diversity of Calliphoridae and Polleniidae (Diptera) in the Himalaya, India

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Abstract: The family Calliphoridae (Diptera: Calyptratae: Oestroidea) is primarily known for its synanthropic, necrophagous, and myiasis–causing species. This study presents an updated checklist of blow fly species recorded in the Himalayan regions of India, Nepal, and Pakistan. The dataset includes 23 genera and 69 Species from Indian Himalayas, 18 genera and 52 species from the Pakistani Himalayas, and 22 genera and 74 species from Nepalese Himalaya. The data is categorised into three elevation zones: the Shivalik range (350–1,200 m), Lesser Himalaya (1200-2,200 m), and Upper Himalaya (2,200 m and above) taking into consideration factors such as vegetation, temperature, and other environmental variables. The Sorensen Similarity Index was utilized to quantify the degree of species overlap and similarity among blow fly communities within these elevation ranges.

Keywords: Calliphoridae, Himalaya, Ameniinae, Bengallinae, Calliphorinae, Chrysomyinae, Luciliinae, Phumosiinae, Rhiniinae, Polleniidae, Sorensen index.

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INTRODUCTION

The upsurge of the youngest, largest, and highest chains of mountains, the Himalaya, from the Mediterranean seabeds of Tethys involved three distinct and widely separated phases of the uplift (Pandit et al. 2014). The upliftment of marine sediments during the post-Eocene epoch gave rise to the present-day "Greater ranges of Himalaya"**.** The second upheaval at the end of the Miocene epoch formed the present-day "Middle or Lesser Himalayan ranges" and the last movement at the end of the Tertiary period led to the formation of the "Shivalik range" of Himalaya (Wadia 1963). Afghanistan, Pakistan, India, Nepal, Bhutan, China, and Myanmar have sovereignty over the Himalayan landscape (Xu et al. 2009; Pandit et al. 2014). The Shivalik ranges and the Lesser Himalayan ranges fall in the Oriental region and witness a subtropical to sub-temperate type of climate. On the other hand, the Greater Himalayan ranges, which lie in the Palaearctic zone, experience a temperate type of climatic conditions. Thus, the complex ecosystem and topography of the Himalaya, coupled with factors such as adaptive divergence, speciation, following immigration, or allopatric speciation, have made it a hotspot of biodiversity (Xu et al. 2009). While extensive research has explored the biodiversity and evolutionary dynamics of plants and vertebrates in the Himalayan region, there is a significant knowledge gap. This gap pertains to our understanding of the biodiversity and distribution of invertebrates, especially insects, which constitute the largest percentage of organisms worldwide.

The origin and diversification of dipteran lineages (true flies) encompass the four largest Mesozoic insect radiations within its sub-order Brachycera, i.e., the "higher Diptera" (Wiegmann et al. 2011). Most hypotheses suggest that the four major Brachyceran lineages (Xylophagomorpha, Tabanomorpha, Stratiomyomorpha (SXT clade), and Muscomorpha) originated in the Jurassic (200 MYA) and radiated rapidly into the diverse extant forms present today. The family Calliphoridae belongs to the clade Schizophora and the group Calyptratae of the infra-order Muscomorpha. According to Wiegmann (2011), the clade Schizophora originated within the Upper Cretaceous (74–98 MYA) and diversified in the Tertiary (65–20 MYA), exploding into numerous families of acalyptrate Diptera between 65–40 MYA, radiation that has occurred within a short period. The calyptrate, on the other hand, comprises the youngest lineage of Diptera, e.g., blow flies, house flies, etc., and first appeared in the fossil record about 40 million years ago (Wiegmann et al. 2011). Schizophoran radiation, which accounts for more than a third of extant fly diversity and 3% of all animal diversity, is the largest insect radiation in the Tertiary (Wiegmann et al. 2011). This period coincides with the formation of the Himalaya. The blow fly species of seven subfamilies have adapted well to the environmental stress of the region and have undergone adaptive radiation. It is also believed that the flies appear to become diverse because of higher rates of speciation and lower rates of extinction (Wiegmann et al. 2011). Compared to other dipteran lineages, the young calyptrate taxa have evolved a variety of life strategies, namely, the development of ptilinum sacs, the capacity to feed in almost any nutrient-rich medium, and have diversified to occupy a broad range of trophic niches (Cerretti et al. 2017).

The family Calliphoridae (Diptera: Calyptratae: Oestroidea) is largely known for its synanthropic, necrophagous, and myiasis-causing species (Courtney et al. 2017). Historically, the group was an assemblage of paraphyletic taxa (Rognes 1997; Kutty et al. 2010) and comprised up to 14 sub-families, viz., Ameniinae, Aphyssurinae, Bengaliinae, Calliphorinae, Melanomyinae, Chrysomyinae, Helicoboscinae, Luciliinae, Mesembrinellinae, Phumosiinae, Polleniinae, Prosthetosominae, Rhiniinae, and Toxotarsinae (Yan et al. 2021). The study of Calliphoridae phylogeny has accelerated in the last decade thanks to the application of molecular methods. Multiple hypotheses and taxonomic actions have been put forth in the study of certain fly families, such as raising polleniids, rhiniids, and mesembrinellids to full family status (Kutty et al. 2010; Marinho et al. 2011, 2017; Singh & Wells 2013; Cerretti et al. 2017, 2019). These hypotheses have faced challenges in terms of robust support, particularly in critical nodes, when relying on traditional multi-locus Sanger sequencing.

A pivotal shift occurred with the adoption of next-generation sequencing (NGS) methods, leading to the emergence of three highly supported phylogenetic hypotheses (Kutty et al. 2019; Buenaventura et al. 2021; Yan et al. 2021). Notably, Yan et al. (2021) proposed a formal reclassification of the family, revisiting the concept of a broad Calliphoridae family that includes various subfamilies: Ameniinae (incorporating the former Helicoboscinae), Bengaliinae, Calliphorinae (encompassing the former Aphyssurinae, Melanomyinae, and Toxotarsinae), Chrysomyinae, Luciliinae, Phumosiinae, Rhiniinae, and Rhinophorinae.

This study focuses on exploring the taxonomic and ecological diversity of the Calliphoridae and Polleniidae groups in the Himalayan region, with a specific emphasis on their elevation ranges. In the Oriental region, these groups are expansive, encompassing approximately 47 genera and 390 species (Kurahashi & Kirk-Spriggs 2006). Within India, there are 128 species belonging to these groups, distributed across 30 genera and eight subfamilies (Bharti & Kurahashi 2009, 2010; Bharti 2011, 2012, 2014a,b, 2015a,b,c, 2018, 2019; Bharti & Bharti 2016; Bharti & Bunchu 2016; Bharti & Verves 2016; Bharti & Singh 2017; Bharti & Rognes 2018). In contrast, the Indian Himalayan region is represented by 23 genera and 69 species. Similarly, Pakistan exhibits a diversity of 18 genera and 57 species (Hassan et al. 2018), with 52 species located on the Pakistani side of the Himalaya. The Nepalese Himalaya house 22 genera and 74 species of blow flies (Kurahashi & Thapa 2002) (Table 1). This comprehensive checklist provides an updated record of blow fly species found across various Himalayan regions in India, Nepal, and Pakistan.

MATERIALS AND METHODS

The Himalayan blow flies (including families Calliphoridae and Polleniidae) checklist is based on original papers (Senior-White et al. 1940; Kurahashi 1989, 1994; Rognes 1993; Wells & Kurahashi 1995; Cerretti 2017, 2019; Hassan et al. 2018), lead author's collection data from northwestern and northeastern Himalaya (Bharti & Kurahashi 2009, 2010; Bharti 2011, 2012, 2014a,b, 2015a,b,c, 2018, 2019; Bharti & Bharti 2016; Bharti & Bunchu 2016; Bharti & Verves 2016; Bharti & Singh 2017; Bharti & Rognes 2018) and Pakistan (Kurahashi & Afzal 2002; Hassan et al. 2018). It includes the currently valid genera and species of the two families reported from India, Pakistan, and the Nepalese Himalaya. The data is divided into three altitude zones: the Shivalik range (350–1,200 m), the Lesser Himalaya (1,200–2,200 m), and the Upper Himalaya (2,200 m and above) with respect to vegetation, temperature, and other environmental factors (Mani 1968). The Sorensen similarity index was calculated to measure the extent of species overlap or similarity among blow fly communities in the three ranges.

RESULTS

Representatives of seven subfamilies, namely Ameniinae (including Helicoboscinae), Bengaliinae, Calliphorinae, Chrysomyinae, Luciliinae, Phomosiinae, and Rhiniinae, are present in the Himalaya. Subfamily Ameniinae is represented by two species of the genus *Catapicephala* (*C. pattoni* from Pakistan and the Nepalese Himalaya and *C. splendens* and *C. pattoni*) and

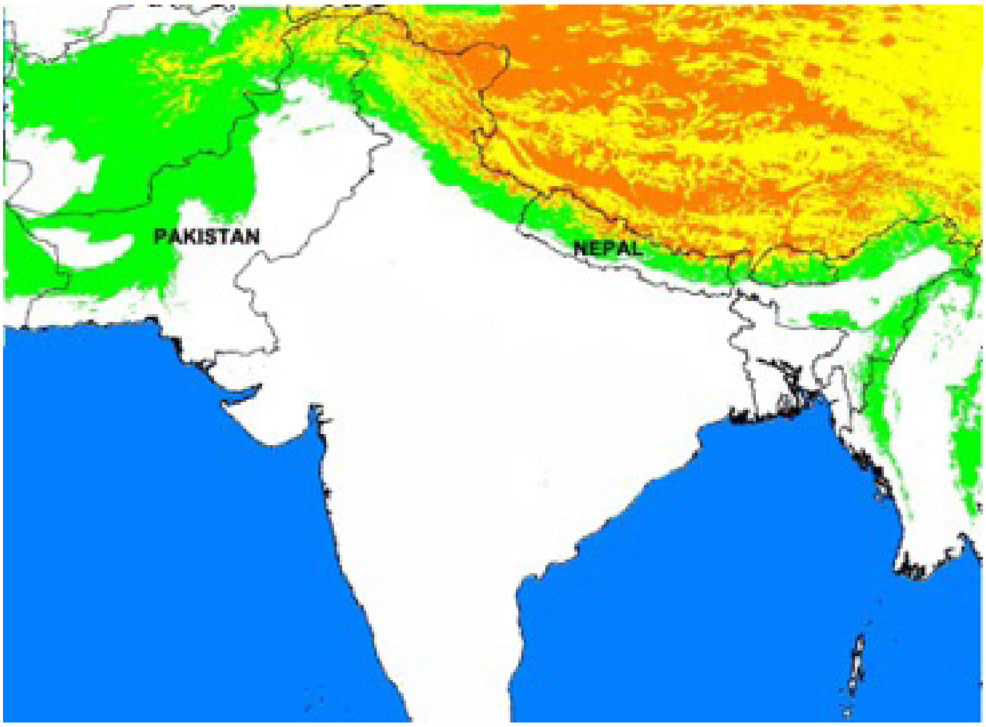

Figure 1. Map showing Himalayan regions spanning across India, Pakistan, and Nepal.

Figure 1. Map showing Himalayan regions spanning across India, Pakistan, and Nepal.

Family	Sub-family	Species	Indian Himalaya	Pakistan Himalaya	Nepalese Himalaya
Calliphoridae	**Ameniinae**	*Catapicephala splendens*	√		
		Catapicephala pattoni	√	√	√
		Gulmargia angustisquama	√		
	Bengaliinae	*Bengalia varicolor*	√	√	√
		Bengalia martinleakei	√	√	√
		Bengalia surcoufi	√	√	√
		Bengalia torosa	√	√	√
		Bengalia unicolor		√	
		Bengalia emarginata			√
		Bengalia escheri	√	√	√
		Bengalia subnitida			√
		Bengalia hastativentris	√		
		Termitoloemus marshalli	√		
		Aldrichina grahami		√	
		Calliphora chinghaiensis		√	√
		Calliphora himalayana		√	√
		Calliphora uralensis	√		
		Calliphora vicina	√	√	√
		Calliphora vomitoria	√	√	√
		Calliphora loewi		√	√
		Calliphora pattoni	√		√
		Cynomya mortuorum	√	√	
		Melinda sugiyamai		√	√
		Melinda scutellata	√	√	√
		Melinda abdominalis	√		
		Melinda bengalensis	√		
		Melinda pusilla indica	√		√
		Melinda nuortevae			√
		Melinda nepalica			√
		Nepalonesia pulchokii			√
		Nepalonesia shinonagai			√
		Onesia sp.		√	
		Onesia kiyoshii		√	
		Onesia menechmiodes		√	
		Onesia flavisquama			√
		Onesia atripalpis	√		
		Onesia khasiensis	√		
		Onesia girii			√
		Polleniopsis sp.		√	
		Polleniopsis himalayana			√
		Polleniopsis nepalica			√
		Polleniopsis pilosa	√		
		Polleniopsis kasmirensis	√		

Family	Sub-family	Species	Indian Himalaya	Pakistan Himalaya	Nepalese Himalaya
		Polleniopsis pulchokii			√
	Chrysomyinae	*Chrysomya megacephala*	√	√	√
		Chrysomya albiceps	√	√	
		Chrysomya nigripes	√	√	√
		Chrysomya phaonis	√	√	√
		Chrysomya pinguis	√	√	√
		Chrysomya putoria	√		
		Chrysomya regalis		√	
		Chrysomya rufifacies	√	√	√
		Chrysomya bezziana	√		
		Chrysomya defixa	√		
		Chrysomya villeneuvi	√		√
		Chrysomya chani	√		√
		Chrysomya thanomthini	√		√
		Protocalliphora azurea		√	
		Protocalliphora maruyamensis		√	
		Protocalliphora terraenovae		√	
		Trypocalliphora braueri			√
	Luciliinae	*Hemipyrellia ligurriens*	√	√	√
		Hemipyrellia pulchra	√	√	√
		Lucilia cuprina	√	√	√
		Lucilia papuensis	√	√	√
		lucilia porphyrina	√	√	√
		Lucilia sericata	√	√	
		Lucilia ampullacea	√		
		Lucilia bazini	√		
		Lucilia calviceps	√	√	
		Lucilia illustris	√		
		Lucilia bismarkensis			√
		Lucilia shenyangensis			√
		Lucilia sinensis			√
	Phumosiinae	*Phumosia testacea*			√
	Rhiniinae	*Borborhinia bivitatta*	√		
		Cosmina prasina	√	√	√
		Cosmina nepalica			√
		Cosmina limbipennis	√		
		Isomyia aurifacies		√	
		Isomyia fulvicornis	√	√	
		Isomyia pseudoviridana	√	√	√
		Isomyia coei			√
		Isomyia electa		√	√
		Isomyia facialis			√
		Isomyia gomezmenori			√
		Isomyia hetauda			√
		Isomyia nepalana			√

Family	Sub-family	Species	Indian Himalaya	Pakistan Himalaya	Nepalese Himalaya
		Isomyia oestracea	√		√
		Isomyia pichoni			√
		Isomyia pictifacies			√
		Isomyia shelpa			√
		Isomyia singhi			√
		Isomyia sivah	√		√
		Isomyia versicolor	√		√
		Isomyia delectans	√		
		Isomyia viridaurea	√		
		Isomyia nebulosa	√		
		Metallea flavibasis	√		
		Metallea setosa	√		√
		Metallea setiventris			√
		Rhyncomya townsendi		√	
		Rhyncomya setipyga			√
		Strongyloneura prolata			√
		Chlororhina exempta	√		
		Idiella divisa	√		√
		Rhinia apicalis	√	√	
		Stomorhina cribrata		√	
		Stomorhina discolor	√	√	√
		Stomorhina procula	√	√	√
		Stomorhina lunata	√	√	
		Stomorhina melastoma	√		√
		Stomorhina xanthogaster	√	√	√
		Stomorhina luteigaster			√
Polliniidae		*Dexopollenia nigriscens*			√
		Dexopollenia testacea	√		√
		Morinia argenticincta	√		√
		Pollenia dasypoda		√	
		Pollenia pediculata		√	
		Pollenia rudis	√	√	√

one species of the genus *Gulmargia* from the Indian Himalaya.

