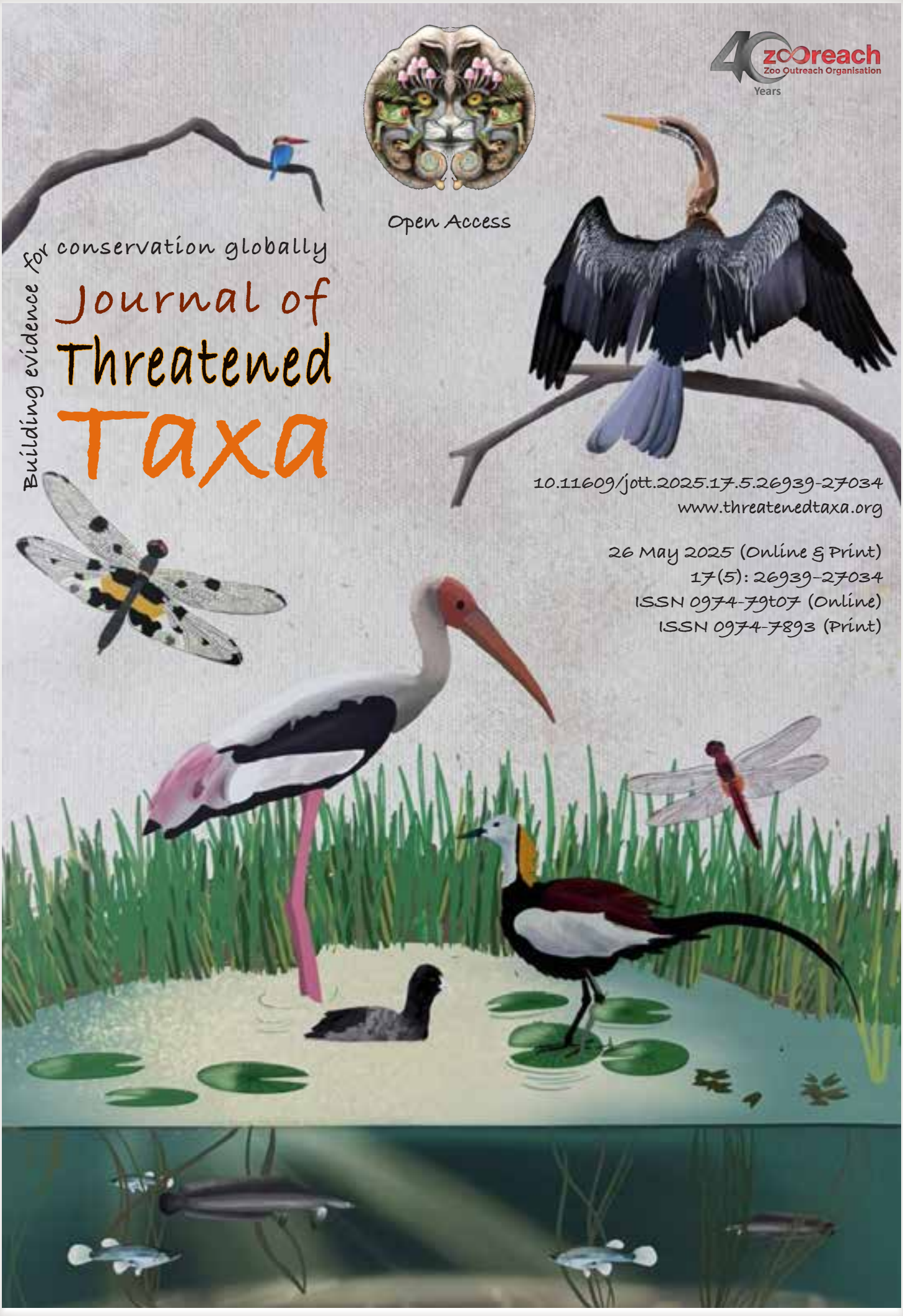

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Cover: A digital art of water birds of Noyyal River and its wetlands in Coimbatore District by Megha A. Kashyap.

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ARTICLE

A review of Tsimlyansk Birch Mouse *Sicista cimlanica* (Mammalia: Rodentia: Sminthidae): distribution, phylogeography, and conservation

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Abstract: All known localities of the Tsimlyansk Birch Mouse are summarized. The area of occupancy of the species is estimated as 123,000 km^2, whereas the extent of occurrence is estimated as 4,000 km^2. The species is proposed as 'Near Threatened' according to IUCN Red List categories and criteria. Analyses of the full mitochondrial cytochrome b gene sequences from four distinct populations indicate that all *Sicista cimlanica* individuals form a monophyletic clade. Having a limited distribution of the Middle Don area in western Russia and eastern Ukraine, this species has an exceptionally high haplotype diversity (h = 0.98), though the nucleotide diversity is considerably low (π = 0.009).

Keywords: Birch mice, cytochrome b, diversity, Don River, genetic diversity, genetic diversity, PCR protocol, pitfall trap.

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INTRODUCTION

Birch mice *Sicista* are characterized by high karyologic and genetic variability within the genus but limited differences in morphology. The Steppe Birch Mouse *Sicista subtilis* species group is one of the best studied examples within *Sicista*. Unlike all other species of the genus, which tend to occupy mesophyte tall grasslands, *S. subtilis* s.l. is adapted to arid and semi-arid environments (Lebedev et al. 2019). The western edge of its range begins from the Pannonian Plain, extending to Siberia in the east.

Originally, most of the currently recognized forms of Steppe birch mice were described as separate species. However, when Ognev (1948) united all of them under one species – *Sicista subtilis* (Pallas, 1773) – his perspective remained dominant for a long time until the 1980s, when cytogenetic studies revealed substantial differences in chromosomes for different populations (Sokolov et al. 1986). The latter authors divided all Steppe birch mice into two species: *S. subtilis* and *S. severtzovi* Ognev, 1935. This taxonomy was accepted by subsequent researchers (Shenbrot et al. 1995). According to Shenbrot et al. (1995), the nominative form *S. subtilis* was distributed from Hungary to Kazakhstan and Siberia, thus covering most of the species group's range, excluding only the area of Middle Don River. The second species – Severtsov's Birch Mouse *S. severtovi* – was believed to occur in the basin of the Don River.

Subsequently, it was found that birch mice from different populations in the Don Basin were characterized by polymorphism both in chromosome numbers (2n) and their fundamental numbers (nFa) (Kovalskaya et al. 2011). It was revealed that *S. severtzovi* from the type locality (Voronezh Region, east of Don River) were different from all other Middle Don populations. Therefore, it was suggested to treat all those forms, previously attributed to *S. severtzovi*, as two undescribed species: S. sp.1 and S. sp.2 (Kovalskaya et al. 2011). It remains unclear why the authors did not include *S. severtzovi cimlanica* Kovalskaya et al. (2000) in their review.

The first genetic studies of the *S. subtilis* species group (Cserkész et al. 2016) used mtDNA cytb and nDNA IRBP genes and included five populations from the Middle Don area (out of 12 studied populations). It was discovered that all birch mice from the Middle Don area formed one clade and were sister to the nominative form (*S. subtilis subtilis*) from the left bank of the Volga River. Thus, it was concluded that birch mice from Middle Don should be attributed to one taxon: *S. subtilis severtzovi*. This assumption was premature since there were no sampled animals from the type locality of *S. severtzovi*.

The next work (Lebedev et al. 2020) was based on mtDNA cytb and COI markers from 28 populations of *S. subtilis* species group, with eight populations from Middle Don area. It was shown that Middle Don Birch Mice were not conspecific with specimens of *S. severtzovi* from the type locality, thus reinforcing the results of cytogenetic studies (Kovalskaya et al. 2011). Authors concluded that the only available name for birch mice from Middle Don was *Sicista cimlanica* Kovalskaya et al., 2000 (Lebedev et al. 2020). This species includes chromosomal forms 'cimlanica', 'S.sp.1' and 'S.sp.2'. The putative range of this species lies within western Russia and eastern Ukraine. Thus far, only a few populations have been genotyped, with the number of animals used for these studies varying from one to three. Nonetheless, this taxonomy has been accepted and *S. cimlanica* is now included as a valid species in the American Society of Mammologists (ASM) Mammal Diversity Database.

In the present study, original data are combined with available material to shed light on within-species polymorphism in *S. cimlanica*, to describe the most accurate species distribution and discuss conservation outputs.

MATERIALS AND METHODS

Animal sampling

Nine birch mice were captured using pitfall traps during field surveys conducted in 2016 and 2019 in the western part of the Tsimla Sands. Pitfalls were set for 1–2 nights in the psammophyte steppe. The animals were examined, and small tissue samples were taken for DNA testing. After that, the mice were released back into the wild. The tissue samples were kept in ethanol. Details on all specimens used in the study are provided in Table 1.

DNA isolation, PCR, and sequencing

Genomic DNA from ethanol-preserved tissues was extracted using a Diatom DNA Prep100 kit (Isogen Laboratory) according to the manufacturer's instructions. To extract full mitochondrial cytochrome b (cytb) genes, a set of universal primers L7/H6 (Montgelard et al. 2002) was used. A polymerase chain reaction (PCR) was conducted in a volume of 25 ml using the Taq 5X Master Mix (New England Biolabs); the reaction mixture contained 5 pM of each primer, 0.1–0.2 mkg of DNA, and ddH2O to the final volume.

Image 1. A—Typical habitat of *Sicista cimlanica*. © Alexey Tikhonov | B—*Sicista cimlanica* from Tsimla Sands. © Mikhail Rusin.

The PCR protocol for all samples was an initial denaturation step at 95°C for 1 min, then 35 cycles of 95°C for 20 s, 55°C for 20 s, and 72°C for 20 s, with a final extension of 72°C for 5 mins. PCR products were visualized using UV light in 1.5% agarose gel stained with ethidium bromide, cut off, and purified using a GeneJET Gel Extraction kit (ThermoFisher Scientific) according to the manufacturer's instructions.

The nucleotide sequence of gene cytb was determined using an ABI PRISM 3500xL automatic sequencer with the BigDye Terminator Chemistry v. 3.1 (Applied Biosystems) and each of the pair of external primers. The resulting nucleotide sequences were manually aligned with the SeqMan (Lasergene) and BioEdit v 7.0.4.1 (Hall 1999) software.

Phylogenetic analyses

Total alignment contained 21 sequences (17 *S. cimlanica* and four outgroups). Sequence MK259967 was not included in the analyses as it was found to be another isolate from the same specimen as MK758100 (Vladimir Lebedev pers. comm. 2021).

Nine sequences generated in this study were deposited in GenBank (Acc. No.: MT295493–MT295501). MEGA X software (Kumar et al. 2018) was used for sequence analysis and distance estimation. The within- and between-group genetic differences were estimated according to the Kimura two-parameter model (K2p) calculated in MEGA X. Haplotype diversity (h) and nucleotide diversity (π) were calculated in DnaSP v.5.10.01.

The substitution model was chosen in MEGA X, and HKY+G (ncat = 5) had the lowest Bayesian information criterion (BIC) score. The maximum likelihood (ML) tree was constructed in MEGA X. Node support values were estimated according to bootstrapping (1,000 replicates). A Bayesian inference (BI) of phylogeny tree was constructed in MrBayes 3.2.7 (Ronquist & Huelsenbeck 2003) and run on the CIPRES gateway (Miller et al. 2010). The following parameters were used: two runs of five million generations, with four chains, sample frequency set at every 2,000 generations. Runs were checked for convergence and effective sample size in Tracer 1.7.1 (Rambaut et al. 2018), and the burn-in rate was set at 300 trees. Both runs were combined manually and annotated with TreeAnnotator 1.10.4 (Suchard et al. 2018).

For analysis of haplotypes, the sequence data were slightly shortened. After trimming the unequal conservative flanks, all the sequences had the same length of 1,122 bp (positions from 14 to 1,135 bp in the alignment), excluding the only sequence MK758099 from GenBank, which had a length of 1,095 bp (positions 26–1,120 bp in alignment). A haplotype network was constructed using Network v. 10.0.0.0 software (Fluxus Technology Ltd).

RESULTS

Genetic structure and diversity

The mitochondrial DNA cytb gene (1,095–1,140 b.p.) from 17 *S. cimlanica*, belonging to four populations, was analysed. Overall, 94 sites (approximately 8% of

the full fragment length) were variable, and 64 of them were parsimony-informative. The mean nucleotide composition was 27.7% (A), 32.9% (T), 13.1% (G), and 26.3% (C).

An unusually high variability in the structure of this marker both over the species range (17 individuals, 15 haplotypes, $\pi = 0.00875 \pm 0.00098$ SD, $h = 0.978 \pm 0.031$ SD), and in the type locality (11 individuals, 9 haplotypes, $\pi = 0.009528 \pm 0.00091$ SD, $h = 0.945 \pm 0.066$ SD) was registered (Table 2). The level of intraspecific variability was approximately 0.9%. There were no shared haplotypes among the four genotyped populations.

The total sample set of haplotypes was found to be distributed among four weakly differentiated haplogroups in accordance with the geographical location of the samples. The haplogroup "Tsimla Sands" represents the type locality, where most of the cytb variants are registered (nine haplotypes). It formed a star-like pattern, which may indicate a recent population expansion. The central haplotype (cim4) is probably ancestral. The three other haplogroups are less studied and therefore fewer haplotypes are known: "Serafimovich" (two haplotypes) from the northern part of the Archedin-Don Sands, "Yamskaya Steppe" (three haplotypes) from Belgorod Region, and "Lugansk" (one haplotype) from Triokhizbenka Sands in Ukraine (Figure 1).

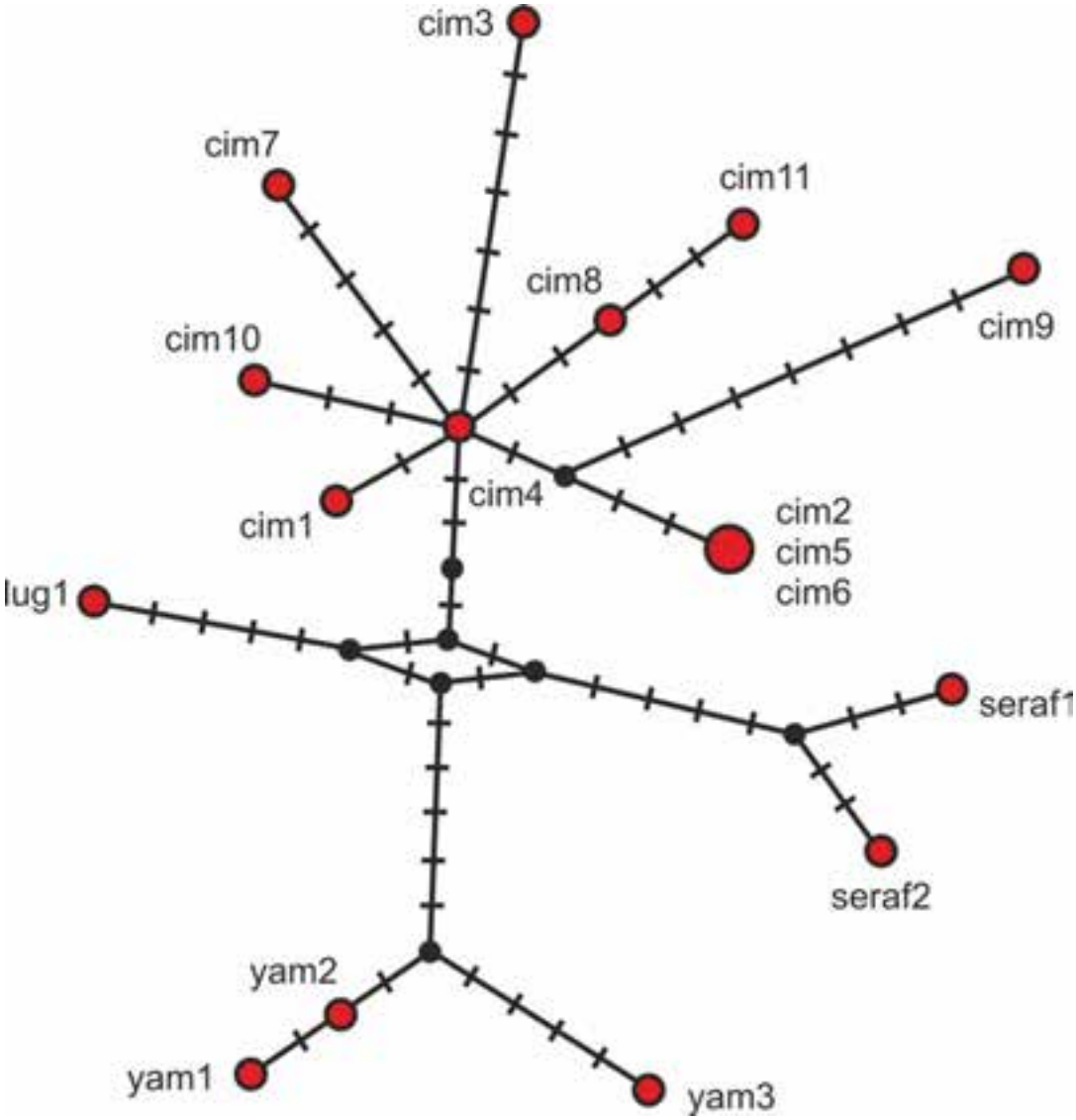

Figure 1. Unrooted haplotype network of absolute distances between mtDNA haplotypes of the cytb gene. Each circle represents a unique haplotype, its size proportional to the haplotype frequency. Black circles represent hypothetical haplotypes. Lines connecting each haplotype represent a single nucleotide substitution, and the hatch marks along those branches represent additional substitutions. Haplotypes with more than one branch connecting them to other haplotypes represent alternative pathways of equal likelihood. Sample names are given in Table 1.

Table 1. A compiled list of verifiable capture localities of birch mice in the middle Don area (both original and previously published data). Designations: ID on Figures — the encoding of the sample names used for illustration | N0 & E0 — coordinates not specified | Karyotype = unknown — local karyotype has not been studied. ZMMU—Zoological Museum of Moscow State University, Moscow, Russia | ZMKNU—Zoological Museum of Kyiv National University | NMNHU—National Museum of Natural History, Kyiv, Ukraine | HNHM—Hungarian Natural History Museum, Budapest, Hungary.

Species	Population	Sampling data	ID on Fig.	GenBank Acc.No.	References
Sicista cimlanica	Tsimla sands, Rostov and Volgograd regions, Russia 2n = 22, NF = 35–36	Eight specimens: holotype S-165916 ZMMU, paratypes S-165917, S-165919, S-165920, S-165923–S-165926, viii.1996; 2 specimens (S-165921 – S-165922, viii.1997; 1 specimen S-195918, 22.vi.1996; 1 specimen S-165927, 24.viii.1996; Rostov part of Tsimla sands, col. G. Tikhonova, N? E?			Kovalskaya et al. 2000
		One specimen: ZMMU S-178462; 2002; col. Yu. Kovalskaya, N? E?			
		Two specimens: NMNHU 10994–10995, col. S. Zolotukhina, 17.vi.1986 and 29.v.1986			Shevchenko & Zolotukhina 2005
		Two specimens: 14–15.viii.2002; ZMMU S-173549, S-173550; col. I. Tikhonov; 48.15N 42.85E	cim11	MK758100	Lebedev et al. 2020
		Two specimens: col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko; 12.vi.2014, 47.818N, 42.663E*	cim10	KP715870	Cserkész et al. 2016
		vouch. ZMMU S-197469; col. M. Rusin & N. Nedyalkov; 11.v.2016, 48.020N 42.413E	cim1	MT295493	this study
		vouch. ZMMU S-197468; col. M. Rusin & N. Nedyalkov; 11.v.2016, 48.022N 42.410E	cim2	MT295494	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 28.vii.2019, 47.934N 42.451E	cim3	MT295495	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 28.vii.2019, 47.935N 42.446E	cim4	MT295496	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 27.vii.2019, 47.935N 42.450E	cim5	MT295497	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 28.vii.2019, 47.935N 42.445E	cim6	MT295498	this study
		vouch. ZMMU S-202215; col. A. Korneev, A. Tikhonov, V. Kilyakova; 27.vii.2019, 47.884N 42.480E	cim7	MT295499	this study
		released; col. A. Korneev, A. Tikhonov, V. Kilyakova; 27.vii.2019, 47.933N 42.450E	cim8	MT295500	this study
		vouch. ZMMU S-202214; col. A. Korneev, A. Tikhonov, V. Kilyakova; 26.vii.2019, 47.886N 42.478E	cim9	MT295501	this study
	Alekseevskie Sands, Volgograd Region, Russia, 2n = 26, NF = 46	One specimen: ZMMU S-183022, col. A. Surov, G. Tikhonova, I. Tikhonov, 28.viii.1999, 50.2N 42.3E			Kovalskaya et al. 2011
	Medveditza riv. right bank, Volgograd Region, Russia, 2n = 26, NF = 46	Three specimens: 49.65N 42.62E			Kovalskaya et al. 2000 Kovalskaya et al. 2011
	Medveditza riv. left bank, Volgograd Region, Russia, 2n = 22, NF = 41	One specimen: 49.94N 43.22E			Kovalskaya et al. 2011
	Archedinskie Sands (north), Volgograd Region, Russia, 2n = 23, NF = 44	One specimen: 49.65N 42.72E	seraf2	MK758099	Kovalskaya et al. 2011 Lebedev et al. 2020
		One specimen taken to HNHM (vauch. publicly not available), 5.vi.2013, col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 49.65N 42.72E	seraf1	KP715865	Cserkész et al. 2016
	Archedinskie sands (south), Volgograd Region, Russia, 2n = 24, NF = 46	Two specimens: 49.24N 44.82E			Kovalskaya et al. 2011
	Ilovlya, Volgograd Region, Russia, 2n = 24, NF = 46	One specimen: 49.25N 44.12E			Kovalskaya et al. 2011
	Yamskaya steppe, Belgorod Region, Russia, 2n = 21–22, NF = 29–31	At least seven specimens: one stored in ZMMU S-178461, col. Yu. Kovalskaya, 2002	yam2 yam3	MK758095 MK758096	Kovalskaya et al. 2011 Lebedev et al. 2020
		Three specimens: col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 13–14.vi.2013, 51.187N 37.637E*	yam1	KP715869	Cserkész et al. 2016
	Aidar river, Belgorod Region, Russia, 2n = 16–18, NF = 28	Two specimens stored in ZMMU S-177985 and S-178332, 2001, col. Yu. Kovalskaya, 49.89N 38.89E			Kovalskaya et al. 2011
		One specimen, 49.89N 38.89E			Oparin et al. 2001

Species	Population	Sampling data	ID on Fig.	GenBank Acc.No.	References
	Oskol River, Belgorod Region (probably same as Yamskaya Steppe?)	Three specimens: ZMMU S-174761–174763, col. Yu. Kovalskaya, 2001, N? E?			
	Stenki Izgorya, Novooskolskiy District, Belgorod Region, Russia, 2n = 22, NF = 30	Three specimens: 50.69N 37.85E			Kovalskaya et al. 2011
	Krasnogorovka, Voronezh Region, Russia, 2n = 18, NF = 28	One specimen: viii–ix.1996, 49.97N 40.8E			Kovalskaya et al. 2000
	Barkalovka, Kursk Region, Russia, 2n = 19–20, NF = 29–30	Two specimens: 51.558N 37.645E			Baskevich et al. 2011
	Bukreevy Barmy, Kursk Region, 2n = 19–20, NF = 28–29	Three specimens: 51.503N 37.347E			Baskevich et al. 2011
	Streletzkaya steppe, Kursk Region, Russia, 2n = 18–20, NF = 28–30	Six specimens: 51.58N 36.12E			Sokolov et al. 1986
	Triokhizbenka Sands, Lugansk Region, Ukraine, karyotype = unknown	Five specimens: 1 specimen taken to HNHM (vauch. publicly not available), col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 1–2.vi.2013, 48.793N 38.956E	lug1	KP715864	Cserkész et al. 2016
		Two specimens: col. V. Timoshenkov 26.iv.2012, 1.x.2012, 48.774N 38.948E			Timoshenkov 2018
	Streltsovskaya steppe, Lugansk region, Ukraine, 2n = 17, NF = unknown	One specimen: col. A. Kondratenko, 1998, 49.29N 40.08E			Zagorodniuk & Kondratenko 2000
		NMNHU 13985, col. A. Kondratenko, 18.vii.1988 NMNHU 14389, 19.v.1991, col. A. Kondratenko NMNHU 14390, col. V. Timoshenkov & A. Kondratenko, 21.v.1991 NMNHU 14391, col. V. Timoshenkov & A. Kondratenko, 18.vii.1988 NMNHU 2700, col. G. Modin, 8.v.1951			Shevchenko & Zolotukhina 2005
		ZMKNU 3415, col. G. Modin, 6.vii.1956			
Sicista severtzovi	Kamennaya Steppe, Voronezh Region, Russia, karyotype = unknown	Holotype ZMMU S-26104, col. S. Obolenskiy, 22.vii.1921, 51.04N 40.72E			
	Krasnoe, Novohkoperskiy district, Voronezh Region, Russia, 2n = 26, NF = 48	Five specimens: ZMMU S-182605-182609, col. Yu. Kovalskaya, 16–17.v.2007, 51.15N 41.47E		MK758097, MK758098	Kovalskaya et al. 2011 Lebedev et al. 2020
Sicista subtilis	Grachi, Yenotaevskiy District, Astrakhan Region, Russia, karyotype = unknown	Two specimens: ZMMU S-197171–197172, col. G. Ryurikov & N. Poplavskaya, 11.vii.2016, 47.827N 46.234E	astrakhan	KY967417	Rusin et al. 2018
	Ilovlya, Volgograd Region, Russia, Karyotype = unknown	Five specimens: One taken to HNHM (vauch. publicly not available), col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 7.vi.2013, 49.23N 44.12E		KP715866	Cserkész et al. 2016
	Kalach Sands, Volgograd Region, Russia, 2n = 24, NF = 44	One specimen, viii–ix.1996, 48.77N 43.51E** One specimen, col. V. Stakheev, 29.iv.2021 48.840N 43.615E, skull transferred to ZMMU			Kovalskaya et al. 2000 V. Stakheev, pers. comm. 2021
	Kamyshin, Volgograd Region, Russia, 2n = 24, NF = 41	One specimen, date not specified, 49.92N 45.23E			Kovalskaya et al. 2011
		One specimen taken to HNHM (vauch. publicly not available), col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 8.vi.2013, 49.92N 45.23E			Cserkész et al. 2016
	Manych, Rostov Region, Russia, karyotype = unknown	Three specimen: ZMMU S-197470–197472, col. M. Rusin & N. Nedyalkov, 18–19.v.2016, 46.94N 43.02E		MK758101 MK758102	Lebedev et al. 2020
	Tuva, Russia, karyotype = unknown	One specimen: ZMMU S-188542; col. A. Surov, 10.viii.2010, 50.57N 95.06E	tuva	KY967415	Rusin et al. 2018
Sicista lorigera	Khomutovskaya steppe, Donetsk Region, Ukraine, 2n = 26, NF = 48	Two specimens, 47.29N 38.18E			Sokolov et al. 1986
	Borisovka, Ostrasyevy Yary, Belgorod Region, Russia, 2n = 26, NF = 48	Six specimens: col. T. Cserkész, M. Rusin, D. Czaban & G. Sramko, 12.vi.2013, 50.560N 36.058E		KP715877	Cserkész et al. 2016

Species	Population	Sampling data	ID on Fig.	GenBank Acc.No.	References
	Provalskaya Steppe, Lugansk Region, Ukraine, 2n = 26, NF = unknown	One specimen, col. A. Kondratenko, 1999, 48.15N 39.89E			Zagorodniuk &Kondratenko 2000
		Eight specimens: NMNHU 13994, col. A. Kondratenko, 25.v.1997 NMNHU 11322–11324, col. V. Marochkina & V. Timoshenkov, 7.v.1997 NMNHU 11396, col. V. Timoshenkov, 9.v.1988 NMNHU 11986–11988, col. A. Kondratenko, 13.viii.1998			Shevchenko & Zolotukhina 2005
Sicista sp.	Taganrog, Rostov Region, Russia, Karyotype = unknown	One specimen: NMNHU 14033, col. G. Guliy, 18.xi.1994, 47.3N 38.9E			Shevchenko & Zolotukhina 2005
	Artemovsk (Bakhmut), Donetsk Region, Ukraine, Karyotype = unknown	Four specimens: NMNHU 12373 14. ix.1960, col. R. Skobichevskiy; NMNHU 9987–9989, col. S. Valkh, 14.vi.1928, 11.v.1928 and 20.v.1929, 48.6N 38.0E			Shevchenko & Zolotukhina 2005
	Novo-Vodolazhskiy District, Kharkiv Region, Ukraine, Karyotype = unknown	One specimen: NMNHU 10699, col. Rudinskiy, 21.iv.1934, 49.6N 35.9E			Shevchenko & Zolotukhina 2005
	Malinovka, Kharkiv Region, Ukraine, Karyotype = unknown	One specimen: NMNHU 9990, col. N. Yumatov, 30.vii.1947, 47.8N 36.7E			Shevchenko & Zolotukhina 2005

Note: * in the original publication (Cserkész et al. 2016), incorrect coordinates were given; here, corrected data are provided; ** we believe that in the original publication (Kovalskaya et al. 2011), coordinates are given with error, therefore we put coordinates which better suit the text description of the locality.

Table 2. Characteristics of cytb gene sequences of *Sicista cimlanica* populations.

Population	*N*	N_{hapl}	N_{uniq}	π (*SD*)	*h* (*SD*)	Tajima's *D*, *P*	Fu's *Fs*, *P*
Tsimla sands (type locality)	11	9	8	0.00528 (0.00091)	0.945 (0.066)	-1.26741, N/s	-2.262, N/s
The other four populations	6	6	6	0.00938 (0.00147)	1.000 (0.096)	-0.14620, N/s	-0.917, N.s.
Total	**17**	**15**	**14**	**0.00875 (0.00098)**	**0.978 (0.031)**	**-1.35731,** N/s	**-4.924,** N/s

N—the number of assayed animals | N_{hapl}—number of found haplotypes | N_{uniq}—number of unique haplotypes | π—nucleotide diversity (averaged over loci) | *h*—haplotype diversity | *SD*—standard deviation | tests of selective neutrality: Tajima's *D* and Fu's Fs; N/s—Not significant, P >0.10.

DISCUSSION

Species distribution and phylogeography

The Tsimlyansk Birch Mouse represents a species with a restricted distribution area, though the exact limits of its range are not yet fully understood. Birch mice, in general, tend to have fragmented distributions occupying narrow species-specific landscapes. While most *Sicista* dwell in mesophyte habitats, *S. subtilis* s.l. is unique in its adaptations to dry environments (Lebedev et al. 2019). Tsimlyansk Birch Mice are no exception within the *S. subtilis* group and are found mostly in dry steppe grasslands. The majority of the known populations occur in psammophyte (sandy) steppes.

These sandy areas were formed as a result of flooding by melting glaciers during the Pleistocene. At least four layers of deposits were created, corresponding to glacier maximums in the Pleistocene: Don 650 kya, Oka 450 kya, Moscow 150 kya and Valdai 20 kya (Brylev 2008). Valdai (last glacial maximum) deposits are the least represented, probably because this glaciation was the weakest in Eastern Europe and had little effect on the region (Brylev 2008). The regular flooding of large areas could have affected both past and present distribution, with regular isolations and local extinctions. The sandy areas form narrow clusters of optimal habitats for *S. cimlanica*, but often they are detached from each other by tributary rivers. Some populations known to exist in other landscapes (from Belgorod and north of Lugansk Regions) are associated with drier vegetation. Nonetheless, the large transformation of natural habitats within most parts of the range of *S. cimlanica* have likely led to increased and widespread isolation and, to the best of our knowledge, this species rapidly declines in human-transformed habitats (e.g., where there is agriculture, settlements, artificial tree-plantations, etc.),

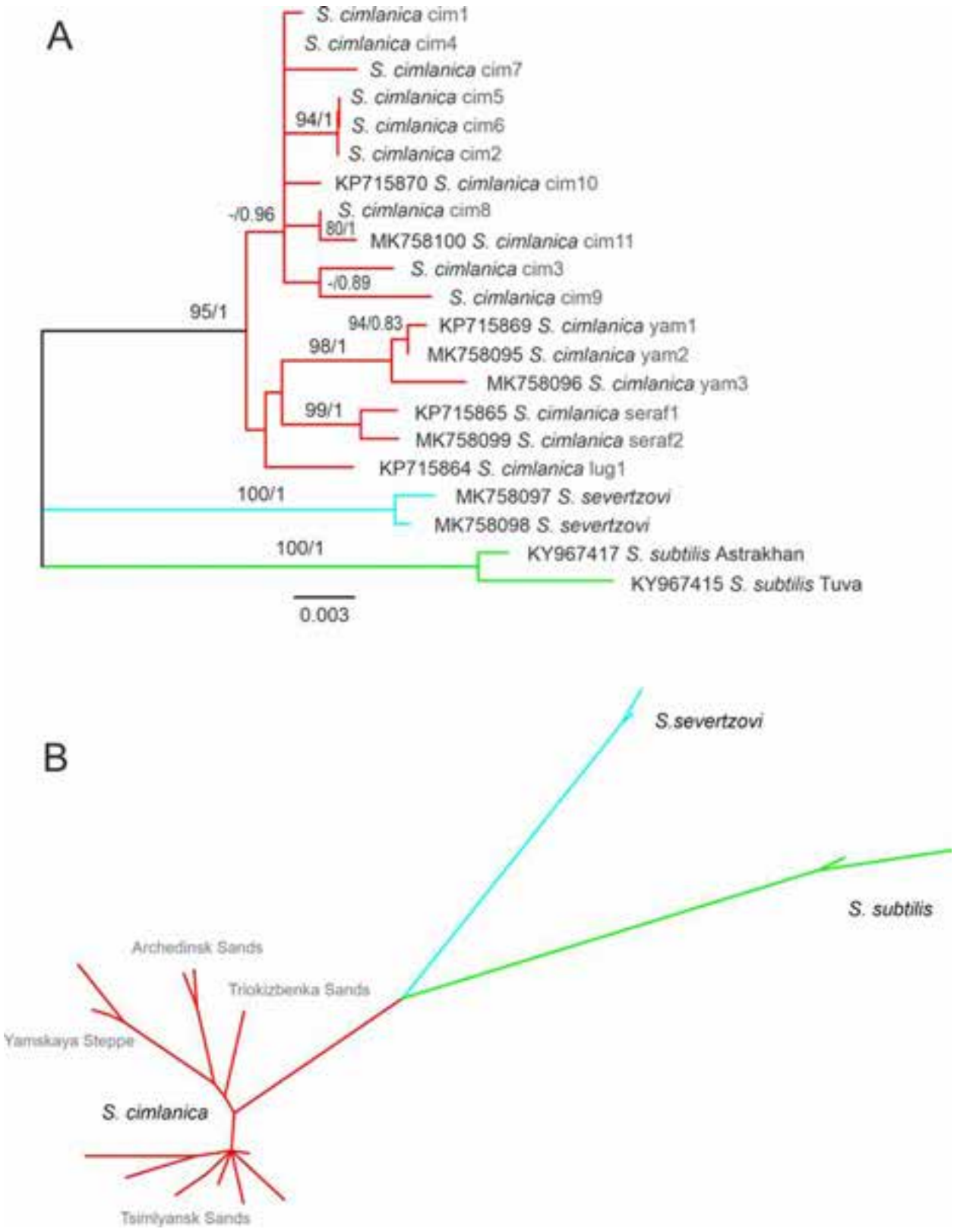

Figure 2. Inter and intraspecific relationships of *Sicista cimlanica* with closest OTUs reconstructed from the cytb gene: A—Unrooted maximum likelihood tree. Values at nodes represent ML bootstrap support from 1,000 replications / Bayesian posterior probabilities. Bootstrap support is only shown for the values exceeding 70%. Sample names are given in Table 1 | B— Radial layout indicating main lineages.

Figure 3. Putative species range of *S. cimlanica* and sampled localities (original data, GenBank sequences and literature data). Sample names correspond to Table 1. Larger red circles represent genotyped populations of *S. cimlanica*, smaller circles — population not genotyped. Dots within populations indicate karyotyped populations. Red star — type locality of *S. cimlanica*. White squares indicate verified populations of *S. lorigera*. Green diamonds — verified populations of *S. subtilis*. Cyan star — *S. severtzovi*.

eventually completely disappearing.

The natural range of *S. cimlanica* may be best described as lying between the Don and Seversky Donets Rivers (Figure 4). All verifiable localities of this species are summarized in Table 1. The southern distribution of this species is most likely limited by the Don River. Consensus on the western border is still lacking. Earlier, it was suggested that the distribution of *S. severtzovi* s.l. expands as far west as the Kyiv Region (Shenbrot et al. 1995). Conversely, further studies have not supported this hypothesis: few known localities from the Lugansk and Belgorod Regions suggest that the border is close to the Seversky Donets River (but does not necessarily follow it). *S. cimlanica* is found only from the left bank of the Seversky Donets (Zagorodnyuk & Kondartenko 2000; Kovalskaya et al. 2011; Cserkész et al. 2016), while on the right bank, only *S. lorigera* is known (Kovalskaya et al. 2011; Lebedev et al. 2020; Zagorodnyuk & Kondartenko 2000).