Thirteen taxa of Bengaliinae are known from the world (Rognes 2011), out of which only two, namely, *Bengalia* and *Termitoloeus,* are known from Nepal, Pakistan, and the Indian Himalaya. The adults of *Bengalia* are predaceous on the immature stages of ants (Rognes 2009), and their larvae feed in termite nests (Rognes 2011). There is also an observation of an adult *Bengalia* sucking the abdomen of a termite (Rognes 2011). *Termitoloeus marshalli* Baranov is the sole species known from the Indian Himalaya that attacks and captures termite mounds and feeds on termite broods. The fly in question is so voracious that it can finish a termite colony in a few months, and this aspect could potentially be used to control the termite menace in India.

Sub-family Calliphorinae (including Aphyssurinae, Melanomyinae, and Toxotarsinae) is represented by the genera *Aldrichina*, *Calliphora*, *Cynomya*, *Melinda*, *Nepalonesia, Onesia,* and *Polleniopsis* from Nepal, Pakistan, and the Indian Himalaya (Kurahashi & Afzal 2002; Bharti 2015a,b, 2018; Bharti & Rognes 2018; Hassan et al. 2018). Representatives of the genus

Cynomya have been reported from Pakistan and the Indian Himalaya (Bharti & Rognes 2018). *Nepalonesia* only comes from the Nepalese Himalaya. The genus *Aldrichina* is represented by single species, *Calliphora, Polleniopsis,* and *Melinda* by seven species, and *Onesia* by six each from the region under study (Table 1). The flies belonging to this group are oviparous or viviparous. Larvae are saprophagous, parasites of snails, or predators of earthworms.

The subfamily Chrysomyinae encompasses 12 genera, including *Phormia* Robineau-Desvoidy, *Protophormia* Townsend, *Protocalliphora* Hough, *Trypocalliphora* Peus, *Phormiata* Grunin, and *Chrysomya* Robineau-Desvoidy, all generally characterized as Holarctic/Paleotropical or belonging to the Old World Chrysomyines. Additionally, it includes *Chrysopyrellia* Seguy, *Cochliomyia* Townsend, *Compsomyiops* Townsend, *Hemilucilia* Brauer, *Paralucilia* Brauer & Bergenstamm, and *Chloroprocta* Wulp, classified as Neotropical. The previous practice of employing arbitrary tribal classifications, as proposed by Rognes in 1991 and later reaffirmed by Singh & Wells in 2013, has been abandoned. In the Himalayan region, this subfamily is represented by the genera *Chrysomya*, *Protocalliphora*, and *Trypocalliphora*. For instance, *Chrysomya* comprises thirteen species distributed across different Himalayan ranges, each playing distinct ecological roles such as scavenging, parasitism, and predation (Bharti & Kurahashi 2009; Bharti 2019).

Subfamily Luciliinae includes two species of the genus *Hemipyrellia* and 11 species of the genus *Lucilia* from the studied area. All species are oviparous, with larvae primarily exhibiting saprophagous behaviour in decaying animal matter. The adults visit flowers, faeces, and dead animals, and many species are involved in human or animal myiasis, inflicting wounds. The genus *Lucilia* is of great medical, hygienic, and forensic importance, with some species suspected of transmitting the poliomyelitis virus to humans (Rognes 1991).

Phumosia testacea is the sole representative of the Phumosiinae subfamily identified in the Nepalese Himalayan region. Records also confirm its presence in southern India, where observations have been made regarding the breeding of these flies within frog egg masses. An interesting aspect of their behaviour is the targeted attack on frog egg nests, with the larvae actively preying upon and consuming the developing embryos. This phenomenon was documented by Senior-White et al. (1940), shedding light on the unique ecological interactions of these flies within their habitat.

Subfamily Rhiniinae is broadly divided into two subfamilies, Cosminiinae and Rhiniinae. It is one of the most diverse and widely distributed families in the Oriental region, with approximately 14 genera. The subfamily includes the genera *Bororhinia*, *Cosmina*, *Strongyloneura, Isomyia, Metallia, Rhyncomya, Chlororhinia, Idiella, Rhinia*, and *Stomorhina* from the Nepalese, Pakistani, and Indian Himalaya. These flies are closely associated with Hymenoptera, Isoptera, and Orthoptera. Some species are predators of locust egg capsules, while others are associated with termites and ant nests (Senior-White 1940; Arce et al. 2019). The subfamily remains relatively unexplored biologically (Dear 1977).

Family Polleniidae, previously considered a part of the Calliphoridae family, has undergone a reclassification based on molecular studies and their breeding habits as parasitoids of soil-dwelling invertebrates. Molecular research conducted by Singh and Wells (2013), Winkler et al. (2015), Cerretti et al. (2017), Blaschke et al. (2018), Kutty et al. (2019), Stireman et al. (2019), and Johnston et al. (2022) has indicated their sister group relationship with Tachinidae, suggesting a phylogenetic distance from Calliphoridae. Furthermore, Cerretti et al. (2014) and Stireman et al. (2019) proposed that a non-molecular synapomorphy could be established based on their breeding habit as parasitoids of soil-dwelling invertebrates, aligning them with sister group tachinids, which also parasitize soil-dwelling insect larvae.

Table 2. Sorensen similarity index shows similarity between different assemblages.

	Shivalik Range	Lower Himalaya	Upper Himalaya
Shivalik Range	_	0.43	0.21
Lower Himalaya		_	0.37
Upper Himalaya			_

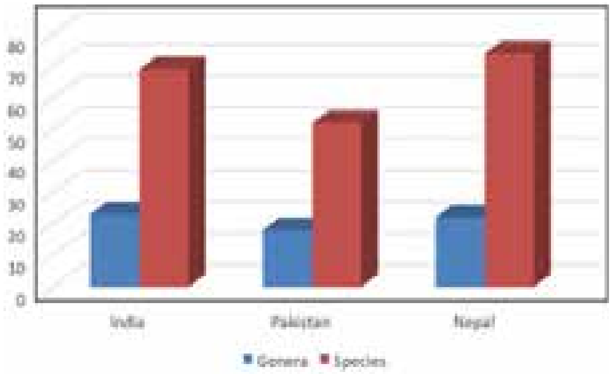

Figure 2. Total species of Calliphoridae and Polleniidae from the Himalaya.

Table 3. List of blowfly species from Pakistan, Nepal, and Indian Himalaya.

Family	Sub-family	Species	Shivalik Range	Lower Himalaya	Upper Himalaya
Calliphoridae	Ameniinae	*Catapicephala splendens*	P	P	A
		Catapicephala pattoni	P	P	A
		Gulmargia angustisquama	A	P	P
	Bengaliinae	*Bengalia varicolor*	P	P	A
		Bengalia martinleakei	A	P	A
		Bengalia surcoufi	P	A	A
		Bengalia torosa	P	P	A
		Bengalia unicolor	A	A	P
		Bengalia emarginata	A	A	P
		Bengalia escheri	P	P	A
		Bengalia subnitida	P	P	A
		Bengalia hastativentris	P	A	A
		Termitoloemus marshalli	P	A	A
		Aldrichina grahami	A	P	A
		Calliphora chinghaiensis	A	A	P
		Calliphora himalayana	A	A	P
		Calliphora uralensis	A	A	P
		Calliphora vicina	P	P	P
		Calliphora vomitoria	P	P	P
		Calliphora loewi	A	P	P
		Calliphora pattoni	A	P	P
		Cynomyamortuorum	A	A	P
		Melinda sugiyamai	P	P	A
		Melinda scutellata	A	P	P
		Melinda abdominalis	A	P	A
		Melinda bengalensis	A	A	P
		Melinda pusilla indica	A	P	P
		Melinda nuortevae	A	A	P
		Melinda nepalica	A	A	P
		Nepalonesia pulchokii	A	P	P
		Nepalonesia shinonagai	A	P	P
		Onesia sp.	A	P	A
		Onesia kiyoshii	A	A	P
		Onesia menechmiodes	P	P	A
		Onesia flavisquama	A	P	P
		Onesia atripalpis	A	A	P
		Onesia khasiensis	A	P	A
		Onesia girii	A	P	A
		Polleniopsis sp.	A	P	A
		Polleniopsis himalayana	A	A	P
		Polleniopsis nepalica	A	P	P
		Polleniopsis pilosa	A	P	A
		Polleniopsis kasmirensis	A	A	P
		Polleniopsis pulchokii	A	A	P
	Chrysomyinae	*Chrysomya megacephala*	P	P	P
		Chrysomya albiceps	P	P	A

Family	Sub-family	Species	Shivalik Range	Lower Himalaya	Upper Himalaya
		Chrysomya nigripes	P	P	P
		Chrysomya phaonis	P	P	P
		Chrysomya pinguis	P	P	P
		Chrysomya putoria	A	P	A
		Chrysomya regalis	A	P	A
		Chrysomya rufifacies	P	P	A
		Chrysomya bezziana	P	P	A
		Chrysomya defixa	P	A	A
		Chrysomya villeneuvi	P	P	P
		Chrysomya chani	P	A	A
		Chrysomya thanomthini	A	P	P
		Protocalliphora azurea	A	P	P
		Protocalliphora maruyamensis	A	A	P
		Protocalliphora terraenovae	A	A	P
		Trypocalliphora braueri	A	A	P
	Luciliinae	*Hemipyrellia ligurriens*	P	P	A
		Hemipyrellia pulchra	P	P	P
		Lucilia cuprina	P	P	P
		Lucilia papuensis	P	P	P
		Lucilia porphyrina	P	P	P
		Lucilia sericata	P	P	P
		Lucilia ampullacea	P	P	A
		Lucilia bazini	P	A	A
		Lucilia calviceps	P	A	A
		Lucilia illustris	P	P	A
		Lucilia bismarkensis	P	P	P
		Lucilia shenyangensis	A	P	P
		Lucilia sinensis	A	P	P
	Phumosiinae	*Phumosia testacea*	A	P	A
	Rhiniinae	*Borborhinia bivitatta*	P	A	A
		Cosmina prasina	P	P	P
		Cosmina nepalica	A	P	A
		Cosmina limbipennis	P	A	A
		Isomyia aurifacies	P	A	A
		Isomyia fulvicornis	P	P	A
		Isomyia pseudoviridana	P	P	P
		Isomyia coei	P	A	A
		Isomyia electa	P	A	A
		Isomyia facialis	P	A	A
		Isomyia gomezmenori	P	P	P
		Isomyia hetauda	A	P	A
		Isomyia nepalana	P	A	A
		Isomyia oestracea	P	P	A
		Isomyia pichoni	A	P	A
		Isomyia pictifacies	P	P	A
		Isomyia shelpa	P	A	A
		Isomyia singhi	P	P	P

Family	Sub-family	Species	Shivalik Range	Lower Himalaya	Upper Himalaya
		Isomyia sivah	P	P	P
		Isomyia versicolor	P	P	A
		Isomyia delectans	P	A	A
		Isomyia viridaurea	P	A	A
		Isomyia nebulosa	P	A	A
		Metallea flavibasis	P	A	A
		Metallea setosa	P	P	P
		Metallea setiventris	A	P	A
		Rhyncomya townsendi	A	P	A
		Rhyncomya setipyga	P	P	A
		Strongyloneura prolata	P	P	A
		Chlororhina exempta	A	P	A
		Idiella divisa	P	A	A
		Rhinia apicalis	P	P	A
		Stomorhina cribrata	A	P	A
		Stomorhina discolor	P	P	P
		Stomorhina procula	P	P	P
		Stomorhina lunata	A	P	P
		Stomorhina melastoma	P	A	A
		Stomorhina xanthogaster	P	P	A
		Stomorhina luteigaster	A	A	P
Polliniidae		*Dexopollenia nigriscens*	A	A	P
		Dexopollenia testacea	A	A	P
		Moriniaargenticincta	A	P	A
		Pollenia dasypoda	A	P	P
		Pollenia pediculata	A	P	A
		Pollenia rudis	A	P	A

Presently, 147 species of Polleniidae are classified under eight genera worldwide (Cerretti et al. 2019). The genus *Pollenia* stands out as the most species-rich, with 95 representatives from Oriental, Australasian, and Palaearctic regions. *Dexopollenia* comprises 21 species, *Morinia* 13, *Melanodexia* 8, and *Xanthotryxus* 7, with *Anthracomyza* Malloch, *Alvamaja* Rognes, and *Nesodexia* Villeneuve each represented by a single species. In the Himalaya, these flies are represented by the genera *Dexopollenia, Morinia*, and *Pollenia* (Table 1). *Morinia* species primarily feed on dead decaying matter, whereas lumbricids serve as hosts and substrates for larval development in *Pollenia* species. Cluster fly larvae exclusively develop on earthworms and do not accept other food sources, although there are occasional reports of alternative hosts such as insect larvae (Yahnke & George 1972; Jewiss-Gaines et al. 2012).

Ecological diversity

Blow flies being ubiquitous seem to occur in almost all the available ecosystems on Earth. Having said so, it is also true that there is a disparity in the distribution of species regarding climate, latitude, and altitude. Like many other groups of plants and animals, blow flies show a strong latitudinal gradient in their diversity, with the highest at the equator and declining towards the poles. Similarly, altitude also has a profound effect on the richness and abundance of Calliphoridae. Generally, species diversity decreases with an increase in altitude. But, in the case of Himalayan blow flies, diversity was maximum at the mid-elevation (MDE), i.e., lower Himalaya (79 species) compared to Shivalik (64) and upper Himalayan ranges (56).

CONCLUSIONS

A comprehensive survey in the regions of Pakistan, Nepal, and the Indian Himalayas has resulted in the recording of a total of 30 genera and 120 species of blow flies. Among these regions, Pakistan exhibits 60% of the generic diversity, while the Nepalese and Indian Himalaya each contribute 70% (Figure 1).

To assess the spatial variability of environmental conditions and describe species composition along environmental gradients, the Himalayan blow fly fauna was categorised into three distinct elevation ranges: 350–1,200 m (Shivalik range, sub-tropical), 1,200–2,200 m (Lower Himalayan ranges, sub-temperate), and 2,200 m onwards (Upper Himalayan ranges, temperate) (Table 3). It's important to note that the first two elevation ranges are situated in the Oriental region, while the Upper Himalayan ranges belong to the Palaearctic region.