The northern border remains unstudied, though it is known to occur in the Kursk Region (Sokolov et al. 1986; Baskevich et al. 2011). The eastern border most likely follows the right bank of the Don River in the Voronezh Region. Further to the south in Volgograd Region, *S. cimlanica* crosses the Don River and can be found on Archedinsk and Alekseevski Sands (Kovalskaya et al. 2011; Cserkész et al. 2016). How far they infiltrate the left bank of the Don River remains unknown. In Ilovlya Sands, Kovalskaya et al. (2011) reported karyotypes that are now attributed to *S. cimlanica* ('S.sp 1'), while Cserkész et al. (2016), based on cytb sequences, identified animals from this locality as *S. subtilis* s.str. These conflicting results could relate to either sympatry or even hybridization (either recent or ancient) of two species in Ilovlya Sands, though more sampling in that region is needed to determine the true nature of this case.

There are several old records of birch mice that

cannot be unambiguously attributed to any species at the current stage of knowledge. They are labeled as *Sicista* sp. in Table 1. Yet, it is rather likely that the population from Malinovka (Kharkiv Region) belonged to *S. cimlanica*, while populations from Taganrog, Bakhmut and Novo-Vodolazhsk most likely belonged to *S. lorigera*. This assumption requires further testing based on genetic or karyological markers.

Due to limited sampling, the phylogeographic structure within the above- described species range cannot be explained thoroughly. At present, the birch mice from Tsimla Sands, Yamskaya Steppe, Trokhizbenka Sands, and Archedinsk Sands were assumed each form their own branch, though none could be named as an ancestral population, as the number of substitutions from each branch to the potential ancestor is equal (Figure 1). Further sampling covering all known populations as well as searching for new populations, especially at the central part of the species range, could answer the questions concerning the phylogeography of *S. cimlanica*.

Genetic diversity in the type locality

Since almost all examined individuals had unique haplotypes (Figure 3), the haplotype diversity (0.98 ± 0.03) was close to its maximum value ≈ of 1. In contrast, the average nucleotide variability for the studied mtDNA region was not high (0.9 ± 0.01%).

To reconstruct the processes of the modern species, range formation, it is necessary to collect more data for testing the hypothesis of sudden population expansion and computer modeling of historical demography processes.

Implications for conservation

The conservation of genetically complex groups of mammals with narrow distributions requires more sophisticated approaches (Csorba et al. 2015). Conservation efforts should focus on below-species level for effectively preserving the breadth of persisting genetic diversity (Garner et al. 2005). Species groups of so-called 'microspecies' often suffer from their wider-species concepts, as one superspecies normally has a wide distribution range with multiple populations. This can result in recognising such superspecies as facing relatively low levels of risk, thus resulting in listings of 'Least Concern' on the IUCN Red List. Each microspecies within these groups often has a different conservation status and can be much more threatened (Csorba et al. 2015). *S. cimlanica* is an example of such 'microspecies' requiring a specialized approach for its conservation.

Despite intensive studies of cytogenetic aspects, *S. cimlanica* remains one of the most poorly-documented taxa in Europe. Only approximately 14–15 populations have been recorded (Table 1) in the past 30 years. Most of these populations are strongly isolated from one another, lying within protected areas such as Tsimlyansk Reserve, Yamskaya Steppe Reserve, Triokhizbenka Sands Reserve, and Central-Chernozem Reserve. In the Streltsovskaya Steppe (Lugansk Region, Ukraine), the Tsimlyansk Birch Mouse was last recorded in 1999 (Zagorodniuk & Kondratenko 2000), and has not been found since, despite intensive small mammal surveys and birch mice monitoring (Mikhail Rusin's original data 2018). This may indicate that small, isolated populations are under threat of extinction even within protected areas. Relatively large populations of *S. cimlanica* are recorded only from Tsimlyansk and Archedinsk sands in the Rostov and Volgograd regions of Russia.

Grasslands – such as those within which this species is found – are among the most transformed ecosystems on earth, though one receiving the poorest level of conservation attention (Hoekstra et al. 2005; Carbutt et al. 2017). Nevertheless, grasslands represent some of the largest biodiversity hotspots on the planet (Habel et al. 2013). High diversity of the genus *Sicista* within limited grassland areas (i.e., the Eastern European Steppe, and especially the Middle Don area) is not surprising if compared to the diversity of plants and other taxa in the same region. Since the *S. subtilis* species group is highly associated with the threatened steppe biome, there is an argument that all members of the group require conservation focus. Until recently, *S. cimlanica* was omitted as a separate species for conservation work and it wasn't until 2021 that it was included in the Red Book of Ukraine (Decree of Ministry of Ecology and Natural Resources of Ukraine № 29 from 19.01.2021), where it is listed as an Endangered species with a single active locality in Ukraine. However, most of its range lies in Russia, where it has no official protection or conservation status yet.

On a range-wide level, the IUCN Red List criteria can be applied to determine a conservation status for the Tsimlyansk Birch Mouse. The putative extent of occurrence – defined by the IUCN (2012) as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass areas in which the taxon occurs – of the Tsimlyansk Birch Mouse is approximately 123,000 km^2. The area of occupancy – a metric representing the area of suitable habitat occupied by the taxon – of known populations is unlikely to exceed 4,000 km. Although this species has a somewhat

limited and isolated distribution, the measures of its extent of occurrence and area of occupancy are outside of the thresholds that must be met for consideration as threatened under criterion B of the IUCN Red List (IUCN 2012). Following Red List terminology, the species does meet two of three required conditions of criterion B: (1) its range is severely fragmented and (2) there has been an observed reduction in the number of populations potentially relating to the transformation of steppe habitat. Therefore, on a global scale, this species can be considered Near Threatened, with a high risk of becoming Vulnerable in the future. This implication has already been adopted in the IUCN Red List (Rusin 2024a).

All known populations would benefit from the conservation of habitats. Thus far, there are no data on how management for birch mice in isolated populations could assist their survival. In any case, the example of Streltsovskaya Steppe, where *S. cimlanica* likely has gone extinct, raises questions regarding the survival of such small, isolated populations. Moreover, the species has suffered from the war in Ukraine, as some habitats (such as in Triokhizbenka Sands) were turned into battlefields, resulting in extensive habitat degradation and loss. The current population status of birch mice in the war-torn regions is unknown.

The sibling species, *S. severtzovi* s. str., is also on a steep path of decline. Decades of intensive surveys discovered only a single small population (Yulia Kovalskaya, pers. comm. 2016). This population, described in Kovalskaya et al. (2011), was checked in 2014, and no birch mice were recorded, with part of the habitat having been destroyed for pig farm construction (T. Cserkész, M. Rusin, D. Csaban, and G. Sramkó, unpublished data). Following IUCN Red List criteria (IUCN 2012), *S. severtzovi* should fall within the Critically Endangered category under criterion B (Rusin 2024b). There are no populations in captivity of this species, which means there is already a high risk of *S. severtzovi* going, or having gone, Extinct. Conservation and research actions for this species are clearly urgently needed.

In summary, a middle Don area is a region of high genetic variability for birch mice with two local endemics — *S. cimlanica* and *S. severtzovi*. Hypothetically, both species evolved in the region during the middle Pleistocene as a result of isolation during various glaciation maximums. Both species remain poorly known, with few active populations known. Conservationists and zoologists should be encouraged to conduct extensive surveys of both *S. cimlanica* and *S. severtzovi*.

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ARTICLE

First characterization of the bacteriological profile of the Mediterranean Pond Turtle *Mauremys leprosa* (Schweigger, 1812) in Reghaïa Lake, Algeria

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Abstract: The Mediterranean Pond Turtle *Mauremys leprosa*, a 'Near Threatened' species, is poorly studied in Algeria and no data exists on its bacteriological carriage. However, knowledge about the microbiota of wildlife species is essential to develop holistic conservation approaches that integrate microbial health, habitat preservation, and species-specific needs. Recent concerns regarding the potential transmission of zoonotic pathogens by turtles have been highlighted in several studies. In this context, the current study analyzed the aerobic cloacal/fecal bacteria associated with the Mediterranean Pond Turtles, which were collected from Reghaïa Lake. Samples collected from 24 turtles allowed the identification of 11 bacterial genera. *Salmonella* was the most frequent isolated genus with a percentage of 22 of the total isolates, followed by *Escherichia* and *Enterobacter*. The diversity of genera isolated from juveniles is relatively low compared with adults. Turtle-bacterial genera relationships were tested by logistic regressions and redundancy analysis (RDA). Results of RDA indicate a statistically significant association (p-value <0.01) between morphological features and bacterial genera frequency. Our results confirm the reputation of freshwater turtles as a reservoir of several zoonotic bacterial pathogens. This microbiota analysis offers a non-invasive, multi-faceted approach to conserving endangered species by linking health, habitat, reproduction, and ecological dynamics. This highlights the importance of establishing an epidemiological surveillance system and an awareness program must be carried out to reduce the health risks associated with owning pet turtles.

Keywords: Chelonian, cloacal microbiota, freshwater ecosystems, health risk, wildlife conservation, zoonotic pathogens.

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INTRODUCTION

Freshwater turtles are key elements of aquatic ecosystems and play various ecological roles as predators and herbivores. Their presence in, and interactions with, the environment are crucial for maintaining the ecological balance and biodiversity of freshwater ecosystems (Wilbur 1997; Garig et al. 2020).

Among the species of chelonians in limnic ecosystems, the Mediterranean Pond Turtle *Mauremys leprosa*, widely distributed in northern Africa, the Iberian Peninsula, and southern France, stands out from other turtles occupying the same range (European Pond Turtle *Emys orbicularis*) due to its high tolerance to salinity, environmental pollution, and its adaptation to anthropogenic changes (Maran 1996; Bertolero & Busack 2017; El hassani et al. 2019). It is listed as Near Threatened in the IUCN Red List of Threatened Species in 2023 (Luiselli 2024), considered Vulnerable in the European Red List of Reptiles (Cox & Temple 2009), and included in Appendix II of the Bern Convention.

However, compared to other species, the Mediterranean Pond Turtle remains one of the least studied species in ecological research (Nowakiewicz et al. 2015). Indeed, only three studies focus on the ecology of this species in Algeria (Bakhouche et al. 2019; Ramedani et al. 2024).

To the best of our knowledge, none have examined the bacterial carriage of the Mediterranean Pond Turtle. Recent concerns regarding the potential transmission of zoonotic pathogens by turtles and reptiles were highlighted in several studies demonstrating the presence of zoonotic fecal bacterial carriage in these animals (Kautman et al. 2016; Hernandez et al. 2021). These facts raise concerns about the potential risks of transmitting dangerous pathogens to humans in Algeria, due to the commercialization of wild species known to be reservoirs of pathogens in several provinces (Tiar et al. 2019).

On the other hand, the close association of hosts and their microbiomes and the functional role of the microbiota provide essential information for ecological aspects of conservation biology (Zhu et al. 2021). Knowledge of microbiota can help identify specific ecological needs of endangered species, such as dietary preferences or habitat types that support a particular microbial community (Redford et al. 2012).

Our study seeks to characterize the aerobic cloacal and fecal microbiota of the Mediterranean Pond Turtle, focusing on identifying potential zoonotic pathogens within this microbial community. By examining the turtle–bacteria interactions, we aim to better understand the role of bacteria as pathogenic agents in wild turtles.

METHODS

Study site

The study was conducted in Réghaïa's Lake which was designated as a RAMSAR site during 2003. The site is located 30 km from the city of Algiers (36.785 ^{0}N, 3.342 ^{0}E). Bordered to the north by the Mediterranean Sea, this lake takes the shape of a basin with a depth of 7–9 m for which Oued de Réghaïa constitutes the main tributary (Image 1). The shores of the lake are slightly sloping. The immediate surroundings consist of fallow fields, wild olive scrub, and mastic trees, as well as few groves of Eucalyptus. It remains in the region the only witness and remnant of the various biogeographical characteristics of the former coastal wetland areas of the Mitidja Plain.

Study population and data collection

All captured turtles were marked to avoid duplicate sampling of the same individuals' turtles during the same study period. The surveys were carried out between March and May 2023, at a rate of two monitoring sessions per week. This period is considered optimal for studying the Mediterranean Pond Turtle of Reghaïa Lake, according to our study already conducted on the site in 2019 (Bakhouche et al. 2019). As the site is classified as a Ramsar site and where many birds nest, we chose to sample only at one station to avoid any disturbance. Capture of individuals was carried out using a net and a fishing line. Once the individuals are captured, they are marked. The marking involves making an incision with a file on the marginal scales of the carapace. Recaptures allow tracking individuals over time through their marking. Biometric measurements allow, on one hand, to compare individuals with each other, and on the other hand, to test relationship between bacterial carriage and species characteristics.

The method used for age estimation is direct count of growth rings. Sexual maturity was assessed based on the external secondary sexual characteristics of the shell and the sexual behavior of individuals.

Comparison of different morphological features was carried out using the Mann-Whitney U test for independent samples. Statistical analysis was conducted using IBM SPSS software version 27.

Image 1. Map showing the location of the site study (Reghaïa Lake) where the turtles were captured.

Analyses of bacterial communities

Samples were taken in situ for each individual captured turtle. Two cloacal swabs were taken per specimen. One was used for selective enrichment and the second for non-selective enrichment. Samples are transported in an isothermal bag at 4 °C, then frozen at (-20) °C until bacterial analysis.

Isolation and bacteriological characterization were carried out at the medical microbiology laboratory of the Higher National Veterinary School of Algiers, following the steps below:

- Pre-enrichment: Inoculation on salmonella-selective Rappaport Vassiliadis Soy (RSV) broth and non-selective Brain Heart Infusion (BHI) broth to amplify bacterial numbers and increase chances of isolation. The swabs are immersed in the liquid medium in a sterile manner, close to the Bunsen burner. The tubes are then incubated at 37 °C for 18–24 hours.

- Isolation of bacteria on selective media: The bacterial isolation was performed on selective media (Microbiology agar, Merck KGaA, Germany) following pre-enrichment and subsequent Gram staining. Inoculation of four prepared culture media and incubation at 37 °C for 24–48 hours (Salmonella-Shigella (SS) agar: for selective isolation of Salmonella. Eosin Methylene Blue (EMB) agar: for the selective isolation of pathogenic *Escherichia coli* and other Enterobacteriaceae. MacConkey agar: for the selective isolation of lactose-positive Enterobacteriaceae and other gram-positive bacteria. Chapman agar: for the selective isolation of Staphylococci and other gram-positive bacteria.)

- Biochemical characterization of isolated bacteria: Biochemical testing was conducted using API E20 test strips (Biomerieux SA, Marcy l'Etoile, France). The table 1 summarizes the various tests that were used.

Turtle-Bacterial genera relationships

To highlight the relationships between the turtles and bacterial genera, we first performed binary logistic regressions using the 'stepwise top-down' method, aiming to maximize the explanation of the dependent variable (bacterial genus) with the lowest number of independent variables (Turtle characteristics). The logistic model belongs to the family of generalized linear models and links, by a linear combination, the environmental variables to the variable to be predicted by means of a logistic link function. It is a statistical tool recommended for binary data (in our case presence/absence of bacterial genera) (Guisan & Zimmermann 2000). We used IBM SPSS version 27 software.

Secondly, we performed a redundancy analysis (RDA) to examine relationships between the bacterial genera frequency and characteristics of Mediterranean Pond Turtle. RDA can highlight associations between specific morphological characteristics (such as weight, shell length) and microbiota composition. It is particularly useful when the data are multivariate, which is our case (several microbial genera and several morphological

traits) and allows us to better understand the interactions between the species, its microbiota and the environment.

To test the relationship significance between mentioned variables, we performed a Monte Carlo permutation test. For the purposes of RDA, we constructed four groups of variables by combining age and sex. The groups are respectively adult male, adult female immature male and the finally immature female. We performed the RDA analysis in XLSTAT (version 2021.2.2, Addinsoft).

RESULTS

Mediterranean Pond Turtle population

A total of 24 individuals were captured, including six females and 18 males. 10 individuals were recaptured at least once. There were no juveniles.

Descriptive statistics for the morphological features of turtles are summarised in the table below. Average length of the carapace is 163 mm. The posterior width of the carapace varies 90.5–122.6 mm with an average of 111.6 mm. The weight shows a very high standard deviation (211.7).

While comparing morphological features between turtle groups the only significant difference (α = 0.03) was observed in the distance between the cloaca and the carapace (immature males and immature females). Other comparison showed no significant difference (Table 3).

Bacterial carriage

Bacterial growth was assessed on 74 bacterial cultures. Identification methods resulted in the identification of 70 strains. Four (4) strains could not be identified by the tests used in our study due to the absence of reaction after subculturing. After elimination of strains corresponding to contaminants, we obtained a pure 60 bacterial strains. A total of 11 bacterial genera were identified (Table 4, Image 1). Salmonella was the most frequent isolated genus with a percentage of 21.66 (13/60) of the total isolates, followed by *Escherichia* and *Enterobacter*. (Figure 1). These are in reality the most abundant bacterial genera in the cloacal flora of the studied population, with prevalences of 54% and 42%, respectively. The prevalence of *Salmonella* spp. and *Klebsiella* spp. is significantly higher in females than in males. *Vibrio*, *Listeria*, *Yersinia*, and *Lactobacillus* were only found in males, while *Proteus* is present only in females (Table 4).

Turtle–Bacterial genera relationships

We obtained a logistic regression model for six bacterial genera, namely: *Lactobacillus*, *Pseudomonas*, *Enterobacter*, *Vibrio*, *Salmonella*, and *Escherichia*.

Table 1. Appropriate biochemical tests for bacterial identification.

Test	Test principle	Reading
Vogues-Proskauer (VP)	Reveals the capacity of bacteria to produce acetone during glucose fermentation. The test is carried out on Clark and Lubs broth already seeded, then incubated at 37 °C for 18 to 24 hours, to which VP1 and VP2 reagents are added respectively, with 10-minute intervals in between (Varghese & Joy 2014).	- Red/pink Coloration (VP +) - Incolore (VP -)
Rouge de méthyle (RM)	Reveals the ability of bacteria to oxidize glucose with the production of acid derivatives. This test is carried out on Clark and Lubs broth, which has already been seeded and then steamed at 37°C for 18 to 24 hours, to which methyl red reagent is added (Varghese & Joy 2014).	- Red Coloration (RM +) - Yellow Coloration (RM -)
Triple sugar iron (TSI)	Highlights the bacteria's ability to ferment the three sugars present in the medium (Glucose, sucrose and lactose). Inoculation is performed by a central prick in the tube and slope inoculation, followed by incubation at 37°C for 24 hours (Varghese & Joy 2014).	- Acidification of the slope (fermentation of sucrose, lactose or both) - Pellet acidification (glucose fermentation) - Black precipitates (H2S production and glucose fermentation)
Citrate	Highlights the ability of bacteria to use citrate as their sole carbon source. This test is performed by plating a slant agar on Simmons citrate tube incubated at 37°C for 24–48 hours (Varghese & Joy 2014).	- Blue Coloration (Citrate +) - Green coloration (Citrate -)
Mannitol motility	Used to detect bacterial mobility and mannitol fermentation. It is performed by central pricking in Mannitol mobility agar incubated at 37 °C for 24–48 hours (Varghese & Joy 2014).	- Yellow Coloration (Mannitol +) - Red Coloration (Mannitol -) - Growth by creating a disturbance from the central prickle (Mobility +) - Growth all along the central sting (Mobility -)
Urease	Demonstrates the ability of bacteria to degrade urea to ammonia using urease. Inoculation of urea indole medium and incubation at 37 °C for 24 hours (Varghese & Joy 2014).	- Pink coloration (Urease +) - No colour change (Urease -)
Indole production	Demonstrates the ability of bacteria to degrade tryptophan to indole. Test performed on urea indole medium after seeding and incubation for 24 hours with the addition of KOVAC's reagent (Varghese & Joy 2014).	- Formation of a red ring on the surface of the medium (Indole +) - No red ring formation (Indole -)

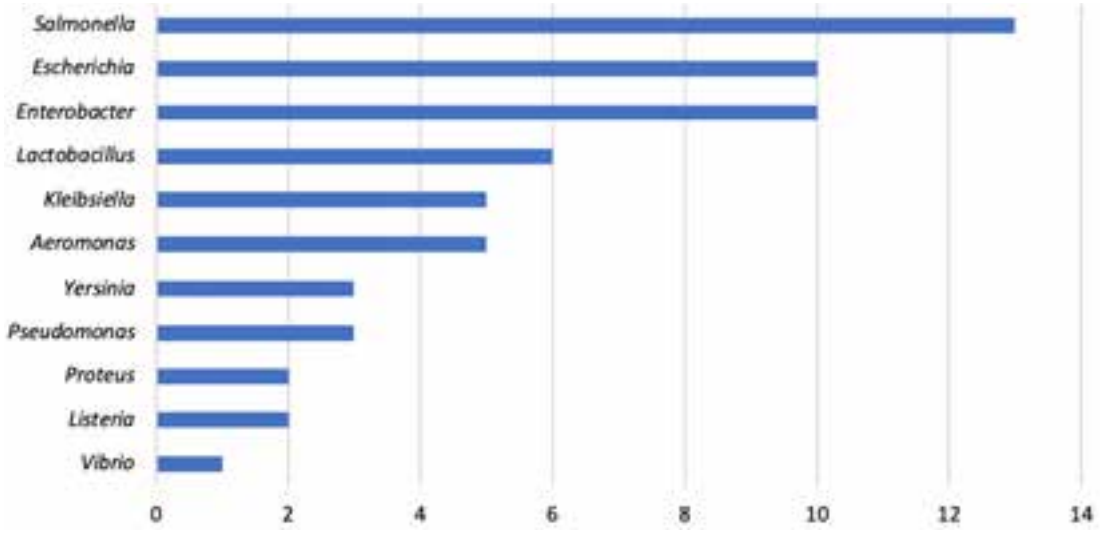

Figure 1. Graph showing frequency of isolation of bacterial genera the Mediterranean Pond Turtle captured in Reghaïa Lake.

Table 2. Descriptive statistics of morphological features of the Mediterranean Pond Turtle.

Parameters (unit)	Code	Min.	Max.	Mean	Standard deviation
Carapace length (mm)	CL	126.75	185.00	163.69	26..22
Anterior width of carapace (mm)	AWC	79.25	109.33	97.98	13.34
Posterior width of carapace (mm)	PWC	90.50	122.57	111.58	14.91
Carapace height (mm)	H	41.75	58.71	52.71	7.76
Tail length (mm)	TL	75.25	87.67	82.07	5.14
Distance carapace–cloaque (mm)	CC	32.00	39.23	36.41	3.15
Weight (g)	W	268.50	727.46	567.65	211.69

Models were invalid for the other bacterial genera (Table 5).

The assessment of models' fit was based on Nalgelkerke's R^2. Like the R^2 for multiple regression, the higher the value of this coefficient, the better the model fit to data. Table 5 shows that models for *Pseudomonas* and *Vibrio* genera give a perfect prediction with a probability of one.

In order to identify variables that most predict presence probability of bacterial genera, we used the Wald statistic. For the *Enterobacter* genus model (Table 5) the 'Age' and 'H' parameters positively influenced (Exp(B) >1) the probability of presence of this genus at the threshold $\alpha \leq 0.05$, whereas there was a negative effect (Exp(B) <1) of the 'CL' ($\alpha \leq 0.05$). The *Pseudomonas* and *Vibrio* models did not show any significant variables.

The 'CL' variable had a negative effect (Exp(B) <1; $\alpha \leq 0.05$) on the presence of *Salmonella* and *Escherichia*. However, 'TL' had a positive effect (Exp(B) >1; $\alpha \leq 0.05$) on the presence of *Escherichia*.

Results of RDA indicate a statistically significant association (p-value <0.01) between morphological features and bacterial genera frequency. The first two axes of the RDA together carry 83.36% of the constrained inertia. F1 axis carry 56% of the constrained inertia and F2 axis carry 27.36% (Figure 2).

MI and FA individuals contributed significantly to the construction of F1 axis and are well represented on this axis. Male individuals contributed to the construction of F2 axis and are also well represented on this axis (Table 6, Figure 2).

The bacterial genera *Lactobacillus*, *Kleibsiella*, *Listeria*, and *Proteus* contributed significantly to the construction of F1 axis and are also well represented on it. The genera *Aeromonas*, *Enterobacter*, *Vibrio*, and *Yersinia* contributed significantly to the construction of F2 axis (Table 6, Figure 2).

Genera *Kleibsiella* and *Proteus* are positively correlated with female individuals. Genera *Vibrio*, *Aeromonas*, *Yersinia*, and *Lactobacillus* are positively

Table 3. Summary of the Mann-Whitney U test.

Male Adult (MA) – Female Adult (FA)							
	CL	AWC	PWC	H	TL	CC	W
Mann-Whitney	25.5	14.5	21.5	22.00	20.5	27.0	22.0
Wilcoxon	130.5	119.5	126.5	127.0	125.5	132.0	127.0
Test statistics	25.50	14.50	21.50	22.00	20.50	27.00	22.0
SD	7.93	7.90	7.89	7.90	7.86	7.88	7.94
Standardised test statistics	0.57	-0.82	0.06	0.13	-0.06	0.76	0.13
Bilateral test	0.57	0.41	0.95	0.90	0.95	0.45	0.90
Male Immature (MI) – Female Immature (FI)							
	CL	AWC	PWC	H	TL	CC	W
Mann-Whitney	7,000	6.00	7.50	5.00	9.00	12.00	6.00
Wilcoxon	17,000	16.00	17.50	15.00	19.00	22.00	16.00
Test statistics	7,000	6.00	7.50	5.00	9.00	12.00	6.00
SD	2,828	2.83	2.78	2.83	2.80	2.83	2.83
Standardised test statistics	,354	0.00	0.54	-0.35	1.07	2.12	0.00
Bilateral test	,724	1.00	0.59	0.72	0.28	0.03	1.00

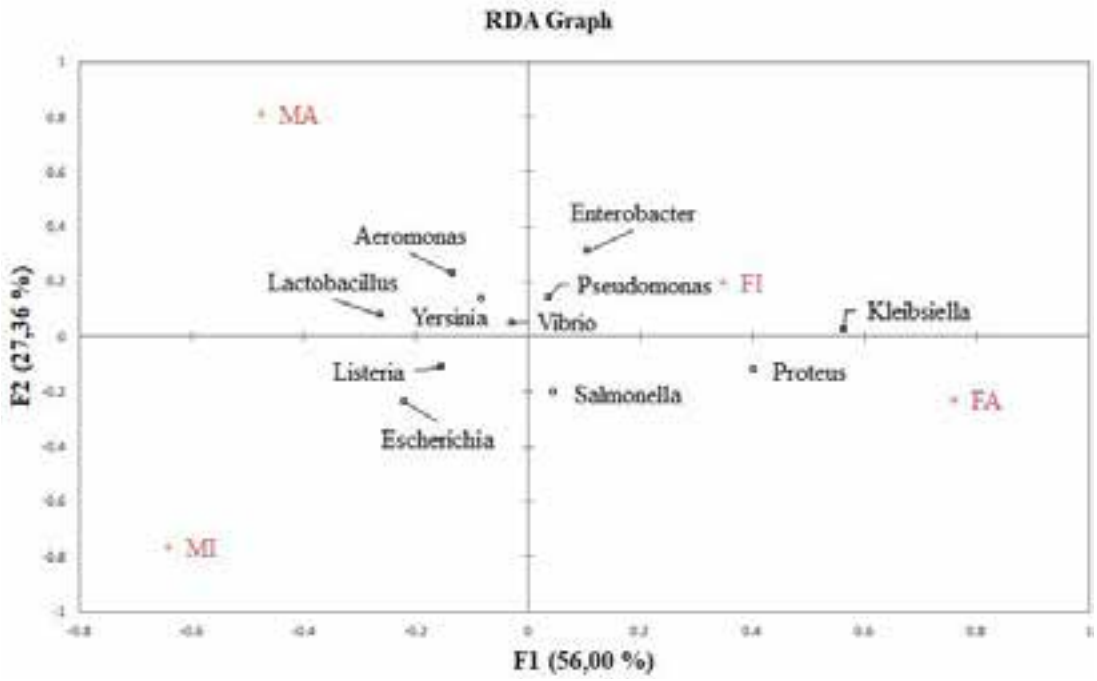

Figure 2. Redundancy analysis (RDA) ordination plot showing the relationship between the bacterial genera frequency and characteristics the individuals of the Mediterranean Pond Turtle at the site of Reghaïa Lake along RDA axes 1 and 2 (explaining 83.36% on the total variation).

correlated with adult males, while genera *Listeria* and *Escherichia* are more associated with immature males (Figure 2).

DISCUSSION

The duration (March–May 2023) and the main objective of the study, which required laboratory work to isolate and characterise bacterial carriage, were the main cause for sampling effort reduction at one site.

Table 4. Bacterial genera identified and their prevalence in the Mediterranean Pond Turtle.

Turtles			Identified bacterial genus										
ID	Sex	Maturity	*Lactobacillus*	*Listeria*	*Aeromonas*	*Pseudomonas*	*Vibrio*	*Kleibsiella*	*Enterobacter*	*Salmonella*	*Escherichia*	*Proteus*	*Yersinia*
1	F	A	0	0	0	0	0	1	0	0	0	1	0
2	F	A	0	0	0	0	0	0	0	1	1	1	0
3	F	A	0	0	0	0	0	1	1	1	0	0	0
4	F	I	0	0	0	1	0	1	0	0	0	0	0
5	F	I	0	0	0	0	0	0	0	1	1	0	0
6	F	I	0	0	0	0	0	1	1	1	0	0	0
7	M	A	1	0	1	0	0	1	1	0	0	0	0
8	M	A	0	0	0	0	0	0	0	0	0	0	1
9	M	A	1	0	0	0	0	0	0	0	1	0	1
10	M	A	0	1	0	1	0	0	0	0	0	0	1
11	M	A	0	0	1	0	0	0	0	1	1	0	0
12	M	A	0	0	1	1	0	0	1	0	1	0	0
13	M	A	0	0	1	0	0	0	0	0	0	0	0
14	M	A	0	0	0	0	0	0	0	0	0	0	0
15	M	A	0	0	1	0	0	0	1	0	0	0	0
16	M	A	1	0	0	0	0	0	1	1	0	0	0
17	M	A	0	0	0	0	0	0	1	1	1	0	0
18	M	A	1	0	0	0	0	0	1	1	0	0	0
19	M	A	0	0	0	0	0	0	0	1	1	0	0
20	M	A	1	0	0	0	1	0	1	1	0	0	0
21	M	I	0	0	0	0	0	0	0	1	1	0	0
22	M	I	0	1	0	0	0	0	1	0	1	0	0
23	M	I	0	0	0	0	0	0	0	1	1	0	0
24	M	I	1	0	0	0	0	0	0	1	0	0	0
Prevalence (%)			25	8.33	20.83	12.5	4.17	20.83	41.67	54.17	41.67	8.33	12.5

It is interesting to note, however, that sex ratio favour males, supporting that described in a study carried out on the same population by Bakhouche et al. (2019).

The Mediterranean Pond Turtle population of lake Reghaïa exhibits high variability in individual weight. This may influence microbiota diversity, particularly due to variation in body spaces, diets, physiological conditions, and social behaviors. Heavier individuals, often having more resources and space for their microbes, may support a more diverse microbiota than smaller individuals (Youngblut et al. 2019; Budd et al. 2020).

Bacteriological analysis led to the identification of 11 bacterial genera (*Lactobacillus*, *Listeria*, *Aeromonas*, *Pseudomonas*, *Vibrio*, *Kleibsiella*, *Enterobacter*, *Salmonella*, *Escherichia*, *Proteus*, *Yersinia*). These results correspond to a basic reptilian microbiota (Colston 2017). The predominance of *Pseudomonadota* could provide information on the diet of Mediterranean Pond Turtle population of lake Réghaïa, as they are associated with a predominantly carnivorous diet.

The growth of two gram+ bacterial genera (*Lactobacillus* and *Listeria*) on Chapman culture media (Salt Mannitol Agar) was made possible by their high tolerance of the medium's salinity. In fact, these genera tolerate concentrations of 12% and 20%, respectively (Cole et al. 1990; Osek et al. 2022), far exceeding the concentration of salt (selective agent) present in the culture media used (7.5%), which enabled these strains to be isolated. The mobility of the *Yersinia* strains isolated excludes the possibility that they belong to the

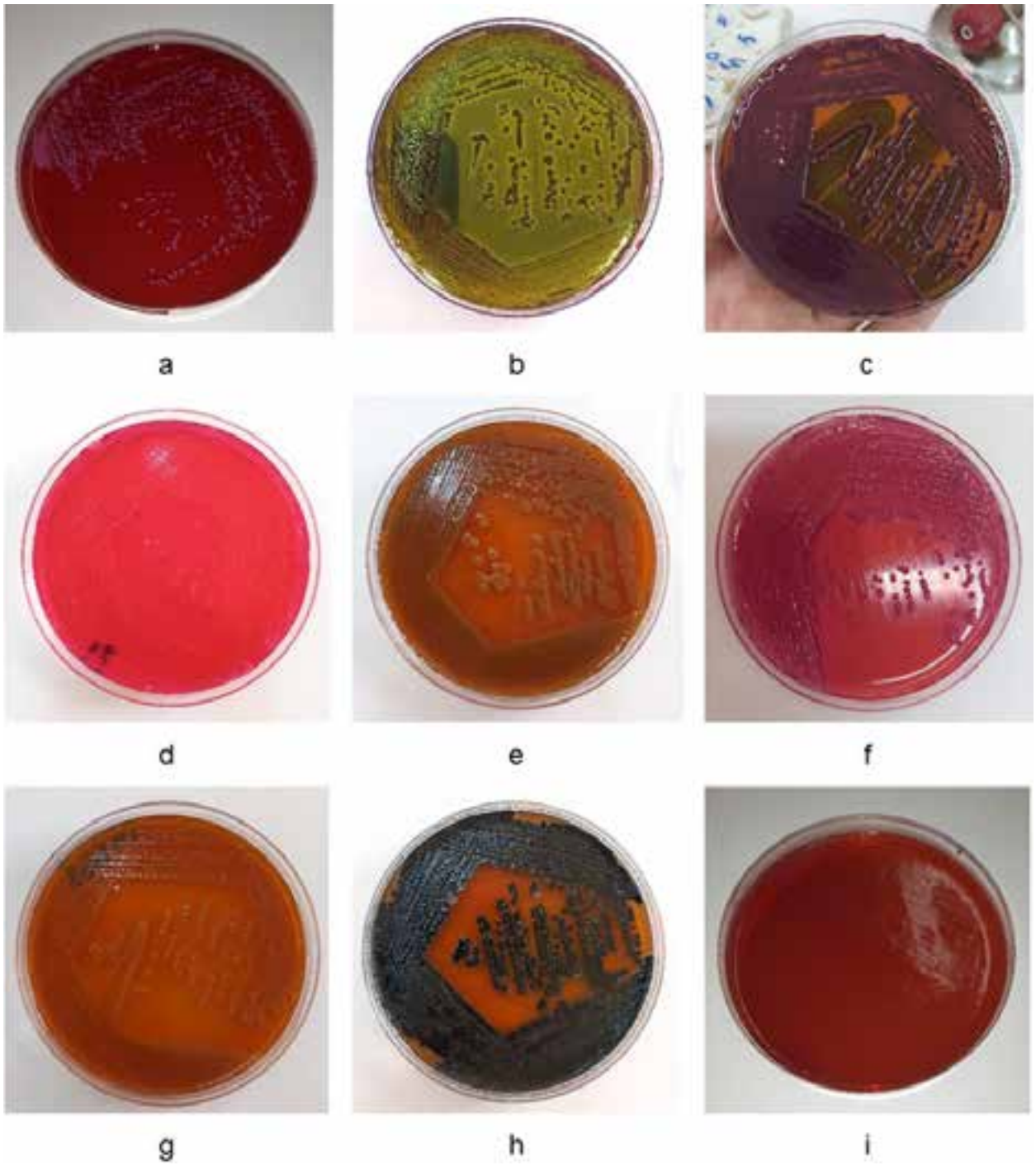

Image 2. Illustrations on some bacterial genera identified by biochemical tests. a—*Aeromonas* on MAC | b—*Escherichia* on EMB agar | c—*Kleibsiella* on EMB agar | d—*Listeria* on MSA | e—*Proteus* on MAC | f—*Pseudomonas* on MAC | g—*Salmonella* (H2S-) on SS | h—*Salmonella* (H2S+) on SS | i—*Yersinia* on MAC. © Rayane Ahcene Reda BELAIDI.

species *Yersinia pestis*, which is an immobile bacterium (Jorgensen et al. 2015). This implies that our strains would belong to the species *Yersinia enterolitica or Yersinia pseudotuberculosis.*

The cloacal bacterial carriage corresponds qualitatively and in proportions to those highlighted in several previous studies of freshwater turtles, in particular *Emys orbituclaris*, which is commonly recognised as syntopic for Mediterranean Pond Turtle (Hacioglu et al. 2012; Marin et al. 2013; Nowakiewicz et al. 2015; Ruzauskas et al. 2016).

The high prevalence of *Salmonella* and *Klebsiella*

Table 5. Logistic regression models of bacterial genera in the Mediterranean Pond Turtle.

		B	E.S	Wald	ddl	Sig.	Exp(B)	R^2 de Nagelkerke
Lactobacillus	Sex	-20.51	16408.71	0.00	1	0.999	0.000	0.231
	Constant	-0.69	0.50	1.92	1	0.166	0.500	
Pseudomonas	Age	4225.05	87867.62	0.00	1	0.962		1.000
	CL	-112.58	2356.29	0.00	1	0.962	0.000	
	AWC	161.06	3308.13	0.00	1	0.961	8.87E+69	
	PWC	-130.33	2688.50	0.00	1	0.961	0.000	
	H	84.24	1814.76	0.00	1	0.963	3.86E+36	
	TL	-100.06	2066.50	0.00	1	0.961	0.000	
	CC	135.82	2822.41	0.00	1	0.962	9.70E+58	
	Constant	12401.99	257536.43	0.00	1	0.962		
Enterobacter	Sex	-2.25	1.48	2.32	1	0.128	0.105	0.392
	Age	7.58	3.67	4.27	1	**0.039**	1956.076	
	CL	-0.29	0.13	5.07	1	**0.024**	0.748	
	H	0.66	0.32	4.29	1	**0.038**	1.939	
	Constant	7.76	5.95	1.70	1	0.192	2343.684	
Vibrio	Age	227.97	42188.59	0.00	1	0.996	1.01E+99	1.000
	CL	-4.96	1330.69	0.00	1	0.997	0.007	
	PWC	-2.13	1513.71	0.00	1	0.999	0.118	
	H	10.85	2657.93	0.00	1	0.997	51704.704	
	Constant	269.77	94378.88	0.00	1	0.998	1.44E+117	
Salmonella	CL	-0.20	0.10	3.97	1	**0.046**	0.820	0.348
	W	0.02	0.01	3.59	1	0.058	1.025	
	Constant	18.98	9.29	4.18	1	0.041	175005394.480	
Escherichia	Sex	-2.99	1.66	3.27	1	0.071	**0.**050	0.495
	CL	-0.30	0.15	3.88	1	**0.049**	0.740	
	PWC	0.46	0.25	3.51	1	0.061	1.585	
	TL	0.37	0.18	4.41	1	**0.036**	1.446	
	CC	-0.35	0.19	3.42	1	0.064	0.703	
	Constant	-19.51	10.40	3.52	1	0.061	0.000	

genera, along with the detection of *Listeria*, *Vibrio*, and *Yersinia* in our study, combined with the known role of freshwater turtles and reptiles as reservoirs of various zoonotic bacterial pathogens (Gaertner et al. 2008; Back et al. 2016; Hernandez et al. 2021), underscores the need to implement a surveillance system at the Reghaïa Lake, which is frequently visited by the public.

The presence of *Pseudomonas* spp. which are bacteria that inhabit soil and water, can also cause disease in plants, animals as well as immunocompromised humans (Wu et al. 2015). It is commonly found in the oral cavity and intestinal tract of reptiles and can cause a number of diseases such as ulcerative stomatitis, pneumonia, dermatitis and septicaemia (Campa et al. 1993; Warwick et al. 2013). In the Silene Nature Park (Latvia), it was the most common bacterium found in all skin areas (cloaca, mouth, and feet) of *Emys orbicularis* individuals (Umbrasko et al. 2020).

Presence of *Enterobacter* genus in Mediterranean Pond Turtle was positively influenced by age and carapace height parameters, which are associated with larger individuals. In *Emys orbicularis*, this genus was more abundant in juveniles in the study conducted by Nowakiewicz et al. (2015).

The presence of *Salmonella* in Mediterranean Pond Turtle is negatively influenced by carapace size. It is therefore associated with small turtles. This poses an even greater risk to children who acquire them as pets. In fact, children run a high risk of contracting salmonellosis associated with small turtles, as they can

Table 6. Results of the redundancy analysis (RDA).

Observations						
	Scores		Contributions		Square cosines	
	F1	F2	F1	F2	F1	F2
Obs1 (MA)	-0,446	0,759	0,168	0,488	0,224	0,650
Obs2 (MI)	-0,601	-0,724	0,305	0,443	0,407	0,591
Obs3 (FA)	0,717	-0,219	0,435	0,041	0,580	0,054
Obs4 (FI)	0,330	0,183	0,092	0,028	0,122	0,038
Response variables						
	Scores		Contributions		Square cosines	
	F1	F2	F1	F2	F1	F2
Lactobacillus	-0,262	0,076	0,103	0,018	0,827	0,070
Listeria	-0,154	-0,107	0,036	0,036	0,671	0,325
Aeromonas	-0,135	0,229	0,027	0,163	0,224	0,650
Pseudomonas	0,039	0,143	0,002	0,064	0,024	0,325
Vibrio	-0,027	0,046	0,001	0,007	0,224	0,650
Kleibsiella	0,563	0,026	0,479	0,002	0,936	0,002
Enterobacter	0,107	0,311	0,017	0,299	0,102	0,868
Salmonella	0,047	-0,204	0,003	0,129	0,046	0,853
Escherichia	-0,221	-0,240	0,074	0,178	0,458	0,541
Proteus	0,404	-0,123	0,247	0,047	0,580	0,054
Yersinia	-0,081	0,138	0,010	0,059	0,224	0,650

be easily handled and placed in the mouth (CDC 2008). Two cases of turtle-associated salmonellosis in children were reported in Japan in 2007 and 2008 (Kuroki et al. 2015).

RDA is a proven multivariate analysis technique for processing species-environment data (Legendre & Legendre 1998). It was used to analyse the determinism of biometric parameters on the presence of bacterial genera. The first group identified comprises the genera isolated only from adult male Mediterranean Pond Turtle, which are generally isolated from freshwater (*Vibrio* and *Aeromonas*), plants and soil (*Yersinia*). Cloacal transmission during copulation could also be a means of acquiring new microbial species (Hidalgo-Vila et al. 2007; Nowakiewicz et al. 2015).

The second group includes the genus *Proteus* and *Klebsiella* specific to adult females in captured Mediterranean Pond Turtle. These bacteria of *Proteus* genus are part of pathogenic or normal microflora and can be symbiotic or change from neutral/commensal to parasitic (Drzewiecka 2016). They can also be interpreted as an indicator of pollution (Al-Bahry et al. 2012). In our case, the individuals captured were apparently in very good health, which suggests that the presence of the *Proteus* genus is due more to environmental pollution, which is well established in the lake. However, the risk remains high, as studies (Oros et al. 2005; Awong-Taylor et al. 2008) have reported a low hatching success rate in Loggerhead Turtles linked to *Proteus* spp. and mortality in marine turtles in the Canary Islands.

The diversity of genera isolated from juveniles is relatively low compared with adults. The same observation has been made in *Emys orbicularis* in Poland (Nowakiewicz et al. 2015). This phenomenon may be linked to a number of nutritional and/or behavioural factors.

These findings highlight the presence of bacterial genera that include species known to be potentially pathogenic to humans, suggesting the need for further investigation into their pathogenicity and potential implications for public health. People living near the Réghaïa wetland, visitors and traders who sell turtles should be more careful when handling them.

Despite its status as a protected species under national regulations (Executive Decree No. 12-235 of 24 May 2012 establishing the list of protected non-domestic animal species in Algeria), Mediterranean Pond Turtle is still one of the illegally traded species (personal unpublished data). Tighter controls should minimise the risk of diseases being transmitted by turtles. A ban

on small turtles' sale (shells less than four inches long) prevented around 100,000 cases of turtle-associated salmonellosis in children in 1980 which constitutes a good example of biodiversity health related conservation (CDC 2008).

This data highlights the importance of setting up an epidemiological and microbiological surveillance system, and strengthens the need to implement environmental protection programmes. In addition, an awareness-raising programme needs to be carried out to raise awareness of significant health risks associated with pet turtle ownership.

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ARTICLE

Morphological and molecular identifications of sea turtles *Lepidochelys olivacea* and *Eretmochelys imbricata* from the Turtle Bay of Cilacap, Indonesia

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Abstract: DNA barcoding is a powerful tool for accurately identifying marine turtle species, especially when morphological identification is challenging, owing to insufficient clues in the samples available. This study focused on two sea turtle samples of dead adults washed ashore and babies hatching out from nests, which pose a challenge for morphological identification. They represented two species, the Olive Ridley Turtle *Lepidochelys olivacea* and Hawksbill Turtle *Eretmochelys imbricata*, from Turtle Bay, Cilacap, Indonesia. For one sample, morphological identification initially suggested it as a Green Sea Turtle *Chelonia mydas*, based on traits like a pair of prefrontal scales, brown carapace colouration, and the absence of serrations on the posterior carapace. The degraded condition of the specimen and shared juvenile traits between *C. mydas* and *E. imbricata* made conclusive identification challenging. Using mtDNA barcoding with the CO1 gene provided more accurate species identification, revealing the sample to be *E. imbricata* with a perfect genetic match in the BLAST search (0% divergence). This result highlights the advantages of molecular approaches when traditional methods fall short. Phylogenetic analysis of *L. olivacea* and *E. imbricata* sequences revealed close clustering of sampled sequences with published sequences from Ghana, Australia, and China. High bootstrap values of 92% for *L. olivacea* and 98% for *E. imbricata* confirmed the molecular identifications of these samples. The study underscores the value of combining DNA barcoding and phylogenetics for marine turtle identification and evolutionary insights, with implications for conservation and species management.

Keywords: Biodiversity, DNA barcoding, dead specimen, % divergence, extraction, genetics, phylogenetic, scutes, taxonomy, threatened.

Indonesian Abstrak: Barkoding DNA merupakan alat yang ampuh untuk mengidentifikasi spesies penyu laut secara akurat, terutama ketika identifikasi secara morfologi sulit dilakukan, karena petunjuk yang tersedia tidak memadai. Penelitian ini difokuskan pada dua sampel yaitu penyu laut dewasa yang mati terdampar di pantai dan anakan yang menetas dari sarang, sehingga menimbulkan tantangan untuk identifikasi secara morfologi. Sampel tersebut mewakili dua spesies, Penyu Belimbing *Lepidochelys olivacea* dan Penyu Sisik *Eretmochelys imbricata*, yang berasal dari Teluk Penyu, Cilacap, Indonesia. Untuk satu sampel, identifikasi morfologis awalnya menunjukkan bahwa penyu tersebut adalah Penyu Hijau *Chelonia mydas*, berdasarkan ciri-ciri seperti sepasang sisik prefrontal, warna karapas coklat, dan tidak adanya gerigi pada karapas posterior. Kondisi spesimen yang terdegradasi dan kesamaan ciri-ciri juvenil antara *C. mydas* dan *E. imbricata* membuat identifikasi konklusif menjadi sulit. Penggunaan barkoding mtDNA dengan gen CO1 memberikan identifikasi spesies yang lebih akurat, yang menunjukkan bahwa sampel tersebut adalah *E. imbricata* dengan kecocokan genetik yang sempurna dalam pencarian BLAST9 (divergensi 0%). Hasil ini menggambarkan keuntungan pendekatan molekuler ketika metode tradisional tidak berhasil. Analisis filogenetik dari sekuens *L. olivacea* dan *E. imbricata* menunjukkan pengelompokan yang erat dari sekuens sampel dengan sekuens yang dipublikasikan dari Ghana, Australia, dan Tiongkok. Nilai bootstrap yang tinggi sebesar 92% untuk *L. olivacea* dan 98% untuk *E. imbricata* mengonfirmasi identifikasi molekuler dari sampel-sampel ini. Studi ini menggarisbawahi nilai penggabungan barkoding DNA dan filogenetik untuk identifikasi penyu laut dan wawasan evolusi, dengan implikasi untuk upaya konservasi dan pengelolaan spesies.

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INTRODUCTION

Sea turtles are globally recognized for their ecological importance, with seven species documented worldwide (Robinson & Paladino 2013). Of these, six species inhabit the waters of Indonesia, reflecting the region's rich biodiversity (Ario et al. 2016). Olive Ridley Sea Turtle *Lepidochelys olivacea*, Green Sea Turtle *Chelonia mydas*, Hawksbill Sea Turtle *Eretmochelys imbricata*, Leatherback Sea Turtle *Dermochelys coriacea*, Flatback Sea Turtle *Natator depressus*, and Loggerhead Sea Turtle *Caretta caretta* are found in Indonesian Waters (Pardede & Yealta 2013; Suraeda et al. 2018). Despite legal protection and their inclusion in Appendix I of the Convention on International Trade in Endangered Species (CITES), illegal trade in sea turtles remains widespread. Transactions often involve parts such as eggs, meat, and shells, complicating taxonomic identification (Nijman & Nekaris 2014; Foran & Ray 2016). One notable site, Turtle Bay in Cilacap Regency, was once iconic for turtle landings but has since been transformed into an industrial and tourist hub, threatening the local turtle populations (Thahira & Wirasmoyo 2022). Although Pertamina (2019) designates the area as a turtle landing site, the lack of sufficient data on sea turtle activity poses a challenge, leaving classification to rely mostly on morphological identification. This study focuses on two species of particular interest: the Olive Ridley *Lepidochelys olivacea* and the Hawksbill Turtle *Eretmochelys imbricata*, both of which are critically affected by habitat disruption and illegal trade.

Morphological characteristics and tracks have traditionally been the primary methods for identifying sea turtle species, with traits such as carapace shape, colouration, head and limb structure, track width, and egg size serving as key indicators. Studies like Rupilu et al. (2019) have detailed how features such as the number of costal scutes on the carapace, inframarginal scutes on the plastron, and prefrontal & postocular scales provide essential markers for distinguishing species. In Indonesia, as well as globally, these characteristics have been widely used to identify sea turtles, offering a foundational approach to understanding their development, evolution, and ecological role (van Dam & Diez 1998). Despite its historical significance in biology and paleontology, morphological identification is not without limitations. A major challenge is the risk of misidentification when body parts are degraded or worn out, making it difficult to rely on a limited set of morphological features for accurate identification.

Molecular techniques have become vital in overcoming the limitations of morphology-based species identification, especially in regions like Indonesia, where illegal sea turtle trade complicates classification. Various markers, such as 16S, 12S, CYTB, and ITS, have been used for accurate species identification, with this study focusing specifically on mitochondrial Cytochrome oxidase 1 (CO1). This molecular approach is particularly useful in cases where body parts are traded, as highlighted by Madduppa et al. (2019). Recent studies, including those by Santhosh et al. (2018) and Ollinger et al. (2020), stress the importance of integrating genetic data with morphological analysis to enhance species identification. Molecular methods are not without challenges, such as contamination or incorrect marker usage, which can lead to misidentification. Despite these weaknesses, genetic analysis remains crucial in understanding sea turtle diversity, particularly in areas like Turtle Bay, and Cilacap, where traditional methods fall short.

Sea turtles are threatened wildlife often found dead and decaying on beaches, posing challenges for species identification, critical to conservation intervention. In such cases, DNA barcoding serves as a powerful tool for accurately identifying marine turtle species, particularly when morphological identification is hindered by the lack of sufficient diagnostic traits. This study employed both morphological and genetic analyses to identify sea turtle species in Turtle Bay, Cilacap, Indonesia. Morphological characteristics such as shell structure, curved carapace length, plastron color, and prefrontal scales were assessed alongside genetic data to reduce biases associated with relying solely on physical traits, especially when dealing with deceased & decayed individuals, juveniles, or embryos. Tissue samples from nesting turtles at sites SP1–SP8 were collected for genetic analysis to complement morphological findings. The objective of this research was to accurately identify the species inhabiting the region, integrating both approaches to enhance conservation, and protection efforts.

METHODS

A combination of morphometric data and genetic studies was used to identify the sea turtle species in the study area. For the genetic studies, clutches retrieved from nesting sites were carefully incubated in the sand within the conservation area at Sodong Beach, Cilacap. For the dead and decaying turtle sample, tissue was carefully obtained from the carapace, avoiding

contamination. The collected sample was designated unidentified GT for Green Turtle *Chelonia mydas* and OR1, OR2, and OR3 for Olive Ridley baby turtles and embryo *Lepidochelys olivacea*. Data collection for this study occurred during the new and full moon (July, August, and September 2023) phases to facilitate taxonomic studies. Tissue samples gathered from the field were preserved at -20 ^{0}C and subsequently subjected to analysis at Genetica Science, Indonesia, employing a DNA barcoding protocol.

Study area

The study was conducted in Turtle Bay, Cilacap, Indonesia, across a designated area spanning SP1 to SP8, with each station covering a distance of 2 km (Image 1). Cilacap Beach, nestled in Cilacap Regency, Central Java, Indonesia, stretches along the Indian Ocean, offering a serene yet vital coastal landscape. Geographically, it sits at about 7.730^{0}S, 108.980^{0}E, surrounded by a mixture of sandy beaches and coastal hills. Positioned roughly 200 km southwestern of Semarang and 300 km southeastern of Jakarta, the area is more than just a beautiful stretch of coastline. Despite the growing industrial activity, the beach holds immense ecological value as a key nesting ground for sea turtles. This makes it a focal point for conservation efforts, where preserving the natural habitat becomes essential for the survival of these endangered species. Global Positioning Systems (GPS) were utilized to ascertain each station's research locations and coordinates. The sampling process involved monitoring during the nesting season at eight designated stations (SP1–SP8) with coordinates as follows: SP1 (7.691 ^{0}S, 109.181 ^{0}E), SP2 (7.691 ^{0}S, 109.191 ^{0}E), SP3 (7.696 ^{0}S, 109.231 ^{0}E), SP4 (7.692 ^{0}S, 109.244 ^{0}E), SP5 (7.698 ^{0}S, 109.260 ^{0}E), SP6 (7.698 ^{0}S, 109.261 ^{0}E), SP7 (7.700 ^{0}S, 109.292 ^{0}E), SP8 (7.700 ^{0}S, 109.287 ^{0}E).

Data collection method

During the nesting season, samples were collected from *L. olivacea* and *E. imbricata*. Monitoring of nesting sea turtles occurred during the early morning hours (0200–0600 h.) using rechargeable torchlights. Eggs retrieved from the nesting sites were buried in sandy soils at the conservation area, Sodong Beach, Cilacap, and allowed to hatch. After approximately 45 days (±15 days), samples were obtained from unhatched eggs and deceased hatchlings (Olive Ridley). Additionally, a sample was obtained from a dead adult Hawksbill Sea Turtle *E. imbricata* carried offshore by ocean currents. They were immersed in a 96% ethanol solution to preserve the samples and stored at -20°C, following established protocols (Roden et al. 2013; Dutton et al. 2014b). The specific methods used to obtain these samples are detailed below.

For the morphological studies, the length and width of the carapace of deceased adult turtles were measured using a measuring tape. Observations were made regarding the number of scutes, carapace colour, shell shape, prefrontal scale, and plastron colour. This information was meticulously recorded in a dedicated research notebook and compared to a reference publication by Pritchard & Mortimer (1999). Genetic studies followed the procedure of using Olive Ridley

Image 1. Map of study sites in Turtle Bay of Cilacap for data collection.

Lepidochelys olivacea eggs as described below.

Collection tubes filled with 96% ethanol solution were prepared. An unhatched egg was selected and thoroughly rinsed with distilled water. Gloves were worn and changed for each sample collection to prevent contamination. After drying the eggshell with a tissue and placing it in a petri dish, an ethanol swab was used for additional cleaning. Using forceps to grasp a part of the egg, the shell was gently opened with sterile scissors. The embryo was carefully collected, transferred to sample collection tubes, and securely sealed using a wooden skewer. The samples were labelled using a pencil. The containers were wrapped and stored in a cool, dry place until they were later transported to the Animal Taxonomy Laboratory in a cooler with ice. Subsequently, they were stored at -20 °C for the next stage of the process.

Table 2 outlines the protocol optimization based on the methods described by Madduppa et al. (2019), with modifications to the extraction and amplification procedures. In the study involving dead Olive Ridley baby turtles, embryo, and Hawksbill Sea Turtles (initially labelled as GT sample, unverified specimen), gloves were worn and changed for each sample collection. The rear end of the flipper was cleaned with an ethanol swab. A small section of the hind flipper was carefully cut using forceps with sterile scissors. The sample was then transferred into a plastic vial filled with ethanol using a wooden skewer. The samples were labelled using a pencil. The containers were wrapped and stored in a cool, dry place until later transported to the Animal Taxonomy Laboratory at the Faculty of Biology, UNSOED, in a cooler with ice. Subsequently, the samples were stored at -20°C for the next stage of the process as described below.

DNA Barcoding was conducted at Genetica Science, Indonesia, using CO1 gene to identify the sea turtle species. The Genomic DNA Extraction was done using the Quick-DNA™ Tissue/Insect Miniprep kit (ZYMO, D6016) following the manufacturer's protocol. A 50 mg tissue sample was added to a ZR BashingBead™ Lysis Tube (2.0 mm). Then, 750 µl of BashingBead™ buffer was added to the tube and tightly capped. It was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 10 min. The ZR BashingBead™ Lysis Tube (2.0 mm) was centrifuged in a microcentrifuge at ≥10,000 x g for one min. Subsequently, 400 µl of the supernatant was transferred to a Zymo-Spin™ III-F filter in a collection tube and centrifuged at 8,000 x g for 1 min. Then, 1,200 µl of genomic lysis buffer was added to the filtrate in the collection tube, and the mixture was thoroughly mixed. Further, 800 µl of the mixture from the previous step was transferred to a Zymo-Spin™ IICR Column one in a collection tube and centrifuged at 10,000 x g for 1 min. The eluate from the collection tube was discarded, and the procedure was reiterated.

Subsequently, 200 µl of DNA pre-wash buffer was introduced to the Zymo-Spin™ IICR column within a fresh collection tube and subjected to centrifugation at 10,000 x g for 1 min. Following this, 500 µl of g-DNA Wash Buffer was applied to the Zymo-Spin™ IICR Column, followed by centrifugation at 10,000 x g for 1 min. Ultimately, the Zymo-Spin™ IICR column was relocated to a sterile 1.5 ml microcentrifuge tube, and 100 µl (with a minimum requirement of 35 µl) of DNA elution buffer was directly administered to the column matrix. The column was centrifuged at 10,000 x g for 30 s to facilitate DNA elution (ZYMO Research Manual 2021).

PCR was performed in a 25 µL reaction volume consisting of 1 µL template DNA, 12.5 µL MyTaq HS Red Mix 2X, and 1.0 µL of each of the four primers (10 µM), 7.5 µL of ddH_2O, and the conditions were set as seen in Table 1. The four primer sequences used were as follows:

VF2_t1: TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC

FishF2_t1: TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC

FishR2_t1: CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA

FR1d_t1: CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA

Data analysis

Species identification followed the reference guidelines provided by Pritchard & Mortimer (1999). Sequences obtained were analyzed using the BOLD system and GenBank databases to confirm species identity, and similarity. Forward and reverse primer sequences were assembled into contigs, creating a single sequence for each sample. These sequences were subjected to a BLAST search on GenBank to identify species. They were also submitted to the NCBI database, where they were assigned the following GenBank accession numbers: OR1 (OR562509.1 and 627 bp), OR2 (OR562510.1, and 648 bp), OR3 (OR562511.1, and 656 bp), and GT (OR562508.1, and 648 bp). The BIN for each closest-matching species was retrieved from BOLD, with OR1, OR2, and OR3 *Lepidochelys olivacea* assigned BIN AAC1248, and GT *Eretmochelys imbricata* assigned BIN ACE8206 (pending compliance with metadata

requirements).

In addition, sequences from 10 individuals of *Lepidochelys olivacea*, 10 individuals of *Eretmochelys imbricata*, and seven individuals of *Lepidochelys kempii* were downloaded from BOLD to construct a phylogenetic tree with the sequences from the study. Sequences were concatenated and edited to remove stop codons. One sequence of *Dermochelys coriacea* was used as the outgroup tree was built using the Neighbor-Joining method in MEGA, illustrating the phylogenetic relationships of species inhabiting Turtle Bay of Cilacap. The results were then discussed for further insights into species positioning.

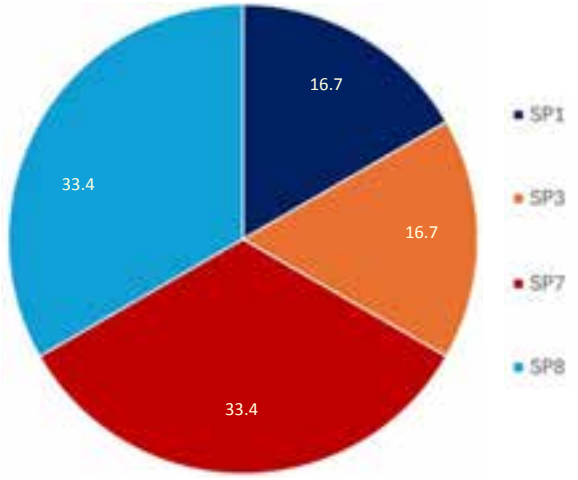

Figure 1. Chart representation of stations with laid eggs of sea turtles in the study area.

RESULTS

Of the eight sampling sites surveyed, eggs were found in four areas (Table 3). The number of clutches retrieved from one nest in a sampling site within the study area ranged between 30 (SP8) and 100 (SP3). These data cannot be used to quantify species identity due to irregularities caused by constant poaching prior to egg retrieval. Eggs were recovered from SP1, SP3, SP7, and SP8, with SP8 having the highest number of clutches (Figure 1). At SP7, nesting was also observed twice, but no eggs were found despite visible turtle tracks (noted as NA), indicating poaching. Figure 1 provides a chart representation of egg retrievals across the sampling stations (SP1–SP8), where four sites had no egg records.

A single dead turtle (Image 2) was labelled as "GT" due to certain features, including a brownish plastron colour, absence of strong or prominent serrations, and a pair of prefrontal scales (Table 4). The morphological data for the OR samples, derived from two deceased baby turtles (Image 2), are also presented in Table 4. These data indicated 5–7 costal scutes, grey carapace colouration, slightly overlapping carapace shape, four prefrontal scales, and a white plastron.

Due to the juvenile stage of the specimens, DNA barcoding was employed to confirm the species identity of these two OR samples apart from the decayed GT sample. Similarity and identity results from BOLD Systems and GenBank are presented in Table 5. Both *Eretmochelys imbricata* and *Lepidochelys olivacea* showed a 100% similarity (0% divergence) to reference sequences, signifying high confidence in species identification. Taxonomic identities, including taxon assignment, and associated probabilities for the two species identified in Turtle Bay, Cilacap, are summarized in Table 6.

Table 1. PCR Conditions details used in the present study.

Steps	Temperature °C	Time	Cycles
Initial denaturation	95	3 min	1
Denaturation Annealing Extension	95 50 72	15 sec 30 sec 45 sec	35
Final extension	72	3 min	1
Hold	4	∞	1

DISCUSSION

This study utilized a combination of fieldwork for data collection and wet lab experiments. The morphological aspect of the study involved a series of body

Table 2. Protocol optimization for tissue sampling following Maduppa et al. (2019).

	Tissue type	Tissue sampling	Preservation	Extraction	Amplification
1	Dead baby turtle	rear-flipper	96% ethanol	ZYMO, D6016	Bioline, BIO-25048
2	Dead GT turtle	Flipper	96% ethanol	ZYMO, D6016	Bioline, BIO-25048
3	Egg Clutches	Embryo	96% ethanol	ZYMO, D6016	Bioline, BIO-25048

Table 3. Number of sea turtle egg clutches retrieved from the study locations.

	Code	Sampling site	Date (found)	Eggs
1	SP1	Sodong	July 2	65
2	SP2	Srandil	-	-
3	SP3	Welahan Wetan	Aug. 21	100
4	Sp4	Widarapayung Kulon	-	-
5	Sp5	Sidayu	-	-
6	Sp6	Widarapayung Wetan	-	-
7	Sp7	Sidaurip	July 9, 11	NA, 96
8	Sp8	Pagubugan	July 7	30, 80

The eggs were retrieved from SP1, SP6, SP7, and SP8, respectively.

Table 4. Morphological identification of samples of a dead turtle (GT sample) and baby turtles (OR) sourced from the study area.

	Features	GT Sample	OR (baby turtles)
1	Costal scutes	4	5–7
2	Carapace color	Brown	Grey
3	Carapace length CCL	66 cm	-
4	Carapace width CCW	57 cm	-
5	Carapace shape	Oval	Slightly overlapping
6	Prefrontal scales	2	4
7	Plastron	White	White

Image 2. Studied samples of two species of sea turtles in Turtle Bay of Cilacap (left): dead and partially decayed sample of *E. imbricata* ("GT") (right): babies of *L. olivacea* (OR). © Mr. Jumawan and Ikegwu, C.M.

measurements and observations. As shown in Table 4, morphometric characters were collected and compared with data from previous studies. Morphological identification involved body measurements and observations based on the guidelines of Pritchard & Mortimer (1999). In Olive ridley, (OR samples), morpho-data were obtained from baby turtles. The recoded costal scutes were 5–7, grey colouration, slightly overlapping carapace, two pairs of prefrontal scales, and white plastron colour. It's morphometrics were consistent with Pritchard & Mortimer (1999), who reported 6–9 costal scutes in *L. olivacea* (with occasional occurrences of five in hatchlings), two pairs of prefrontal scales, and a slightly overlapping carapace in juveniles.

For the GT sample, which was a dead and decayed specimen with degraded body parts, morphological identification proved challenging. Initially, the sample was tentatively identified as *Chelonia mydas* due to certain features such as the presence of four costal scutes—a trait shared by both Green and Hawksbill Turtles. Additionally, the measured curved carapace length (CCL) was 66 cm, classifying the specimen as juvenile, since *C. mydas* & *E. imbricata* reach sexual maturity at >85 cm, and 78.8 cm CCL, respectively (Liles et al. 2011; Zárate et al. 2013). The juvenile state complicated the classification as either *C. mydas* or *E. imbricata* based solely on CCL data. Most external morphological characteristics aligned with those of *C. mydas*, despite the initial uncertainty. These included the presence of a pair of prefrontal scales, which is a distinguishing feature of Green Turtles compared to Hawksbill Turtles. The observed specimen had a pair of prefrontal scales, supporting its tentative classification as a Green Turtle. Regarding plastron colour, the remaining intact parts were brown, consistent with Pritchard & Mortimer's (1999) description of immature *C. mydas*

Table 5. Similarity (BOLDsystems) and identity (GenBank) and accession number details.

	Sample Initial	Similarity %	Identity %	Reference Species
1	GT	100 100 100 100	100 100 100 100	*Eretmochelys imbricata* JX454970 *E. imbricata* GQ152886 *E. imbricata* KU254594 *E. imbricata* KP221806
2	OR1	100 100 100 100	100 100 100 100	*Lepidochelys olivacea* JX45991 *L. olivacea* NC_028634 *L. olivacea* JX454979 *L. olivacea* JX454987
3	OR2	100 100 100 100	100 100 100 100	*L. olivacea* JX454991 *L. olivacea* NC_028634 *L. olivacea* JX454979 *L. olivacea* JX454987
4	OR3	100 100 100 100	100 100 100 100	*L. olivacea* JX454991 *L. olivacea* NC_028634 *L. olivacea* JX454979 *L. olivacea* JX454987

Table 6. Identification summary for *Eretmochelys imbricata* and *Lepidochelys olivacea*.

	Linnaean rank	*Eretmochelys* sp.	*Lepidochelys* sp.	Probability %
1	Phylum	Chordata	Chordata	100
2	Class	Reptilia	Reptilia	100
3	Order	Testudines	Testudines	100
4	Family	Cheloniidae	Chelonidae	100
5	Genus	*Eretmochelys*	*Lepidochelys*	100
6	Species	*Eretmochelys imbricata*	*Lepidochelys olivacea*	100

as having brown colouration with radiating streaks. This characteristic varies significantly in adults, ranging from plain to streaked or spotted in shades of brown, buff, or other earth tones, further supporting the identification as *C. mydas*.

In terms of carapace shape, both Green and Hawksbill Turtles have an oval carapace. In contrast, Hawksbill Turtles possess strongly serrated posterior margins, whereas Green Turtles have a broadly oval, non-serrated carapace. The observed carapace lacked prominent serrations, but given the specimen's degraded condition, it is possible that the absence of strong serrations resulted from damage, complicating definitive identification. Overall, while the morphological evidence pointed toward *C. mydas*, the degraded state of the specimen and overlapping characteristics with *E. imbricata* highlighted the challenges of relying solely on morphological identification.

In contrast, molecular analysis provided more definitive results. Tissue samples were collected following the protocols of Dutton (1996) and Carreras et al. (2007) for DNA extraction and amplification (Table 2). The CO1 mitochondrial DNA gene was used for species identification through DNA barcoding, a method praised for its precision, and wide applicability (Madduppa et al. 2019). DNA barcoding allowed for accurate species identification even in the absence of complete morphological data, as demonstrated by Abreu-Grobois et al. (2006). This method has been successfully applied across various taxa, including fish larvae (Nuryanto et al. 2023a), fish from family Sparidae (Nuryanto et al. 2023b), Antarctic Fish (Belchier & Lawson 2013), and sea turtles (Bahri et al. 2017). Furthermore, Hernandez et al. (2013) supported taxonomic sufficiency at the genus level for analyzing assemblage diversity.

For the GT sample, DNA barcoding contradicted the initial morphological identification. While certain morphological characters initially suggested it was *Chelonia mydas*, the genetic analysis revealed it to be *Eretmochelys imbricata*, as confirmed by a 100% (0% divergence) match in the BLAST search, although the BIN (ACE8206) on BOLD is still undergoing metadata validation. This outcome demonstrates the advantages of molecular approaches over traditional methods, particularly when morphological data is incomplete or unreliable. Additionally, studies such as those by Pereira et al. (2013), Lim et al. (2016), Briñoccoli et al. (2020), and Mohammed-Geba et al. (2021) have shown that intraspecific genetic similarities ranging from 92.5% to 99% can serve as species borders in various research contexts (Nuryanto et al. 2017; Winarni et al. 2023). Čandek & Kurtner (2015) further emphasized the importance of geographic locality information for accurate species delineation.

The CO1 target was used in this study to assess the similarity and identity of sea turtles in Turtle Bay, Cilacap. Amplification of two tissues (two baby turtles), an embryo for OR, and tissue from an unidentified GT, from different individuals yielded 100% similarity (0% divergence) to CO1 sequences in BOLD Systems and GenBank for *L. olivacea*, and from one tissue yielded *E. imbricata* (Table 5). Nuryanto et al. (2023) reported that 99% genetic similarity is a reliable species delineation threshold, consistent with findings from other studies (Ratnasingham & Hebert 2013; Nuryanto et al. 2018; Amatya 2019; Kusbiyanto et al. 2020).

In the context of phylogenetic studies, Yang & Rannala (2010) revealed that phylogenetic analysis employs molecular techniques to identify and analyze the connections among closely related species in systematics and taxonomy. The current study provides a phylogenetic

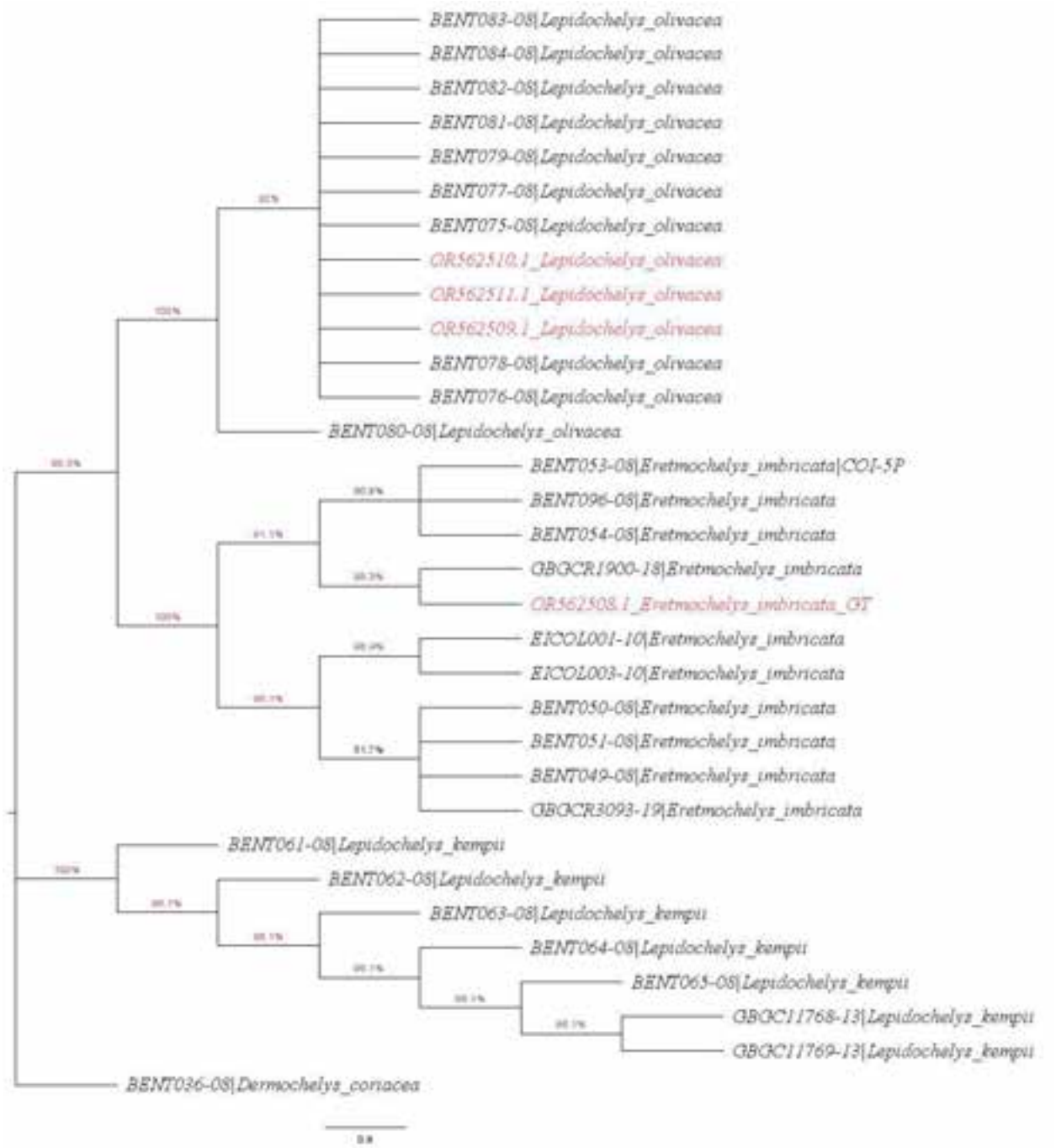

Figure 2. Strongly resolved Neighbor-Joining tree of CO1, showing molecular identifications of *Lepidochelys olivacea* and *Eretmochelys imbricata* samples studied nested within the published sequences of these species.

overview of *Lepidochelys olivacea* and *Eretmochelys imbricata* found in Indonesia. The phylogeny was largely consistent with other marine phylogenies (Bowen et al. 1993; Dutton et al. 1996). Phylogenetic analysis, supported by high bootstrap values, clearly showed that all sequences of *L. olivacea* obtained in Indonesia could be grouped into a single genetic lineage. Specifically, OR1, OR2, and OR3 identified as *L. olivacea* clustered with sequences BENT083-08, BENT084-08, and BENT082-08 from Ghana, as well as BENT081-08, BENT079-08, BENT077-08, BENT075-08, BENT078-08, and BENT076-08 from Australia, achieving a bootstrap value of 92%. At the larger clade level, the bootstrap support is 100%. Meanwhile, for GT, the sequence in the present study

falls within a well-supported *Eretmochelys imbricata* clade, with a 91.1% bootstrap value for its subclade, indicating strong support, while another subclade within *E. imbricata* from BOLD has 96.1% support. The larger *E. imbricata* clade is highly supported at 100%. Additionally, the sequence in the present study forms a sister species relationship with GBGCR1900-18 from Chinese waters, with a strong bootstrap support of 98.3%, highlighting their close genetic similarity.