The evaluation of faunal similarity between these three assemblages used the incidence-based Sorensen Similarity Index (Sorensen 1948): 2a/(2a+b+c), where 'a' represents the number of shared species, 'b' the number of unique species in the first assemblage, and 'c' the number of unique species in the second assemblage. Findings indicate that the Shivalik range shares approximately 43% faunal similarity with the Lower Himalayan ranges but only about 21.42% with the Upper Himalayan ranges. Conversely, the lower and upper ranges exhibit a 37.11% similarity in their species assemblages (Table 2). Additionally, 11 genera were identified that are common to all three elevational ranges, differing only in the composition of species along different gradients in the Himalaya. The highest generic similarity was observed between the lower and upper ranges of the Himalaya, accounting for 56.6% similarity. Specifically, the genera *Morinia* and *Phumosia* were unique to the lower ranges, while *Dexopollenia, Trypocalliphora*, and *Cynomya* were unique to the upper Himalayan ranges.

These findings contribute valuable insights into the distribution and diversity of blow flies across the Himalayan region, shedding light on the unique characteristics of each elevation range.

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SHORT COMMUNICATION

First photographic evidence of mange manifestation in Panna Tiger Reserve, India

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Abstract: We report the first ever photographic evidence of mange-infested Golden Jackal *Canis aureus* from Panna Tiger Reserve, central India. The infected animals were photo-captured during the ongoing camera trap sampling in 2019 as a part of a long-term study on the ecology of reintroduced tigers and co-predators. This new record triggers wildlife health and monitoring issues and, subsequently, the importance of restricting the disease outbreak and treatment measures among other associated species within the protected area.

Keywords: *Canis aureus*, carnivore, disease, Golden Jackal, Madhya Pradesh, Mange, Vindhya Hills.

Sarcoptic mange is a commonly widespread and highly contagious skin disease found in wild mammals (McCarthy et al. 2004; Currier et al. 2011). The microscopic mite, *Sarcoptes scabiei*, is the causative agent that infests the skin of its host epidermis by burrowing tunnels (Fuller 2013). Females lay eggs there; subsequently, nymph starts to burrow new tunnels by cutting, secreting, and infesting the epidermal skin (Arlian et al. 1984, 1989), resulting in uncontrolled itching, hair loss, erythema (redness of the skin due to inflammation), and secondary skin infection, which may further lead to death of the host animal (Radi 2004; Oleaga et al. 2008; Nakagawa et al. 2009). These epizootics have been reported in various host species and well documented in Europe, America, Australia, Africa and Asia (Zumpt & Ledger 1973; Mörner 1992; Kraabøl et al. 2015; Fraser et al. 2016; Old et al. 2018; Niedringhaus et al. 2019). The mite spreads from infested animals to a new host through both direct (rubbing) and indirect contact by sharing common dens and resting places (Devenish-Nelson et al. 2014; Almberg et al. 2015; Ezenwa et al. 2016); moreover, transmission from adult to offspring also occurs (Fthenakis et al. 2001).

Mange is well-documented for domestic animals, though limited knowledge is available for wild animals, especially in the Indian subcontinent. In India, the infestation of mange is common in domestic animals (Chhabra & Pathak 2011) such as cats (Sivajothi &

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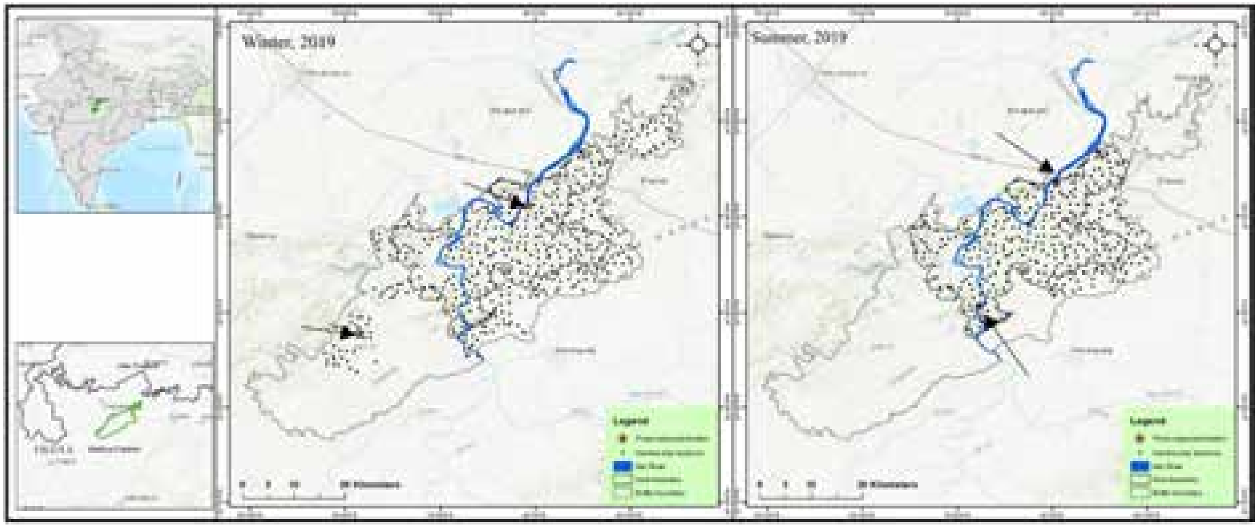

Figure 1. Location of the study area and the season-wise camera trap station locations (black dots) during winter 2019 (left) and summer 2019 (right). The marked areas (red star, indicated by arrow) are the photo-captured sites of mange-infested Golden Jackal.

Reddy 2015), goats (Sreenivasan & Rizvi 1946), and cattle (Tikaram & Ruprah 1986; Gill et al. 1989). Sarcoptic mange was also found in pigs in India (Das et al. 2010; Laha 2015). Earlier, mange-infested Golden Jackal was documented in western India (Dubey et al. 2016).

Study Area and Methods

The study was carried out in Panna Tiger Reserve (PTR), situated in the Vindhyan hill range, under biogeographic province 6A Deccan Peninsula – Central Highlands (Rodgers et al. 2002). PTR spreads over 1,574 km^2 area with two distinct administrative units; core zone (542 km^2) and buffer zone (1,032 km^2), and covers Panna, Chhatarpur and Damoh districts. PTR is categorized as tropical dry-deciduous forests (Champion & Seth 1968); and Teak *Tectona grandis*, Tendu *Diospyros melanoxylon*, Khair *Acacia catechu*, and Kardhai *Anogeissus pendula* are the major dominated trees in this landscape. Tiger is the apex predator in this ecosystem, while Leopard *Panthera pardus* represents a co-predator. The forest carries a good number of other carnivores like Indian Wolf *Canis lupus*, Striped Hyena *Hyaena hyaena*, Asiatic Wild Dog *Cuon alpinus*, Golden Jackal *Canis aureus*, Indian Fox *Vulpes bengalensis*, Jungle Cat *Felis chaus*, Asiatic Wildcat *Felis silvestris*, and Rusty Spotted Cat *Prionailurus rubiginosus*. Chital *Axis axis*, Sambar *Rusa unicolor,* and Wild Boar *Sus scrofa* are the major prey species, followed by Nilgai *Boselaphus tragocamelus*, Chinkara *Gazella bennettii*, and Chousingha *Tetracerus quadricornis*. The core area is highly protected, while the buffer area of PTR holds 63 villages, most of it being a human-dominated landscape.

We deployed a pair of automated camera traps in 2 km^2 grid network. A total of 476 and 338 stations were active during the winter (survey period January 2019 to March 2019) and summer (survey period May 2019 to June 2019) season of 2019 (Figure 1) as a part of the study of monitoring released tiger and associated carnivore populations in PTR. The camera trap stations were active for at least 30 days on a 24-hour basis to ensure demographic closure (Kendall 1999), and traps were checked at an interval of 5–7 days.

Results

A total of 476 (14,500 trap nights) and 338 (11,719 trap nights) camera trap stations yielded 2,145 and 757 independent captures of Golden Jackals during winter and summer, respectively. Among those, we found three independent captures of Golden Jackals from two different locations, both in summer and winter. The photo-captured stations were 5.5 km and 2.52 km away (linear distance) from the village during winter, while in summer, the locations were 4.06 km and 500 m away from the nearby village. The captured individuals (Image 1) had severe erythema, alopecia (excessive hair/ fur loss) and infection (Bornstein et al. 1995; Nimmervoll et al. 2013).

Discussion and Management Implication

The mite, *Sarcoptes scabiei*, was intentionally introduced during the early 20th century to reduce and control the Wolf and Coyote population in the USA; it was first reported in wolves in Yellowstone National

Image 1. Images of mange-infested Golden Jackal during the camera trap sampling in 2019 in Panna Tiger Reserve, central India.

Park in January 2007. Mange-infested animal death is common (Wydeven et al. 2003; Smith & Almberg 2007) and can spread quickly in the population (Almberg et al. 2012). Therefore, identification of affected individuals is necessary and proper treatment should be provided (Rowe et al. 2019). Mange-infested jackal has the potential to infect other jackals and other species through direct and indirect contact (Alasaad et al. 2012; Valldeperes et al. 2021). Affected individuals are genetically compromised, which may lead to severe detrimental effects in population level (DeCandia et al. 2021). Since transmission may occur from human to domestic animals or domestic to wild animals; thus, the 'One Health' approach should be executed to monitor human-domestic-wildlife health (Lerner & Berg 2015; Mackenzie & Jeggo 2019).

A tiger had died in PTR due to canine distemper virus (CDV; Shetty 2019). Thereafter, a vaccination drive was implemented for domestic animals to restrict the transmission of CDV from domestic animals to wild animals (Nayak et al. 2020). Taking clue from our present observations, we strongly recommend that PTR must adopt a wildlife disease surveillance strategy to reduce and restrict any pathogen transmission. Routine monitoring of the health of domestic and wild population, where feasible (including blood sample collection) and water quality analysis of waterholes should be exercised. Usage of the thermal camera can play an important role in identifying and monitoring the affected individuals, as they emit severe heat loss signatures due to extensive alopecia.

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SHORT COMMUNICATION

New locality record of Forest Spotted Gecko *Cyrtodactylus* (*Geckoella*) cf. *speciosus* (Beddome, 1870) (Reptilia: Squamata: Gekkonidae) from Thanjavur, in the eastern coastal plains of Tamil Nadu, India

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Abstract: The study of species distributions is critical for gaining insights into biogeographic patterns and for the protection of threatened species. Here, I report on the new distributional record of the Forest Spotted Gecko (*Cyrtodacylus* cf. *speciosus*) from Thanjavur in the coastal plains of Tamil Nadu. This observation marks the first documented occurrence of this species outside its typical hilly habitat in southern India.

Keywords: Eachankottai Village, Erode Gecko, population, range extension, road cruising surveys.

The Forest Spotted Gecko or Erode Gecko *Cyrtodactylus speciosus* (Beddome, 1870) was described initially as *Gymnodactylus speciosus* without precise locality information from "a tope near Erode," in Erode District, Tamil Nadu, India (Beddome 1870). Smith (1935) listed this species as a color variant of *Gymnodactylus collegalensis* Beddome, 1870 and noted that its range is limited to low-elevation sites in the hills of southern India. Agarwal et al. (2016) subsequently recognized this species as *Cyrtodactylus speciosus* based on comparisons with the damaged type specimen and molecular data collected from individuals at Coimbatore North Taluk, Coimbatore District, Tamil Nadu. This species is yet to be collected from the type locality, Erode. *Cyrtodactylus speciosus* is also reported from three other locations in the southern Eastern Ghats (Jawadhu, Shevaroy, and Kolli hills; Table 1). Further, this species or a closely related lineage is known to occur in Sirumalai Hills, Dindigul District, Tamil Nadu (Ganesh & Arumugam 2016). However, these records were based on photo vouchers alone, whereas persevered voucher specimens and tissue samples for genetic data would have ideally been the preferred case. Here, based on photo vouchers, I report the easternmost locality of *Cyrtodactylus* cf. *speciosus* from Thanjavur, in the coastal plains of Tamil Nadu.

On 12 July 2014 at 1952 h, I encountered a live adult *Cyrtodactylus* cf. *speciosus* crossing a road near Eachankottai Village, Thanjavur, Tamil Nadu (10.6490°N, 79.1492°E; Figure 1). This individual was identified to be a male (based on hemipenal bulge) with a snout-vent length (SVL) of 47 mm. I encountered another male gecko with a SVL 43 mm on 10 June 2022 at 2037 h from the same road (10.6529°N, 79.1500°E; Image 1).

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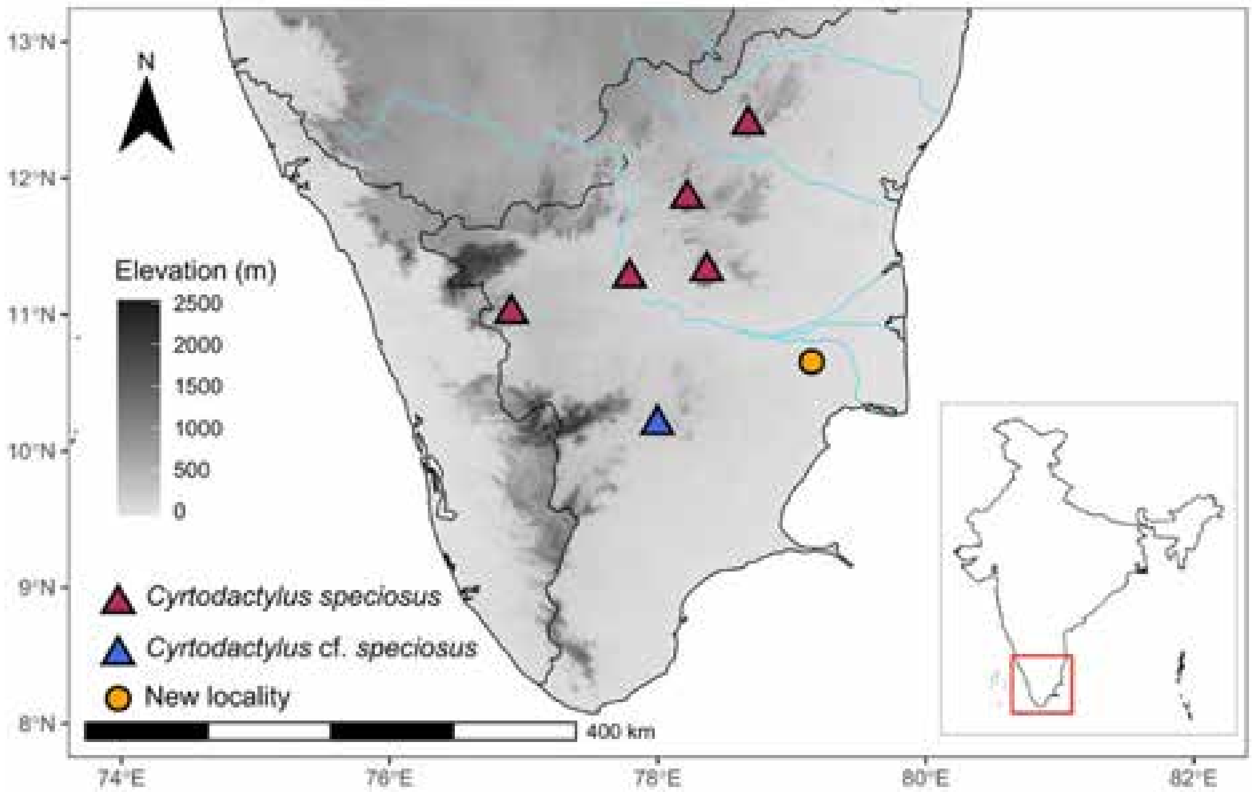

Figure 1. Map showing known locality records for: *Cyrtodactylus speciosus*—maroon triangle | *Cyrtodactylus* cf. *speciosus*—blue triangle | new locality—orange circle.