For *L. olivacea*, despite being from different geographical locations, the clustering in a single clade implies low genetic divergence, indicating a close evolutionary relationship. This suggests divergence from a common recent ancestor, even though they are separated by distance. The single individual sequenced from Indonesia identified as *E. imbricata* also clustered with a species from China, further implying low genetic divergence.

Despite the findings, there may be limitations in this study due to the restricted number of morpho-characters analyzed and the limited data available to demonstrate the complete relationship of *E. imbricata* with other species, as only one individual was identified. This study contributes to systematic biology by demonstrating the importance of integrating morphological and molecular techniques for species identification, particularly in cases where morphological data alone are insufficient. By using DNA barcoding alongside traditional morphological methods, the research highlights how molecular tools, such as the CO1 gene, can provide more precise identification of species, addressing the limitations of morphology-based approaches. The identification of *Eretmochelys imbricata* through genetic analysis, despite initial misclassification based on morphology, underscores the growing need for molecular methods in systematics.

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ARTICLE

Pollen morphology of Annonaceae from the Bicol Region, Philippines

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Abstract: Annonaceae is one of the most prominent families with diverse pollen morphology. The present study aimed to investigate and describe the pollen morphology of 12 species of Annonaceae from the Bicol Region, Philippines. The pollen grains were subjected to acetolysis and described using a scanning electron microscope, and the hierarchical cluster analysis was done to cluster the pollen grains with similar characters. The 12 Annonaceae species share characters such as monads, inaperturate, and isopolarity, but they formed three distinct clusters based on pollen size. Their polar axis (PA) and equatorial diameter (ED) are statistically significant due to their variability, with PA ranging 36.41±15.86 µm and ED 28.27±14.27 µm. The polar length-to-equatorial diameter (PA/ED) ratio is 1.32, with mostly sub-prolate shape. Exine ornamentations showed significant variability among the 12 Annonaceae species with echinate, rugulate, scabrate, psilate, and verrucate. The pollen morphology of the two endemic species, namely *Friesodielsia lanceolata* (Merr.) Steenis and *Goniothalamus elmeri* Merr. is first reported here. Collections of more endemic Philippine Annonaceae species are deemed necessary to comprehensively analyze their pollen characters, which are helpful for infrageneric relationships within the family.

Keywords: Acetolysis, endemic species, equatorial diameter, exine ornamentation, palynology, polar axis, pollen size, pollen shape, pollination, SEM.

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Author contributions: AORD—contributed to the field collections, data analysis, discussion of results, and conclusion of the manuscript. GJDA—led the discussion, editing, and paper review for the manuscript.

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INTRODUCTION

Annonaceae is a pantropical family comprising 110 genera and approximately 2,500 species (Chatrou et al. 2012; Xue et al. 2021). The family members are characterized by exstipulate, distichous leaves, trimerous perianth of two whorls of petals, numerous stamens, and free carpels (van Heusden 1992; Chatrou et al. 2012; Couvreur et al. 2012). Moreover, the family has the greatest basal angiosperm diversity at both macromorphological and pollen morphological levels (Doyle & Le Thomas 2012). Annonaceae provide forest and ecosystem services, such as provisioning, regulating, cultural, and supporting services (Handayani & Yuzammi 2021; Erkens et al. 2023). They are widely distributed in Asia-Pacific regions such as Thailand, Malaysia, Borneo, and the Philippines (Turner 2011; Johnson et al. 2021).

The palynology of Annonaceae has gained interest because it is a significant source of evidence for systematics and phylogenetic analysis (Doyle & Le Thomas 1997; Xue et al. 2011; Ragho 2020*).* The minute pollen grains contain a remarkable degree of information in their highly resistant sporopollenin wall (Davey et al. 2015), which provides additional plant identification and classification characters to solve differences in problematic groups (Okechukwu et al. 2021); solve complicated taxonomic interrelationships (Saunders et al. 2018).

Several studies on pollen morphology, such as *Neo-uvaria* Airy Shaw, showed inaperturate, monads with scabrate or micro-echinate exine ornamentations (Chaowasku et al. 2011); the pollen grains of some Thai *Artabotrys* R.Br. were described as monad pollen, inaperturate, apolar, and medium to large grains. The pollen shapes were divided into two groups, subprolate & euprolate, and different exine ornamentations with rugulate & perforate-fossulate exine sculptures (Eiadhong & Insura 2014); species from China have small, medium-sized, and large to very large pollen grains, elliptic, subspherical in monads for *Artabotrys*, *Fissistigma* (Merr.) Steenis, *Miliusa* Lesch. ex A.DC., *Trivalvaria* (Miq.) Miq., *Uvaria* L., and *Polyalthia* Blume, and tetrads for *Annona* L., *Goniothalamus* Hook.f. & Thomson, *Mitrephora* Hook.f. & Thomson, and *Polyalthia rumphii* (Blume ex Hensch.) Chaowasku (Gan et al. 2015); three species of *Annona* L. have been studied, namely *Annona squamosa* L. and *Annona senegalensis* L. were tetragonal with a globose shape, rugulate exine ornamentation, inner structure, and *Annona muricata* L. was rhomboidal with an ellipsoidal shape, reticulate exine ornamentation (Okechukwu et al. 2021). The pollen morphology studies reaffirm the great diversity among and within genera in Annonaceae (Shao & Xu 2017).

While pollen morphology of Annonaceae species from America and Africa has been studied well (Couvreur et al. 2008; Turner 2011; Azeez & Folorunso 2014; Shao & Xu 2018), research in the Philippines remains limited. The Philippines has 33 genera and 147 species recognized, and 97 are endemic (Pelser et al. 2011 onwards). For the diversity of Annonaceae species, floral inventories and collections are deemed necessary to study their pollen morphology, which would supplement the identification and classification of the Annonaceae. Pollen studies of the Philippine Annonaceae need to be investigated well, especially among the endemic species. The present study was the first attempt to study pollen morphology on the 12 species of Annonaceae collected from the Bicol Region, Philippines, utilizing a scanning electron microscope (SEM); thus, it is important to provide detailed descriptions and present a better understanding of pollen diversity.

MATERIALS AND METHODS

Plant collection

The flowers of Annonaceae were collected during the explorational surveys for Annonaceae in the four protected areas (PAs) in the Bicol Region, Philippines, during the flowering month of July in 2019 and 2021. The PAs include Abasig-Matogdon-Mananap Natural Biotic Area (AMMNBA) covering Mt. Mananap in San Vicente, Mt. Matogdon in San Lorenzo Ruiz, and Labo, Camarines Norte; Bulusan Volcano Natural Park (BVNP), Sorsogon; Mt. Isarog Natural Park (MINP) in Panicuason, Naga City, Camarines Sur, and Mt. Mayon Volcano Natural Park (MMVNP) in Barangay Mayon, Albay (Image 1). Gratuitous Permits were secured from the Department of Environment and Natural Resources (DENR) Region V.

SEM preparation of pollen grains

The flowers of the Annonaceae species collected from the Bicol Region (Dioneda & Alejandro 2022, 2023) and pollen grains were placed in microtubes with labels and stored in the refrigerator at 50 °C. The present study used the acetolyzation procedure by Halbritter et al. (2018a). The pollen grains were placed in a small test tube with a mixture of nine parts acetic anhydride and one part concentrated sulfuric acid and heated for four minutes at 100 °C. The acetolyzed mixtures were placed in a water bath while heating. After heating, the liquid was decanted, and the residues were washed with acetic

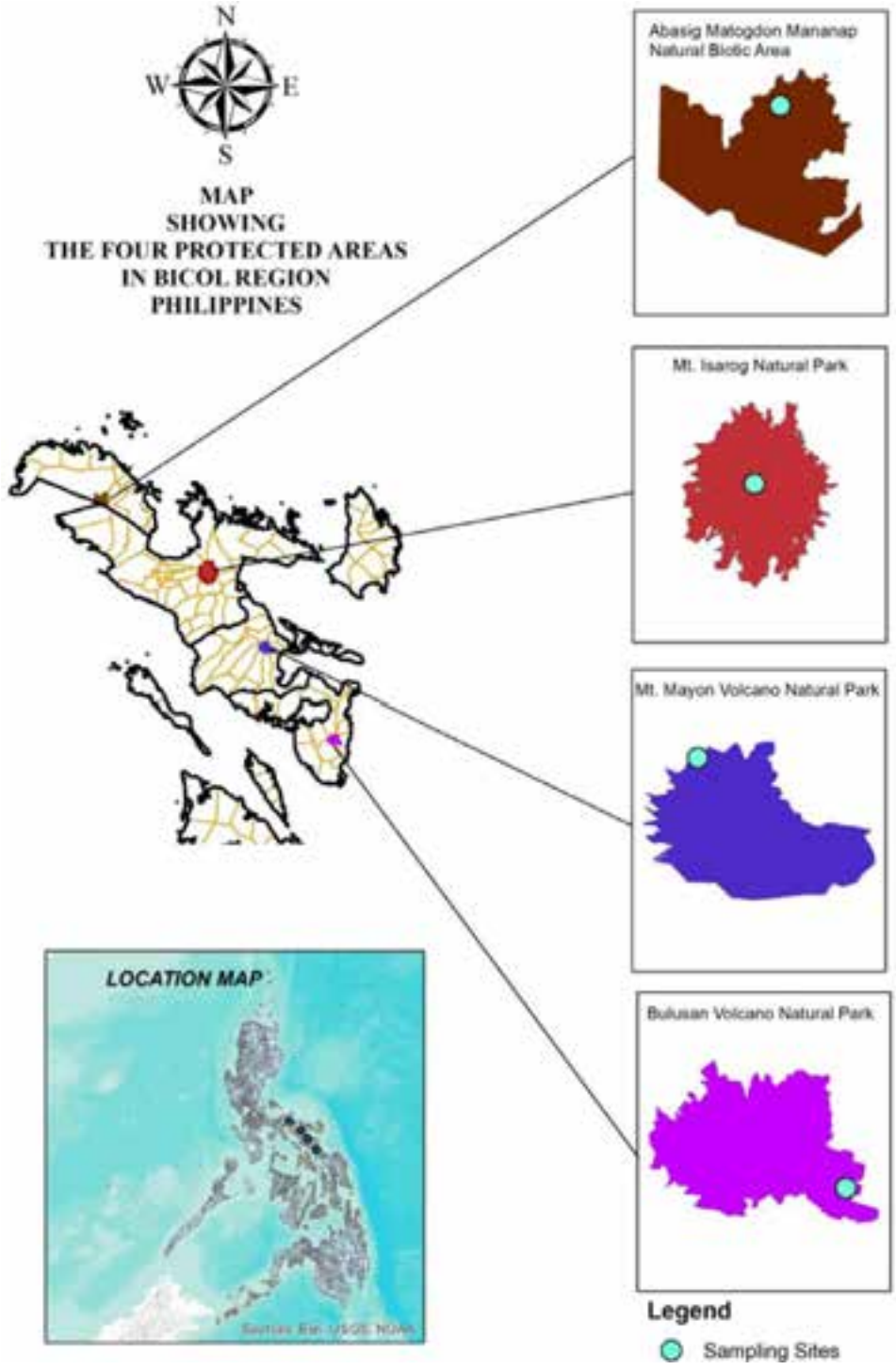

Image 1. The map shows the study site, the Bicol Region, and the four PAs. The collection sites were marked in green circles inside the PAs of AMMNBA (brown), MINP (red), MMVNP (blue), and BVNP (purple). The map was prepared using ARC-GIS (DENR-Region V 2023).

acid three times. Then, more water was added to wash further. The mixture was filtered, and the pollen grains were air-dried. The air-dried pollens were placed on the stub with carbon tape and inside the SEM chamber (HITACHI TM 3000). For each species, 20 pollen grains were measured, and intricate images of pollen were observed using the higher resolution of SEM (2000–3000 magnification or more). The SEM observation was held at the Analytical Services Laboratory, Research Center for the Natural and Applied Sciences (RCNAS), University

Table 1. Pollen shape classes and suggested relationships between polar axis (PA) and equatorial diameter (ED) (Erdtman 1952).

Pollen shape classes	PA / ED	100 x PA / ED
Peroblate	<4/8	< 50
Oblate	4/–6/8	50–75
Suboblate	6/8–7/8	75–88
Oblate spheroidal	7/8–8/8	88–100
Prolate spheroidal	8/8–8/7	100–114
Subprolate	8/7–8/6	114–133
Prolate	8/6–8/4	133–200
Perprolate	>8/4	>200

of Santo Tomas, España, Manila. Photomicrographs of the examined pollen grains were taken for further identification.

Pollen descriptions and measurements

The pollen characters used to describe the pollen grains were pollen shapes, sizes, exine sculpture or ornamentations, distribution, and apertures (El-Amier 2015; Halbritter et al. 2018b). Punt et al. (2007) and Halbritter et al. (2018c) were used for pollen descriptions and terminology. Pollen shape and size terminology followed Erdtman (1952).

The polar axis (PA) and equatorial diameter (ED) were measured. The equatorial measurement of the pollen grains was done by measuring the grain from one side of the equator to the opposite side. Polar measurement was done by measuring one pole to the other to indicate pollen shape accurately. The mean value of both axes was computed. The shape of the pollen was determined by the ratio of PA/ED (Table 1). The PA and ED were subjected to statistical analysis using IBM SPSS software v. 3, and Hierarchical cluster analysis was used to cluster Annonaceae species with similar characters and generate the dendrogram to visually represent the relationships between characters.

RESULTS AND DISCUSSION

General description of pollen grains of Annonaceae species from the Bicol Region

The pollen morphology of Annonaceae from the Bicol Region was notably varied. Mostly, the pollen grains were monads, a few were dyads, and tetrads. The pollen sizes ranged from small (10–30 µm), medium-sized (30–50 µm), and large to very large (50–100 µm). The polar axis (PA) value ranges 36.86 ± 15.86 µm, and the equatorial diameter (ED) value ranges 28.27 ± 14.27 µm. The total mean value of the polar axis is 35.27 µm, the total mean value of the equatorial axis is 27.22 µm, and the total mean PA/ED ratio is 1.32, with a generally sub-prolate shape. The exine ornamentations also varied in echinate, rugulate, scabrate, psilate with micro-perforations, and verrucate. Detailed morphometry is presented in Tables 2 & 3.

Species-level pollen descriptions

Tribe Miliuseae

Meiogyne cylindrocarpa (Burck) Heusden, 1994 (Image 2)

Pollen unit: monad, pollen size: small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: sub-prolate, aperture: inaperturate, PA mean value: 30.94 ± 4.20 µm with ED mean value 25.37±3.38 µm, PA/ED ratio: 1.22, ornamentation: rugulate.

Monoon grandifolium (Elmer) B.Xue & R.M.K.Saunders, 2012 (Images 2 & 3)

Pollen unit: monad, small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: sub-prolate, aperture: inaperturate, PA mean value 35.47 ± 9.33 µm, ED mean value 28.27 ± 6.73 µm, PA/ED ratio 1.32, ornamentation: granular-rugulate.

Phaeanthus ophthalmicus (Roxb. ex G.Don) J. Sinclair, 1955 (Images 2&3)

Pollen unit: monad, pollen size: small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: prolate, aperture: inaperturate, PA mean value 34.71 ± 8.57 µm, ED mean value 24.64 ± 5.43 µm, PA/ED ratio 1.41, ornamentation: scabrate.

Polyalthia obliqua Hook.f. & Thomson, 1855 (Images 2&3)

Pollen unit: monad, pollen size: small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: prolate, aperture: inaperturate, PA mean value 27.46±5.92 µm, ED mean value: 20.41±6.11 µm, PA/ED ratio 1.39, ornamentation: verrucate.

Popowia pisocarpa (Blume) Endl. ex. Walp., 1842 (Image 2)

Pollen unit: monad, pollen size: small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: sub-prolate, PA mean value 31.85 ± 10.58 µm, ED mean value 27.17 ± 12.41 µm, PA/ED ratio 1.17, ornamentation: scabrate.

Tribe Uvarieae

Fissistigma latifolium (Dunal) Merr., 1919 (Image 2)

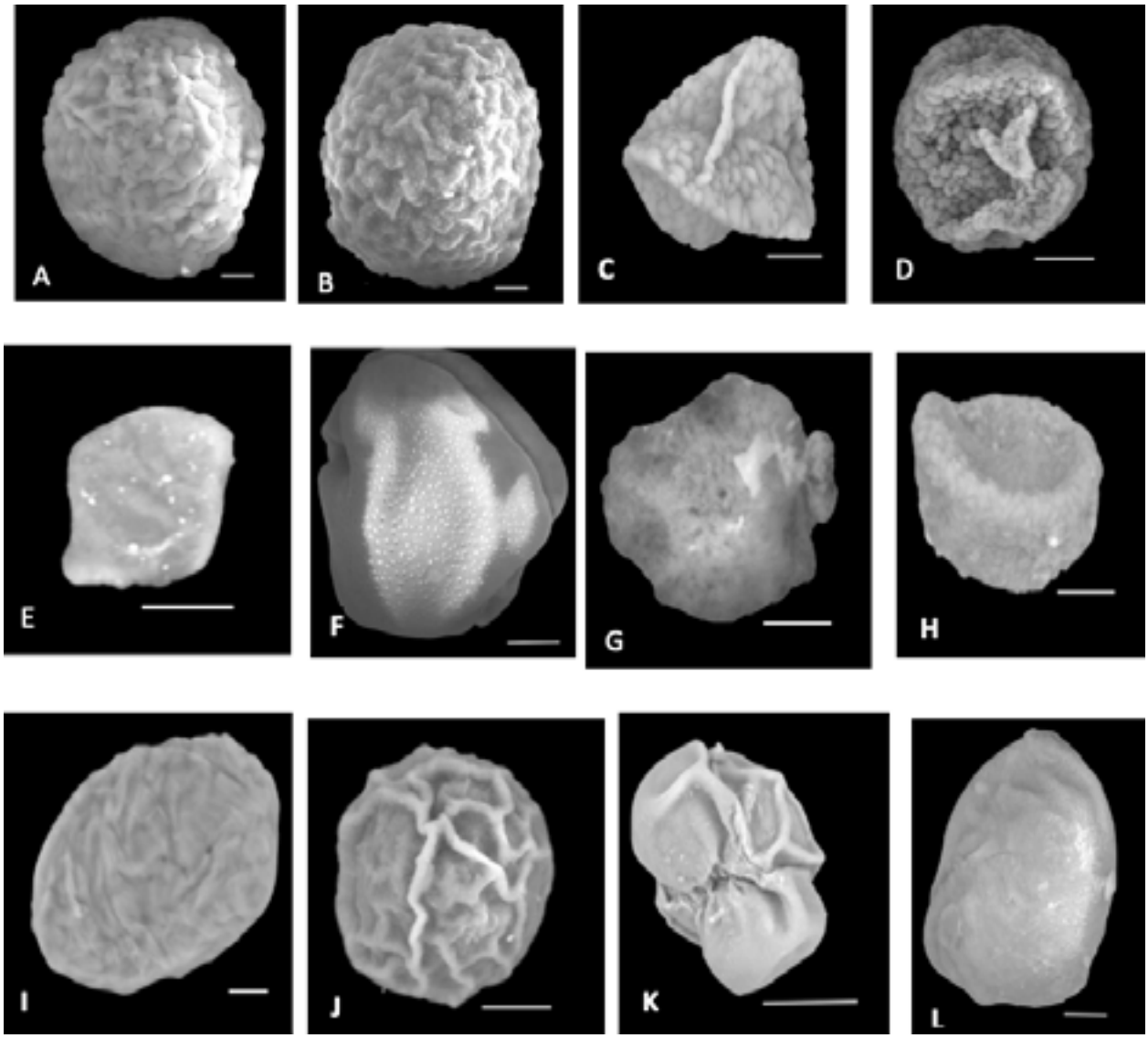

Image 2. SEM views of twelve pollen grains: A—*Meiogyne cylindrocarpa* | B—*Monoon grandifolium* | C—*Phaeanthus ophthalmicus* | D—*Polyalthia obliqua* | E—*Popowia pisocarpa* | F—*Cananga odorata* | G—*Drepananthus acuminatus* | H—*Fissistigma latifolium* | I—*Friesodielsia lanceolata* | J—*Uvaria monticola* | K—*Goniothalamus elmeri* | L—*Artabotrys suaveolens*. © Retuerma-Dioneda. Scale bar: K 100 µm; F 50 µm; C, D, E, G, H, I, J, L 30 µm; A, B 20 µm.

Pollen unit: monad, pollen size: small to medium-sized (10–30 µm), polarity: isopolar, pollen shape: prolate; aperture: inaperturate, PA mean value 24.47±5.39 µm, ED mean value 18.15 ± 5.10 µm, PA/ED ratio 1.35, ornamentation: verrucate.

Friesodielsia lanceolata (Merr.) Steenis, 1964 (Image 2)

Pollen unit: monad, pollen size: small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: prolate, aperture: inaperturate, PA mean value 33.55±7.70 µm, ED mean value 24.10 ± 7.82 µm, PA/ED ratio 1.39, ornamentation: echinate.

Uvaria monticola Miq., 1865 (Images 2 & 3)

Pollen unit: monad, pollen size: medium to large (30–50 µm), polarity: isopolar, pollen shape: prolate, aperture: inaperturate, PA mean value 32.20 ± 2.46 µm, ED mean value 28.81 ± 2.51 µm, PA/ED mean value 1.12, ornamentation: coarsely rugulate.

Tribe Canangeae

Cananga odorata (Lam.) Hook.f. & Thomson, 1855 (Images 2)

Pollen unit: dyad, pollen size: large to very large-sized (50–100 µm), polarity: isopolar, pollen shape: sub-prolate, apertures: inaperturate; PA value 73.14 ± 14.91

Table 2. SEM pollen morphometry of the 12 Annonaceae species from the Bicol Region, Philippines (Erdtman 1952).

Name of species	PA value	ED value	PA/ED ratio
Tribe Miliusae			
Meiogyne cylindrocarpa (Burck) Heusden	30.94 ± 4.20	25.37 ± 3.38	1.22
Monoon grandifolium (Elmer) B.Xue & R.M.K.Saunders	35.47 ± 9.33*	26.84 ± 6.73*	1.32
Phaeanthus ophthalmicus (Roxb. ex G.Don) J.Sinclair	34.71 ± 8.57*	24.64 ± 5.43*	1.41
Polyalthia obliqua Hook.f. & Thomson	27.46 ± 5.92	20.40 ± 6.11	1.35
Popowia pisocarpa (Blume) Endl. ex. Walp.	31.85 ± 10.58*	27.17 ± 12.41*	1.17
Tribe Uvarieae			
Fissistigma latifolium (Dunal) Merr.	24.47 ± 5.33	18.15 ± 5.10	1.35
Friesodielsia lanceolata (Merr.) Steenis	35.55 ± 7.70	24.1 ± 7.82	1.39
Uvaria monticola Miq.	32.20 ± 2.46	28.81 ± 2.51	1.12
Tribe Canangeae			
Cananga odorata (Lam.) Hook.f. & Thomson	73.14 ± 14.91*	61.05 ± 15.75*	1.20
Drepananthus acuminatus (C.B.Rob.) Survesw & R.M.K.Saunders	29.40 ± 8.72*	19.31 ± 6.27*	1.52
Tribe Annoneae			
Goniothalamus elmeri Merr.	33.62 ± 10.95*	24.28 ± 7.51*	1.38
Tribe Xylopiaeae			
Artabotrys suaveolens (Blume) Blume	34.71 ± 8.57*	23.43 ± 8.27*	1.48
Mean values	36.41 ± 15.86*	28.27 ± 14.27*	1.32**

*—significant | **—not significant.

µm, ED mean value 61.05 ± 15.75 µm, PA/ED ratio 1.20, ornamentation: psilate with microperforations.

Drepananthus acuminatus (C.B.Rob.) Survesw. & R.M.K. Saunders, 2010 (Image 2)

Pollen unit: monad, pollen size: small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: prolate, aperture: aperturate; PA mean value 29.40±8.72 µm, ED mean value 19.31±6.27 µm, P/E ratio 1.52, ornamentation: scabrate

Tribe Annoneae

Goniothalamus elmeri Merr., 1912 (Image 2)

Pollen unit: tetrad, pollen size: small to medium-sized (20–100 µm), polarity: isopolar, pollen shape: prolate, aperture: inaperturate, PA mean value 33.62 ± 10.95 µm, ED mean value 24.28 ± 7.51 µm, PA/ED ratio 1.38, ornamentation: coarsely rugulate.

Tribe Xylopieae

Artabotrys suaveolens (Blume) Blume, 1830 (Image 2)

Pollen unit: monad, size (pollen unit): small to medium-sized (20–30 µm), polarity: isopolar, pollen shape: prolate, apertures: inaperturate; PA mean value 34.71 ± 8.57 µm, ED mean value 23.43 ± 8.27 µm, PA/ED ratio 1.48, ornamentations rugulate.

DISCUSSION

The pollen morphology of basal angiosperms like the Annonaceae is highly diverse (Lu et al. 2015). This diversity of pollen types gained interest because it provides insights into the evolutionary history. Annonaceae, one of the most prominent primitive families, produces a variety of pollen types, including monads, dyads, tetrads, and polyads composed of eight, 16, or 32 grains (Walker 1971). The variety of pollen grains in Annonaceae in terms of size, aperture number, shape, and exine ornamentations was observed in many studies (Eiadthong & Insura 2014; Shao & Xu 2018). According to Lora et al. (2014), pollen production in monads is plesiomorphic in angiosperms, but the aggregation into tetrads has arisen independently at different times during the evolution of flowering plants. Aggregated forms offer advantages in situations involving infrequent pollinators, short pollen viability, pollen transfer periods, and protection from desiccation. Furthermore, aggregated pollen is considered an advanced character in Annonaceae, surpassing the traditional monad form

Table 3. Pollen morphology of the 12 species of Annonaceae pollen grains from Bicol Region, Philippines (Punt et al. 2007; Halbritter et al. 2018b).

Name of species	Pollen size (µm)	Pollen size	Pollen shape	Pollen unit	Pollen aperture	Exine ornamentation
Tribe Miliusae						
Meiogyne cylindrocarpa (Burck) Heusden	20–30	Small–medium	Sub-prolate	Monad	Inaperturate	Rugulate
Monoon grandifolium (Elmer) B.Xue & R.M.K.Saunders	20–30	Small–medium	Sub-prolate	Monad	Inaperturate	Rugulate
Phaeanthus ophthalmicus (Roxb. ex G.Don) J. Sinclair	20–30	Small–medium	Prolate	Monad	Inaperturate	Scabrate
Polyalthia obliqua Hook.f. & Thomson	20–30	Small–medium	Prolate	Monad	Inaperturate	Verrucate
Popowia pisocarpa (Blume) Endl. ex. Walp	20–30	Small–medium	Sub-Prolate	Monad	Inaperturate	Scabrate
Tribe Uvarieae						
Fissistigma latifolium (Dunal) Merr.	10–30	Small–medium	Prolate	Monad	Inaperturate	Verrucate
Friesodielsia lanceolata (Merr.) Steenis	20–30	Small–medium	Prolate	Monad	Inaperturate	Echinate
Uvaria monticola Miq.	30–50	Medium–large	Prolate-Spheroidal	Monad	Inaperturate	Rugulate
Tribe Canangeae						
Cananga odorata (Lam.) Hook.f. & Thomson	50–100	Medium–very large	Sub-Prolate	Dyad	Aperturate	Psilate-microperforation
Drepananthus acuminatus (C.B. Rob.) Survesw & R.M.K.Saunders	20–30	Small–medium	Prolate	Monad	Aperturate	Scabrate
Tribe Annoneae						
Goniothalamus elmeri Merr.	20–100	Medium–very large	Prolate	Tetrad	Inaperturate	Rugulate
Tribe Xylopiaeae						
Artabotrys suaveolens (Blume) Blume	20–30	Small–medium	Prolate	Monad	Inaperturate	Rugulate

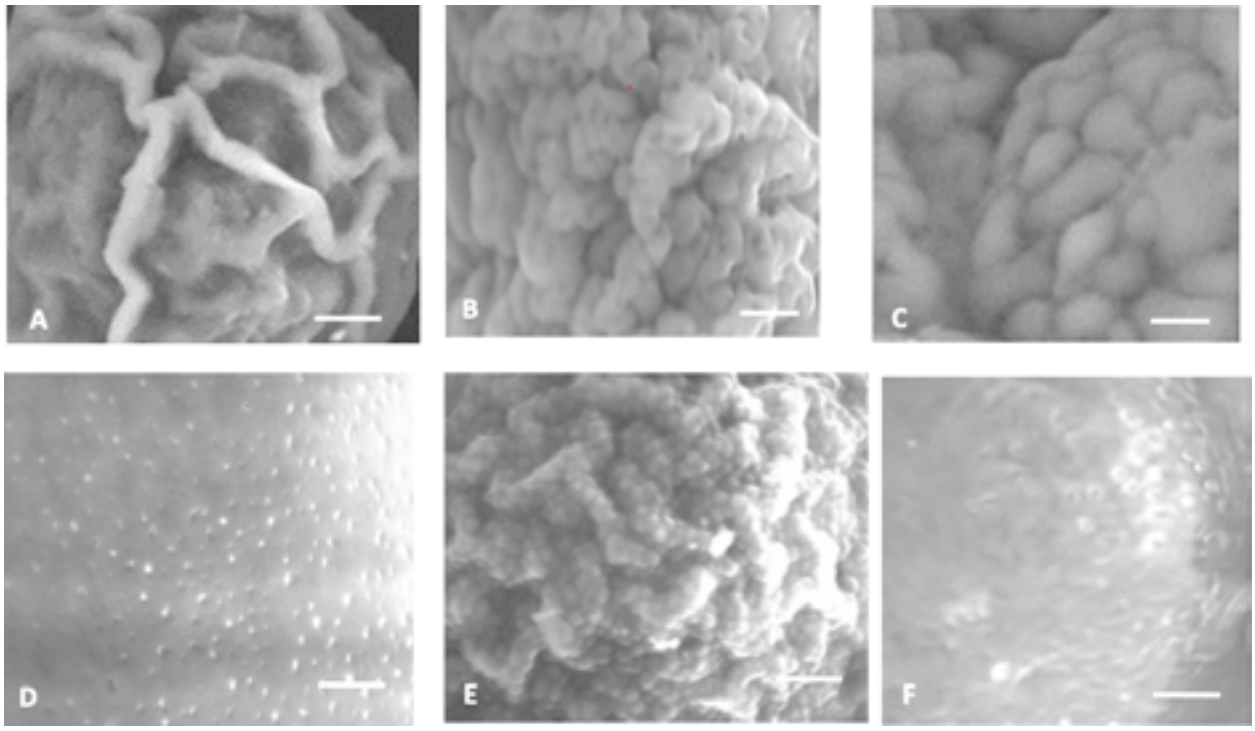

Image 3. The exine ornamentation: A—*Uvaria monticola*, rugulate | B—*Polyalthia obliqua*, verrucate | C—*Phaeanthus ophthalmicus*, scabrate | D—*Cananga odorata*, psilate- microperforations | E—*Monoon grandifolium*, granular rugulate | F—*Friesodielsia lanceolata*, echinate. © Retuerma-Dioneda. Scale bar: 6 µm.

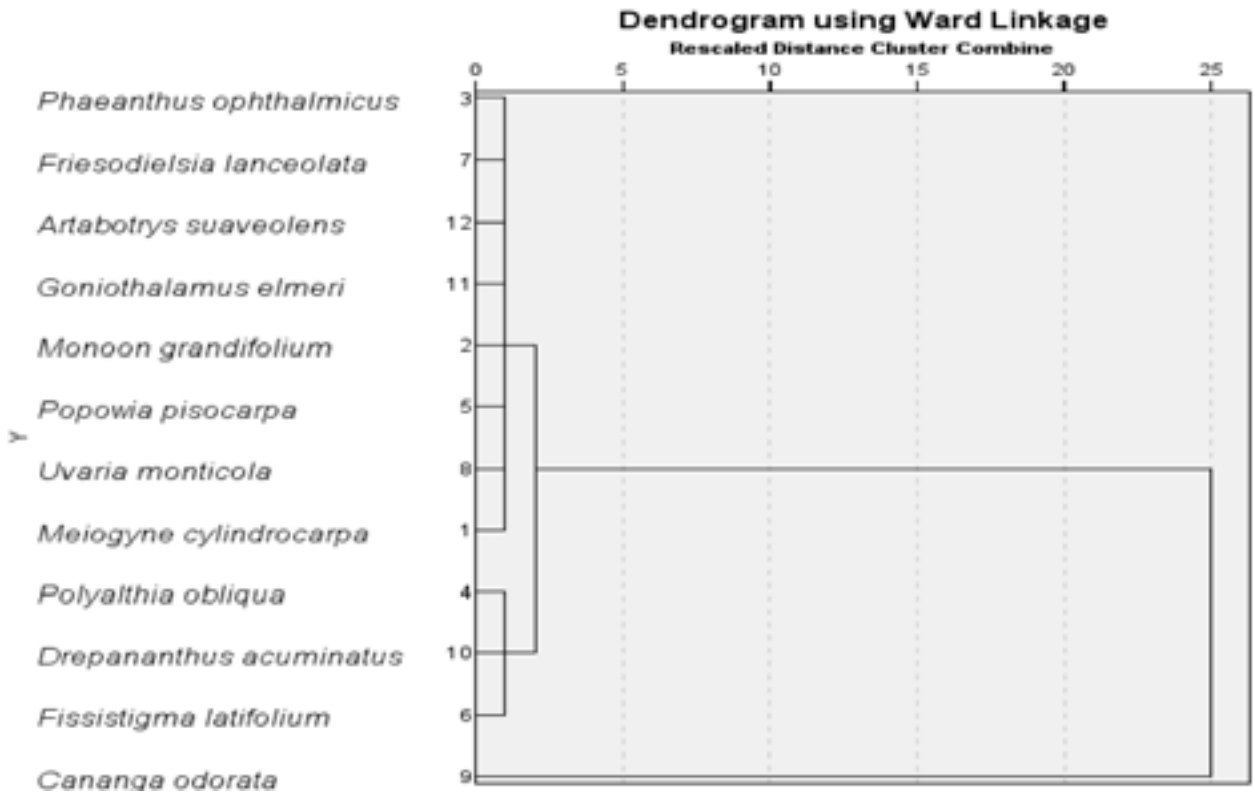

Figure 1. Dendrogram derived from hierarchical clustering of the pollen samples using Ward's method.

(Azeez & Folorunso 2014).