Table 1. Published locality records and corresponding districts where *Cyrtodactylus speciosus* and *Cyrtodactylus* cf. *speciosus* have been reported from Tamil Nadu.

Locality	District	Reference
'Tope near Erode'	Erode	Beddome 1870
Coimbatore North Taluk	Coimbatore	Agarwal et al. 2016
Jawadhu hills	Tiruvannamalai	Ganesh & Arumugam 2016
Shevaroy hills	Salem	Ganesh & Arumugam 2016
Kolli hills	Namakkal	Ganesh & Arumugam 2016
Sirumalai hills (*Cyrtodactylus* cf. *speciosus*)	Dindigul	Ganesh & Arumugam 2016
Eachankottai village (*Cyrtodactylus* cf. *speciosus*)	Thanjavur	Present note

Both individuals were visually similar to *C. speciosus,* as described by Agarwal (2016) and Agarwal et al. (2016). The characters noted in the two individuals include two interorbital spots, an elongated interparietal streak longer than two interoccipital spots, a deeply notched post-occipital collar (separated from the postorbital streak in one of the two individuals; Image 1), and two brown dorsal bands between limb insertions with the black border of the bands slightly notched (Image 1). The geckos made squeaking calls when handled for the first time, as observed in other *Geckoella* group (Agarwal et al. 2016). The geckos were photographed, measured, and released at the capture site. Other geckos observed in this location include the widespread, common, and human-commensal ones, viz., *Hemidactylus* cf. *parvimaculatus, H. triedrus*, *H. frenatus,* and *H. leschenaultii*. *Hemidactylus scabriceps*, another dryland specialist that shares ecological characteristics of *C. speciosus* (strictly ground-dwelling and nocturnal)*,* is also known to occur in Thanjavur (Srikanthan et al. 2018). However, I have not seen *H. scabriceps* from this location. The only nearby sighting of *H. scabriceps* is about ca. 20 km from the current location (10.7958°N, 79.0589°E).

The habitat adjacent to the road where the geckos were found consists mainly of open arid scrublands and thorn forests—Deccan thorn scrub forest ecoregion—according to Olson et al. (2001). These are bordered by plantation trees along both sides of the road (Image 2). This land area is a part of the district's exotic cattle breeding farm, established by the Government of Tamil Nadu in 1954, and part of it has remained unaltered since

Image 1. Voucher photographs of *Cyrtodactylus* cf. *speciosus* from Eachankottai Village, Thanjavur District, Tamil Nadu. The top individual was photographed in 2014, and the bottom individual was photographed in 2022. © Gopal Murali.

Image 2. Habitat of *Cyrtodactylus* cf. *speciosus* from Eachankottai Village, Thanjavur, Tamil Nadu showing the unmanaged open arid scrub forest habitat where the geckos were seen. © Gopal Murali.

then. Regions immediately surrounding the farm are dominated greatly by agricultural land, mostly cashew and sugarcane fields. I further conducted extensive road cruising surveys in nearby potentially suitable habitats within a ca. 25 km radius surrounding the sighted locality. However, I did not find any *Cyrtodactylus* spp. This indicates that species is probably sensitive to habitat modification and that the population is likely confined to the unmanaged arid scrublands within the cattle farm, encompassing about 5.6 km^2 in total, with native scrubland occupying no more than 2.6 km^2. The currently reported locality does not strictly fall under any protected area network. However, the entire farm area is fenced (Image 2), and outsiders are not allowed without permission. Nevertheless, this region seemed to have been occasionally used by locals for cattle grazing. The present sighting of such an apparently habitat-specific gecko (Agarwal et al. 2016) suggests that such habitat patches like in Eachankottai require greater protection, as they harbor greater biodiversity than the surrounding farmlands, in as far as is known.

Cyrtodacylus speciosus has been assigned to the 'Endangered' category by the IUCN, and the species population status is assessed to be in decline due to habitat loss and fragmentation (Achyuthan et al. 2021). This new easternmost locality for *C.* cf. *speciosus* is ca. 112 km south-west of the nearest known locality at Kolli Hills, Tamil Nadu, and 173 km from the type locality Erode (Figure 1). Nevertheless, it remains unclear if *C. speciosus* is actually a single widespread species or a group of closely related species (Agarwal et al. 2023a). All members of the *C. collegalensis* complex, except *C. varadgirii* and the recently described *C. chengodumalaensis*, have been documented only in hilly landscapes of southern India (Agarwal et al. 2023b). The newly documented location is situated at an elevation of 24 m, about 65 km from the nearest hillock. Further, this location is ~45 km from the nearest coastline. The current distribution locality thus marks the first occurrence record of this species complex outside their typical hilly terrain in southern India in addition to imprecise type locality ('Erode'). It is possible that the *Cyrtodactylus* cf. *speciosus* population reported here could be an entirely distinct lineage. Hence, further genetic sampling may help assess its evolutionary relationship with other arid zone *Cyrtodactylus* species and help resolve the biogeographic history of this species complex.

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OPEN ACCESS

SHORT COMMUNICATION

Preliminary observations of moth (Lepidoptera) fauna of Purna Wildlife Sanctuary, Gujarat, India

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Abstract: Purna Wildlife Sanctuary is located in the Sahyadri range in Dang District of Gujarat State. A survey of the sanctuary was conducted to explore moth fauna from the area, as no previous work is available on this group. Thus, an attempt has been made to study the moth fauna based on collections made from April 2019 to March 2021 under the various ranges of the sanctuary. During the studies, a list of 42 species referable to 39 genera and nine families have been provided.

Keywords: Dang, moths, Gujarat, Sahyadri, sanctuary

Gujarat is the fifth largest state of India and is situated on the western coast with a coastline of 1,600 km under the Kathiawar peninsula. There are 33 districts in Gujarat. Purna Wildlife Sanctuary (WS) (Dang District, Gujarat), known as a hotspot for its biodiversity, is situated on the extreme northern side of the Western Ghats. It has tropical moist deciduous forests with various flora and fauna in it. It comprises of two protected areas - Purna WS and Vansda National Park (NP). They are known to protect the precious fauna of the area, but limited information is available on the invertebrate fauna from the sanctuary. Purna WS is rich in its fauna because of its different terrain, landscapes, and forest.

Purna WS is located at Dang District of Gujarat under the coordinates 20.91793°N, 73.7007°E with an area of 160.84 km^2. It has southern moist deciduous forests and southern dry deciduous forests (Champion & Seth 1968; Singh et al. 2000), with a normal rainfall of 1,600 mm annually. The topography of the WS is undulant with an altitudinal range of 130–1,100 m. Thus, the WS has a varied range of flora and fauna.

Moths play an important role as indicators of the health of an ecosystem (Bachanda et al. 2014). Most moth larvae are herbivorous and are predators of vegetables & crops, thus playing ecological roles throughout the life cycle (Scriber & Feeny 1979) while adults and larvae are food sources for other animals, and some are night pollinators (Holt 2002; Hahn & Bruhl 2016).

In class Insecta, moths are among the most varied groups (Soggard 2009). There are almost 1,65,000 species of moths throughout the world (Khan 2018), out of which about 12,000 species are described from India (Cotes & Swinhoe 1887–1889; Hampson 1893, 1894, 1895, 1896; Bell & Scott 1937; Chandra 2007; Chandra & Nema 2007; Smetacek 2011; Gurule & Nikam 2011, 2013; Uniyal et al. 2013; Sondhi & Sondhi 2016). Four-hundred-and-one species of moths have been recorded from Gujarat (Nurse 1899; Mosse 1929; Gupta & Thakur 1990), but no information is available on the moths

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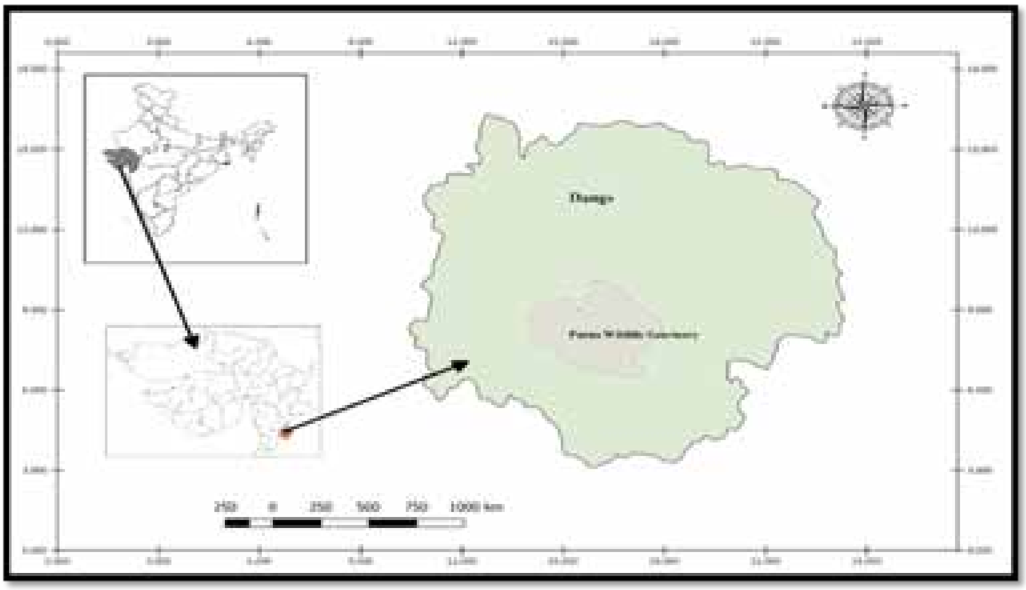

Figure 1. Map of the surveyed area of the Purna Wildlife Sanctuary.

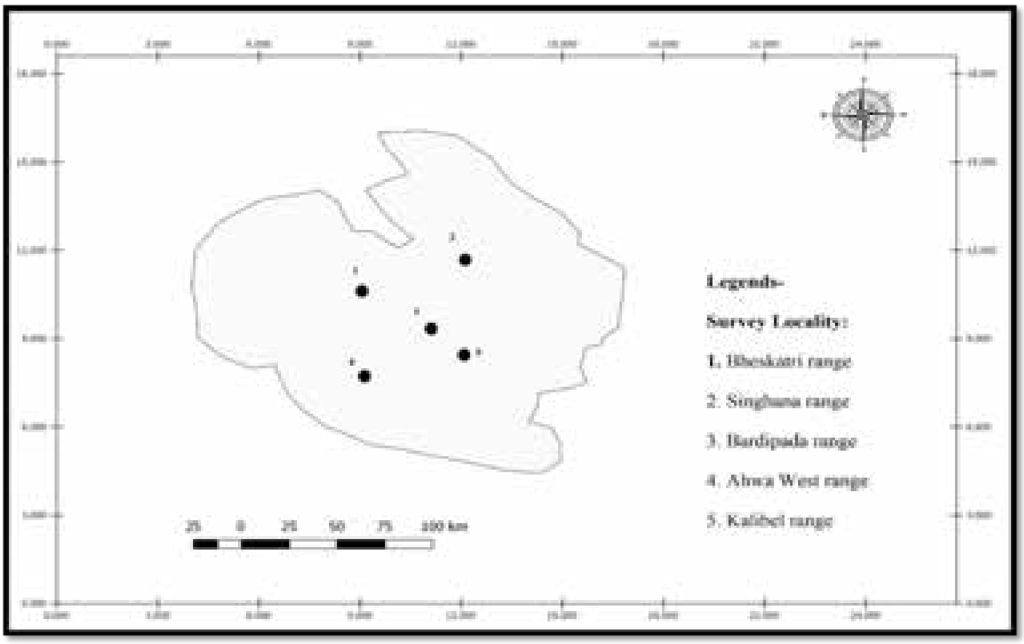

Figure 2. Survey localities of the Purna Wildlife Sanctuary.

from the Purna WS and therefore the present study was conducted for the first time.

Collection and identification

Survey of Purna WS was carried out from 2019–2022. Various localities were visited, viz.: Bardipada range, Bheskatri range, Kalibel range, and Singhana range of Dang & Ahwa districts of Gujarat (Table 1). For the collection, night traps for 5–6 hours were used for

Table 1. Collection of data from various localities of the study area.

	District	Sites surveyed	Individuals collected
1	Dang	Bardipada range	153
2		Bheskatri range	26
3		Kalibel range	141
4		Singhana range	48
5	Ahwa	Ahwa West range	39
Total			**407**

Table 2. List of preliminary observation moth fauna from Purna Wildlife Sanctuary.

	Scientific name	Status	Image numbers according to Annexure 1
Superfamily: Pyraloidea **Family:** Crambidae			
1	*Botyodes asialis* Guenée, 1854	Common	1
2	*Conogethes punctiferalis* (Guenée, 1854)	Rare	
3	*Cydalima laticostalis* (Guenée, 1854)	Common	
4	*Diaphania indica* (Saunders, 1851)	Common	
5	*Parotis marginata* (Hampson, 1893)	Rare	2
Superfamily: Noctuoidea **Family:** Erebidae			
6	*Achaea janata* (Linnaeus, 1758)	Common	3
7	*Amata cyssea* (Stoll, [1782])	Rare	
8	*Anomis flava* (Fabricius, 1775)	Rare	
9	*Argina astrea* (Drury, 1773)	Common	4
10	*Arna bipunctapex* (Hampson, 1891)	Rare	5
11	*Asota caricae* (Fabricius, 1775)	Common	6
12	*Asota ficus* (Fabricius, 1775)	Common	7
13	*Chalciope mygdon* (Cramer, [1777])	Common	8
14	*Creatonotos gangis* (Linnaeus, 1763)	Common	9
15	*Eudocima phalonia* (Linnaeus, 1763)	Common	
16	*Lymantria serva* (Fabricius, 1793)	Rare	10
17	*Lyncestis amphix* (Cramer, [1777])	Rare	11
18	*Nepita conferta* (Walker, 1854)	Rare	
19	*Orvasca subnotata* Walker, 1865	Rare	
20	*Perina nuda* (Fabricius, 1787)	Common	
21	*Spilarctia* sp.	Rare	12
22	*Spirama helicina* (Hübner, 1824)	Common	13
23	*Sphrageidus similis* (Füssli, 1775)	Common	14
24	*Syntomoides imaon* (Cramer, [1779])	Common	15
25	*Thyas coronata* Fabricius (1775)	Common	16
26	*Thyas honesta* Hübner, [1824]	Common	17
27	*Trigonodes disjuncta* (Moore, 1882)	Common	18
28	*Utetheisa lotrix* (Cramer, [1777])	Common	19
Family: Noctuidae			
29	*Spodoptera litura* (Fabricius, 1775)	Common	20
Superfamily: Geometroidea **Family:** Geometridae			
30	*Biston suppressaria* (Guenée, [1858])	Rare	21
31	*Hypomecis* sp.	Rare	22
Superfamily: Lasiocampoidea **Family:** Lasiocampidae			
32	*Trabala ganesha* Roepke, 1951	Rare	23
33	*Trabala vishnou* (Lefebvre, 1827)	Rare	24
Superfamily: Pyraloidea **Family:** Pyralidae			
34	*Cadra cautella* (Walker, 1863)	Rare	25
Superfamily: Bombycoidea **Family:** Saturniidae			
35	*Actias selene* (Hübner, [1807])	Rare	26
36	*Antheraea paphia* (Linnaeus, 1758)	Rare	27
Superfamily: Bombycoidea **Family:** Sphingidae			
37	*Daphnis nerii* (Linnaeus, 1758)	Common	28
38	*Marumba dyras* (Walker, 1856)	Common	29
39	*Nephele hespera* (Fabricius, 1775)	Common	30
40	*Psilogramma* sp.	Common	31
41	*Theretra nessus* (Drury, 1773)	Rare	32
Superfamily: Zygaenoidea **Family:** Limacodidae			
42	*Parasa lepida* (Cramer, 1799)	Rare	

trapping moths per night.