The 12 species of Annonaceae have shared pollen characteristics like single pollen grains, monads, and inaperturate pollen, except dyads for *Cananga odorata* and tetrad for *Goniothalamus elmeri*. The present study confirms that the genus *Cananga* is often observed as a dyad; although some authors reported that some pollen grains of *Cananga* were possibly in tetrads, the links between pollen pairs might have been loose within the tetrad and resulted in dyad pollen grains (Walker 1971; Xu & de Craene 2012; Li et al. 2023). On the other hand, pollen grains of *Goniothalamus elmeri* were tetrahedral tetrads, and different types were seen as tetragonal in *G. sawtehii* C.E.C.Fisch, *G. tamirensis* Pierre ex Finet & Gagnep., and *G. undulatus* Ridl. at the same time, *G. wynadensis* (Bedd.) Bedd. has two types of decussate and tetragonal tetrads (Jayan & Sreekala 2023).

In terms of the hierarchical cluster analysis, the pollen grains from the Bicol Region formed three clusters (Figure 1). The species *Cananga odorata* belongs to cluster I; it has dyad pollen grains, with the most significant variation in the PA and ED, pollen size of 50–100 µm, and psilate with micro-perforation exine ornamentations. In cluster II, eight species share characters of pollen grains as monads, with sizes ranging from small to medium (20–30 µm), namely, *Artabotrys suaveolens* (Blume) Blume, *Friesodielsia lanceolata* (Merr.) Steenis, *Goniothalamus elmeri* Merr., *Phaeanthus ophthalmicus* (Roxb. ex G.Don) J. Sinclair, *Meiogyne cylindrocarpa* (Burck) Heusden, *Monoon grandifolium* (Elmer) B.Xue & R.M.K.Saunders, *Popowia pisocarpa* (Blume) Endl. Ex Walp., and *Uvaria monticola* Miq. The species *Goniothalamus elmeri* was found nearer the cluster's centre with prolate shapes, while the *Uvaria monticola* was found farther from the cluster centre with prolate-spheroidal shapes. On the other hand, in cluster III, three species, namely *Drepananthus acuminatus* (C.B.Rob.) Survesw. & R.M.K.Saunders, *Fissistigma latifolium* (Dunal) Merr., and *Polyalthia obliqua* Hook.f. & Thomson shares pollen characters of monads, inaperturate, and prolate, but differs in the sizes of the PA, ED, and exine ornamentations.

Pollen sizes greatly vary among the species, from small to very large grains. The PA and ED are statistically significant due to a wide range of variability (Table 2). The species *Fissistigma latifolium* has smaller pollen sizes from small to medium (10–20 µm) and medium to large (30–50 µm) in *Uvaria monticola* and *Cananga*

odorata, while very large as tetrahedral-type tetrad (100 µm) but as monads (20 µm) for *Goniothalamus elmeri*. The variability in pollen size of Annonaceae is highly homoplastic and influenced by the preparation method, which appears to be shrunken using SEM rather than in the light microscope (Halbritter et al. 2018b). Although grain size seems unstable, it plays a role in the systematics (Lee 1984). According to Ejsmond et al. (2011), the desiccation intensity of pollen grains may decrease the pollen sizes due to climatic factors such as temperature, potential evapotranspiration, and altitude, which may significantly affect pollen grains' sizes. Environmental stresses like heat, drought, cold, and humidity affect pollen production & viability. During anthesis, the pollen grains of some plants enter a metabolically "inactive state" to support survival during pollen dispersal. The pollen grains lose water and reach a state of complete or partial desiccation tolerance, depending on environmental conditions (Pacini & Dolferus 2019). Furthermore, pollen size is also affected by the mineral content of the soil, shoot defoliation, and climate change. Higher concentrations of soil nitrogen and phosphorus have been reported to increase pollen grains' size, yield, and germinability (Lau & Stephenson 1994).

The prominent pollen shapes were prolate, sub-prolate, and prolate-spheroidal (Table 3). The prolate shape (1.34–1.99) was observed in the six species, namely *Artabotrys suaveolens*, *Drepananthus acuminatus*, *Fissistigma latifolium*, *Friesodielsia lanceolata*, *Goniothalamus elmeri*, *Phaeanthus ophthalmicus*, and *Polyalthia obliqua*, while sub-prolate shape (1.15–1.33) was observed in *Cananga odorata*, *Meiogyne cylindrocarpa*, *Monoon grandifolium*, *Popowia pisocarpa*, and prolate-spheroidal shape (1.01–1.14) was found only in *Uvaria monticola*. *Friesodielsia desmoides* (Craib) Steenis has spheroidal shape compared to *F. lanceolata* with prolate, and *Artabotrys hexapetalus* (L.f.) Bhandari has perprolate shape compared to *Artabotrys suaveolens* with a prolate shape. The PA/ED ratio was not statistically significant because it showed no variability among the species. Moreover, most of the 12 pollen grains are isopolar, with identical proximal and distal poles. Most species are inaperturate with no visible aperture or indication of a pole, except for *Drepananthus acuminatus*, which has a small opening (Image 2). This small opening is a disulcate aperture*, as* confirmed by Xu & de Craene (2012). Hence, pollen aperture is a significant criterion for identifying and describing pollen (Waha & Merawetz 1988). Nevertheless, recognizing apertures' position, shape, and nature is often problematic. The pollen grain is surrounded by a resistant wall called exine; certain regions of the pollen surface receive little or no exine deposition, leaving an aperture that serves as a site for pollen tube exit (Sarwar & Takahashi 2012; Zhang et al. 2017).

The pollen wall structure, exine, is one of the characteristics used for identification (Sari et al. 2015). Rugulate ornamentation was observed in the pollen grains of *Artabotrys suaveolens, Goniothalamus elmeri*, *Meiogyne cylindrocarpa*, *Monoon grandifolium*, and *Uvaria monticola*. The scabrate ornamentations were seen in *Phaeanthus ophthalmicus*, *Popowia pisocarpa, and Drepananthus acuminatus.* In addition*,* verrucate was observed in *Polyalthia obliqua* and *Fissistigma latifolium,* and the psilate with micro-perforation was found only in *Cananga odorata.* In the study of Shao & Xu (2017), *Goniothalamus laoticus* (Finet & Gagnap.) Bân has psilate ornamentations and *Polyalthia bullata* King rugulate with different exine ornamentations; *Artabotrys hexapetalus* has microrugulate and coarsely rugulate in *Fissistigma oldhamii* (Hemsl.) Merr. exine ornamentations (Xu & de Craene (2012) (Table 4). The above results corroborate the diverse exine ornamentations in Annonaceae species. Some studies also reaffirm the variety of exine ornamentations in some species such as the Asian genus of *Friesodielsia* which were heterogeneous (Walker 1971) with echinate-perforate with well-developed spines but differs from the African genus with coarsely verrucate pollen ornamentations, *Uvaria* with coarsely rugulate observed in *Uvaria grandiflora* (Lesch. Ex DC) and scabrate in *Uvaria macrocarpa* (Dunal)Vahl (Xu & de Craene 2012) and some species in the tribe Xylopieae with coarsely fossulate-perforate ornamentations (Shao & Xu 2017). Despite the differences among species, exine ornamentation patterns are one of the important characteristics of pollen, which is significant in the study of genetic evolution and systematic taxonomy. The ornamentation exine of pollen grains is highly conserved and genetically stable (Xu & de Craene 2012; Shao & Xu 2017).

Interestingly, the surface ornamentation of pollen grains correlates with pollination types by interacting with pollinators and how the pollen can efficiently be transferred from one flower to another (Sannier et al. 2009). The pollen ornamentation, such as spines and rough surfaces, can make pollen more sticky and less likely to fall off on the pollinator's body, allowing it to better adhere to the pollinators like insects or animals, increasing the number of flowers it can reach (Hasegawa et al. 2021). The identified exine ornamentations with complex patterns in the present study include some pollen grains with echinate, rugulate, scabrate, and

verrucate ornamentations, indicating that animals have been pollinating flowers of Annonaceae. The plants pollinated by animals develop complex patterns with various decorations on their pollen surface. The various spines, ridges, and papilla on their exine surface may help pollen grains attach to animal pollinators, influencing how pollen grains are dispersed and interact with the pollinators. This finding corroborates that the majority of pollinators of the family Annonaceae are beetles included in the family of Nitidulidae, Staphylinidae, Chrysomelidae, and Curculionidae and other insect groups like thrips, flies, bees, and cockroaches have also been identified as pollinators in some Annonaceae species such as *Popowia pisocarpa* and *Xylopia aromatica* (Lam.) Mart. (Gottsberger 1999; Momose et al. 2006; Lau et al. 2016).

Furthermore, the exine ornamentations can deter pollen consumption by flower visitors than the target pollinators (Lynn et al. 2020). In addition, the exine ornamentations facilitate the pollen-stigma interaction, pollen hydration, and release of pollen tubes for fertilization (Mach 2012). In contrast, the psilate with micro-perforations in *Cananga odorata* indicates that it was pollinated by wind or water, and a smooth surface may improve the aerodynamics of pollen. Hence, water is not a common method of pollination in Annonaceae (Lau et al. 2016).

Two endemic species were included in the study, namely, *Friesodielsia lanceolata* and *Goniothalamus elmeri,* and they were compared with the study of Xu & de Craene (2012) and Shao & Xu (2017) (Table 4). The species *Friesodielsia lanceolata* pollen grains are prolate monads, echinate, PA of 35.55 µm, and differ from *F. desmoides* pollen grains are spheroidal monads with no visible aperture, and echinate-perforate with well-developed spines and a mean PA of 29 µm, while *Goniothalamus elmeri* shares characteristics with *G. laoticus* of tetrahedral tetrad pollen, inaperturate and PA of ±100; however, they differ in exine ornamentations of rugulate and psilate, respectively. Although these species have the same genus, pollen sizes, shapes, and exine ornamentations differ.

CONCLUSION

The pollen morphology of the 12 Annonaceae studied exhibited high diversity, and they shared characters such as monad, inaperturate, and isopolar characters, but formed three clusters based on pollen size. Most have sub-prolate pollen shapes. They varied significantly

Table 4. Comparison of pollen characters of pollen grains from Bicol Region, Philippines with the pollen grains from Thailand (Xu & de Craene 2012; Shao & Xu 2017).

Pollen types	*Friesodielsia lanceolata**	*Friesodielsia desmoides*	*Goniothalamus elmeri**	*Goniothalamus laoticus*	*Polyalthia obliqua**	*Polyalthia bullata*	*Artabotrys suaveolens**	*Artabotrys hexapetalus*	*Fissistigma latifolium**	*Fissistigma oldhamii*
Pollen unit	monad	monad	tetrad	tetrad	monad	monad	monad	Monad with single furrow	monad	Monad
Pollen shape	prolate	spheroidal	Tetrahedral	tetrahedral	prolate	prolate	prolate	Perprolate	prolate	Prolate spheroidal
Pollen aperture	Inaperturate	Inaperturate	inaperturate	Inaperturate	Inaperturate	inaperturate	Inaperturate	Inaperture	Inaperturate	inaperturate
Pollen ornamentation	Microechinate	Echinate-perforate with spines	rugulate	psilate	verrucate	rugulate	rugulate	Microrugulate	verrucate	Coarsely rugulate
Polar axis (µn)	35.55	29	100	105	27.46	48	34.71	56	24.47	35

Legend: * present study

in PA & ED axis and size from small to very large, and exine ornamentations include echinate, rugulate, psilate with micro-perforations, scabrate, and verrucate. The present study was the first attempt to investigate 12 species of Annonaceae collected from the Bicol Region, Philippines. Pollen morphology of two endemic species is first reported here. Therefore, increasing the collection of endemic Philippine Annonaceae is recommended to search for new pollen characters that generate a comprehensive analysis of infrageneric relationships and family classifications.

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ARTICLE

Plant composition and species diversity in Delhi NCR of India

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Abstract: The present study provides a first major step in documenting and conserving the local plant diversity in Delhi NCR, which witnessed high species richness within a relatively small, localized area and harbors many high-value medicinal plants used in Ayurveda and other Indian traditional systems of medicine. The local hotspot areas that serve as a unique ecosystem for flora are being depleted day by day as a result of anthropogenic pressure. Hence, there is a need to conserve the species hotspots involving local communities who always interact with these ecosystems. The present study documents 272 plant species belonging to 204 genera and 69 families from the Delhi NCR of India. The aquatic and terrestrial vegetation in the study area was surveyed frequently throughout the year in different seasons, and plant collections were made. The majority of species documented were flowering plants, which comprised 216 dicots, 53 monocots, and three species belonged to lycophytes and fern groups. Fabaceae was the most dominant family with 39 plant species, followed by Poaceae (31 species), Asteraceae (25 species), Convolvulaceae (14 species), and Amaranthaceae and Malvaceae (12 species each). Genus *Ipomoea* is recorded to have the highest number of species (7), followed by *Euphorbia* and *Cyperus*, having five species each. Overall, the herbaceous community of species was greater in numbers than other life forms.

Keywords: Biodiversity documentation, diverse habitats, high-value herbs, local flora, rare plants, sustainable utilization, village panchayat, western Uttar Pradesh, wild plants.

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Author contributions: AKT carried out the field survey, compiled data, captured photographs and prepared the manuscript. JKS, as a principal investigator, mentored the whole research work, reviewed, and edited the manuscript. Both authors approved the final submitted manuscript.

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INTRODUCTION

Floristic surveys and subsequent documentation play an important role in the conservation of biodiversity (Sharma et al. 2018). Repeated surveys in any area in different seasons increase the chances of documenting rare and unique species, but the absence of knowledge about the species present and taxonomic identification of individual species makes it difficult to assess the richness of plant diversity.

Western Uttar Pradesh, within Delhi NCR (National Capital Region) has some demographic, economic, and cultural patterns that are distinct from other parts of Uttar Pradesh, and more closely resemble those of Haryana & Rajasthan states. A major part of western Uttar Pradesh falls within National Capital Region (NCR) of India and is experiencing rapid economic growth due to intensive agriculture and infrastructure development; hence, it is imperative to document and conserve the floristic wealth of these areas before the plant species are threatened and lost (Sharma et al. 2018).

Very few studies (Singh 1969; Vardhana 2007; Ahamed & Gupta 2010; Shishodia 2013; Malik 2015) are available supporting the floristic studies of western Uttar Pradesh, which come under Delhi NCR. The first major step in documenting and conserving the local plant diversity is the survey of the flora of that region. There is a need for proper inventory and documentation of all plants available in the western Uttar Pradesh NCR region, which will help formulate a much-needed strategy for conservation.

The present study has been carried out in Delhi NCR of western Uttar Pradesh. The study provides baseline information on floristic diversity with some unique, rare, and new species record that will help in updating the flora of India. The study also highlights many high-value medicinal herbs growing in the area. The present work is the first exhaustive study of its kind in Delhi NCR and is vital for the conservation of local flora and also for increasing awareness among local people.

MATERIAL AND METHODS

Study area

The study area is the Chithara Village Panchayat including the campus of Shiv Nadar University, Gautam Buddha Nagar District in western Uttar Pradesh (Figure 1); it is the eastern part of Delhi NCR. This region falls in the Gangetic plains, which are one of the most fertile regions of the country and equally rich in wild plant diversity. Geographically, Chithara lies between 28.516–28.55 °N and 77.55–77.58 °E, and is one of the 56 Panchayats under Dadri Block Panchayat/Tehsil and covers an area of 7.7 km^2. The village has a population of 7,656 of which 4,098 are males while 3,558 are females as per Population Census 2011. The village is divided into mohallas largely based on communities. Gujjar and Jatav communities are prominent in the village.

Chithara is a sub-urban area with a semi-arid climate. The climate is typically monsoonal with three distinct seasons, namely, summer (March–June), rainy (July–September), and winter (October–February). It experiences the hottest weather in June, with maximum temperature around 45^{0}C. The coldest month is January, with minimum temperature around 5^{0}C. August is usually the wettest month during the rainy season.

The soil is fertile alluvial, whose pH ranges between 7.64–9.38 with reasonably good maximum water holding capacity. Nutrient-wise the soil contained organic carbon (0.299–0.400%), which is within the desirable range of soils of upper Gangetic Plain. The village is surrounded by agricultural fields and several wetlands. The wild date palm tree, *Phoenix sylvestris* (L.) Roxb., is scattered as individual trees, in small groups, and in large groves. The village has an agriculture-based economy, mainly having wheat, and paddy cultivation.

Methods

The biodiversity documentation of Chithara Village Panchayat was carried out during 2015–2019. Field surveys covered different types of habitats such as agricultural fields, canal bunds, roadsides, village streets, wild date palm groves, wetlands, wastelands, grasslands (Sharma et al. 2018). The aquatic and terrestrial vegetation of the area was surveyed frequently throughout the year, covering different seasons. Photographs of each species were taken in the field for identification purposes. The plants were identified with the help of available floras (Duthie 1960; Maheshwari 1966; Sharma & Dhakre 1995; Vardhana 2007) and herbaria of the Botanical Survey of India, Northern Regional Office, Dehradun, and the Forest Research Institute, Dehradun. The specimens of identified plants were deposited in the BSI herbarium, Dehradun and CESE Department, Shiv Nadar University (SNU/CESE). The taxonomic identity of the plant specimens was verified from POWO (2024). The medicinal properties and medicinal uses of each plant species were studied, and among the list, 10 highly important medicinal plants were identified, which are known for their outstanding medicinal properties and are used in Ayurveda and

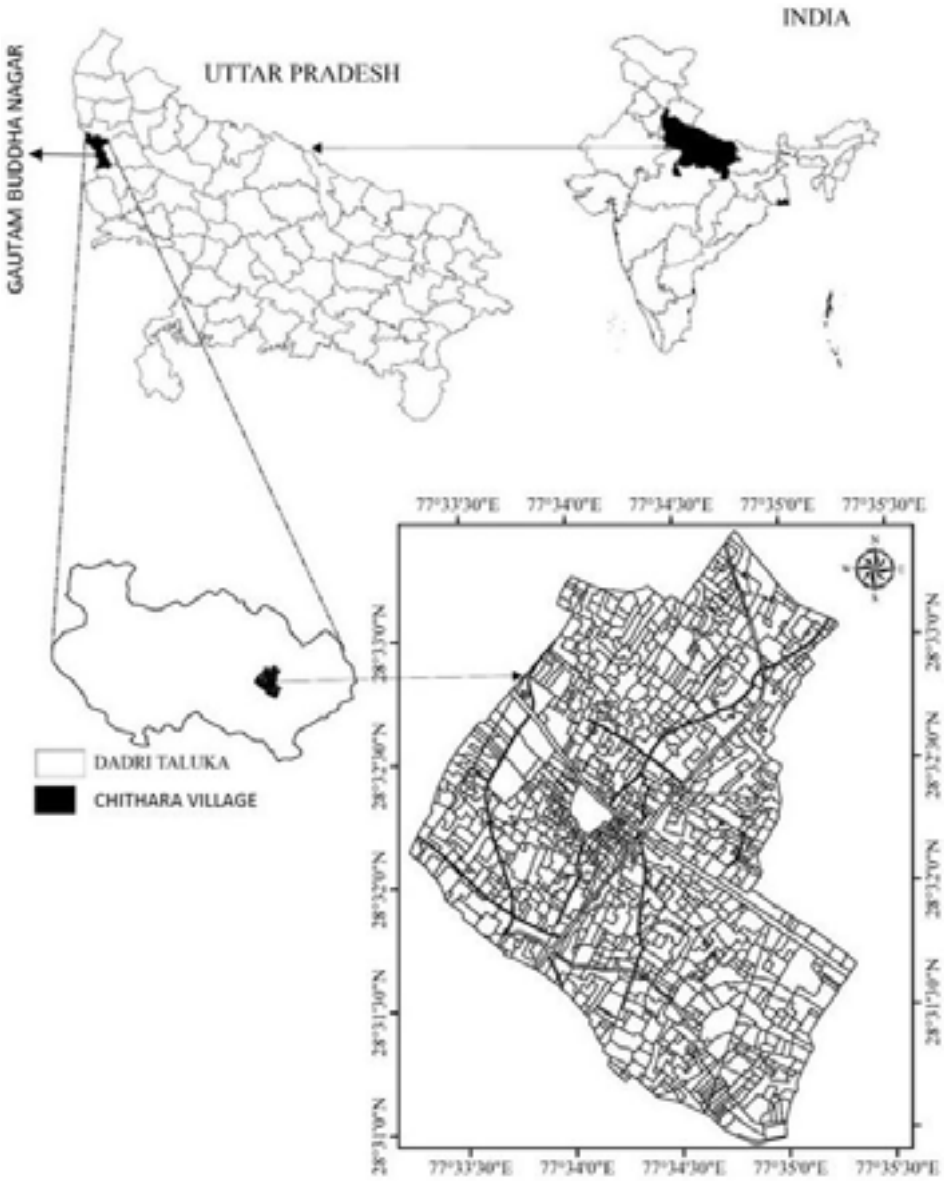

Figure 1. Location map of study area.

other traditional systems of medicine (Sharma et al. 2018). Plant species with very restricted distribution were considered locally rare.

RESULTS AND DISCUSSION

The present study documented a total of 272 plant species belonging to 204 genera and 69 families. The majority of species were flowering plants (269 plants: dicots—216 species, monocots—53 species) and only three species belonged to lycophytes and fern categories (Table 1).

In terms of percentage, 79% of species were dicotyledons, 20% were monocotyledons, and 1% were lycophytes & fern categories (Figure 2). There was a preponderance of tall grasses such as *Saccharum spontaneum* L., *Saccharum bengalense* Retz., and *Phragmites karka* (Retz.) Trin. ex Steud., particularly in the wetland areas, which is very typical of uncultivated lands in the region of Gangetic plains.

Previously published study reveals that the flora of Delhi had been extensively explored by Maheshwari (1963) who reported a total of 531 species from the region (Table 2). The present study reported 272 plant species (Table 1), which look-like small in number with respect to earlier work, but in terms of the geographic area, it is a very high proportion, which is only 7.7 km^2

Table 1. List of the plant species in Chithara Village Panchayat, Delhi NCR.

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
	Herbs			
1	*Achyranthes aspera* L.	Amaranthaceae	Chirchita, Latjira	SNU/CESE/188
2	*Acmella paniculata* (Wall. ex DC.) R.K.Jansen	Asteraceae	Toothache Plant	SNU/CESE/277
3	*Ageratum conyzoides* (L.) L.	Asteraceae	Billygoat-weed	SNU/CESE/073
4	*Ageratum houstonianum* Mill.	Asteraceae	Floss Flower	SNU/CESE/422
5	*Alhagi maurorum* Medik.	Fabaceae	Camelthorn-bush	SNU/CESE/435
6	*Alternanthera paronychioides* A.St.-Hil.	Amaranthaceae	Smooth Chaff Flower	SNU/CESE/392
7	*Alternanthera philoxeroides* (Mart.) Griseb.	Amaranthaceae	Alligator Weed	SNU/CESE/285
8	*Alternanthera pungens* (Kunth)	Amaranthaceae	Chaff-Flower	SNU/CESE/101
9	*Alternanthera sessilis* (L.) R.Br. ex DC.	Amaranthaceae	Sessile Joyweed	SNU/CESE/152
10	*Alysicarpus monilifer* (L.) DC.	Fabaceae	Necklace-pod Alyce Clover	SNU/CESE/130
11	*Alysicarpus vaginalis* (L.) DC.	Fabaceae	Alyce Clover	SNU/CESE/159
12	*Amaranthus viridis* L.	Amaranthaceae	Jangali Chaulai	SNU/CESE/002
13	*Ammannia baccifera* L.	Lythraceae	Blistering Ammannia	SNU/CESE/236
14	*Anisomeles indica* (L.) Kuntze	Lamiaceae	Kala Bhangra	SNU/CESE/251
15	*Arabidopsis thaliana* (L.) Heynh.	Brassicaceae	Thale Cress	SNU/CESE/316
16	*Arenaria serpyllifolia* L.	Caryophyllaceae	Thyme-leaved Sandwort	SNU/CESE/362
17	*Argemone mexicana* L.	Papaveraceae	Satyanashi	SNU/CESE/038
18	*Artemisia scoparia* Waldst. & Kitam.	Asteraceae	Redstem Wormwood	SNU/CESE/168
19	*Avena sterilis* L.	Poaceae	Jangali Jai	SNU/CESE/325
20	*Bacopa monnieri* (L.) Wettst.	Plantaginaceae	Water Hyssop (Brahmi)	SNU/CESE/220
21	*Barleria prionitis* L.	Acanthaceae	Vajradanti	SNU/CESE/300
22	*Bidens pilosa* L.	Asteraceae	Black-jack	SNU/CESE/071
23	*Blainvillea acmella* (L.) Philipson	Asteraceae	Para Cress Flower	SNU/CESE/208
24	*Blumea laciniata* (Wall. ex Roxb.) DC.	Asteraceae	Cutleaf Blumea	SNU/CESE/423
25	*Blumea obliqua* (L.) Druce	Asteraceae	Common Floss Flower	SNU/CESE/269
26	*Blumea viscosa* (Mill.) V.M.Badillo	Asteraceae	Sticky Blumea	SNU/CESE/427
27	*Boerhavia diffusa* L.	Nyctaginaceae	Punarnava	SNU/CESE/341
28	*Brachiaria ramosa* (L.) Stapf	Poaceae	Browntop Millet	SNU/CESE/190
29	*Brachiaria reptans* (L.) C.A.Gardner & C.E.Hubb.	Poaceae	Running Grass	SNU/CESE/085
30	*Caesulia axillaris* Roxb.	Asteraceae	Pink Node Flower	SNU/CESE/280
31	*Cannabis sativa* L.	Cannabaceae	Bhang	SNU/CESE/148
32	*Capsella bursa-pastoris* (L.) Medik.	Brassicaceae	Shepherd's Purse	SNU/CESE/317
33	*Cardamine hirsuta* L.	Brassicaceae	Hairy Bitter Cress	SNU/CESE/292
34	*Carthamus oxyacantha* M.Bieb.	Asteraceae	Wild Safflower	SNU/CESE/434
35	*Celosia argentea* L.	Amaranthaceae	Safed Murga	SNU/CESE/054
36	*Cenchrus ciliaris* L.	Poaceae	Buffel Grass	SNU/CESE/346
37	*Centaurium pulchellum* (Sw.) Druce	Gentianaceae	Pink Centaury	SNU/CESE/349
38	*Centella asiatica* (L.) Urb.	Apiaceae	Indian Pennywort (Brahmi)	SNU/CESE/383
39	*Ceratophyllum demersum* L.	Ceratophyllaceae	Coon's Tail	SNU/CESE/353
40	*Chenopodium album* L.	Amaranthaceae	Bathua	SNU/CESE/304
41	*Chenopodium murale* L.	Amaranthaceae	Jangali Bathua	SNU/CESE/416
42	*Chloris barbata* Sw.	Poaceae	Feather Finger Grass	SNU/CESE/439
43	*Chrysopogon zizanioides* (L.) Roberty	Poaceae	Khas-khas	SNU/CESE/386

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
44	*Cirsium arvense* (L.) Scop.	Asteraceae	Creeping Thistle	SNU/CESE/347
45	*Cleome viscosa* L.	Cleomaceae	Pili Hurhur	SNU/CESE/030
46	*Commelina benghalensis* L.	Commelinaceae	Kankawwa	SNU/CESE/231
47	*Commelina forskaolii* Vahl	Commelinaceae	Kankawwa	SNU/CESE/089
48	*Convolvulus prostratus* Forssk.	Convolvulaceae	Shankhpushpi	SNU/CESE/343
49	*Corchorus aestuans* L.	Malvaceae	Chonch	SNU/CESE/077
50	*Corchorus olitorius* L.	Malvaceae	Pat-Sag	SNU/CESE/384
51	*Corchorus tridens* L.	Malvaceae	Horn-fruited Jute	SNU/CESE/061
52	*Crotalaria medicaginea* Lam.	Fabaceae	Trefoil Rattlepod	SNU/CESE/045
53	*Crotalaria mysorensis* Roth	Fabaceae	Mysore Rattlepod	SNU/CESE/209
54	*Croton bonplandianus* Baill.	Euphorbiaceae	Ban Tulsi	SNU/CESE/005
55	*Cyanotis axillaris* (L.) D.Don ex Sweet	Commelinaceae	Kana	SNU/CESE/174
56	*Cyanthillium cinereum* (L.) H.Rob.	Asteraceae	Sahadevi	SNU/CESE/193
57	*Cynodon dactylon* (L.) Pers.	Poaceae	Doob	SNU/CESE/006
58	*Cyperus alopecuroides* Rottb.	Cyperaceae	Foxtail Flatsedge	SNU/CESE/118
59	*Cyperus compressus* L.	Cyperaceae	Mothi	SNU/CESE/387
60	*Cyperus difformis* L.	Cyperaceae	Small-Flowered Nutsedge	SNU/CESE/047
61	*Cyperus iria* L.	Cyperaceae	Rice Flatsedge	SNU/CESE/385
62	*Cyperus rotundus* L.	Cyperaceae	Motha	SNU/CESE/019
63	*Dactyloctenium aegyptium* (L.) Willd.	Poaceae	Makra Ghas	SNU/CESE/048
64	*Datura innoxia* Mill.	Solanaceae	Safed Dhatura	SNU/CESE/007
65	*Desmodium gangeticum* (L.) DC.	Fabaceae	Shalaparni	SNU/CESE/175
66	*Desmodium triflorum* (L.) DC.	Fabaceae	Tipatiya	SNU/CESE/218
67	*Desmostachya bipinnata* (L.) Stapf	Poaceae	Dabh, Kush	SNU/CESE/367
68	*Dichanthium annulatum* (Forssk.) Stapf	Poaceae	Marvel Grass	SNU/CESE/391
69	*Digera muricata* (L.) Mart.	Amaranthaceae	Lahsuva	SNU/CESE/368
70	*Digitaria ciliaris* (Retz.) Koeler	Poaceae	Wild Crabgrass	SNU/CESE/194
71	*Dysphania ambrosioides* (L.) Mosyakin & Clemants	Amaranthaceae	Sugandha Vastooka	SNU/CESE/096
72	*Echinochloa colona* (L.) Link	Poaceae	Shama	SNU/CESE/136
73	*Echinochloa crus-galli* (L.) P.Beauv.	Poaceae	Sanwak	SNU/CESE/371
74	*Eclipta prostrata* (L.) L.	Asteraceae	Bhringaraj	SNU/CESE/021
75	*Eichhornia crassipes* (Mart.) Solms	Pontederiaceae	Jal Kumbhi	SNU/CESE/232
76	*Eleusine indica* (L.) Gaertn.	Poaceae	Jangali Marua	SNU/CESE/112
77	*Eragrostis amabilis* (L.) Wight & Arn.	Poaceae	Bharbhusi	SNU/CESE/393
78	*Eragrostis japonica* (Thunb.) Trin.	Poaceae	Pond Lovegrass	SNU/CESE/242
79	*Erigeron bonariensis* L.	Asteraceae	Flax-leaf Fleabane	SNU/CESE/100
80	*Eriochloa procera* (Retz.) C.E.Hubb.	Poaceae	Tropical Cupgrass	SNU/CESE/401
81	*Euphorbia heterophylla* L.	Euphorbiaceae	Lesser Green Poinsettia	SNU/CESE/273
82	*Euphorbia hirta* L.	Euphorbiaceae	Bari Dudhi, Asthma Weed	SNU/CESE/051
83	*Euphorbia hypericifolia* L.	Euphorbiaceae	Dudh Mogra	SNU/CESE/196
84	*Euphorbia prostrata* Aiton	Euphorbiaceae	Choti Dudhi	SNU/CESE/008
85	*Euphorbia serpens* Kunth	Euphorbiaceae	Dudhi	SNU/CESE/004
86	*Evolvulus nummularius* (L.) L.	Convolvulaceae	Musakarni	SNU/CESE/394
87	*Fimbristylis dichotoma* (L.) Vahl	Cyperaceae	Tall Fringe Rush	SNU/CESE/124

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
88	*Fimbristylis ferruginea* (L.) Vahl	Cyperaceae	West Indian Fimbry	SNU/CESE/079
89	*Fumaria indica* (Hausskn.) Pugsley	Papaveraceae	Pittpapra	SNU/CESE/307
90	*Gnaphalium purpureum* L.	Asteraceae	Purple Cudweed	SNU/CESE/319
91	*Gomphrena celosioides* Mart.	Amaranthaceae	Prostrate Globe-amaranth	SNU/CESE/062
92	*Gonostegia pentandra* (Roxb.) Miq.	Urticaceae	Narrow-Leaf Pouzolz's Bush	SNU/CESE/097
93	*Grangea maderaspatana* (L.) Poir.	Asteraceae	Mustaru	SNU/CESE/358
94	*Heliotropium ellipticum* Ledeb.	Boraginaceae	Hathisund	SNU/CESE/010
95	*Hemarthria compressa* (L.f.) R.Br.	Poaceae	Whip Grass	SNU/CESE/125
96	*Hydrilla verticillata* (L.f.) Royle	Hydrocharitaceae	Hydrilla	SNU/CESE/354
97	*Hydrocotyle sibthorpioides* Lam.	Araliaceae	Lawn Pennywort	SNU/CESE/126
98	*Mesosphaerum suaveolens* (L.) Kuntze	Lamiaceae	Vilaiti Tulsi	SNU/CESE/249
99	*Imperata cylindrica* (L.) Raeusch.	Poaceae	Blady Grass	SNU/CESE/041
100	*Indigofera astragalina* DC.	Fabaceae	Silky Indigo	SNU/CESE/400
101	*Indigofera linifolia* (L.f.) Retz.	Fabaceae	Ratnamala	SNU/CESE/250
102	*Indigofera tinctoria* L.	Fabaceae	Neel	SNU/CESE/149
103	*Indigofera tsiangiana* Metcalf	Fabaceae	Birdsville Indigo	SNU/CESE/432
104	*Justicia japonica* Thunb.	Acanthaceae	Common Small Justicia	SNU/CESE/139
105	*Lathyrus aphaca* L.	Fabaceae	Jangali Matar	SNU/CESE/330
106	*Launaea procumbens* (Roxb.) Ramayya & Rajagopal	Asteraceae	Peeli Duddhi	SNU/CESE/011
107	*Lemna perpusilla* Torr.	Araceae	Minute Duckweed	SNU/CESE/411
108	*Lepidium didymum* L.	Brassicaceae	Jangali Hala	SNU/CESE/302
109	*Leptochloa panicea* (Retz.) Ohwi	Poaceae	Sprangletop	SNU/CESE/441
110	*Leucas cephalotes* (Roth) Spreng.	Lamiaceae	Guma	SNU/CESE/197
111	*Lindernia ciliata* (Colsm.) Pennell	Linderniaceae	Fringed False Pimpernel	SNU/CESE/086
112	*Lindernia crustacea* (L.) F.Muell.	Linderniaceae	Brittle False Pimpernel	SNU/CESE/087
113	*Ludwigia hyssopifolia* (G.Don) Exell	Onagraceae	Ban Long	SNU/CESE/198
114	*Ludwigia octovalvis* (Jacq.) P.H.Raven	Onagraceae	Ban Long	SNU/CESE/226
115	*Lysimachia loeflingii* F.J.Jiménez & M.Talavera	Primulaceae	Krishnaneel	SNU/CESE/106
116	*Malva parviflora* L.	Malvaceae	Panirak	SNU/CESE/320
117	*Malvastrum coromandelianum* (L.) Garcke	Malvaceae	Kharenti	SNU/CESE/018
118	*Mazus pumilus* (Burm.f.) Steenis	Phrymaceae	Japanese Mazus	SNU/CESE/023
119	*Mecardonia procumbens* (Mill.) Small	Plantaginaceae	Baby Jump Up	SNU/CESE/083
120	*Medicago monantha* (C.A.Mey.) Trautv.	Fabaceae	Single-Flowered Medick	SNU/CESE/335
121	*Medicago lupulina* L.	Fabaceae	Black Medic	SNU/CESE/294
122	*Medicago polymorpha* L.	Fabaceae	Toothed Medic	SNU/CESE/424
123	*Melilotus albus* Medik.	Fabaceae	Safed Ban Methi	SNU/CESE/337
124	*Melilotus indicus* (L.) All.	Fabaceae	Ban Methi	SNU/CESE/287
125	*Melochia corchorifolia* L.	Malvaceae	Chitrabeej	SNU/CESE/050
126	*Mollugo nudicaulis* Lam.	Molluginaceae	Naked-stem Carpetweed	SNU/CESE/164
127	*Murdannia nudiflora* (L.) Brenan	Commelinaceae	Kansura	SNU/CESE/177
128	*Nepeta hindostana* (B.Heyne ex Roth) Haines	Lamiaceae	Billi Lotan	SNU/CESE/200
129	*Nicotiana plumbaginifolia* Viv.	Solanaceae	Jangali Tambakoo	SNU/CESE/012
130	*Nymphaea pubescens* Willd.	Nymphaeaceae	Kumud	SNU/CESE/276
131	*Nymphoides cristata* (Roxb.) Kuntze	Menyanthaceae	Kumudini	SNU/CESE/275

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
132	*Oldenlandia corymbosa* L.	Rubiaceae	Daman Pappar	SNU/CESE/013
133	*Oxalis corniculata* L.	Oxalidaceae	Amrul, Khatti-buti	SNU/CESE/084
134	*Oxalis debilis* var. *corymbosa* (DC.) Lourteig	Oxalidaceae	Khatti-mithi	SNU/CESE/338
135	*Panicum antidotale* Retz.	Poaceae	Blue Panic Grass	SNU/CESE/405
136	*Parthenium hysterophorus* L.	Asteraceae	Gajar Ghas	SNU/CESE/014
137	*Paspalum distichum* L.	Poaceae	Knotgrass	SNU/CESE/440
138	*Paspalum scrobiculatum* L.	Poaceae	Kodo	SNU/CESE/063
139	*Dicliptera paniculata* (Forssk.) I.Darbysh.	Acanthaceae	Atrilal	SNU/CESE/267
140	*Persicaria barbata* (L.) H.Hara	Polygonaceae	Bearded Knotweed	SNU/CESE/398
141	*Persicaria lanigera* (R.Br.) Soják	Polygonaceae	Pink Knotweed	SNU/CESE/024
142	*Phalaris minor* Retz.	Poaceae	Mandusi	SNU/CESE/326
143	*Phragmites karka* (Retz.) Trin. ex Steud.	Poaceae	Narkul	SNU/CESE/406
144	*Phyla nodiflora* (L.) Greene	Verbenaceae	Jal Buti	SNU/CESE/070
145	*Phyllanthus amarus* Schumach. & Thonn.	Phyllanthaceae	Bhuiaonla	SNU/CESE/132
146	*Phyllanthus maderaspatensis* L.	Phyllanthaceae	Hajarmani	SNU/CESE/043
147	*Phyllanthus tenellus* Roxb.	Phyllanthaceae	Mascarene Island Leaf-flower	SNU/CESE/437
148	*Physalis angulata* L.	Solanaceae	Rasbhari	SNU/CESE/037
149	*Physalis peruviana* L.	Solanaceae	Rasbhari	SNU/CESE/410
150	*Pistia stratiotes* L.	Araceae	Water Lettuce	SNU/CESE/395
151	*Pluchea lanceolata* (DC.) C.B.Clarke	Asteraceae	Rasna	SNU/CESE/064
152	*Poa annua* L.	Poaceae	Annual Bluegrass	SNU/CESE/421
153	*Polygonum plebeium* R.Br.	Polygonaceae	Small Knotweed	SNU/CESE/308
154	*Portulaca oleracea* L.	Portulacaceae	Kulpha, Noni	SNU/CESE/351
155	*Portulaca quadrifida* L.	Portulacaceae	Chicken Weed	SNU/CESE/381
156	*Potamogeton crispus* L.	Potamogetonaceae	Curly-Leaf Pondweed	SNU/CESE/412
157	*Ranunculus sceleratus* L.	Ranunculaceae	Jaldhaniya	SNU/CESE/352
158	*Rorippa palustris* (L.) Besser	Brassicaceae	Chamsuru	SNU/CESE/315
159	*Rumex dentatus* L.	Polygonaceae	Jangali Palak	SNU/CESE/419
160	*Rumex hypogaeus* T.M.Schust. & Reveal	Polygonaceae	Three Corner Jack	SNU/CESE/314
161	*Rungia pectinata* (L.) Nees	Acanthaceae	Comb Rungia	SNU/CESE/413
162	*Saccharum bengalense* Retz.	Poaceae	Munj, Sarkanda	SNU/CESE/404
163	*Saccharum spontaneum* L.	Poaceae	Kaans	SNU/CESE/379
164	*Sagittaria guayanensis* Kunth	Alismataceae	Guyanese Arrowhead	SNU/CESE/199
165	*Scoparia dulcis* L.	Plantaginaceae	Ghoda Tulsi	SNU/CESE/001
166	*Senna obtusifolia* (L.) H.S.Irwin & Barneby	Fabaceae	Chakwar	SNU/CESE/147
167	*Senna occidentalis* (L.) Link	Fabaceae	Kasundi	SNU/CESE/016
168	*Sesamum indicum* L.	Pedaliaceae	Safed Til	SNU/CESE/184
169	*Sesbania bispinosa* (Jacq.) W.Wight	Fabaceae	Dhaincha	SNU/CESE/065
170	*Setaria pumila* (Poir.) Roem. & Schult.	Poaceae	Bandra	SNU/CESE/128
171	*Setaria verticillata* (L.) P.Beauv.	Poaceae	Laptuna	SNU/CESE/374
172	*Sida acuta* Burm.f.	Malvaceae	Baraira	SNU/CESE/068
173	*Sida cordata* (Burm.f.) Borss.Waalk.	Malvaceae	Kharenti	SNU/CESE/203
174	*Sida cordifolia* L.	Malvaceae	Bala	SNU/CESE/215
175	*Silene conoidea* L.	Caryophyllaceae	Cone Catchfly	SNU/CESE/310
176	*Sisymbrium irio* L.	Brassicaceae	Khubkalan	SNU/CESE/322

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
177	*Solanum americanum* Mill.	Solanaceae	Makoy	SNU/CESE/286
178	*Solanum surattense* Burm. f.	Solanaceae	Kateli	SNU/CESE/032
179	*Sonchus asper* (L.) Hill	Asteraceae	Dudhi	SNU/CESE/311
180	*Sonchus brachyotus* DC.	Asteraceae	Dudhali	SNU/CESE/117
181	*Sorghum halepense* (L.) Pers.	Poaceae	Jangali Jowar	SNU/CESE/205
182	*Spergula arvensis* L.	Caryophyllaceae	Ban Dhania	SNU/CESE/425
183	*Spermacoce articularis* L.f.	Rubiaceae	Guthari	SNU/CESE/058
184	*Sphenoclea zeylanica* Gaertn.	Sphenocleaceae	Goose Weed	SNU/CESE/204
185	*Spirodela polyrhiza* (L.) Schleid.	Araceae	Common Duck Meat	SNU/CESE/414
186	*Stellaria media* (L.) Vill.	Caryophyllaceae	Buch-Bucha	SNU/CESE/296
187	*Stuckenia pectinata* (L.) Börner	Potamogetonaceae	Sago Pondweed	SNU/CESE/433
188	*Symphyotrichum squamatum* (Spreng.) G.L.Nesom	Asteraceae	Annual Saltmarsh Aster	SNU/CESE/456
189	*Tephrosia pumila* (Lam.) Pers.	Fabaceae	Indigo Sauvage	SNU/CESE/380
190	*Tephrosia purpurea* (L.) Pers.	Fabaceae	Sharpunkha	SNU/CESE/069
191	*Tephrosia villosa* (L.) Pers.	Fabaceae	Hoary Tephrosia	SNU/CESE/388
192	*Trianthema portulacastrum* L.	Aizoaceae	Vishakhapara	SNU/CESE/017
193	*Tribulus terrestris* L.	Zygophyllaceae	Gokhru	SNU/CESE/442
194	*Tridax procumbens* (L.) L.	Asteraceae	Ghamra	SNU/CESE/099
195	*Trifolium tomentosum* L.	Fabaceae	Tipatiya Ghaas	SNU/CESE/283
196	*Triumfetta rhomboidea* Jacq.	Malvaceae	Chinese Bur	SNU/CESE/234
197	*Typha domingensis* Pers.	Typhaceae	Patera	SNU/CESE/390
198	*Utricularia stellaris* L.f.	Lentibulariaceae	Bladderwort	SNU/CESE/408
199	*Vallisneria spiralis* L.	Hydrocharitaceae	Tape Grass	SNU/CESE/431
200	*Verbascum chinense* (L.) Santapau	Scrophulariaceae	Gadar Tambaku	SNU/CESE/144
201	*Verbascum thapsus* L.	Scrophulariaceae	Jangali Tambaku	SNU/CESE/134
202	*Veronica agrestis* L.	Plantaginaceae	Field Speedwell	SNU/CESE/288
203	*Veronica anagallis-aquatica* L.	Plantaginaceae	Water Speedwell	SNU/CESE/324
204	*Vicia sativa* L.	Fabaceae	Matri	SNU/CESE/298
205	*Xanthium strumarium* L.	Asteraceae	Sankhahuli	SNU/CESE/257
	Shrubs			
206	*Abutilon indicum* (L.) Sweet	Malvaceae	Kanghi	SNU/CESE/157
207	*Calotropis gigantea* (L.) Dryand.	Apocynaceae	Safed Aak	SNU/CESE/365
208	*Calotropis procera* (Aiton) Dryand.	Apocynaceae	Aak, Madar	SNU/CESE/026
209	*Capparis sepiaria* L.	Capparaceae	Wild Caper Bush	SNU/CESE/102
210	*Clerodendrum phlomidis* L.f.	Verbenaceae	Arni	SNU/CESE/247
211	*Ficus palmata* Forssk.	Moraceae	Jangali Anjir	SNU/CESE/115
212	*Ipomoea carnea* Jacq.	Convolvulaceae	Behaya	SNU/CESE/271
213	*Jatropha gossypiifolia* L.	Euphorbiaceae	Bellyache Bush	SNU/CESE/436
214	*Justicia adhatoda* L.	Acanthaceae	Arus, Arusa	SNU/CESE/333
215	*Lantana camara* L.	Verbenaceae	Raimuniya	SNU/CESE/080
216	*Mimosa rubicaulis* Lam.	Fabaceae	Himalayan Mimosa	SNU/CESE/207
217	*Phyllanthus reticulatus* Poir.	Phyllanthaceae	Kale Madhu Ka Per	SNU/CESE/116
218	*Ricinus communis* L.	Euphorbiaceae	Arandi	SNU/CESE/213
219	*Solanum torvum* Sw.	Solanaceae	Bhankatiya	SNU/CESE/142

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
220	*Tamarix indica* Willd.	Tamaricaceae	Jhau	SNU/CESE/396
221	*Urena lobata* L.	Malvaceae	Bachita, Lapetua	SNU/CESE/171
222	*Withania somnifera* (L.) Dunal	Solanaceae	Ashwagandha	SNU/CESE/145
223	*Ziziphus nummularia* (Burm.f.)Wight & Arn.	Rhamnaceae	Jhar Beri	SNU/CESE/240
	Climbers/Creepers			
224	*Abrus precatorius* L.	Fabaceae	Ratti	SNU/CESE/382
225	*Cajanus scarabaeoides* (L.) Thouars	Fabaceae	Showy Pigeonpea	SNU/CESE/222
226	*Cayratia trifolia* (L.) Domin	Vitaceae	Three-Leaved Wild Vine	SNU/CESE/135
227	*Cissampelos pareira* L.	Menispermaceae	Patha	SNU/CESE/191
228	*Citrullus colocynthis* (L.) Schrad.	Cucurbitaceae	Colocynth	SNU/CESE/407
229	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Kundru	SNU/CESE/109
230	*Convolvulus arvensis* L.	Convolvulaceae	Hirankhuri	SNU/CESE/305
231	*Cucumis melo* L.	Cucurbitaceae	Kachariya	SNU/CESE/039
232	*Cuscuta chinensis* Lam.	Convolvulaceae	Amar Bel	SNU/CESE/034
233	*Cuscuta reflexa* Roxb.	Convolvulaceae	Amar Bel	SNU/CESE/301
234	*Ipomoea aquatica* Forssk.	Convolvulaceae	Kalmi Sag	SNU/CESE/137
235	*Ipomoea coptica* (L.) Roth ex Roem. & Schult.	Convolvulaceae	Egyptian Morning Glory	SNU/CESE/091
236	*Ipomoea eriocarpa* R. Br.	Convolvulaceae	Tiny Morning Glory	SNU/CESE/210
237	*Ipomoea obscura* (L.) Ker Gawl.	Convolvulaceae	Obscure Morning Glory	SNU/CESE/131
238	*Ipomoea pes-tigridis* L.	Convolvulaceae	Tiger Foot Morning Glory	SNU/CESE/163
239	*Ipomoea triloba* L.	Convolvulaceae	Three-lobe Morning Glory	SNU/CESE/252
240	*Merremia hederacea* (Burm. f.) Hallier f.	Convolvulaceae	Ivy Woodrose	SNU/CESE/244
241	*Mukia maderaspatana* (L.) M.Roem.	Cucurbitaceae	Bilari	SNU/CESE/127
242	*Operculina turpethum* (L.) Silva Manso	Convolvulaceae	Nisoth, Pithori	SNU/CESE/245
243	*Oxystelma esculentum* (L. f.) Sm.	Apocynaceae	Dudhialata	SNU/CESE/166
244	*Rhynchosia capitata* (Roth) DC.	Fabaceae	Kulata, Kulthi	SNU/CESE/211
245	*Rhynchosia minima* (L.) DC.	Fabaceae	Kulata, Kulthi	SNU/CESE/212
246	*Teramnus labialis* (L.f.) Spreng.	Fabaceae	Van Udad	SNU/CESE/217
247	*Trichosanthes cucumerina* L.	Cucurbitaceae	Jangali Chachinda	SNU/CESE/377
	Trees			
248	*Acacia nilotica* (L.) Delile	Fabaceae	Babool	SNU/CESE/361
249	*Albizia lebbeck* (L.) Benth.	Fabaceae	Siris	SNU/CESE/289
250	*Azadirachta indica* A.Juss.	Meliaceae	Neem	SNU/CESE/348
251	*Butea monosperma* (Lam.) Taub.	Fabaceae	Dhak, Palash	SNU/CESE/279
252	*Cordia dichotoma* G.Forst.	Boraginaceae	Lasoda	SNU/CESE/003
253	*Crateva adansonii* ssp. *odora* (Buch.-Ham.) Jacobs	Capparaceae	Barna, Barni	SNU/CESE/095
254	*Dalbergia sissoo* DC.	Fabaceae	Shisham	SNU/CESE/418
255	*Diospyros montana* Roxb.	Ebenaceae	Bistendu	SNU/CESE/357
256	*Ficus religiosa* L.	Moraceae	Peepal	SNU/CESE/031
257	*Holoptelea integrifolia* Planch.	Ulmaceae	Chilbil	SNU/CESE/438
258	*Leucaena leucocephala* (Lam.) de Wit	Fabaceae	Subabul	SNU/CESE/256
259	*Melia azedarach* L.	Meliaceae	Bakain	SNU/CESE/098
260	*Mitragyna parvifolia* (Roxb.) Korth.	Rubiaceae	Kaim	SNU/CESE/092
261	*Morus alba* L.	Moraceae	Shahtoot	SNU/CESE/042
262	*Phoenix sylvestris* (L.) Roxb.	Arecaceae	Jangali Khajoor	SNU/CESE/201

	Botanical name	Family	Vernacular/ Common name	Herbarium catalogue no.
263	*Prosopis cineraria* (L.) Druce	Fabaceae	Khejri	SNU/CESE/282
264	*Prosopis juliflora* (Sw.) DC.	Fabaceae	Vilayati Babul	SNU/CESE/180
265	*Salix tetrasperma* Roxb.	Salicaceae	Bed-laila	SNU/CESE/290
266	*Sesbania sesban* (L.) Merr.	Fabaceae	Rawasan	SNU/CESE/066
267	*Streblus asper* Lour.	Moraceae	Sihora	SNU/CESE/430
268	*Syzygium cumini* (L.) Skeels	Myrtaceae	Jamun	SNU/CESE/360
269	*Ziziphus jujuba* Mill.	Rhamnaceae	Ber	SNU/CESE/246
	Lycophytes and Fern			
270	*Ampelopteris prolifera* (Retz.) Copel.	Thelypteridaceae	Walking Fern	SNU/CESE/094
271	*Equisetum ramosissimum* Desf.	Equisetaceae	Branched Horsetail	SNU/CESE/113
272	*Marsilea quadrifolia* L.	Marsileaceae	Chaupatiya	SNU/CESE/140

Figure 2. Percentage distribution of species under different categories.

in comparison to large geographical area of NCT of Delhi (1,483 km^2).

Documentation of such a large number of species, which constitutes about 50% of flora of Delhi in such a small geographical area, clearly showed that the diverse habitats such as agricultural fields, roadsides, village streets, wetlands, canal bunds, wastelands, grasslands, and wild date palm groves, in the study area contributed to the rich plant diversity coupled with fertile soil conditions. The study area recorded many medicinal herbs growing as weeds (Tripathi et al. 2019). Sometimes, medicinal herbs growing as weeds contribute significantly to enhance the local plant diversity (Tripathi et al. 2020). The study area witnessed some prominent groves of wild date palms *P. sylvestris* associated with some other trees, herbs, and shrubs.

The floristic diversity in Delhi, and the National Capital Region (NCR) under western Uttar Pradesh was studied from time to time (Maheshwari 1963; Singh 1969; Vardhana 2007; Ahamed & Gupta 2010; Shishodia 2013; Malik 2015) (Table 2). Compared to all these studies carried out in a specific area/district , the present study has been carried out in a village panchayat in Delhi NCR encompassing high plant diversity.

Family and genera dominance

With respect to families, Fabaceae was the most dominant with 39 plant species followed by Poaceae (31 species), Asteraceae (25 species), Convolvulaceae (14 species), and Amaranthaceae & Malvaceae (12 species each) (Figure 3). Euphorbiaceae & Solanaceae contributed eight species each, while Cyperaceae & Brassicaceae had seven, and six species, respectively. These families contributed approximately 60% of total plants. The dominance of these families was also reported by various authors (Duthie 1960; Vardhana 2007). Out of 272 vascular plant species, dicotyledons were represented by 56 families, 160 genera, 216 species; monocotyledons by 10 families, 41 genera, 53 species, and lycophytes & fern by three families, three genera, and three species (Figure 4). Among the dicots, the most dominant family was Fabaceae represented by 24 genera, and 39 species. Of the 10 families represented in monocotyledons, Poaceae was the most versatile family with the highest number of species belonging to 25 genera and 31 species, contributing more than 50 % species of the total monocots (Table 1). Genus *Ipomoea* had the highest number of seven species. This was followed by *Euphorbia* (Euphorbiaceae) and *Cyperus* (Cyperaceae) having five species each. There were 46 genera under 23 families with at least two species each. Fourteen families had a single species each while six

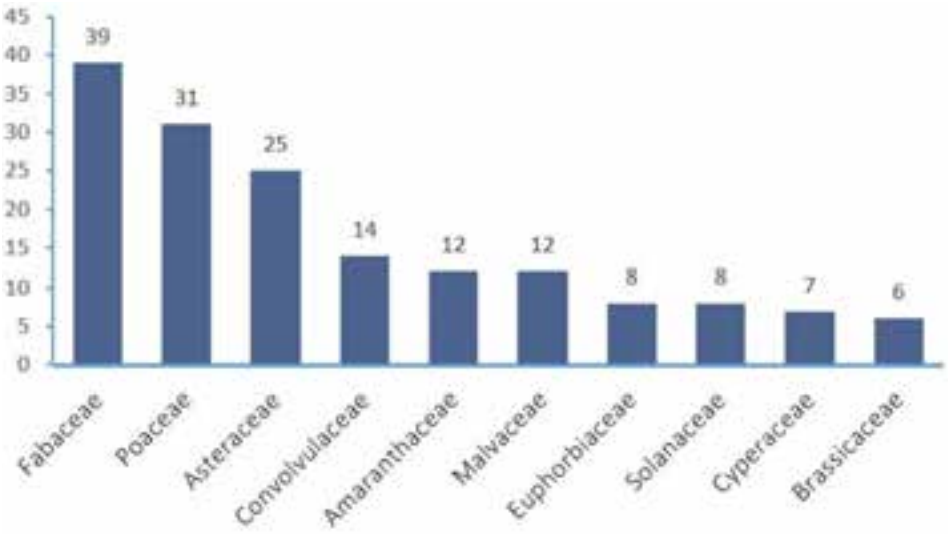

Figure 3. Top ten dominant families with number of species.

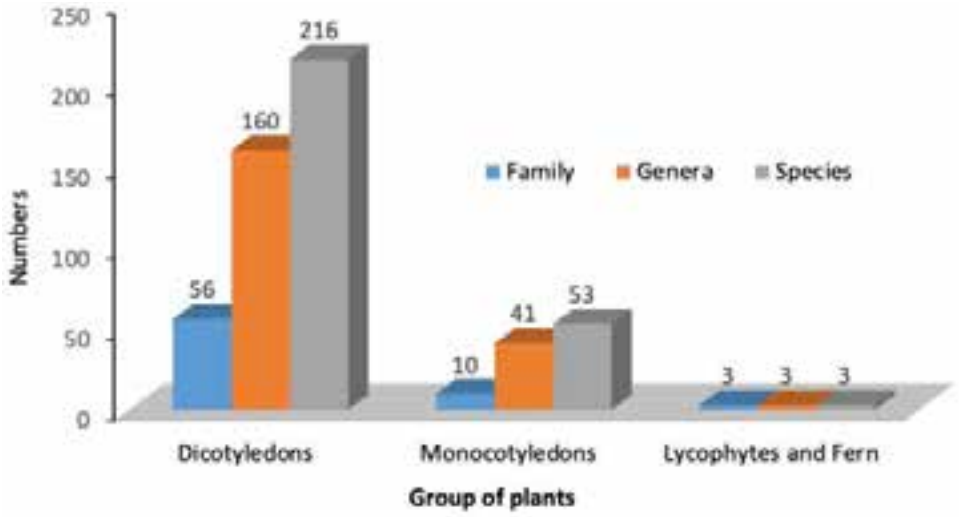

Figure 4. Family, genera and species under different categories.

families had two species each.

Medicinally important plants

The study area witnessed many high-value medicinal plants that play a specific role in ayurveda and other systems of medicine. The list includes *Bacopa monnieri* (L.) Wettst. (Brahmi), *Boerhavia diffusa* L. (Punarnava), *Cannabis sativa* L. (Bhang), *Cissampelos pareira* L. (Patha), *Coccinia grandis* (L.) Voigt (Kundru), *Eclipta prostrata* (L.) L. (Bhringaraj), *Euphorbia hirta* L. (Bari Dudhi), *Justicia adhatoda* L. (Arus), *Sida cordifolia* L. (Bala), *Solanum americanum* Mill (Makoy), *Tribulus terrestris* L. (Gokhru), and *Withania somnifera* (L.) Dunal (Ashwagandha) which were found to be very important as they possess medicinal properties for treating a large number of diseases in traditional system of medicines in India and other parts of the world (Image 1). The whole plant, including root, stem, and leaves is utilized traditionally for curing various ailments such as abdominal pain, anxiety, cold, cough, bronchitis, asthma, diarrhea & dysentery, dull memory, epilepsy, eye troubles, fever, inflammation, jaundice, rheumatism, skin diseases, cardiovascular problems, sexual dysfunction, and various urinary disorders (Sharma et al. 2018). These plants have been reported to contain numerous important phytochemicals such as alkanes, amino acids, flavonoids, organic acids, polyphenolic compounds, steroids, tannins, and terpenes, which

Table 2. Floristic details of studies undertaken in the National Capital Territory Delhi (NCT Delhi), and National Capital Region (NCR) under western Uttar Pradesh.

Study area	Floristic study			
	Total plant species recorded	No. of plant families	No. of genera	Reference
Chithara, NCR (7.7 km²)	272	69	204	Present study
NCT Delhi (1,483 km²)	531	-	-	Maheshwari (1963)
Baghpat (NCR)	566	102	371	Ahamed & Gupta (2010)
Bulandshahr (NCR)	376	-	-	Singh (1969)
Ghaziabad (NCR)	1,654*	171	868	Vardhana (2007)
Meerut (NCR)	862	118	521	Shishodia (2013)
Muzaffarnagar (NCR)	577	77	321	Malik (2015)

*included wild, cultivated, and non-native species.
All floristic details include non-native and invasive species along with native species to the region.

qualify their various medicinal properties (Tripathi et al. 2020). Certain plant species have been reported to yield potent drugs such as vasicine and vasicinone in *Justicia adhatoda,* punarnavine in *Boerhavia diffusa,* bacosoids in *Bacopa monnieri,* protodioscin in *Tribulus terrestris* and withanolides in *Withania somnifera* (Sharma et al. 2018). Sustainable utilization and conservation of these multi-purpose herbs for medicinal purposes is inevitable. Globally, traditional medicine, mostly herbal medicine, is known as a major healthcare provider in rural as well as remote areas, and a large number of people in developing, and underdeveloped countries depend on such medicine for their health (Sen & Chakraborty 2017).

Rare and newly recorded species

The species whose occurrence was very restricted and found only at one or two sites were considered rare species. Among the list, three species, such as *Crateva adansonii* subsp. *odora* (Buch.-Ham.) Jacobs, *Mitragyna parviflora* (Roxb.) Korth., and *Streblus asper* Lour. were rare occurrences in the study area.

The survey documented a few unique species, which were recorded for the first time in this region, such as *Rumex hypogaeus* T.M.Schust. & Reveal (earlier *Emex australis* Steinh.), *Phyllanthus tenellus* Roxb., *Equisetum ramosissimum* Desf., and *Symphyotrichum squamatum* (Spreng.) G.L.Nesom. *R. hypogaeus* belonging to family Polygonaceae, was found growing gregariously in a few places on the SNU campus of the Chithara village and it was a new record for Uttar Pradesh and upper Gangetic plains (Tripathi et al. 2018). *P. tenellus* (Phyllanthaceae), an annual herb that is common in southern India, has expanded its distribution to northern India. *E. ramosissimum* (ssp. *ramosissimum* and ssp. *debile*) was also recorded from the study area; both subspecies were found for the first time from western Uttar Pradesh and NCR Delhi (Sharma et al. 2018). In addition, *S. squamatum*, an erect herb of the Asteraceae, was also recorded for the first time in India from the study area (Tripathi & Sharma 2019).

As a result of repeated surveys in different seasons in heterogeneous habitats of the study area, including agricultural fields, wetlands, canal bunds, wild date palm groves, and roadsides, it became possible to document many rare, and new record species. Due to anthropogenic pressure, the local hotspot areas for biodiversity, such as wild date palm groves & wetlands, which provide habitat to numerous species, are being depleted day by day. Therefore, there is a need to conserve the species hotspots and to create general awareness among local communities.

CONCLUSION

As a first step towards conservation and sustainable utilization of biodiversity, work on biodiversity documentation was undertaken in Chithara Village Panchayat, where so far no efforts have been made to prepare the People's Biodiversity Register as mandated in the Biological Diversity Act (2002). The extensive floristic survey of the village resulted in high species richness, which represents the diversity within a relatively small, localized area with potentially varied habitats within it. A rise in anthropogenic disturbances could threaten important plant habitats in the future, hence, local hotspot areas, such as wetlands, and wild date palm groves that serve as a unique ecosystem for

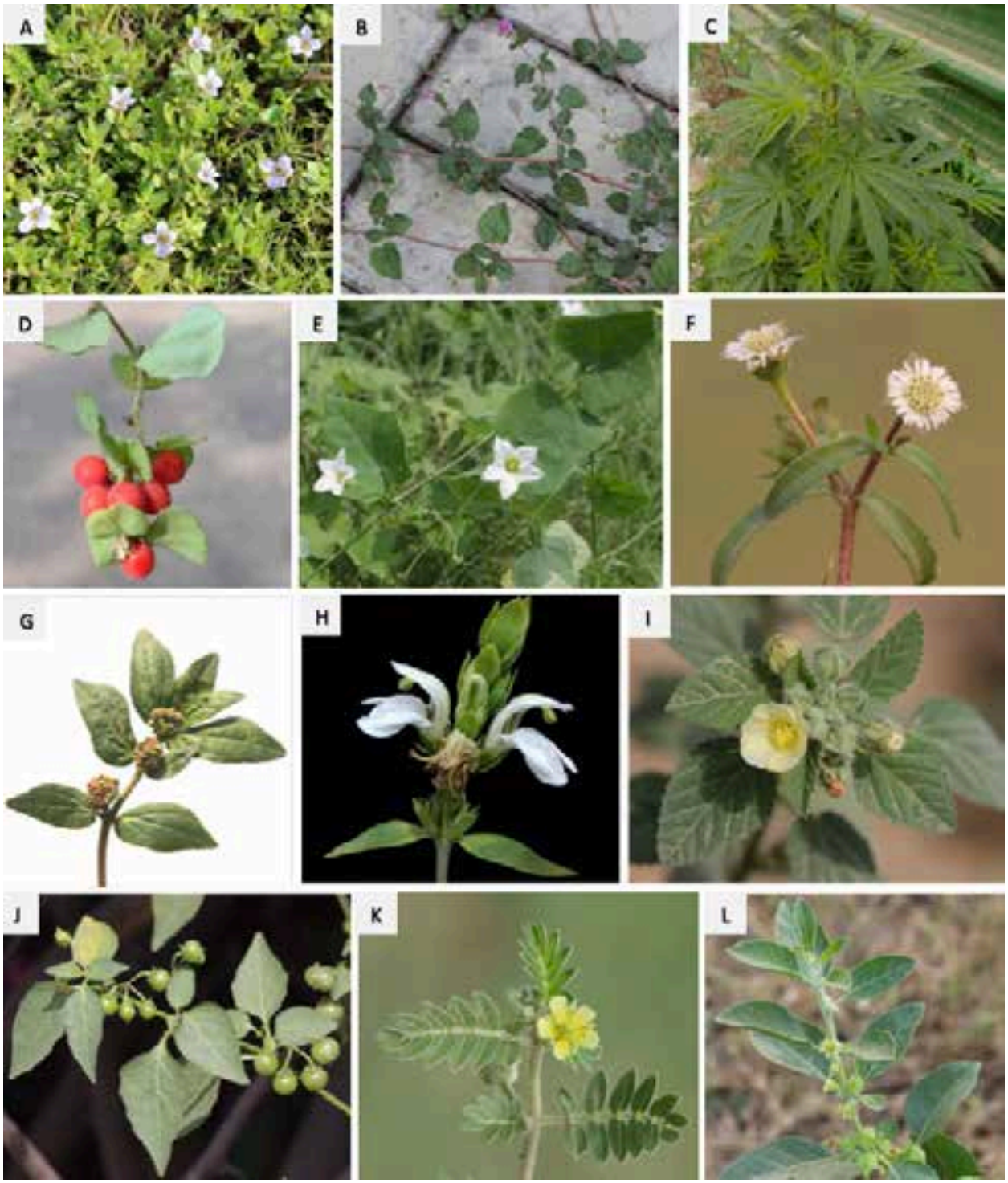

Image 1. Some high value medicinal plants recorded from study area: A—*Bacopa monnieri* | B—*Boerhavia diffusa* | C—*Cannabis sativa* | D—*Cissampelos pareira* | E—*Coccinia grandis* | F—*Eclipta prostrata* | G—*Euphorbia hirta* | H—*Justicia adhatoda* | I—*Sida cordifolia* | J—*Solanum americanum* | K—*Tribulus terrestris* | L—*Withania somnifera*. © Authors.

flora and fauna, should be restored and community-based conservation and management of flora need to be emphasized since local communities always interact with village ecosystems. The study indicates that rural India remains rich in wild plant diversity, including high-value medicinal herbs, and may still harbor many unrecorded species. Hence, long-term, extensive documentation of flora at the village panchayat level should be prioritized.

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COMMUNICATION

Wallowing site observations of the Philippine Tamaraw *Bubalus mindorensis* Heude, 1988 (Artiodactyla: Bovidae) using camera trap data

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Abstract: Due to the combination of anthropogenic and natural stressors, the endemic Philippine Tamaraw *Bubalus mindorensis* was divided into four distinct subpopulations. Currently, the largest population is found within the protected Mts. Iglit-Baco Natural Park. Wallowing is one of the most important behavioral adaptations of *B. mindorensis* to address heat stress but it has remained understudied. With the species facing a continuous threat of extinction, the future conservation plan for the species needs to be carefully strategized, bringing attention to further understand its wallowing behaviour in the wild. Using camera trap technology, the wallowing activity of Tamaraws was photo-captured in a wallowing site of Mts. Iglit-Baco Natural Park. The site was continuously monitored for three years utilizing two sets of cameras capturing opposite sides for a total of 517 independent wallowing events over 1096 sampling days. The photographic data reveal a solitary and diurnal behaviour of Tamaraw wallowing. The correlation of detections with climate variables reveals temperature as a direct driver of *B. mindorensis* wallowing. This study presents the baseline data of the wallowing behavior of the otherwise data deficient species and brings attention towards its management and conservation practices.

Keywords: Endemic, ethology, habitat use, mammalogy, protected area, wildlife monitoring, thermoregulation.

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INTRODUCTION

The Philippine Tamaraw *Bubalus mindorensis* is the largest endemic land mammal in the Philippines, and it is found only on the island of Mindoro, Philippines. The species is listed as Critically Endangered under the Department of Natural Resources (DENR) Administrative Order 2019–09, the Philippine Red List Committee, and The International Union for Conservation of Nature (IUCN) and is also listed in the Appendix I of the Convention on International Trade in Endangered Species (CITES) (Maala 2001). Historically, it was believed to be widespread on the island; however, the combination of anthropogenic (land conversion, illegal logging, migration, and hunting) and natural (rinderpest outbreak) stressors caused a significant decrease in its numbers from an estimated 10,000 individuals in 1900 to 154 individuals by 2000 (Ishihara et al. 2007; Boyles et al. 2016; Tabaranza et al. 2022; Schütz et al. 2023). At present, the *B. mindorensis* is reported to be confined within four sites (Image 1A) on the island: (1) Aruyan-Malate Tamaraw Reservation Area, (2) Upper Amnay watershed region, (3) Mt. Patrick region, and (4) Mts. Iglit-Baco National Park (MIBNP) (Gil et al. 2021; Schütz et al. 2023).

Mts. Iglit-Baco Natural Park (MIBNP) is a protected area proclaimed under the National Protected Areas System (NIPAS) act in 1992 and was recognized as an ASEAN heritage site in 2003 due to the rich biodiversity of native and endemic species inhabiting the mountain (Bonenfant et al. 2023). It is located in the south-central portion of the island, which coincides with the ancestral domain of the Taobuid indigenous communities (Schütz et al. 2023). The park is known for holding over 80% of the entire Philippine Tamaraw population and has been the focal point of the Tamaraw conservation efforts since its establishment (Long et al. 2018; Bonenfant et al. 2023). One instance is the established agreement between the wildlife managers and the indigenous communities that prevents the hunting of Tamaraws, harvesting of resources, or establishment of settlements within a 16 km^2 area inside the Park which they refer as the "2016 IP No Hunting Agreement Zone" which became the Strict Protection Zone when the Protected Area Management Plan was formulated, deemed vital for conserving the species in the mountain (Bonenfant et al. 2023; Schütz et al. 2023). Although protection against killing by poachers and traditional hunting from indigenous communities was enhanced, the confinement of this endemic mammal has led to several problems; one of which is the increase in hunting pressure, especially at the border of the strict protection zone where residing indigenous communities are allowed to set traditional traps (Bonenfant et al. 2023). Another challenge for the confined species is the overgrowth in population, resulting in crowding, slowing the population growth, as well as forcing a source-sink dynamic due to the limited habitat range that may be detrimental to future conservation efforts (Bonenfant et al. 2023).

Wallowing is a behavioural adaptation displayed by several mammals including bisons, pigs, and buffaloes which is done by submerging their bodies in mud/water puddle to cover a thick coat of mud on their body (Coopedge & Shaw 2000; Bracke et al. 2011). This behavior is mainly done to alleviate heat stress, improving the overall well-being in buffaloes. In fact, wallowing was shown to be the most effective cooling strategy of buffaloes, significantly increasing skin temperature, milk production, and overall productivity under heat stress (Aggarwal & Singh 2008; Petrocchi et al. 2023). Despite its ecological significance to the species, wallowing behavior remains understudied since most of *B. mindorensis* studies have been focused on population estimates, distribution and occurrence (Ishihara & Kanai 2010; Ishihara et al. 2015; Gil et al. 2021; Bonenfant et al. 2020, 2023), while behavioral studies remain outdated, and limited (Custodio et al. 1996; Cebrian et al. 2014; Tabaranza et al. 2022). The only study of the species' wallowing is primarily described from a small population of captive Tamaraws (Momongan & Walde 1993). Little is known about the Tamaraw wallowing in the wild besides the earlier descriptions (Custodio et al. 1996; Cebrian et al. 2014; Tabaranza et al. 2022).

Given these challenges, understanding behavioral ecology in response to the rapid shift in climate is critical in preserving natural behaviours and ensuring the species' survival. Especially in MIBNP where intense drought remains a recurring threat to the bovine (Perez et al. 2022). Hence, we report the wallowing observations from comprehensively monitoring a wallowing site in the wild through a camera-trap survey conducted in Mts. Iglit-Baco Natural Park from 2016–2018. The objective of the study is to (i) determine the *B. mindorensis* daily and monthly wallowing patterns and (ii) correlate climatological variables with the wallowing patterns observed. The results of the study will pioneer a descriptive study for the species' wallowing behavior in the wild, as well as highlight its importance to their natural ecology.

MATERIALS AND METHODS

Study site

Here, we report the observed activities of *B. mindorensis* from a three-year comprehensive camera trapping survey (2016–2018) by monitoring a wallowing site (849 m) within the safe zone of the MIBNP (Images 1B, 1C). Here the study area is the wallowing site found at the edge of the grasslands and forest within the 16 km² of SPZ, which contains two mud puddles and is surrounded by shrubs and tall grasses (Image 2). The camera trap survey utilized two cameras, capturing opposite sides of the site.

Camera-trap survey

The initial camera-trap survey was conducted from 04 March 2016 to 24 October 2018 by The World Wide Fund for Nature (WWF-Philippines) in collaboration with the Hubbs-SeaWorld Research Institute (HSWRI), the MIBNP- Protected Area Management Office (MIBNP-PAMO), and Tamaraw Conservation Program (TCP) under the Department of Natural Resources (DENR), and the Far Eastern University (FEU). Due to the critical status and limited distribution of the species, the team opted for targeted sampling in a forest within the no-hunting zone of MIBNP to understand its behavior.