Observation and collection of moths was done using a mercury vapor bulb of 200 W on a white sheet. A collection permit for moths was received from the Gujarat Forest Department vide letter no. WLP/RES/28/C/119-120/2020-21, dated 01/09/2020.

Collected specimens were labeled with locality labels in the field. Later on, they were sorted out, relaxed, pinned, and after identification up to the species level, they were labeled in the laboratory. Their identification was done with the help of identification keys, standard reference books, and available literature (Hampson 1894, 1895; Gurule et al. 2010, 2011; Gurule & Nikam 2013; Gurule 2013). Further, the specimens are deposited at the National Zoological Collection of Desert Regional Centre, Jodhpur.

Results

Four-hundred-and-seven moth specimens were collected and further identified to 42 species under 39 genera and nine families (Table 2, Annexure 1). During the study, it was found that Erebidae is a dominant family of moths followed by Sphingidae, Crambidae, Saturniidae, Geometridae, Lasiocampidae, Noctuidae, Limacodidae, and Pyralidae in the Purna WS.

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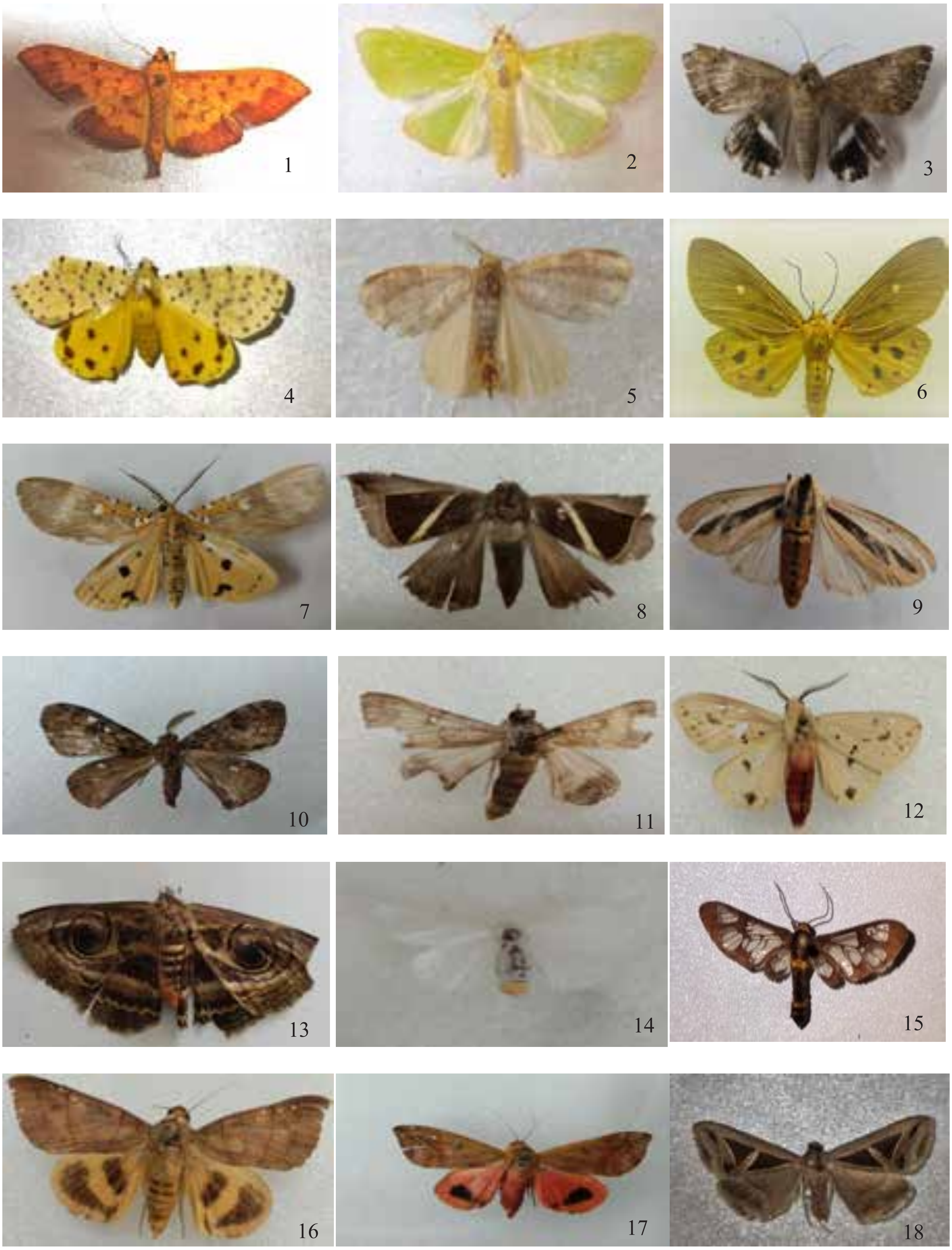

Annexure 1. Photoplate of the species collected from the study sites. 1—*Botyodes asialis* Guenée, 1854 | 2—*Parotis marginata* (Hampson, 1893) | 3-—*Achaea janata* (Linnaeus, 1758) | 4—*Argina astrea* (Drury, 1773) | 5—*Arna bipunctapex* (Hampson, 1891) | 6—*Asota caricae* (Fabricius, 1775) | 7—*Asota ficus* (Fabricius, 1775) | 8—*Chalciope mygdon* (Cramer, [1777]) | 9—*Creatonotos gangis* (Linnaeus, 1763) | 10—*Lymantria serva* (Fabricius, 1793) | 11—*Lyncestis amphix* (Cramer, [1777]) | 12—*Spilarctia* sp. | 13—*Spirama helicina* (Hubner, 1824) | 14—*Sphrageidus similis* (Fussli, 1775) | 15—*Syntomoides imaon* (Cramer, [1779]) | 16—*Thyas coronata* Fabricius (1775) | 17—*Thyas honesta* Hubner, [1824] | 18—*Trigonodes disjuncta* (Moore, 1882). © Preeti Choudhary.

Annexure 1 (cont.). Photoplate of the species collected from the study sites. 19—Utethesia lotrix Cramer, [1777]) | 20—*Spodoptera litura* (Fabricius, 1775) | 21—*Biston suppressaria* (Guenee, [1858]) | 22—*Hypomecis* sp. | 23—*Trabala ganesha* Roepke, 1951 | 24—*Trabala vishnou* (Lefebvre, 1827) | 25—*Cadra cautella* (Walker, 1863) | 26—*Actias selene* (Hübner, [1807]) | 27—*Antheraea paphia* (Linnaeus, 1758) | 28—*Daphnis nerii* (Linnaeus,1758) | 29—*Marumba dyras* Walker, 1856 | 30—*Nephele hespera* (Fabricius, 1775) | 31—*Psilogramma* sp. | 32—*Theretra nessus* (Drury, 1773). © Preeti Choudhary.

Note: There are 10 species of moths, whose photographs have not been provided in the Annexure because the species were represented by single specimens and have been thoroughly investigated/ identified with the help of stereo zoom motorized microscope and were not good enough to take photographs of dorsal/ventral view. The species have been identified by studying the key characters including other morphological features as demanded by the study. Further, the images provided in this manuscript are without scale.

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SHORT COMMUNICATION

On the occurrence of *Audouinella chalybea* (Roth) Bory, 1823, a rare freshwater red algae (Florideophyceae: Acrochaetiales: Audouinellaceae) from eastern Himalaya, India

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Abstract: *Audouinella chalybea* (Roth) Bory has been recorded from Phamrong falls of Sikkim Himalaya. Well developed plants of the alga were found attached to the stones and pebbles in the running outlets of the falls. The plants were found anchored to the substratum by spine like base attachment cells. Such structure has not been recorded in earlier studies. Both monosporangia and tetrasporangia have been recorded in our plants. This is the first report of the species from eastern Himalaya and appears to be the second report from India.

Keywords: Himalayan hill alga, new report, Phamrong falls, Rhodophyta, Sikkim.

The genus *Audouinella* Bory is one of the infrequently recorded freshwater red alga known from running waters throughout the globe (Desikachary et al. 1990; Kumano 2002; John et al. 2011; Wehr et al. 2015). The thallus of this alga is tufty in appearance mostly up to 50 mm in height. Although blue coloured species are included in genus *Audouinella* Bory, many authorities doubts it to be “chantransia stage” of Batrachospermales as no carposporangia or gametangia have been observed (Necchi et al. 1993a,b; Necchi & Zucchi 1997; Pueschel et al. 2000; Sheath & Sherwood 2011). On the other hand Desikachary et al. (1990) have considered all freshwater species as *Audouinella* Bory while marine species as *Acrochaetium* Nageli.

The genus in India is represented by 12 species (Ganesan et al. 2018; Koley et al. 2020). During systematic investigations on the freshwater red algae of eastern Himalaya the authors recorded a good population of *Audouinella chalybea* (Roth) Bory from Sikkim Himalaya.

Material and Methods

The specimens were collected from Phamrong falls of Sikkim. The alga was found growing on rocks under running water along with mosses & blue green algae in the month of April. The pH recorded at the time of collection was acidic (around 4.5–5) & temperature 19^{0}C. The samples were preserved in 4% formalin solution. GWF solution (Glycerine:Water:Formalin::1:1:1) (Bando 1988) was used as mountant for the study. Preliminary observations were made under Olympus GB Microscope & Photomicrographic images were taken using Zeiss Axioscope A1 microscope attached with Axiocam 504 model digital camera.

Systematic description

Audouinella chalybea (Roth) Bory, 1823
(Kumano 2002, p. 51, pl. 26, figs. 5–6)

Editor: Anonymity requested. **Date of publication:** 26 October 2023 (online & print)

Citation: Keshri, J.P. & J. Mal (2023). On the occurrence of *Audouinella chalybea* (Roth) Bory, 1823, a rare freshwater red algae (Florideophyceae: Acrochaetiales: Audouinellaceae) from eastern Himalaya, India. *Journal of Threatened Taxa* 15(10): 24131–24134. https://doi.org/10.11609/jott.8177.15.10.24131-24134

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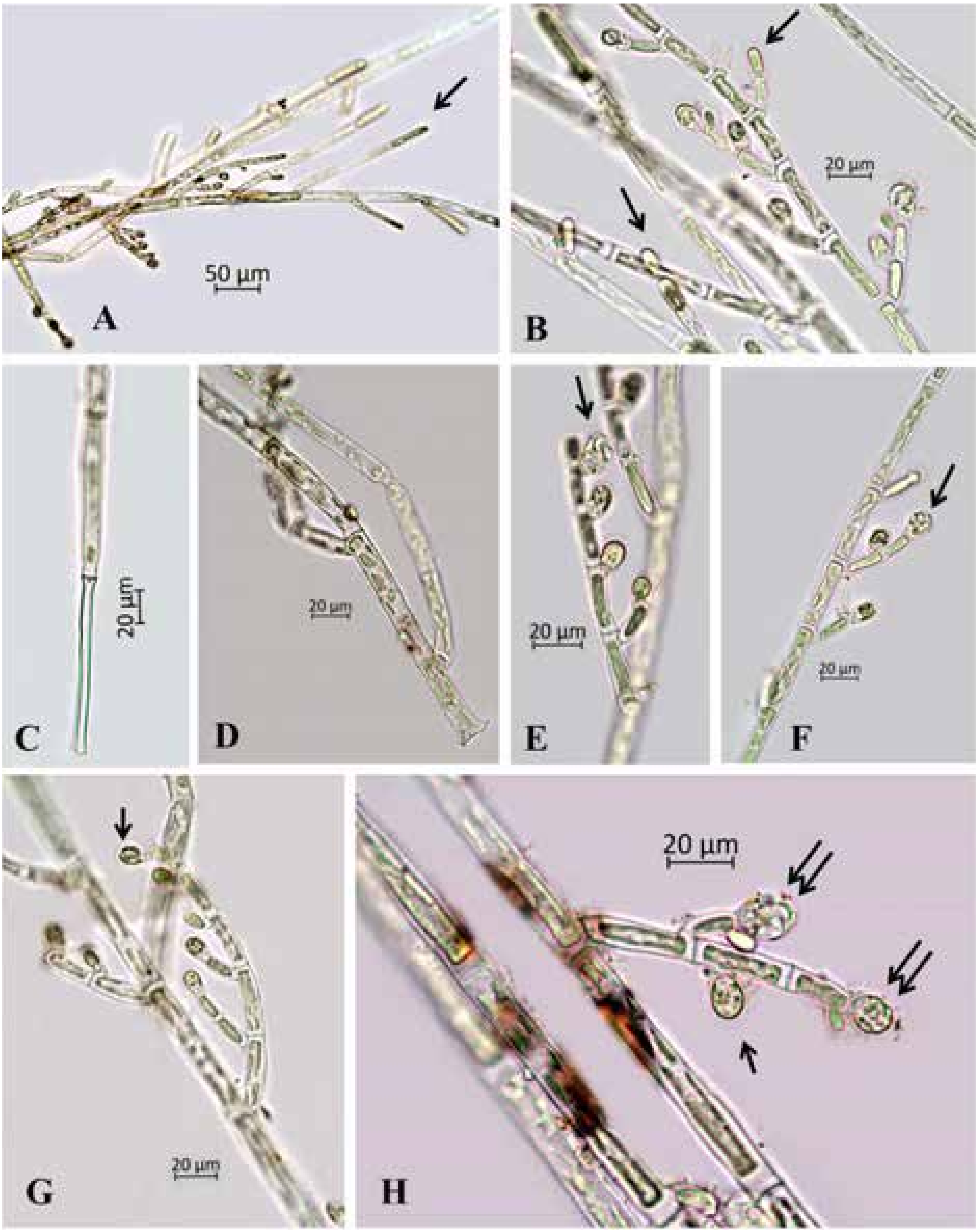

Image 1. ***Audouinella chalybea*: A & B—Part of thallus showing apical rounded cell | C—Arrow showing basal cell of the thallus | D—Branching pattern: Enlarged view | E—Part of thallus showing both types of sporangia (tetrasporangia arrow marked) | F & G—Part of thallus showing sporangia | H—Part of thallus showing both monosporangia (single arrow) & tetrasporangia (double arrow). © Jai Prakash Keshri.**

Basionym: *Conferava chalybea* Roth, 1806
Synonyms: *Trentepohlia pulchella B chalybea* C.Agardh, 1824
Trentepohlia aeruginosa C.Agardh, 1824
Chantransia chalybea (Lyngb.) Fries, 1825
Pseudochantransia chalybea (Roth) Brand, 1909

Thallus found growing on pebbles in running water of stream adhered to substratum possibly be spine like hyaline basal cell 107.75 µm long & 7.67–8.18 µm broad (Image 1C); penicillate forming bushy growth up to 3 mm in height, bluish-green in colour; well branched, branching unilateral & alternate both, up to 3rd order, mostly approaching height of the main axis; main axis distinct, cells of the main axis 7–13 µm in diameter, 28–80 µm long (4–7 times longer than broad); terminal cells rounded never acuminate; cells uninucleate with parietal chloroplast, dissected in mature cells as spiral ribbons; cell wall thin 1.03–1.30 µm; monosporangia abundant mostly unilaterally inserted towards the main axis on secondary and tertiary branched, globular to ellipsoid 10.05–12.43 µm in diameter and 15.75–16.35 µm long; tetrasporangia 13.37–15.19 µm in diameter and 16.21–21.24 µm long growing mostly at the tip of branches.