The survey used two camera models at two different angles: the Bushnell camera capturing one side of the site, which recorded the date and time from 12 March 2016 to 30 July 2017. The Reconyx Ultrafire XR6 camera captured photos from 01 August 2017 to 24 October 2018, which recorded the time stamp, date, temperature, and moon phase on the opposite side of the site. The camera was strategically placed in tree trunks, 1.5 m above ground, ideal for capturing large-sized mammals. Image retrieval and maintenance were conducted once

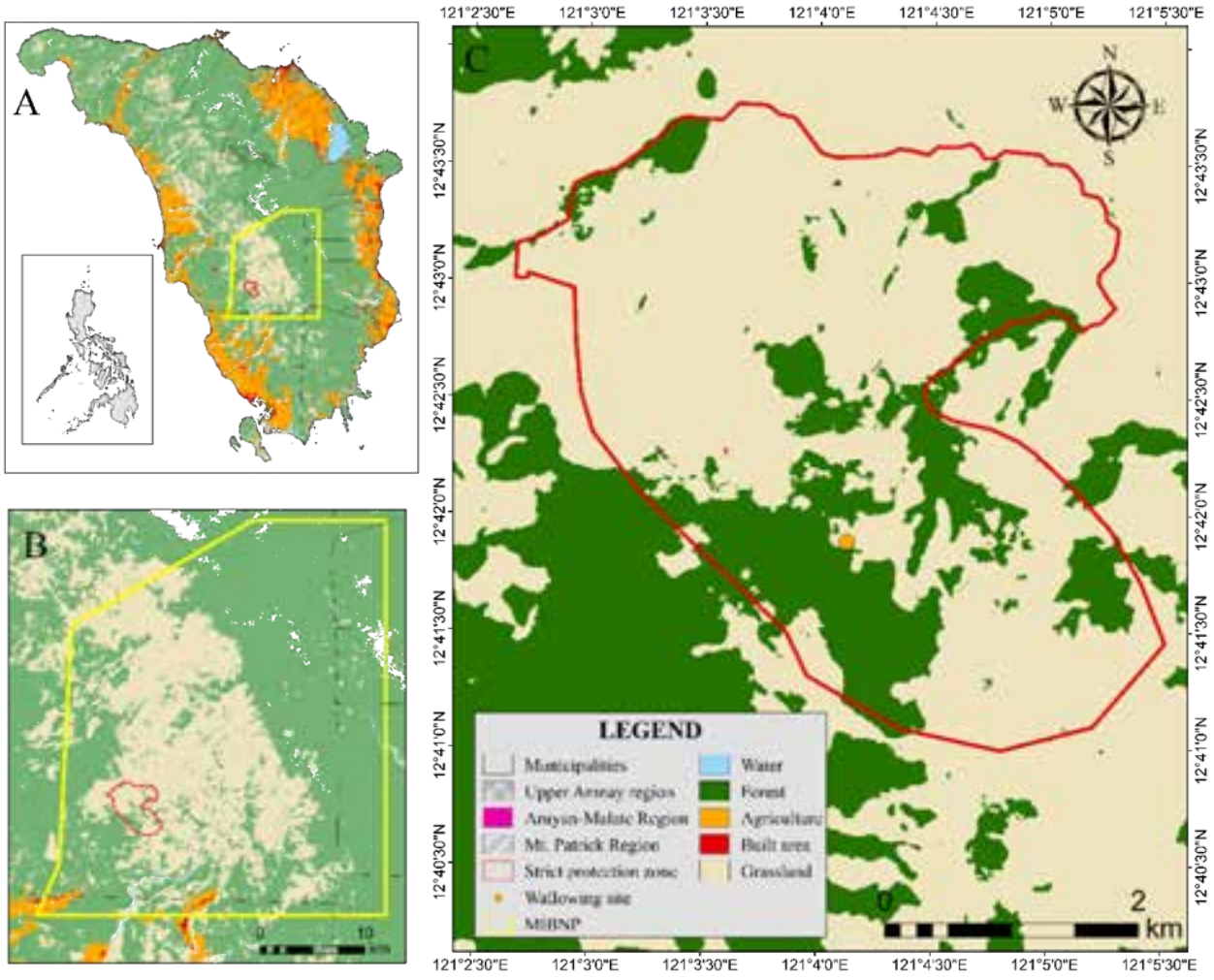

Image 1. Study site map showing: A—Tamaraw reserves within Mindoro Island | B—land cover maps for MIBNP | C—the camera trapped wallowing site within the agreed no-hunting zone.

every month by the WWF-Philippines staff, the TCP & the MIBNP-Park Area Management Office (PAMO) rangers. The cameras were set on high sensitivity, which captures three photographs per trigger and a one-second interval per picture. Photographs were recorded with an image size of 5 megapixels and a 640x480 image resolution.

Image processing

The camera trap data was organized per year, with subfolders arranged by months to determine the seasonality of the observations. The photos were tagged in the metadata using DigiKam ver. 8.4.0. To minimize potential errors in identification, data on the age class & sex were excluded from the image tagging, and only activity, species wallowing behaviour & number of individuals per wallowing observations were tagged. The detection of the images was defined at a 30-minute interval, which is estimated based on the time spent for each Tamaraw activity before moving away from the detection radius of the camera. This ensures that independent events are captured, and it also minimizes potential duplicate counts of individuals and behavior recorded. The statistical analysis was done through R software version 4.4.1 (Kumar & Tiyagi 2024). The function "recordTable" of *camtrapR* package (Niedballa et al. 2016) was used to extract the metadata from the images into a CSV file. The metadata includes the *timestamp*, the *tags*, the *minDeltaTime*, and the *camera station*.

Climate data

The nearest available meteorological station of the Philippine Atmospheric, Geophysical, and Astronomical Services Administration (PAGASA) to MIBNP is located in San Jose City, south of the island. The elevation, geographic, and topographic difference between the available station and the study site may reflect different conditions, potentially affecting the outcome of the analysis. The climatological data of San Jose, Mindoro station, spanning from 01 January 2016 to 31 December 2018, was requested from The Climate and Agrometeorological Data Section (CADS) of PAGASA. The three-year data include the minimum, maximum, and mean temperature, rainfall, humidity, and wind speed.

Statistical analysis

The statistical analysis and visualization were done through the R software using the reshape2, ggplot2, and corrplot packages. A correlation heatmap was used to determine the correlation of wallowing observations with the available climate data which includes rainfall, relative humidity, max temperature, minimum temperature, mean temperature, wind speed, and wind direction.

RESULT AND DISCUSSION

Tamaraw ethogram

The survey lasted for 1,096 days, capturing a total of 9,560 photographs. Using 30-minute intervals, a total of 517 independent wallowing detections were observed. The image tagging revealed 18 different activities exhibited at the wallowing site (Table 1). The activities were further classified into six distinct categories including (1) feeding & drinking, (2) movement & navigation, (3) social interactions, (4) rest & relaxation, (5) wallowing transition, and (6) hygiene maintenance.

Table 1. A constructed ethogram of *Bubalus mindorensis* observed activities.

Activity	Description
Feeding and drinking	
1. Foraging	The head is lowered, chewing food
2. Drinking	The head is lowered in front of a body of water, consuming water
3. Suckling	Juvenile feeding from adult mammary glands
Movement and navigation	
1. Traversing	The head is straightforward, traveling across the landscape
2. Investigating environment	Standing, the head is held up high, either moving left or right
3. Running	Traversing rapidly
Social Interactions	
1. Mounting	A male, positioned on top of a female
2. Nose-to-nose touching	Standing, physical contact between the noses of two individuals
3. Sparring	Two individuals, heads are lowered, physical contact of horns
4. Mock aggression	One or two individuals, heads are lowered, no physical contact of horns
Rest and relaxation	
1. Idling	Individual/s standing, stationary
2. Resting	Individual/s lying down on the grass, stationary
3. Wallowing	Individual/s submerged/standing on top of a mud puddle,
4. Stretching	Extending limbs or entire body
Wallowing transitions	
1. Enter wallow	Moving into a mud puddle
2. Exit wallow	Moving out of a mud puddle
Hygiene and Maintenance	
1. Scratching	Rubbing or scraping the body against a surface or its feet
2. Urinating	Releasing urine, usually in a different stance

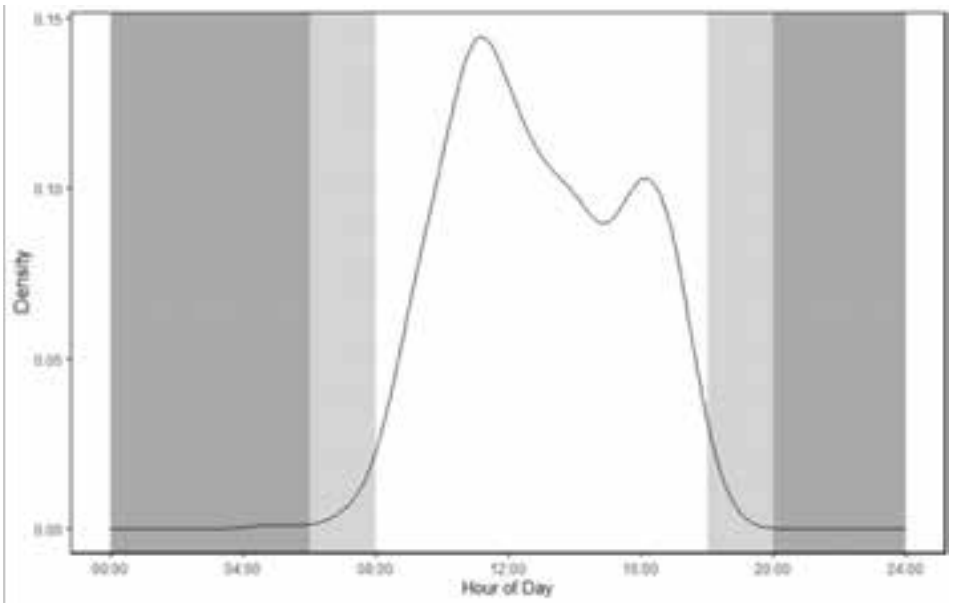

Figure 1. Kernel density estimate of *Bubalus mindorensis* wallowing patterns. The darker shaded area represents nighttime, while the lighter shaded area represents dusk and dawn, respectively.

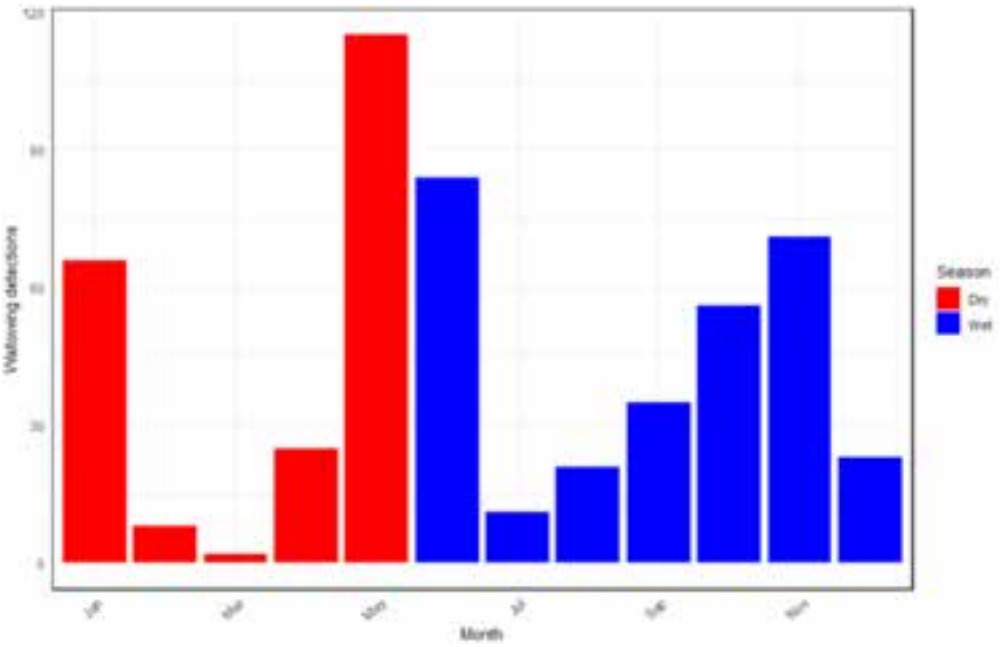

Figure 2. Monthly Philippine Tamaraw wallowing detections differentiated by red (dry season) and blue (wet season) bars (Total records, n = 517).

This preliminary ethogram provides the basic activities that are observed from *B. mindorensis* at wallowing sites which should be considered when strategizing conservation plans for the species. For example, the observed wallowing behavior denotes the importance of mud holes for their natural ecology; therefore, such landscape features must be present in their potential expansion ranges to ensure their survival and overall well-being. Furthermore, the ethogram reports behaviors that have never been described for the species such as sparring and mock aggression, which are commonly observed between juveniles, and bulls with their juveniles.

Image 2. The *Bubalus mindorensis* wallowing observations (© WWF-PHILS): A—one side of the site from the Reconyx camera | B—another side from the Bushnell camera.

Wallowing site activity patterns

Photo analysis revealed a distinct diurnal pattern (Figure 1). Tamaraw wallowing was primarily observed at 0700–1800 h, with two peak periods at mid-day, at 1000–1200 h, and another at 1500–1700 h. No wallowing was observed at night, contrasting the observations of Momongan & Walde (1993) on captive *B. mindorensis* individuals, which reported wallowing during midnight. This suggests that thermoregulation is the primary purpose of wallowing and not ectoparasite protection.

The monthly observations reveal higher counts of wallowing during the longer wet season compared to the dry season (Figure 2). Wallowing was observed to peak in May, when the dry season transitions to the wet season, which was further confirmed by the high correlation between minimum temperatures, and wallowing detections (Figure 3). The observation suggests that perhaps *B. mindorensis* wallowing is induced by the availability of mud puddles, which are limited when these mud puddles are sometimes dry during the hotter months of January to early May. Low wallow observations during the dry season may indicate that these bovines search for other wallowing site instead or rather seek shade in forest during hotter hours when the mud holes dry up, which are normally observed behavior of buffaloes (Katwal et al. 2024)

The correlation of climatological variables detections reveals temperature (minimum temperature, maximum temperature, and mean temperature) as the primary driver of wallowing. This can simply validate the

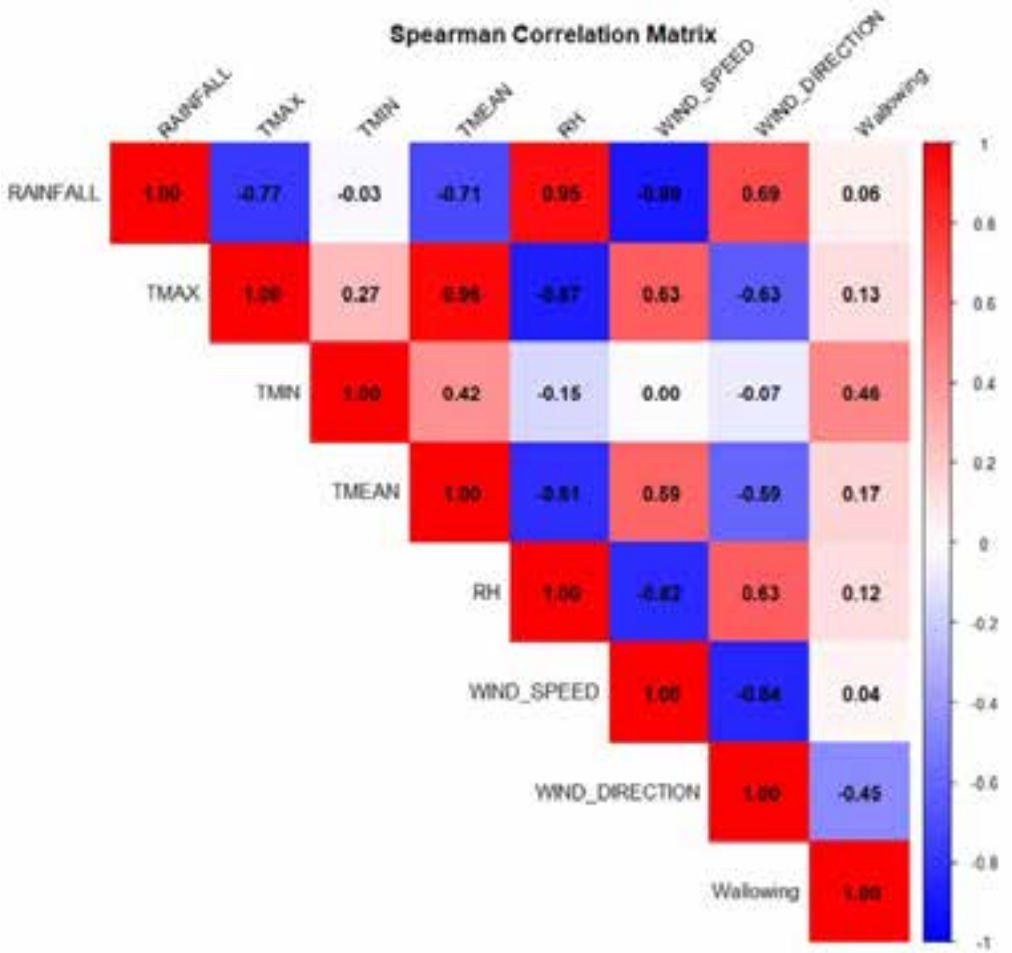

Figure 3. Spearman correlation heatmap of Philippine Tamaraw wallowing occurrences with the climatological variables. TMAX—maximum temperature | TMIN—minimum temperature | TMEAN—mean temperature | RH—relative humidity | WIND_SPEED—wind speed | WIND_DIRECTION—wind direction.

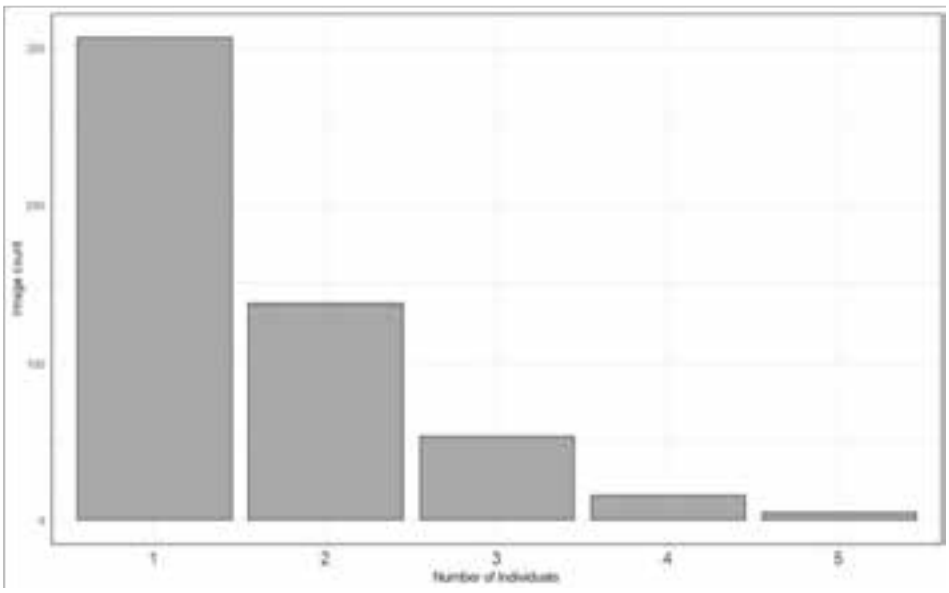

Figure 4. Tamaraw individuals observed at wallowing events over 517 independent records from 1096 days of observation in the strict Tamaraw protection zone of Mts. Iglit-Baco Natural Park.

importance of wallowing as a thermoregulatory mechanism in *B. mindorensis*, inferring that increase in ambient temperature induces the wallowing behavior as long as the mud puddles are readily available in their habitat.

Further analysis of individuals during the Tamaraw wallowing (Figure 4) events reveals a preference for solitary wallowing. This is in line with the solitary nature of Tamaraws, where both adult bulls and cows are observed to be lone individuals (Custodio et al. 1996). Occurrences of wallowing with multiple (3–5) individuals were rare, which may also be attributed to the agonistic behavior between bulls, resulting in the competition for mud puddles. However, this behavior was only associated with breeding season where bull fights are common in order to assert dominance in a herd and displace the losing individual (Custodio et al. 1996)

CONCLUSION

The study provides a preliminary descriptive information regarding the wallowing pattern of *B.* mindorensis on a single wallowing site in MIBNP. It is highly recommended for future that Philippine Tamaraw behavioral studies be conducted on multiple sites to extrapolate its general wallowing patterns in the wild. Nevertheless, the present results highlight the importance of wallowing on the behavioral ecology of *Bubalus mindorensis*, particularly wallowing as their primary thermoregulation during heat stress as suggested by its diurnal pattern. The seasonal patterns show the importance of mud puddle availability to induce the behavior, which should be immediately identified and preserved by the park managers. The preservation of these landscape features should be considered when expanding their range as well as translocating to ensure their survivability. The correlation of climate variables further validate temperature as the primary driver that induces wallowing behaviour and is a critical consideration for future conservation planning of *B. mindorensis.*

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OPEN ACCESS

COMMUNICATION

Diversity and abundance of ants from tehsil Salooni of District Chamba, Himachal Pradesh: a research perspective to study ants as bioindicators

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Abstract: This study evaluated the diversity and abundance of ants in Salooni Tehsil, Chamba District, Himachal Pradesh. Thirty species from five subfamilies were reported using different collection modules, including pitfall traps, baits, soil core, leaf litter, manual, and winkler method. Myrmicinae was the most diversified subfamily, with seven genera, while the genus *Camponotus* of the subfamily Formicinae was found to be most abundant with eight species. The study determined that the region is home to two invasive species (*Monomorium pharaonsis* and *Trichomyrmex destructor*). Due to which it is concluded that the area is prone to many anthropogenic activities, and in the near future, these invasive species can replace many native species of the region. This study also compiled functional groups of ants, which include generalised Myrmicinae, opportunists, subordinate Camponotinii, hot climate specialists, cold climate specialists, tropical climate specialists, cryptic species, and specialist predators. The richness of an environment is represented by the diversity of insects. Because ants maintain the stability of ecosystems, it is important to protect their habitats in order to increase biodiversity in the years to come. The data compiled here represents a turning point in the attempt to understand the geographic variety and dispersion of ant species and also the role of ants as bioindicators.

Keywords: Anthropogenic activities, biodiversity, bioindicator, cryptic species, ecosystem, functional group, invasive species, opportunists, specialist predators, tropical climate specialists.

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Author contributions: JSR—Study design and article drafting; MS—field work.

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INTRODUCTION

Ants are one of the most abundant and diverse groups of insects surviving on Earth. Ants exhibit intricate social behaviours and are present in nearly all terrestrial ecosystems, ranging from rainforests to deserts. Ants serve as efficient bioindicators as they are sensitive to environmental changes and are found in various kinds of environments. They react fast to disturbances such as habitat destruction, pollution, or climate change, making them important for monitoring ecosystem health. Ants are categorized as eusocial among social insects because of their traits, which include sharing generational responsibilities within the colony, and cooperative brood care. Ants are identified as three separate castes: male, female (queen), and sterile workers (Wilson 1971).

Ants are classified as members of the Formicidae family and order Hymenoptera. Among all eusocial insects, ants have the highest ecological dominance (Ward et al. 2015). As of now, 22 subfamilies, 505 genera, and 16,856 valid species of ants are recognized globally (Bolton 2025). These are represented by 10 subfamilies distributed across 108 genera and 865 species in India (Bharti 2025). In recent years, many scientists worldwide have studied the ant fauna. Bharti (2008) compiled a comprehensive checklist and taxonomic review of Indian ant fauna from the Himalayan and sub-Himalayan regions. Their findings include 115 ant species out of 202 crossed an altitude of 2,000 metres; 71 species out of these 115 are endemic to Himalaya. Bharti et al. (2016a) provided a comprehensive and critical list of Indian ant species with up-to-date state-wise distribution. Bharti et al. (2016b) conducted research on ants as bioindicators in Shivalik mountains of Himalayas. A total of 181 species spanning across 59 genera were recorded from Shivalik ranges. Bharti et al. (2017) represented one of the most comprehensive surveys of ant fauna in northwestern Shivalik region. In this study, 179 species group taxa were listed for 61 genera belonging to eight subfamilies. A total of 828 valid species and subspecies names belonging to 100 genera were listed from India. Neupane & Subedi (2018) studied the diversity of ants in the winter and summer seasons in the area of Shivapuri–Nagarjun National Park (SNNP). Using various sampling methods, a total of 817 individual ants, belonging to five sub-families, 16 genera, and 23 morphospecies, were reported. Fontanilla et al. (2019) conducted research on taxonomic and functional ant diversity and identified a total of 263 species in southwestern China. Castro et al. (2020) examined three dimensions of the taxonomic (TD) and functional (FD) (α and β) diversities of ants in a mountainous environment. Brassard et al. (2021) investigated high ant diversity in urban areas. Schmidt et al. (2022) conducted research on ant diversity studies in Brazil and suggested that a global perspective on diversity studies may be achieved by recreating their work in different parts of the world. Li et al. (2023) examined ant species diversity in the central and northern parts of the western Sichuan Plateau in China. A total of 22,645 ant specimens representing 40 species grouped in 18 genera and four subfamilies were collected. Laakel et al. (2024) compiled an ant inventory in Bejaia city urban and suburban areas in order to address the demand for further data regarding ant biodiversity in Algeria's urban environment. Rilta & Sharma (2024) conducted research focused on the diversity and abundance of ants from the tehsil Nerwa of Shimla District. A total of 33 species belonging to 22 genera of four subfamilies were collected. Rilta & Narwal (2025) presented research work focused on ant diversity and community composition from north-western Himalayas. A total of 35 species of ants belonging to 22 genera, and five subfamilies were recorded.

Comprehensive and extensive data were collected on the diversity and abundance of ants with the aim to investigate local ant fauna in the study area and the role of ants as bioindicators.

MATERIALS AND METHODS

Study area

Salooni is a tehsil that is located in the district Chamba, which is at an altitude of 1,829 m. The tehsil is surrounded by the Pir Panjal in the north and the Dhaula Dhar ranges in the south. It provides magnificent sights of the gushing river and snow-capped mountains. The study area is located between 32.500–33.000 ^{0}N & 75.750–76.250 ^{0}E. The human population of the area is 81,556, distributed across 576.5 km^{2} (Vaid & Pathania 2024). There is an abundance of flora and fauna in the valley, which highlights its rich biodiversity. This field has not yet been the subject of any prior research.

Collection tours have been conducted to various localities falling in the tehsil Salooni. The sampling was carried out for three months accounting for both the summer and winter seasons. Localities covered during these tours are labelled in the maps (Figure 1).

Sampling method

For collection of material, all protocol proposed by Agosti et al. (2000) have been followed, which includes:

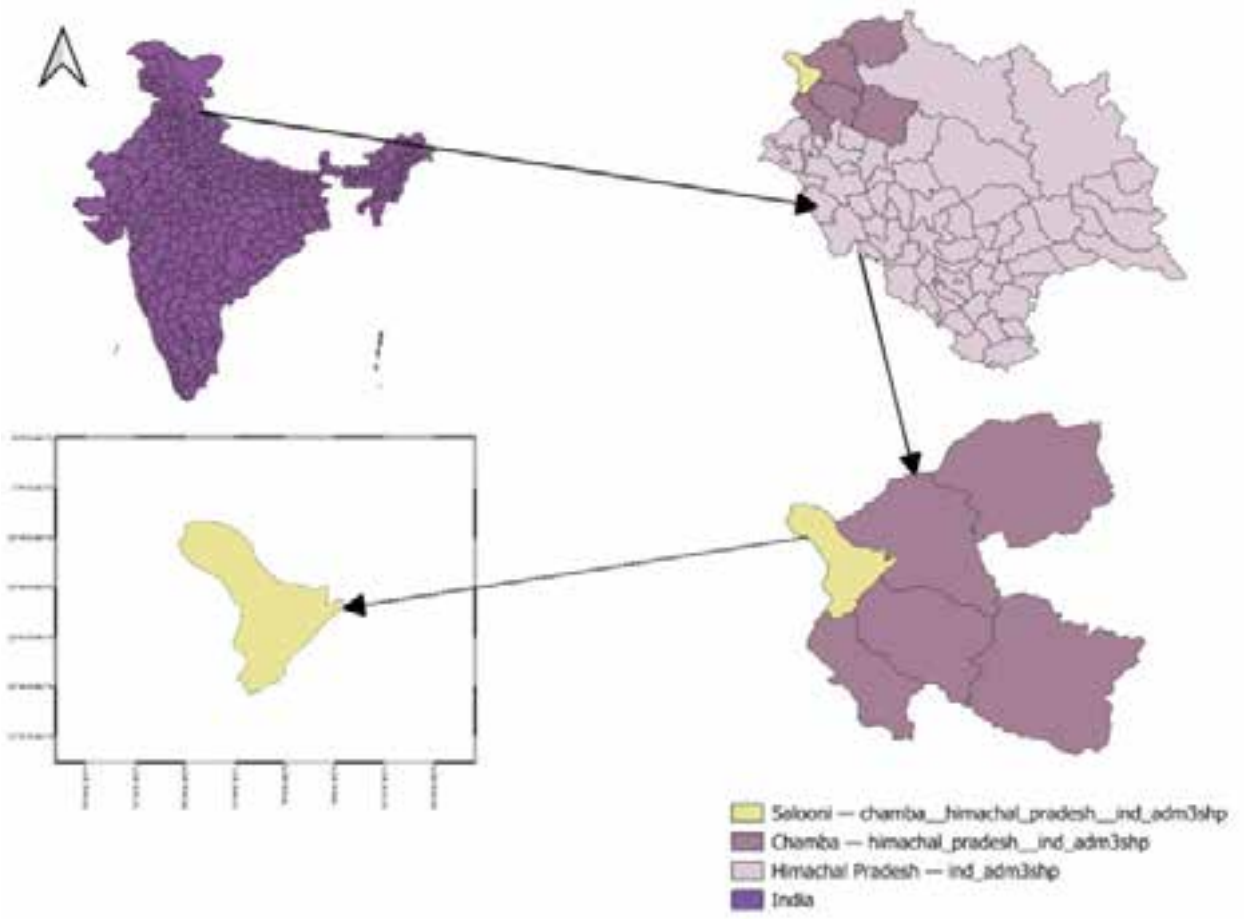

Figure 1. The geographical location of tehsil Salooni, District Chamba, Himachal Pradesh

· Mini Winkler sacs having wire sieve with square holes of 1×1 cm were used to collect ants from leaf litter. Ants were extracted from sifted litter after a period of 48 hours.

· Pitfall traps consisting of test tubes were used. Each test tube was partly filled with 5% ethylene glycol solution, and was buried with the rim flush with the soil surface.

· Arboreal baits were used for sampling of tree ants.

· The soil core method was used for hypogaeic ants, where soil cores, each of 20 × 20 × 15 cm depth, were taken. These were sifted through a hand sieve pan to collect ants.

· Beating vegetation method (to dislodge ants from vegetation onto sheets) was also carried out.

· Light trap, which consisted of a white sheet, and fluorescent bulb was used for the collection of reproductive castes of ants.

· Finally, the ants were also collected by hand picking method, by searching rotten logs, stumps, dead & live branches, twigs, low vegetation, and termite mounds.

Collection preservation and identification

Both morning and evening hours were used to collect ant samples (Gadagkar et al. 1993). The collected material was preserved using 90% alcohol. The ant specimens were then mounted on triangles for research in accordance with accepted practices in ant taxonomy. To aid in identification, their legs were moved ventrally, away from the body, and the mandibles of certain specimens were opened. The ants were then point mounted on triangle "points" on their right side, between the mesocoxa, and metacoxa. Following their separation from debris and mounting, these specimens were appropriately labelled with the following details: Country, state, location, date, method of collection, and ecological data. All the collected material was identified up to species level with the help of Linnaeus (1758), Fabricius (1787), Foerster (1850), Jerdon (1851), Smith (1858), Mayr (1862), Mayr (1879), Emery (1895), Forel

(1902), Bingham (1903), Forel (1904), Donisthorpe (1938), Menozzi (1939) Bolton (1994), Bharti & Wachkoo (2013), Bharti et al. (2016a), Bharti (2024), Bolton (2024), and then compared with the reference collection already hosted in the laboratory.

The taxonomic analysis was conducted on RSMr-10 stereo zoom microscope. Relevant data has been attached to the arranged catalogue of the acquired content. Voucher specimens have been deposited in the Himachal Pradesh University Ant Collection (HPUAC) in Shimla, India.

RESULTS

A total of 646 ant specimens (Figure 3) representing 30 species, belonging to 19 genera of five subfamilies (Figure 2) were collected (Table 1). In this study subfamily Formicinae and Myrmicinae contributed highest in terms of number of species and number of specimens (Figure 4,5). The study also accounts list of two introduced species (Table 2). The ant fauna prevalent in the region is highly diverse. The study also compiled functional groups of ants in this study, which include generalised Myrmicinae, opportunists, subordinate Camponotinii, hot climate specialists, cold climate specialists, tropical climate specialists, cryptic species, and specialist predators (Table 3). The study also mentions the species of ant that has already been reported from higher regions of Himachal Pradesh (Table 4).

DISCUSSION

The primary findings of the study are the various ant species and records of invasive species, importance of the area's diverse biodiversity. Nonetheless, the existence of invasive species also creates a sense of unease that is common among local ants. Monitoring

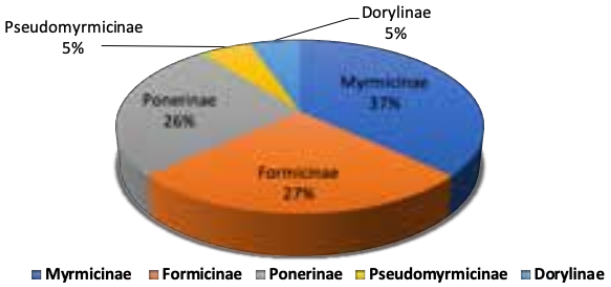

Figure 2. Subfamily representation of 19 ant genera.

Table 1. List of ants collected from tehsil Salooni.

Subfamilies: 5, Genera: 19, Species: 30		
Dorylinae	*Aenictus*	*Aenictus peguensis* (Emery, 1895)
Formicinae	*Camponotus*	*Camponotus albosparsus* Bingham, 1903
		Camponotus arrogans (Smith, 1858)
		Camponotus compressus (Fabricius, 1787)
		Camponotus kattensis Bingham, 1903
		Camponotus nirvanae Forel, 1893
		Camponotus oblongus (Smith, 1858)
		Camponotus opaciventris Mayr, 1879
		Camponotus sp. minor
	Formica	*Formica polyctena* Foerster, 1850
	Lepisiota	*Lapisiota lunaris* (Emery, 1893)
	Polyrhachis	*Polyrhachis menelas* Forel, 1904
	Lasius	*Lasius himalayans* Bingham, 1903
Myrmicinae	*Aphaenogaster*	*Aphaenogaster* JR01
		Aphaenogaster smythiesii Forel, 1902
	Crematogaster	*Crematogaster brunnaea contemta* Mayr, 1879
		Crematogaster sagei Forel, 1902
	Messor	*Messor himalayanus* (Forel, 1902)
	Monomorium	*Monomorium pharaonsis* (Linnaeus, 1758)
	Myrmica	*Myrmica aimonissabaudiae* Menozzi, 1939
		Myrmica smythiesii Forel, 1902
	Pheidole	*Pheidole indica* Mayr, 1879
		Pheidole spathifera aspatha Forel, 1902
	Trichomyrmex	*Trichomyrmex destructor* (Jerdon, 1851)
Ponerinae	*Anochetus*	*Anochetus cryptus* Bharti & Wachkoo, 2013
	Brachyoponera	*Brachyoponera luteipus* (Mayr, 1862)
	Leptogenys	*Leptogenys lucidula* Emery, 1895
	Odontoponera	*Odontoponera denticulata* (Smith, 1858)
	Pseudoneoponera	*Pseudoneoponera rufipes* (Jerdon, 1851)
Pseudomyrmecinae	*Tetraponera*	*Tetraponera rufonigra* (Jerdon, 1851)

Table 2. List of invasive ant species of tehsil Salooni.