Discussion

Our specimen possesses notable characteristics of the species that was not mentioned in the plants described by Misra & Dey (1959). They have not observed the tetrasporangia. Moreover, occurrence of spine like hyaline basal cell is a new observation. This species was reported only once by Misra & Dey (1959) from Uttar Pradesh. Numerous sporangia were shown in the plant but no mention of tetrasporangia or other characteristics have been clearly spelt. Although the species is widely distributed recorded but has been recorded mostly from warm temperate regions (Hu & Wei 2006; Eloranta & Kwandras 2007; Eloranta et al. 2011; Ganesan et al. 2018; Guiry & Guiry 2022), it is surprising that no subsequent report of the species have been made from India. We have found well developed plants of the alga. The needle like basal attachment region recorded in present investigation has not been found in any other relevant literature. Therefore the possibility of the plant to be 'Chantransia Stage' comes under question mark because it should have a thalloid structure. Tetrasporangia were also found common. It may be the plant represents the diploid (sporophyte) phase of the plant. It is possible that the plants are maintaining its life cycle only in one stage in Himalayan streams due to scarcity of opportunities of sexual reproduction. Study of the ploidy level of the plant and detail investigations may put new light in the understanding the taxonomic identity of the taxon.

The authors feel that plenty of freshwater red algae including this species may be obtained from several localities of Indian region specifically from Himalayan streams & rivers.

Conclusion

The authors experienced that Himalayan streams and hills are rich in freshwater algal diversity but they are never abundant on their sites. Only experienced phycologists may locate the plants. So it appears that our knowledge of freshwater red algae is poor possibly due to lack of proper exploration and not due to the scarcity of occurrence. This is because few good papers have come up in last two decades.

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Erratum

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There is a mix up in the affiliations for authors two and three.

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SHORT COMMUNICATION

Addition of four invasive alien plant species to state flora of Mizoram, India

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Abstract: Four alien plant species that have been naturalized in the state of Mizoram are reported for the first time as an addition to the state flora. These are *Achimenes longiflora* DC. & *Chrysothemis pulchella* (Donn ex Sims) Decne. from Gesneriaceae family and *Cuscuta campestris* Yunck. & *Stylosanthes guianensis* (Aubl.) Sw. from Convolvulaceae & Fabaceae families, respectively. The present report of the occurrence of these four invasive alien plant species in the state will allow for early detection, risk assessment, and effective management to mitigate against their potential negative impacts on the native ecosystem and biodiversity.

Keywords: IAPS, Indo-Burma hotspot, native plants, naturalized, northeastern India, taxonomy.

Invasive alien plant species are exotic or non-indigenous plants which have been introduced to intentionally or unintentionally by human activities outside their natural range and have acclimatised themselves in the new ecosystem. Some of the alien plant species established in such a way become invasive by invading and outcompeting the native plant species affecting the natural biological diversity (Chaudhary et al. 2022). So, identification of the alien plant species is very important as they are agents of change that threaten the native biological diversity. They are known to be one of the greatest threats to biological diversity globally in many ecosystems. They are the second most important factor after habitat destruction for species endangerment and extinction (Clout 1997; Mc Neely & Strahm 1997; Wilcove et al. 1998; Hadjisterkotis et al. 2000).

Mizoram is a hilly state located in the northeastern part of India. The state is endowed with rich diversity of flora and fauna as it is a part of Indo-Burma biodiversity hotspot which encompasses many endemic plant taxa with many of them highly threatened. When the alien plant species become naturalized, some of them become invasive due to disturbance created by human activities and affects the native population. This study is crucial because introduced alien species can have a significant impact on the local ecosystem, often outcompeting native plants for resources such as light, water, and nutrients. This can lead to a loss of biodiversity and changes in the structure and function of the ecosystem. By studying introduced plant species, researchers can better understand their ecological impacts and develop strategies to manage them.

Materials and Methods

During random survey works conducted (2021

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to 2023) in and around Aizawl District, Mizoram, four naturalized plant species in the state could be identified and reported as new addition to state flora. The specimens of these four taxa were collected during their flowering period from different localities of Aizawl District. The characters and measurements of the plants were done and after critical examination of characters, perusal of literatures, and consultation of online herbarium (POWO 2023; Tropicos 2023; WFO 2023), the plants were identified. All the collected plants were processed for herbarium preparation following Jain & Rao (1977) and the herbarium vouchers were deposited in Mizoram University Herbarium (MZUH), Department of Botany.

Results

Taxonomic Treatment

Achimenes longiflora DC. Prodr. 7: 536 (1839) [Gesneriaceae] (Image 1A & B)

Herbaceous, up to 70 cm tall, brown to maroon stem, pubescent. Leaves arranged in whorl, green upper surface and maroon lower surface, pubescent, margin serrate. Petiole 1.3–1.7 cm long, pubescent, green. Peduncle absent. Pedicel 1.5 cm long, densely pubescent, maroon. Calyx 1.8 × 0.2 cm long, divided up to base, lanceolate, pubescent, re-curved at tip. Inflorescence 2–4 flowered, axillary. Corolla 4.8 cm long, tube glabrous, yellow to maroon, 5 lobes, purple, inner corolla having dark brown patches. Stamen 4, glabrous, 2 cm long, anther inserted, all 4 anthers fused at one point. Staminode white, one in number, 0.1 cm long. Pistil 2.8 cm long, stigma densely pubescent, bilobed white, 0.1 cm long, style densely pubescent.

Common name: Cupid's Bow

Vernacular name: Not found

Flowering & Fruiting: July–November

Mode of Propagation: Rhizomes and seeds

Habitat & ecology: Adapted to a tropical climate, with high humidity and consistent temperatures throughout the year. In cultivation, it can be grown in a range of temperatures, but it prefers warm, humid conditions and will not tolerate frost.

Native range: Tropical regions of Mexico and Central America

Distribution: India (Meghalaya & Sikkim), Central America, Caribbean.

Economic Importance: The plant is commonly cultivated as ornamental plant in the botanical gardens, ecotourism sites, parks and landscapes. It has huge potential for development into new cultivars by horticulturists for their commercialization and providing economic values to growers and florists.

Species examined: India, Mizoram, Aizawl District, Lungleng, 23.6656^{0}N 92.6635^{0}E, 980 m elevation, 07 November 2022, Lal Tlanhlui & Margaret Lalhlupuii, 129815 (MZUH).

Notes: The plant is introduced into various parts of the world due to its beautiful flowers and become naturalized in the non-native habitats. The vigorous growth and the ability for prolific reproduction through seeds and vegetative means can outcompete the native plants affecting the natural flora and fauna by affecting even the pollinators.

Chrysothemis pulchella (Donn ex Sims) Decne., Rev. Hort. (Paris) sér. 3, 3: 242 (1849) [Gesneriaceae] (Image 1C & D)

Perennial, 20–80 cm long, stem sparsely pubescent, succulent, green. Leaves opposite decussate, 11–24 × 4.6–11.2 cm long, pubescent on upper surface but glabrous on lower surface, margin serrate, base oblique, secondary venation 6–9, rough. Petiole 0.5–3 cm long, succulent, green. Bract pubescent. Pedicel 1 cm long, pubescent. Calyx 5, brick red, teeth pubescent, serrate. Inflorescence corolla 2.5 cm long, pubescent at lower tube, yellow, 5 lobes, inner lobe having maroon streaks. Stamen 4, glabrous, filament coil at the upper end. Pistil glabrous, 1.5 cm long, ovary 0.3 cm long.

Common name: Sunset Bells /Yellow Mellow

Vernacular name: Not found

Flowering & Fruiting: July–October

Mode of Propagation: Seeds

Habitat & Ecology: The plant is often cultivated for ornamental and decorative uses. It is also commonly found to be naturalized in proximity to gardens, alongside roads and near houses as an escapee.

Native Range: Mexico to Tropical America

Distribution: India (Kerala, Manipur); Central America and South America (Introduced in various parts of countries as ornamental plant).

Economic Importance: It is commonly cultivated as ornamental plant in gardens, landscapes as well as their use in cut flower industry. When the plant becomes naturalized in a new environment, it can become invasive and have negative impacts to the native biodiversity.

Species examined: India, Mizoram, Aizawl District, Mizoram University, 23.7386^{0}N, 92.6701^{0}E, 890 m elevation, 23 September 2021, Lal Tlanhlui & Margaret Lalhlupuii, 129812 (MZUH).

Notes: The plant is generally introduced for its ornamental purposes. However, its aggressive growth can have adverse effects to the non-native environment.

Cuscuta campestris Yunck. Mem. Torrey Bot. Club 18: 138 (1932) [Convolvulaceae] (Image 1E, F & G)

Vine, annual (perennial herb if on perennial host), rootless, obligate stem parasitic climber with filiform stems attached to the host by numerous haustoria, leafless. Stem cylindrical, solid, thread like less than 1 mm in diameter, abundantly branched twinning, glabrous. Along the stem, groups of 5–15 suckers (haustoria) are regularly found aligned. Inflorescence consists of dense glomerules, evenly spaced along the stems, comprising of many flowers, 0.7 cm long. Flowers 0.4 cm long, white or yellowish white, pedicel 0.4–0.5 cm long. Calyx consists of 5 ovate sepals, 0.1 cm long, fused at base with rounded lobes, corolla is campanulate (bell shaped) 0.2 cm long, lobes 5 nos., sharp, persistent, tube is same length as lobe. Stamens 5, ovary glabrous, 0.1 cm in diameter, style bifid, stigma globular.

Common name: Field dodder; golden dodder; yellow dodder

Vernacular name: 'Japanhlo ral'

Flowering & Fruiting: September–December

Mode of Propagation: Seeds and stem fragments

Habitat & Ecology: Found as a parasite to many herbaceous plants like *Acmella ciliata* (Kunth) Cass., *Polytoca wallichiana* (Nees ex Steud.) Benth. and very commonly with the invasive alien plant species (IAPS) *Mikania micrantha* Kunth with even the vernacular name Japanhlo ral means enemy to this plant (Image 1F). Locally abundant in moist open grassland along streams associated with *Clinopodium umbrosum* (M. Bieb.) Kuntze, *Chlorophytum nepalense* (Lindl.) Baker, *Oplismenus composites* (L.) P. Beauv., *Pedicularis gracilis* Wall. ex Benth. etc.

Native Range: North America, Caribbean, and western South America.

Distribution: India (Andhra Pradesh, Gujarat, Jammu & Kashmir, Madhya Pradesh, Orissa, Tamil Nadu, Uttar Pradesh, Assam, Meghalaya); Africa, Asia, Australia, Europe, North America, Pacific Island, South America.

Economic Importance: *Cuscuta* spp. including *C. campestris* have become a serious issue for many agricultural crops and other economically important plants that lead to great reduction in their yield. However, *C. campestris* was reported to be an effective biocontrol agent against another invasive alien plant species (IAPS) Mikania *micrantha* (Yu et al. 2008), which is also commonly seen as a host plant in the present study.

Specimens examined: India, Mizoram, Aizawl District, Mizoram University, 23.7386^{0}N, 92.6722^{0}E, 888 m elevation, 18 October 2022, Lal Tlanhlui & Margaret Lalhlupuii, 129811(MZUH).

Notes**:** Although commonly distributed in different parts of Mizoram, the plant is generally misidentified as *Cuscuta reflexa* Roxb. which is already reported from the state. The present report is important for various ecosystems and conservation efforts for the native plants as the plant is under obnoxious IAPS affecting the native plant diversity.

Stylosanthes guianensis (Aubl.) Sw., Kongl. Vetensk. Acad. Handl. 1789: 296 (1789) [Fabaceae] (Image 1G & H)

Herb, spreading shrub, up to 100 cm tall, invasive weed; stem green, pubescent, round; leaf 3 foliate, leaf margin entire, pubescent, pinnate, leaflets sub-sessile, stipules sheathing, inflorescence 2–4 flowered, bract pubescent primary bracts 0.9–2.1 cm long, the outer densely covered with mostly spreading long bristles; secondary bracts 2–5 mm long, 0.7 mm wide; bracteole 1.9–4 mm long, lanceolate, green, calyx pale green, lobes elliptic or oblong, glabrous, corolla yellow, 3–5 mm, vexillum with maroon streaks, keel yellow. Pods ovoid, glabrous, brown, 2 × 1.4 mm, one article, beak 0.4 mm, inflexed, the article ovoid, 2–3 mm long, 1.8 mm wide, glabrous or minutely short pubescent near the apex; beak minute, 0.1–0.4 mm long, inflexed. Seeds pale brown, glabrous compressed-ellipsoid, beaked or pointed near the hilum, shiny.

Common name: Stylo

Vernacular name: Not found

Flowering & Fruiting: September – December

Mode of Propagation: Seeds

Habitat & Ecology: Commonly seen growing along roadsides and open grasslands.

Native Range: Mexico to southern Tropical America

Distribution: India (Kerala, Manipur); Taiwan, China, North America, Central America, South America.

Economic Importance: Tropical perennial forage crop with high forage yield.

Specimens examined: India, Mizoram, Aizawl District, Mizoram University, 23.7375^{0}N, 92.6622^{0}E, 854m elevation, 18 December 2022, Lal Tlanhlui & Margaret Lalhlupuii, 129801(MZUH).

Notes: The plant is commonly introduced to different parts of the world as an important forage and fodder legume and found naturalized in many tropical regions.

Discussion and Conclusion

The four naturalized alien plant species *Achimenes*

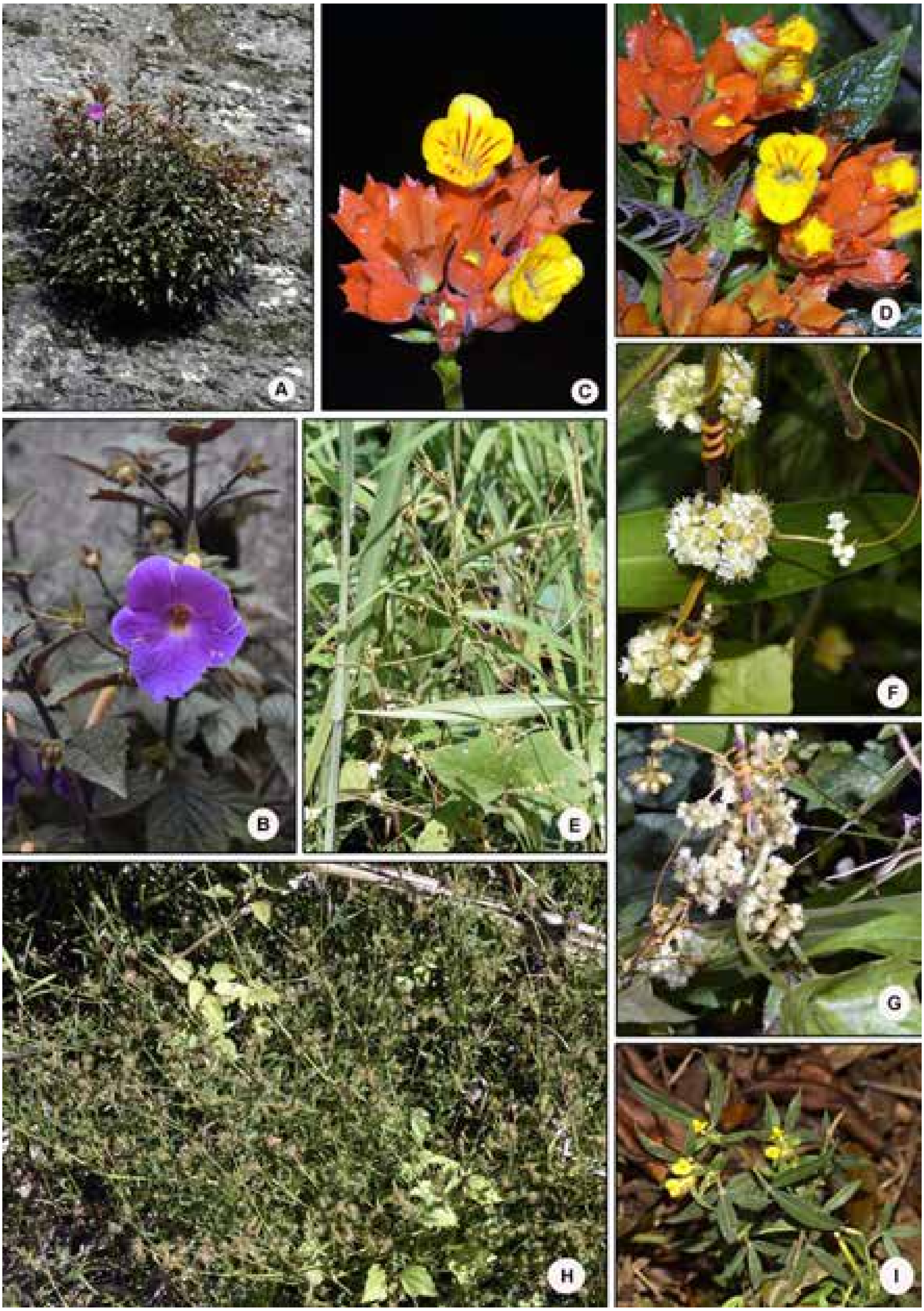

Image 1. A & B—*Achimenes longiflora* (A—habit| B—close up) | C & D—*Chrysothemis pulchella* (close up). E, F & G—*Cuscuta campestris*: E—Habit | F—Close up on the host plant | G—Close up on host *Mikania micrantha*. H & I—*Stylosanthes guianensis*: H—Habit | I—Close up. © Lal Tlanhlui & Margaret Lalhlupuii.

longiflora, *Chrysothemis pulchella*, *Cuscuta campestris* and *Stylosanthes guianensis* with the first two taxa belong to Gesneriaceae and the other two from Convolvulaceae and Fabaceae family respectively are reporting for first time from Mizoram, northeastern India. Except for *Cuscuta campestris*, the other three taxa represent the first generic new record from the state. Also, *C. campestris* is a well-known IAPS which are obligate stem parasite and agricultural pest creating huge economic losses due to its ability to infest wide range of host plants from economically important cultivated plants (Baráth 2021). The two taxa *Achimenes longiflora* and *Chrysothemis pulchella* are generally introduced as ornamental plants which later on become naturalized to the new place and this introduction for horticultural purpose is an important parameter for naturalization and invasion process necessary for becoming IAPS (Rojas-Sandoval & Acevedo-Rodríguez 2015). Many species of *Stylosanthes* including *Stylosanthes guianensis* have been introduced to different parts of India as a forage and fodder legume (Chandra et al. 2006). However, *Stylosanthes* species in particular *S. guianensis* have been identified from Australia as a conservation threat due to their aggressive nature and ability to invade areas beyond pastures (Maass & Sawkins 2004). As Mizoram being a part of Indo-Burma hotspot, which is recognized as one of the most important biodiversity hotspot, early identification and detection of these alien naturalized plants in the state will be crucial to prevent the establishment and their spread to the native ecosystem and for promoting sustainable biodiversity conservation.

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NOTE

First sighting record of Western Reef-Heron *Egretta gularis* (Bosc, 1792) (Aves: Pelecaniformes: Ardeidae) from Jammu & Kashmir, India

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The Western Reef-Heron or Western Reef-Egret *Egretta gularis* is a medium-sized heron that was formerly considered a subspecies of the Little Egret *E. garzetta*. In addition, the dimorphic subspecies of the Little Egret from Madagascar was previously regarded as a subspecies of the Western Reef Heron (del Hoyo et al. 2020). This heron exhibits a slender physique, medium size, and occurs in white or dark plumage. It possesses a long and slender neck, along with a thin bill, dark legs, yellow feet, and notable plumes on the head, breast, and back. Notably, the male individuals are larger in size compared to the females (Dubois & Yésou 1995; Willoughby 2001).

Two distinct morphs can be identified in the Western Reef-Heron. The dark morph is characterized by its dark gray coloration, featuring a white chin and throat. On the other hand, the white morph often leads to confusion with Little Egret and can be differentiated primarily by bill color and structure. Western Reef-Heron is widely distributed in coastal regions across Africa, predominantly near muddy flats, sandy shores, and mangroves (del Hoyo et al. 2020). It is also increasingly appearing as a vagrant species in southern Europe (Dubois & Yésou 1995). It is primarily distributed in coastal regions, with its presence almost exclusively confined to these areas. In western Africa, it can be found from Mauritania to Gabon. It is also observed along the eastern African coast, ranging as far south as Kenya and Tanzania and extending northwards to the Red Sea and the Persian Gulf (del Hoyo et al. 2020). Furthermore, it is found along the western, southern, and southeastern coasts of India. In India, the species has been previously spotted at numerous locations such as Rajasthan (Sharma et al. 2015; Chhangani & Charan 2015) and peninsular India (Byju et al. 2023). During the non-breeding season, it can also be found along the coast of Sri Lanka (Dies et al. 2001; De Juana 2002; Dowsett et al. 2008; del Hoyo et al. 2020). Hybrids between Little Egret and Western Reef-Heron have also been spotted in India and Sri Lanka (Koparde & Yésou 2017). This heron primarily forages in shallow waters, utilizing a slow walking technique to search for fish, amphibians, and

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Image 1–4. Photographic evidence of Western Reef-Heron in Wular Lake. © Showkat Maqbool.

invertebrates (Dubois & Yésou 1995).

On 18 June 2023, the first-ever documented sighting of a Western Reef-Heron was reported from Jammu & Kashmir. The fifth author of this article, spotted the bird at Wular Lake at 34.3436^0 N, 74.3837^0 E at 1357 h in the Saderkoot Payeen area. This sighting expands the avifaunal diversity of Jammu & Kashmir, adding another species to the region.

Remarkably, the Western Reef-Heron remained in the vicinity of Wular Lake until 13 July 2023, providing ample time for observation and study. It stood out distinctly from other avian inhabitants of the lake and could be easily observed feeding along the lake's bank. This particular bird belonged to the adult dark morph category, displaying identifiable characteristics as depicted in Image 1–4. This sighting serves as a noteworthy contribution to our understanding of the avian population and ecological dynamics of the region.

The dark morph of the Western Reef-Heron exhibits a range of coloration, varying from gray-black to pale charcoal gray. Most individuals possess a white chin and throat, along with occasional white markings on the leading edge of the wing and variable white patches on the upper wing. Conversely, the white

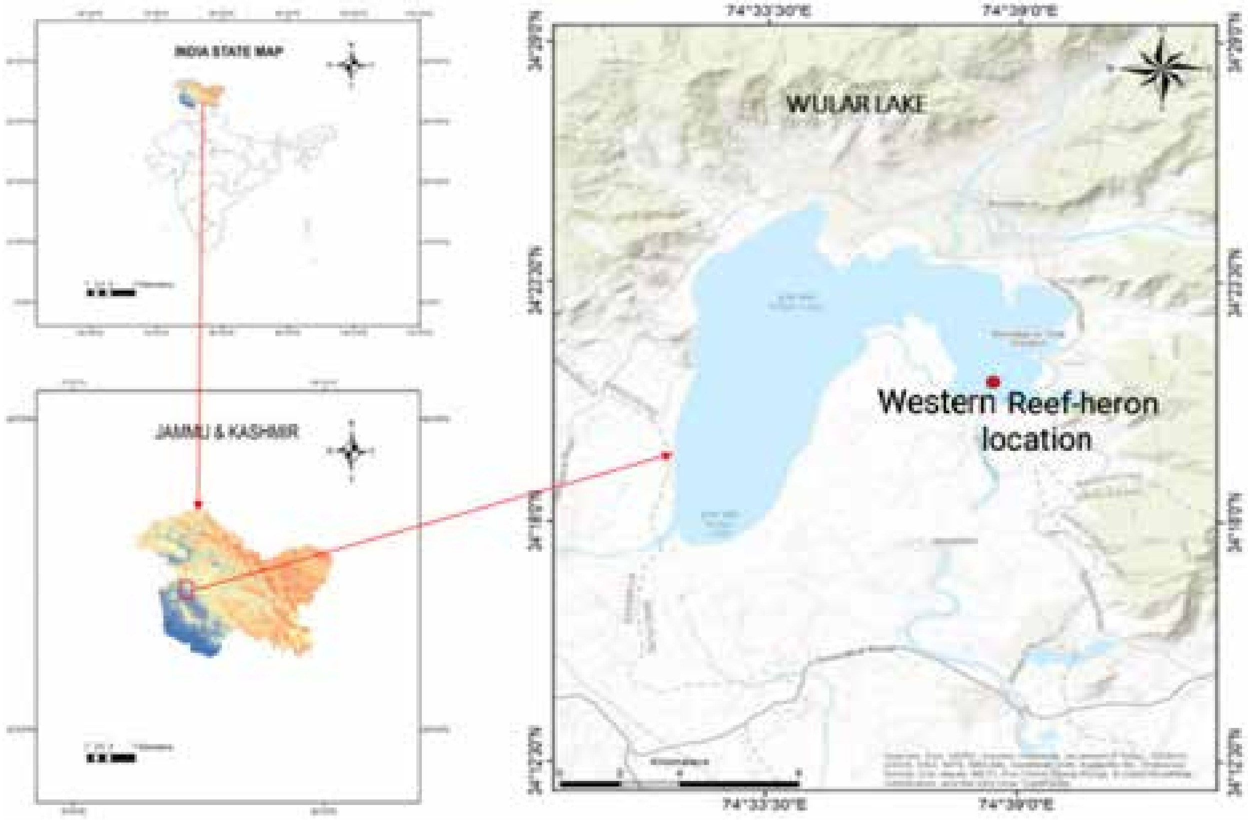

Image 5. Map of Wular Lake with pinpoint location where the Western Reef-Heron was sighted.

morph is rare within the nominate subspecies, with an estimated occurrence of less than 1% in Senegambia. It is predominantly found in the southern part of the species' range and on São Tomé, with these individuals typically displaying scattered gray feathers (Dubois & Yésou 1995). While records of intermediate plumage between both morphs exist, it appears to be more frequently observed in the *E. g. schistacea* subspecies. During the breeding season, both morphs develop long plumes on the nape, scapulars, and breast (Dubois & Yésou 1995; Kushlan & Hancock 2005).

Previously, no documented sighting of the Western Reef-Heron had been recorded in the diversity-rich region of Wular Lake. However, for the first time, it was spotted at Wular Lake in the Saderkoot Payeen area (Image 5). Situated at an elevation of 1,580 m, Wular Lake is one of Asia's largest wetlands, its location is centred on the coordinates 34.3334^0 N, 74. 6354^0 E. The lake is formed by the convergence of the flowing River Jhelum, its major feeding route, and its tributaries. It takes the form of an oxbow lake in the northwestern part of Kashmir, approximately 35 km away from Srinagar city. Functioning as a vast absorption basin for floodwaters, the lake plays a vital role in maintaining the water flow necessary for agriculture, hydropower generation, and recreational activities such as kayaking and rafting in the Kashmir valley. The deepest section of the lake is located on its western side, opposite the hills of Baba Shakur Din, reaching a maximum depth of 5.8 m. The sole outlet of the lake is the river Jhelum, situated towards the northeast. The catchment area of the lake consists of sloping hills from the Zanskar ranges in the western Himalayas, with runoff flowing through various nallahs, including Erin and Madhumati. The low-lying regions of Sonawari can be found on the eastern and southern banks of the river, which were prone to frequent flooding before the construction of multiple embankments along the Jhelum. Historically, farmers cultivated paddy fields and maintained willow, poplar, and fruit tree plantations in the reclaimed lake area. Paddy fields have been established in the low-lying areas on the western side, along the Sopore-Watlab corridor. The area where this bird species was spotted is a marshy area with no deep waters. This may be the reason why the bird occupied and lived in such a part of Wular Lake. Herons usually prefer shallow areas where they can wait for long hours to catch prey (mostly fish) as they need a substrate to settle on. This explains the bird species favouring this particular area for feeding for over 25 days, as such an area is optimum for the bird's survival. In 1986, the Indian government recognized Wular Lake as a wetland of national importance, followed by its designation as a wetland of international significance by the Ramsar Convention in 1990 (Bhat & Pandit 2014).

The lake has emerged as a significant hotspot for its diversity of bird species. Many species have been recently sighted in Wular Lake that were previously absent from the Kashmir valley. Earlier this year, Long-tailed Ducks *Clangula hyemalis* and Smew *Mergellus albellus* were observed in the Kashmir valley after a gap of 84 and 116 years, respectively (Yousuf et al. 2023b, inpress). At the same time, Horned Grebes *Podiceps auratus* were sighted for the first time in the Wular Lake of Jammu & Kashmir in early 2023 (Zargar et al. 2023). Similarly, in 2022, the White-tailed Eagle *Haliaeetus albicilla* was sighted in Wular Lake for the first time from Jammu & Kashmir (Yousuf 2023a). We hope and predict the arrival of numerous other bird species at this exquisite Ramsar site in the Kashmir valley in the years to follow.

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NOTE

Rare desmid genus *Bourrellyodesmus* Compère (Chlorophyceae: Desmidiales: Desmidiaceae) in India with description of a new species (*Bourrellyodesmus indicus* Das & Keshri sp. nov.) from eastern Himalaya, India

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According to Algaebase (2022) *Bourrellyodesmus* Compère is represented by nine species. The genus was established by Compère (1976) to accommodate certain species of *Arthrodesmus* Ralfs. Compère (1976) however reconstructed just one species of *Arthrodesmus* Ralfs (*A. heimii* Bourrelley) to *Bourrellyodesmus heimii* (Bourrelley) Compère on the basis of the presence of thickened membrane in the middle of the semicells, covered with warts or scrobiculations and having unispinous angles put as diagnostic character of the genus (Compère 1976). Later on Bicudo & Compère (1978) proposed four more combinations, viz., *B. excrescens* (Scott & Grönblad) C.Bicudo & Compère, *B. jolyanus* (C. Bicudo & Azevedo) C.Bicudo & Compère, *B. spechtii* (Scott & Prescott) C.Bicudo & Compère, and *B. sumatranus* C.Bicudo & Compère as good species under this genus. Faustino & Bicudo (2004) added another species to this list, namely, *B. guarrerae* Faustino & C.Bicudo from São Paulo, Brazil. Ramos et al. (2022) redesignated two taxa of *Xanthidium* Ehrenberg ex Ralfs viz. *X. tenuissimum* Kurt Förster var. *amazonense* Kurt Förster and *Xanthidium tenuissimum* Kurt Förster var. *constrictum* Kurt Förster to *B. amazonensis* (Kurt Förster) G. Ramos, C.Bicudo & Moura and *B. constrictus* (Kurt Förster) G. Ramos, C. Bicudo & Moura, respectively on the basis of their observations about the taxa. The characters of the taxa actually tallies with the genus *Bourrellyodesmus.* This year Santos et al. (2022) described a new species namely as *B. comperei* Santos & Moura from Caatinga domain, northeastern Brazil.

During our extensive investigations to the desmid flora of Sikkim Himalaya (Das & Keshri 2016), we had observed this curious genus. Although this taxon although resembles *Bourrellyodesmus*, it does not tally with any described species of the genus. It is also interesting that this genus is being recorded for the first time from the Indian subcontinent.

To the native people of Sikkim mountain lakes are of enormous value for their existence. They worship these water bodies and so the live forms inhabit therein. Among several lakes present in this state, 'Betang-cho' or 'Elephanta lake' is unique with respect to its water microflora. Above 150 taxa of phytoplanktons were identified from a single lake (Das & Keshri 2012,

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2016). This high altitude lake is situated in East Sikkim District with an altitude about 4,150 m from sea level and between 27.3337–27.3261 E & 88.8439–88.8487 N. Being situated in such high altitude the area nearby the lake is almost bare. Only a few small herbs and grasses grow here and there. A few algal masses grow in benthic or semi-benthic conditions. Phytoplankton frequently found along with these aquatic or semi-aquatic weeds. In the winter season the lake is completely covered with ice.

Samples were collected simply by hand from different accessible spots of the lake. Totally, nine collections were made. Several samples, i.e., visible algal mat or lumps floating on the surface of the water and also in association with aquatic herbs were collected and immediately fixed on the spot by 5% formalin aqueous solution. In another bottle water sample was preserved with 1% Lugols' iodine to study the microscopic phytoplanktons. Detailed ecological observation recorded in field notebook with water temperature and pH of the water measured by standard thermometer and standard universal pH indicator of Merck (Das & Keshri 2016). Observations were made in the laboratory under Olympus GB compound microscope with GWF as mountant medium (Bando 1988). Photomicrographs were also taken using Zeiss Axiostar plus research microscope with Nikon SLR camera attachment system.

Bourrellyodesmus indicus Das & Keshri sp. nov.
(Image 1 A–C)

Material examined

Holotype: No. DD-380(c), 06 November 2009, India, Sikkim, East Sikkim, Betangcho Lake, (27.3337–27.3261 N & 88.84397–88.8487 E), deposited in the Herbarium of Phycology Section, CAS in Botany, The University of Burdwan, West Bengal (BURD), coll. D. Das & J.P. Keshri.

Cells 1.1-1.3 times broader than long, semicells elliptical, apical margin rounded, curved downward continued to the spines, basal margin convex, angles with 1 solid convergent, short spine; sinus deep, open, V-shaped; cells elliptical in apical view, cell wall minutely punctate, each semicell with a lump of small facial granules in the centre; chloroplast and pyrenoid not

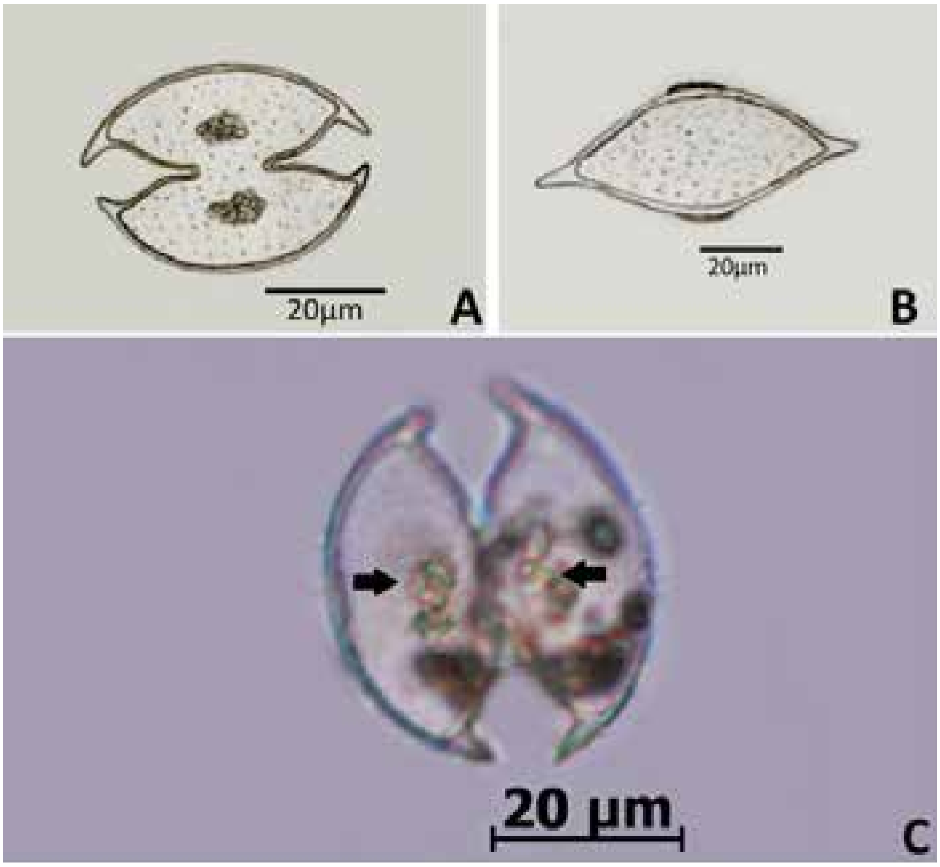

Image 1. A & B—Free hand drawing of _Bourrellyodesmus indicus_ sp. nov. (A—front view | B—top view) | C—Photograph of _Bourrellyodesmus indicus_ sp. nov. © A&B—Debjyoti Das, C—Jai Prakash Keshri.

Table 1. Comparison between existing species of *Bourrellyodesmus* with the proposed species.

	Shape of the semicells	Ornamentation on the upper lateral angles	Sinus	Lateral spine	Ornamentation on the face of the semicell
B. amazonensis (Kurt Förster) G.J.P. Ramos, C.E.M. Bicudo & C.W.N. Moura	Hexagonal	No ornamentation	Open, shallow	Short, blunt, parallel	Small rounded granule, single
B. constrictus (Kurt Förster) G.J.P. Ramos, C.E.M. Bicudo & C.W.N. Moura	Trapiziform	No ornamentation	Open, V-shaped	Short, blunt, parallel	Small rounded granule, single
B. comperei M.A. Santos & C.W.N. Moura	Trapiziform	1 tiny granules in each side of the semicell	Closed, deep	Short, blunt, downward	Small intumescence, single
B. spechtii (A.M.Scott & Prescott) C.E.M.Bicudo & Compère	Subrectan-gular	No ornamentation	Closed	Long, vertical	Small intumescence, single
B. sumatranus C.E.M.Bicudo & Compère	Pyramidal with convex apex	No ornamentation	Closed	Short, acute, parallel to slightly convergent	Rounded median protuberance, single
B. excrescens (Scott & Grönblad) C.E.M.Bicudo & Compère	Elliptic	No ornamentation	Open, V-shaped	Short, acute, parallel to slightly divergent	Rounded tubercle, single
B. heimii (Bourrelley) Compère	Elliptic	No ornamentation	Open, V-shaped	Blunt, convergent	Large granules with triangular scrobiculation, several in number
B. guarrerae Faustino & C.E.M.Bicudo	Elliptic to sub-circular	No ornamentation	Open, acute angled	Acute, subparallel	Small rounded granules, single
B. jolyanus (C.E.M.Bicudo & Azevedo) C.E.M.Bicudo & Compère	Elliptic	2 rounded granules in each side of the semicell	Open, V-shaped	Acute, long, convergent	Small median swelling, single
***B. indicus* sp. nov.**	Elliptic	No ornamentation	Open, V-shaped	Blunt, convergent	Lump of small facial granules

observed; zygospore was also not found. Cell dimension: length 34–36 µm, width without spines 30–32 µm, with spines 39–41 µm. isthmus 11–12 µm. spines length 4–6 µm.

Habitat: New taxa rarely found in the study area, in association with other filamentous algae *Spirogyra* attached to the surface of the submerged aquatic plants. pH: 6, water temperature 10ºC at the time of collection.

Differential diagnosis: In respect to shape of the semicell present taxa resembles *B. excrescens* (Scott & Grönblad) C.Bicudo & Compère and *B. heimii* (Bourrelley) Compère as these species possess elliptical semicells with rounded or convex apex. Although *B. guarrerae* Faustino & C.Bicudo possess elliptic or circular semicells but lateral spines are subparallel here and also cell dimension is smaller. *B. jolyanus* (C.Bicudo & Azevedo) C.Bicudo & Compère despite having elliptical semicells but also possess rounded granules at the upper lateral margins which are absent in *B. indicus*.

Our taxa differs from *B. excrescens* (Scott & Grönblad) C.Bicudo & Compère in having a large emarginate tubercle on the faces of both sides of the semicells and downwardly curved spines.

B. heimii shows close resemblance to the present taxa having similar semicell shape and nature of the spines. Main dissimilarities of *B. heimii* have been noticed is the presence of just a lump of small facial granules on each side of semicell in place of several large granules alternating with triangular scrobiculations. *B. indicus* is also smaller.

A comparative account of the taxa is appended in Table 1.

Genus *Bourrellyodesmus* is being reported for the first time form India as well as eastern Himalayan alpine region. As this region is among the major hotspots of the world, further and more detail observations my reveal more such plant sciences.

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Threats faced by *Humboldtia bourdillonii* Prain (Magnoliopsida: Fabales: Fabaceae), an endangered tree endemic to the southern Western Ghats, India

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Forests cover nearly a third of the world's surface (Lee & Jarvis 1996), containing almost 60,000 tree species (BGCI 2022). According to the first Global Tree Assessment Report in 2021, almost 1/3rd of these tree species are threatened with extinction, of which 142 have already recorded as 'Extinct' (BGCI 2021). Of all the tree species 58% are single-country endemics (Beech et al. 2017). The report says that there are 2,603 tree species in India, of which 650 are endemic and 469 are under the threat of extinction (BGCI 2021). Western Ghats is one of the biodiversity hotspots in India with high floristic diversity and endemism (Jose et al. 2023).

The genus *Humboldtia* comprises nine species (Kumar et al. 2022), all of which are endemic to the Western Ghats, except *H. laurifolia* which is endemic to Sri Lanka. *Humboldtia bourdilloni* Prain is an Endangered species (World Conservation Monitoring Centre 1998) endemic to the southern Western Ghats, India. It is locally known as 'Adimundan' and belongs to the family Leguminosae and subfamily Caesalpinioideae. It is a medium-sized tree that grows up to 20 m in evergreen forests in the altitudinal range of 200–1,250 m. The species was first described by David Prain, based on the collections of T.F. Bourdillon from Peermede Ghats in 1894, then for the next 108 years there was no report or data about this species. In 2002, the Kerala Forest Research Institute (KFRI) research team rediscovered this species from the Periyar Tiger Reserve. Now this species is facing serious ecological and man-made threats in its natural habitat.

We conducted extensive forest surveys in the southern Western Ghats region from June 2021 to March 2023 to study the population and ecology of *H. bourdillonii* in its natural habitat (Image 1). The information from floristic literature and herbariums helped us to plan the field surveys. The major population sites located were Kulamavu, Vagamon, and Arjunankotta-Poonkavanam forests in the Periyar Tiger Reserve of the Peermede plateau. The population studies showed that *H. bourdillonii* has an area of occupancy of less than 0.06 km^2 and an area of occurrence is approximately 2 km^2. The number of mature trees is less than 200.

There are irregularities observed in the flowering and fruiting of *H. bourdillonii* mainly owing to climate change. Generally, the flowers are produced in November–January, and fruiting is observed in January–May (Balan et al, 2019). The flowers are pollinated by wind, ants, and honey bees *Apis indica*. However, the intensity of pollinators has recently decreased. Young fruits are largely consumed by the Malabar Giant Squirrel *Ratufa indica*, which is also endemic to the Western Ghats. Insect infestation heavily affects the reproductive biology of *H. bourdillonii*. Jumping thrips

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Image 1. *Humboldtia bourdillonii*: A—mature tree | B—steep slippery terrain habitat with streams | C—view of flowers | D & E—damaged pods | F—reduction in seeds | G—processed seeds for seedling production. © Jithu K. Jose & Anuraj K.

multiply and colonize the young inflorescence and suck the sap of young fruits. Sixty percent of seeds are lost due to the damage caused by the weevils. Weevils penetrate the fruit wall and lay eggs in the cotyledons of the young embryo. The larvae grow at the expense of the cotyledons and the adults emerge out as the seeds get dispersed. The entire metamorphosis of the insect occurs within the fruit. The attack of weevils is more prominent during the months of April and May. The seed dispersal is carried out by dehiscing pods, the blasted seeds are scattered around the mother tree indicating the short-distance gene flow within the population. This short-distance gene flow has affected the genetic diversity of this species (Rathmacher et al. 2010). The pre-monsoon rainfall is beneficial to seed establishment but recent abnormal monsoon flooding (started in 2018) wipes the seeds and hinders the soil seed bank of the species. The recalcitrant nature of the seed is also a cue factor affecting the regeneration of the species.

The conversion of forest areas in Vagamon and Peermede Ghats into tea and cardamom plantations has heavily affected the populations of *H. bourdillonii*.

This conversion was started during the 19th century British rule. Vagamon is a major tourist spot in Kerala, so tourism development has also negatively affected this species. The impact of recent abnormal flooding in Kerala triggered a number of landslides that affected the Vagamon Hill population of the species. Both locations (Vagamon and Peermede) of this species are identified as landslide-prone areas by the Kerala State Disaster Management Authority (KSDMA; Balan et al. 2019). In Kulamavu forest areas, commissioning the Idukki Dam reservoir may submerge the populations of the target species. So, the conservation of this endangered, endemic species is the need of the hour.

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Articles

Communications

Review

Short Communications

Notes

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