Species	Invasive species
Monomorium pharaonsis (Linnaeus, 1758)	Introduced
Trichomyrmex destructor (Jerdon, 1851)	Introduced

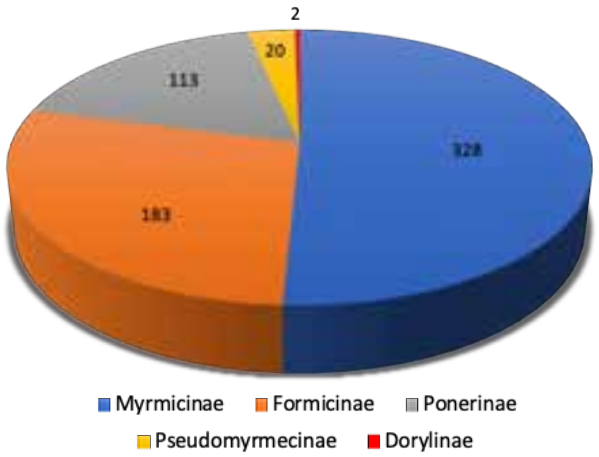

Figure 3. Subfamily representation of 646 ant specimens.

their spread provides valuable information about environmental degradation, particularly in rapidly urbanizing or agriculturally expanding regions (Andersen 1995). An analysis was conducted on the functional group structure of ants inhabiting the area and proposed the ant functional group concept, which shows how ants respond to stressful circumstances and disruptions of environment at a biogeographical scale to identify them as bio-indicators of anthropogenic problems at local scales. The findings of this study reinforce the utility of ants in monitoring environmental changes, particularly in relation to habitat disturbance, pollution and land-use transformation. This approach, which has been in use more recently elsewhere in the world (Andersen 1997), includes the following groups of ants (Table 3). An abundance of ant species has flourished as a result of topographic changes, former climatic regimes, and present-day microclimatic fluctuations. The resulting biodiversity is likely to exhibit a greater level of

Table 3. Different functional groups including their respective genus.

Functional groups	Genus
Generalised Myrmicinae	*Crematogaster*
	Messor
	Monomorium
	Pheidole
Opportunists	*Odontoponera*
	Myrmica
	Lepisiota
	Formica
	Aphaenogaster
Subordinate Camponotini	*Polyrhachis*
	Camponotus
Hot climate specialists	*Monomorium*
Cold climate specialists	*Monomorium*
	Lasius
Tropical climate specialists	*Aenictus*
	Tetraponera
Cryptic species	*Lepisiota*
Specialist predators	*Anochetus*
	Leptogenys

Table 4. List of ant species that has already been reported from higher regions of Himachal Pradesh.

Species name	Location	References
Camponotus albosparsus	Himalayan Region, Nerwa	Bharti (2008), Rilta & Sharma (2024)
Camponotus kattensis	Himalayan region, Nerwa, Shimla	Bharti (2008), Rilta & Sharma (2024), Rilta & Narwal (2025)
Camponotus compressus	Himalayan region, Andretta, Bakhra, Kotla	Bharti (2008), Bharti et al. (2017)
Crematogaster sagei	Himalayan Region, Nerwa, Shimla	Bharti (2008), Rilta & Sharma (2024), Rilta & Narwal (2025)
Monomorium pharaonsis	Himalayan Region, Chanaur, Renuka, Guga, Shivalik region, Nerwa	Bharti (2008), Bharti et al. (2016b), Bharti et al. (2017), Rilta & Sharma (2024)
Leptogenys lucidula	Himalayan Region, Shimla	Bharti (2008), Rilta & Narwal (2025)
Lasius himalayans	Himalayan Region, Shimla	Bharti (2008), Rilta & Narwal (2025)
Messor himalayanus	Himalayan region, Andretta, Bilaspur, Mandi, Nerwa, Shimla	Bharti (2008), Bharti et al. (2017), Rilta and Sharma (2024), Rilta & Narwal (2025)
Myrmica smythiesii	Himalayan region	Bharti (2008)
Myrmica aimonissabaudiae	Himalayan region	Bharti (2008)
Tetraponera rufonigra	Himalayan region	Bharti (2008)
Aphaenogaster cavernicola	Himalayan region	Bharti (2008)
Aphaenogaster smythiesii	Himalayan region, Shimla	Bharti (2008), Rilta & Narwal (2025)
Pheidole indica	Himalayan Region, Una, Terrace, Nerwa, Shimla	Bharti (2008), Bharti et al. (2017), Rilta & Sharma (2024), Rilta & Narwal (2025)

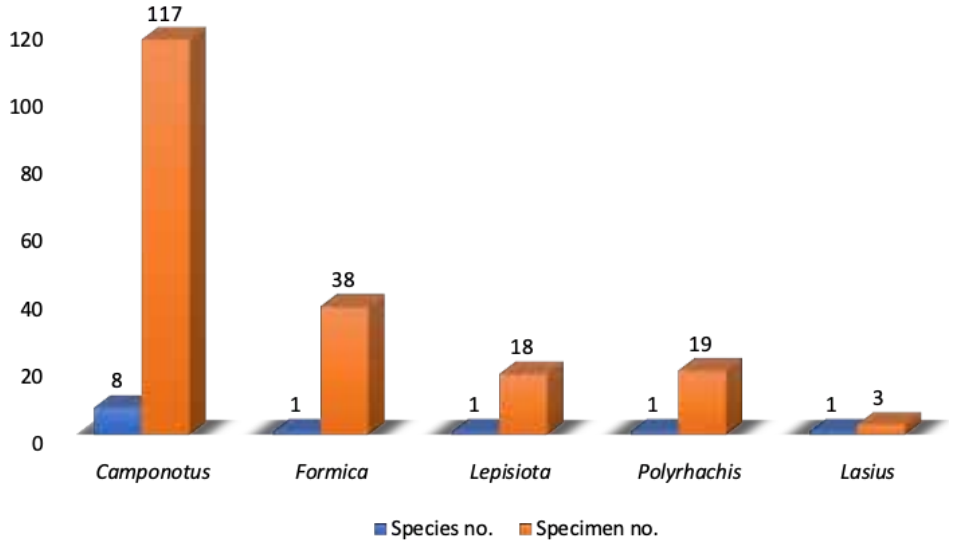

Figure 4. Generic richness of subfamily Formicinae in terms of no. of species and number of specimens collected.

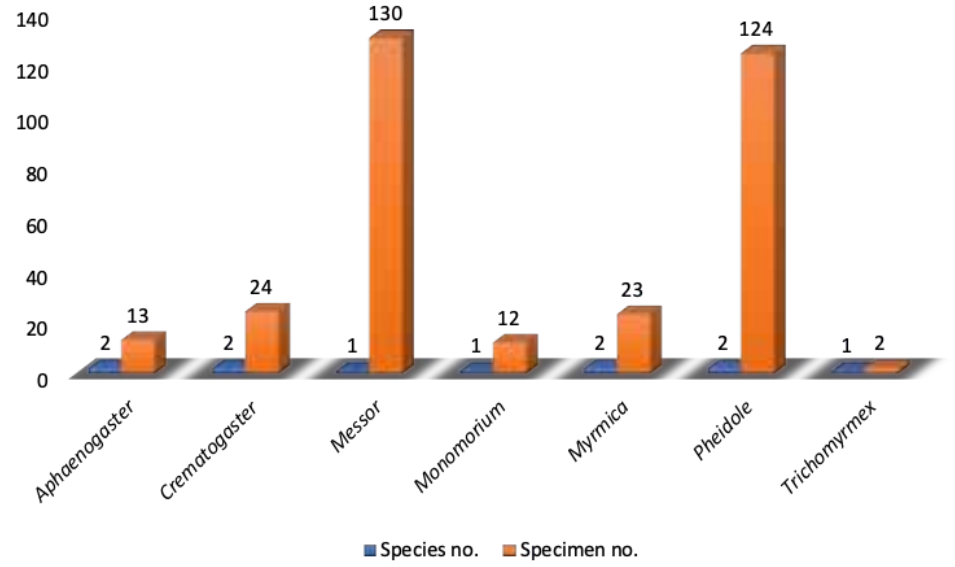

Figure 5. Generic richness of subfamily Myrmicinae in terms of no. of species and number of specimens collected.

specialization and environmental adaptation.

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OPEN ACCESS

COMMUNICATION

Studies on *Heliotropium rottleri* Lehm. (Heliotropiaceae) – a threatened endemic plant of Tamil Nadu, India

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Abstract: *Heliotropium rottleri* Lehm. (Heliotropiaceae; previously Boraginaceae) is endemic to Coimbatore region. Its taxonomic status is ambiguous and unresolved because of mistaken identity of *H. marifolium* J. Koenig ex Retz. and *H. scabrum* Retz. by later botanists. So far, no efforts have been taken to assess its distribution, population size, and conservation. While working on *Heliotropium* L. genus in Tamil Nadu, we have collected it from four localities in the erstwhile undivided Coimbatore district (two in Udumalpet taluk, one in Palladam taluk) in January 2021 and identified with Madras Herbarium, Botanical Survey of India Southern Regional Centre. Our study indicates that it is now Critically Endangered as the habitats are very rapidly altered and changing due to real estate business. Unless urgent actions are taken, certainly this species will disappear. Therefore, it is suggested to take immediate action to conserve this species in its natural habitat.

Keywords: Coimbatore, Critically Endangered, curved raceme, divaricate branches, endangered, leaf margin revolute, perennial, Thiruppur.

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Author details: KADER, S.A., associate professor, is a field botanist and actively engaged in plant diversity survey. G. GOPAL, Ph.D. research scholar, is currently involved in vegetative propagation and floristic studies.

Author contributions: KSA has contributed the data, images, prepared manuscript etc. for the present study; GG has provided transported facility for the last field visit and helped in taking measurement and herbarium preparation.

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INTRODUCTION

The cosmopolitan genus *Heliotropium* L. (Heliotropiaceae; previously Boraginaceae) comprises of about 255 accepted species (POWO 2024) distributed mostly in the arid and semi-arid habitats of the world (Luebert et al. 2016). In British India, Wight (1850) had first reported six species and one variety, namely: *H. coromandelianum* Retz., *H. linifolium* Lehm., *H. marifolium* Retz., *H. rottleri* Lehm., *H. scabrum* Retz., *H. supinum* L. var. *supinum*, and *H. supinum* var. *malabaricum*. Later, Clarke (1885) had documented 16 species and four varieties in the '*Flora of British India'* (Hooker 1885). Furthermore, *H. keralense* was discovered by Sivarajan & Manilal (1972). Presently, there are about 22 *Heliotropium* sensu lato species, two subspecies and four varieties in India (Meena et al. 2020). *Euploca* Nutt. was delimited from *Heliotropium* L. based on presence of bracts in the inflorescence, fruit breaking up into four nutlets, nutlets with pits on endocarp and curved embryo (Dheeren 2021).

Heliotropium rottleri was first published by Lehmann in 1818 from India without specifying precise locality in *'Plantae e Familiae Asperifoliarum Nuciferae'*. Later, Wight (1850) and Gamble (1921) had collected this species from Coimbatore and the former provided a description and an illustration. Despite, its taxonomic status, it has been considered as unresolved (WFO 2023) because later botanists, namely, Ramamoorthy (1976), Matthew (1983), Sasidharan (2011), and Rao et al. (2019) have confused its identity with *H. marifolium* J.Koenig ex Retz. and *H. scabrum* Retz. Based on the above literature, Rao et al. (2019) have wrongly reported it from Andhra Pradesh, Telangana, and Kerala in *'Flora of Peninsular India'*. In fact, these three species are distinct and have been collected from Tamil Nadu during the revisionary studies by us. According to Wight (1850) *H. rottleri* was frequent in Coimbatore. In 1964, Chandrabose collected it from R.S. Puram and the subsequent workers have collected it from the foot-hills of Kuridimalai and Thadagam (all in Coimbatore) but now it is not found in Coimbatore city and rare in Kuridimalai and Thadagam. Ahmedullah & Nayar (1987) categorized its threat status as Endangered in southern Deccan, Coimbatore. While working on the genus *Heliotropium* in Tamil Nadu, we have collected *H. rottleri* from Masagoundanputhur and Raavanapuram (Udumalpet taluk), Puliyampatti, Kamanayakkan Palayam (Palladam taluk) and in adjacent Thiruppur district, Tamil Nadu during January 2021. Recently, Ancy et al. (2024) have published *H. rottleri* as *Euploca wightiana* sp. nov. Therefore, the aim of the present paper is to provide a complete botanical description and to establish the correct identity of *H. rottleri,* and the current status in Tamil Nadu.

MATERIALS AND METHODS

Extensive field survey was carried out for *Heliotropium rottleri* in its type locality and its neighbourhood regions. Specimens were studied at Presidency College, Chennai. Photographs were taken and taxonomical characters were recorded. The collected plant materials were processed following standard herbarium methods and made into herbarium specimens. The specimens were identified using relevant literature such as Hooker (1885) and Gamble (1921), and compared with authentic specimens deposited in the Madras Herbarium (MH). For threat status assessment, the number of individuals was recorded during each visit to the locations. The primary and secondary information required for assigning the criteria as per IUCN Red List Categories and Criteria were collected, following the IUCN guidelines (Standards and Petitions Working Group IUCN 2006).

RESULTS

Botanical Description

Habitat: Marginal waste lands in the plains with grassy, rocky, and calcareous soils. Habit: Erect stiff undershrubs; branches stout, divaricate, covered with white appressed strigose hairs (Image 1a). Leaves: Simple, alternate, scattered; petioles 2–4 mm long, lamina ovate-lanceolate acute with revolute margins, 6–15 × 3–5 mm, white-strigose (Image 1b,c). Inflorescence: Racemes terminal, curved, on divaricate branches, stiff; peduncles 4.6–8.4 cm long with distant leaves (Image 1d). Flowers: c. 2 mm across at mouth (Image 1e), pedicels 1–3 mm long (Image 1f). Calyx: 5-lobed, lobes basally connate, green, ovate-elliptic acuminate, white-strigose, accrescent (Image 1g). Corolla: Campanulate, 5-lobed, white with yellow centre (Image 1e). Fruits: 4-lobed depressed nutlets, c. 4 mm in diam. c. 3 mm thickness, completely covered with short grey adpressed hairs (Image 1g).

Flowering and Fruiting period: Almost throughout the year.

Associate species: *Acacia leucophlea* (Roxb.) Willd., *Abutilon pannosum* (G.Forst.) Schltdl., *Azadirachta indica* A.Juss., *Barleria buxifolia* L., *B. cuspidata* F.Heyne ex Nees, *Dichrostachys cinerea* (L.) Wight & Arn.,

Image 1. *Heliotropium rottleri* Lehm: a—Habitat | b—Habit | c—Leaf upper surface | d—Leaf lower surface | e—Flower | f—Fruits. © Kader, S.A.

Table 1. Morphological differences between *Heliotropium rottleri* Lehm., *H. marifolium* J.Koenig ex Retz., and *H. scabrum* Retz.

	Morphological characters	*Heliotropium rottleri*	*H. marifolium*	*H. scabrum*
1	Habit	Perennial, erect undershrub attaining about 30 cm height; branches divaricate and curved.	Procumbent annual herb; branches divaricate, and straight.	Prostrate woody annual herb; branches radiating from the root stock.
2	Leaves	Shortly petiolate, small, ovate-lanceolate acute with revolute margins, white-strigose, alternate but distant on stem.	Sub-sessile, ovate-lanceolate acute with flat margins, hispid, less scabrous, alternate, dense on stem.	Shortly petiolate, ovate-lanceolate acute with revolute margins, strigose-hirsute, scabrous, alternate and dense on stem.
3	Inflorescence	2.5–5 cm long stiff curved raceme.	Simple spikes of 2.5–5 cm long; bracts conspicuous. In *H. marifolium* ssp. *wallichii,* the spike is forked.	Subcapitate among leaf-like bracts.
4	Flowers	Pedicellate, 2 mm across, distantly arranged.	Sessile, 1 mm across.	Sessile, 2 mm across.

Image 2. *Heliotropium marifolium* Retz. subsp. *wallichii.* © Kader, S.A.

Image 3. *Heliotropium scabrum* Retz. © Kader, S.A.

Parthenium hysterophorus L., *Passiflora foetida* L., *Pergularia daemia* (Forssk.) Chiov., *Prosopis juliflora* (Sw.) DC., *Senna auriculata* (L.) Roxb., and grasses.

A comparative account of *Heliotropium rottleri* Lehm. (Image 1), *H. marifolium* J.Koenig ex Retz. (Image 2) and *H. scabrum* Retz. (Image 3) are given in Table 1.

DISCUSSION

Although, *Heliotropium rottleri* Lehm. (Image 1) resembles *H. marifolium* J.Koenig ex Retz. (Image 2) and *H. scabrum* Retz. (Image 3) in flower, bracts and appressed hairs its habitat and habit differ. *Heliotropium rottleri* typically inhabits marginal waste lands in the plains with grassy rocky and calcareous soils; usually grows gregariously reaching up to 30 cm height and easily recognized by its whitish round growth habit. *Heliotropium marifolium* ssp. *wallichii* is a perennial

Image 4. *Heliotropium marifolium* Retz. subsp. *rottleri.* © Kader, S.A.

decumbent plant with, divaricate straight slender fleshy branches, flat leaf margins and flowers in forked little curved spikes as shown in Image 2, while *H. scabrum* is an annual procumbent plant with twiggy branches, revolute-margined leaves and flowers in sub-capitate inflorescence among leaf-like bracts as shown in Image 3. Furthermore, *H. marifolium* var. *rottleri* is a different plant (Image 4). Despite, Ancy et al. (2024) have recently published *H. rottleri* Lehm. as *Euploca wightiana*, we have already reported *H. rottleri* Lehm. from Coimbatore and Thiruppur districts and its conservation status (Kader & Akram 2020; Kader & Gopal 2022).

According to Wight (1850), and Ahmedullah & Nayar (1987), *H. rottleri* is found only in Coimbatore region of Tamil Nadu, in India. Our study also supports the views of earlier reports that *H. rottleri* Lehm. is strictly confined to Coimbatore and Thiruppur districts. So far, no efforts have been taken to assess its distribution, population size and conservation. Our study based on survey indicates that it is Critically Endangered as the habitats are very rapidly altered and changing due to real estate business (one habitat is located along the National Highway and the other near main road). Populations in one location is declined to 50% and have been extirpated in other location within three years (between 2021 and 2023). The present population is of less than 500 mature individuals, of which about 100 individuals are restricted in a very small area (less than 1 km^2) at Raavanapuram. Data collected during the present study indicates that *Heliotropium rottleri* fulfils the necessary criteria (Appendix 1) to place it in the 'Critically Endangered' category as it faces a high risk of extinction. Therefore, it is strongly suggested here that implementation of effective in situ conservation measures are necessary to prevent this habitat-specific narrowly endemic species from extinction. We appeal the competent authorities to intervene and take immediate action to conserve this species in its natural habitat.

CONCLUSION

The present study revealed that *Heliotropium rottleri* Lehm., *Euploca marifolium* (J. Koenig ex Retz.) Ancy and P. Javad, *E. marifolia* var. *rottleri* (Lehm.) Ancy and P.Javad, and *H. scabrum* Retz. are different species and all occur in Tamil Nadu. Furthermore, *H. rottleri* Lehm. occur only in Coimbatore and Thiruppur districts of Tamil Nadu, and its reports of occurrence in other southern Indian states are based on misidentification. Finally, recently published *Euploca wightiana* Ancy et al., from Coimbatore is relegated as a synonym under *H. rottleri* Lehm.

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Appendix 1. IUCN Red List Assessment: *Heliotropium rottleri* Lehm.

Kingdom: Plantae
Division: Tracheophyta
Class: Dicots
Order: Boraginales
Family: Heliotropiaceae
Genus: *Heliotropium*
Species: *rottleri*
Authority: Lehmann, J.G.C.

Common name: Nil

Taxonomic notes: The species was described by Lehmann in 1818 from India without specifying precise locality and published in *'Plantae e Familiae Asperifoliarum Nuciferae'*. Later, Wight (1850) had collected this species from Coimbatore and provided a description and an illustration. It is a low erect perennial plant reaching only about 30 cm height, having characteristic divaricate curved stiff branches covered with whitish hairs and small ovate-lanceolate very shortly-petioled revolute-margined leaves.

ASSESSMENT INFORMATION

Red List Category and Criteria (Version 3.1): Critically Endangered B1ab(iii)+2ab(iii)
Justification: *Heliotropium rottleri* Lehm. is assessed as Critically Endangered as it is restricted only with the extent of occurrence less than 50 km^2 area. In 2006, only two mature individuals per hectare were recorded in Masagoundanputhur; in 2020, more than 250 individuals per hectare area was recorded in Raavanapuram, and about 25 individuals per acre in Kamanayakkan Palayam. But in 2022 no individuals was recorded in Masagoundanputhur; less than less than 100 individuals were recorded in Raavanapuram as the locality was converted into industrial purpose; and in Kamanayakkan Palayam site was completely used for house construction.

GEOGRAPHIC RANGE / DISTRIBUTION INFORMATION

Range description: The species is restricted to Coimbatore and Thiruppur Districts of Tami Nadu, India.
Countries of occurrence: Native to India (Tamil Nadu State).
Extent of Occurrence (EOO): EOO is approximately 1 km^2 area considering the present population at Raavanapuram. The present population is at about 60 km away from the type locality.
Area of Occupancy (AOO): AOO is 1,143 km^2.
Number of locations: The species is currently restricted to four locations. The species is extirpated from its type locality and other two locations. No other populations have been observed until now.

POPULATION INFORMATION

Population: The species is estimated to have less than 500 mature individuals, of which about 100 individuals are restricted to Raavanapuram.
Population trend: The population appears to be declining at present. Over the last three years road widening, industrial development, real estate business and house constructions have caused severe damage to the population. Populations in one location is declined to 50% and have been extirpated in other location.

HABITAT AND ECOLOGICAL INFORMATION

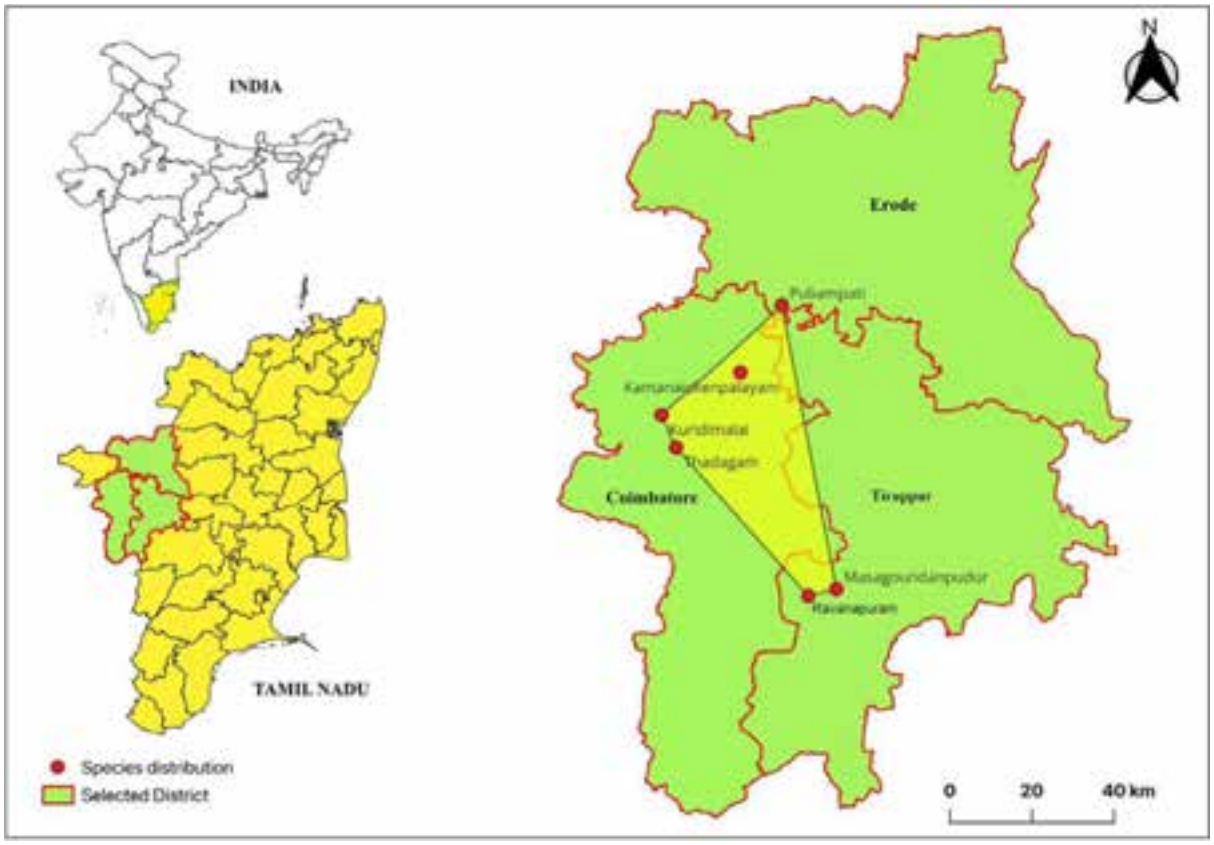
Habitat and ecology: Marginal fallow lands in the plains with grassy, rocky, and calcareous soils. The habitat is shared by *Acacia leucophlea* (Roxb.) Willd., *Abutilon pannosum* (G.Forst.) Schltdl., *Azadirachta indica* A.Juss., *Barleria buxifolia* L., *B. cuspidata* F.Heyne ex Nees, *Dichrostachys cinerea* (L.) Wight & Arn., *Parthenium hysterophorus* L., *Passiflora foetida* L., *Pergularia daemia* (Forssk.) Chiov., *Prosopis juliflora* (Sw.) DC., *Senna auriculata* (L.) Roxb., and grasses.

INFORMATION ON THREAT

Threats: The main threats to the remaining population are road widening and developmental activities. The present available population is near the highway and thus the road widening can cause severe damage to the population.
Additional threats: Real estate business and other developmental activities.

USE AND TRADE INFORMATION

Use: Local people do not collect the species as there is no usage value for the plant.
Livelihoods and sustenance: Communities are not dependent on this species for their livelihoods or sustenance.
Trend in off take from the wild: Not yet observed.
Trend in off take from cultivation: It is not cultivated.
Commercial value: The species has no known local, domestic, national or international commercial value.



INFORMATION ON CONSERVATION ACTIONS

Conservation actions: Until now no actions.
Research in Place: There is no systematic research in place other than causal surveys.
Research needed: Systematic surveys, monitoring, propagation studies, effects of threats on population, and in situ conservation.
Monitoring in place: There is no monitoring off the species, population or habitat in place.
Monitoring needed: Population and site monitoring is essential and must be implemented at the earliest.
Education in place: No formal or informal education about the species is in place.
Education needed: Outreach programmes about the species to local communities and forest department are crucial.

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OPEN ACCESS

SHORT COMMUNICATION

First post-tsunami report of Coconut Crab *Birgus latro* (Linnaeus, 1767) (Malacostraca: Decapoda: Coenobitidae) in Car Nicobar Island, Nicobar Archipelago

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Abstract: Remote islands in the Indo-Pacific are crucial habitats for *Birgus latro*, the world's largest terrestrial arthropod. The 2004 tsunami severely impacted its populations in the Nicobar Archipelago, with no sightings on Car Nicobar Island. This study documents the first post-tsunami opportunistic record, highlighting the species' resilience and extending its northernmost distribution in the Nicobar group of islands.

Keywords: Andaman & Nicobar Islands, Anomura, coastal ecosystem, crustacean, distribution, habitat loss, island biodiversity, recovery, tropical ecology.

The Coconut Crab *Birgus latro* (Linnaeus, 1767) is the largest land arthropod and one of the decapod crustaceans, most adapted to terrestrial life (Lavery et al. 1996). With a leg span reaching up to 1 m and a weight of up to 5 kg, it can live as long as 60 years (Sato & Yoseda 2013; Cumberlidge et al. 2022). These crabs possess a highly developed olfactory sense (Stensmyr et al. 2005) and are opportunistic scavengers, feeding on a wide variety of foods, including fallen fruits & nuts of Pandanas, Barringtonia, Areca Nut, Coconut, and animal remains (Reyne 1939; Daniel & Premkumar 1968). The IUCN Red List status of B. latro was updated from 'Data Deficient' to 'Vulnerable', based on its distribution, threats, and population trends (Cumberlidge et al. 2022). In India, it is protected under the Schedule I–A of the Wildlife Protection Act, 1972.

Birgus latro is distributed widely across the Indo-Pacific region, particularly inhabiting the remote rocky shores of small oceanic islands and atolls. Within the Andaman & Nicobar Islands, it is primarily found throughout the Nicobar Archipelago and on a few islands in the Andaman Archipelago (Hume 1874; Alcock 1905; Daniel & Premkumar 1968; Sivaperuman et al. 2023). In the Nicobar group of islands, it has been documented from Great Nicobar, Little Nicobar, Kamorta, Nancowry, Katchal, Teressa, Chowra, Trinket, Bambokka, Tillangchong, and several smaller islands (Alcock 1905; Daniel & Premkumar 1968; Sankaran et al. 2005; Sivakumar 2010; Patankar & D'souza 2012; Zaibin et al. 2012; Sivaperuman et al. 2023) (Image 1). In Car Nicobar, the northernmost island in the Nicobar Archipelago, its presence was first documented by Hume (1874), and no further report of this species is available (Patankar & D'souza 2012; Sivaperuman et al. 2023).

Coconut Crabs in coastal habitats are vulnerable to habitat loss from anthropogenic activities and geological events, such as tsunamis (Sivakumar 2010; Caro et al. 2021). The 2004 Indian Ocean tsunami extensively

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Competing interests: The authors declare no competing interests.

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devastated coastal habitats across the Andaman & Nicobar Archipelago (Sankaran et al. 2005). A rapid post-tsunami wildlife assessment provided an overview of the surviving coastal flora and fauna, with preliminary observations on the distribution of *B. latro* (Sankaran et al. 2005; Sivakumar 2010). Post-tsunami records from Kamorta, Menchal, and Great Nicobar confirmed the continued presence of *B. latro* in the Nicobar Islands (Patankar 2007). Although Hume's (1874) record is the only documented pre-tsunami report of *B. latro* from Car Nicobar Island, no post-tsunami records have been documented. Efforts to locate Coconut Crabs during the post-tsunami surveys on Car Nicobar were unsuccessful (Patankar & D'souza 2012), which may have led to the exclusion of this island in the subsequent study by Sivaperuman et al. (2023).

This paper presents the first post-tsunami incidental sighting of *B. latro* on Car Nicobar Island, thereby providing an updated distributional record. On 13 April 2024, a single *B. latro* specimen was observed (Geographical coordinates: 9.1640 ^{0}N, 92.7990 ^{0}E) and photographed from Car Nicobar Island (Images 1 & 2). The absence of pleopods identified the specimen as male (carapace length: 121 mm; cephalothoracic width: 52 mm), weighing approximately 1,220 g. Locally known as 'Nyioṅv' (/nju:/) in 'Pu' (Car Nicobar), a dialect of the Nicobarese language. The Coconut Crab was found in a subsurface limestone karst, a small cave located 3.36 km inland from the east coast of Car Nicobar (Image 1). The presence of coconut fruit and anthropogenic litter inside (the cave is a recreation spot, resulting in the spillover of food waste) the cave may have attracted the Coconut Crab (Image 2). Discarded food packaging and plastic waste pose significant hazards, as crabs can become

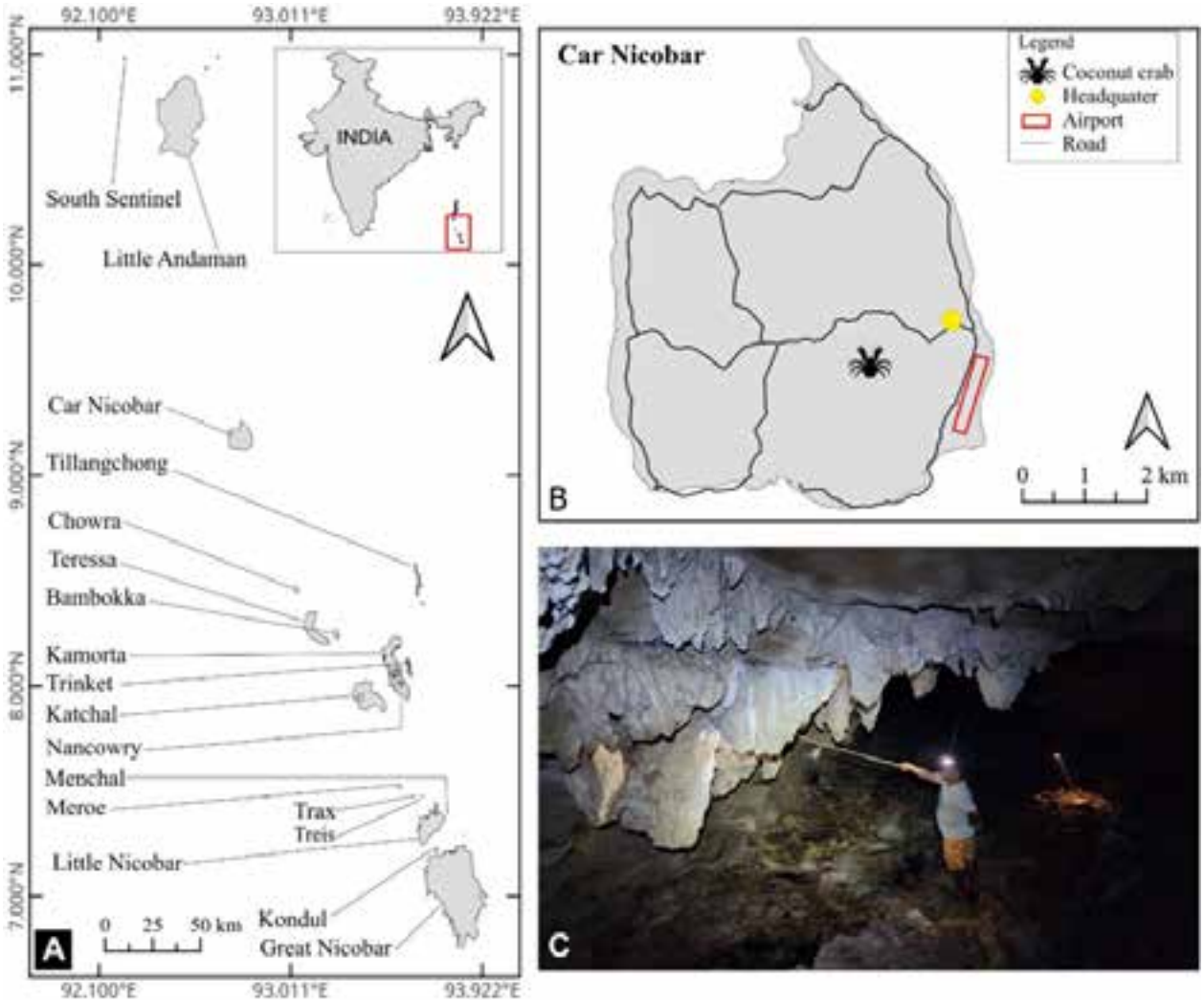

Image 1. Study area map: A—reported geographic distribution of Coconut Crab *Birgus latro* from the Andaman & Nicobar Islands | B—Car Nicobar Island | C—limestone karst. © Dhanesh Ponnu.

Table 1. Pre- and post-tsunami distributional records of Coconut Crabs *Birgus latro* from the Andaman & Nicobar Islands. (+)—present as per local ecological knowledge of Nicobari Tribe | ( )—not been surveyed yet | (-)—surveyed but not found | *—Distribution referring to Nicobar Islands without specifying a particular island.

Island	Island subgroup	Island	Observations	Reference	
				Pre-tsunami records	Post-tsunami records
Andaman group	South Andaman	South Sentinel	1905, 1976	Alcock 1905; Altevogt & Davis 1976	( )
		Little Andaman	1905, 2015–2018	Alcock 1905	Sivaperuman et al. 2023
Nicobar group	Car Nicobar	Car Nicobar	1874	Hume 1874	(**This study**) (-) Patankar & D'souza 2012
	Nancowry	Teressa	2015–2018	(+)	Sivaperuman et al. 2023
		Chowra	2011	(+)	Zaibin et al. 2012
		Bambokka	2015–2018	(+)	Sivaperuman et al. 2023
		Kamorta	2015–2018	(+)	Patankar 2007; Sivaperuman et al. 2023
		Trinket		(+)	( )
		Katchal	2006, 2015–2018	(+)	Sivakumar 2010; Sivaperuman et al. 2023
		Nancowry	2015–2018	(+)	Sivaperuman et al. 2023
		Tillangchong	2005, 2006, 2015–2018	(+)	Sankaran et al. 2005; Sivakumar 2010
	Great Nicobar	Little Nicobar	2015–2018	(+)	Sivaperuman et al. 2023
		Menchal	2005, 2006, 2008, 2015–2018	Altevogt & Davis 1975	Sankaran et al. 2005; Sivakumar 2010; Sivaperuman et al. 2023; Patankar 2007; Patankar & D'souza 2012
		Meroe	1975, 2015–2018	Altevogt & Davis 1975	Sivaperuman et al. 2023
		Treis	2005	(+)	Sankaran et al. 2005
		Trax	2005	(+)	Sankaran et al. 2005
		Kondul	2015–2018	( )	(-) Sivaperuman et al. 2023
		Great Nicobar	1966, 2007	Daniel & Premkumar 1968	Patankar 2007
			1932	*Man 1932	

entangled or ingest harmful materials. As adult *B. latro* individuals prefer residing inland or the interior forest (Sato & Yoseda 2013), it becomes crucial to include interior forests and inland caves in survey efforts. Surveying these inland populations remains challenging due to the cryptic nature and nocturnal behaviour of the species. Moreover, *B. latro* appears to persist in other areas of Nicobar Islands where earlier surveys, such as Patankar & D'souza (2012) and Sivaperuman et al. (2023), were unsuccessful in detecting its presence. Pre- and post-tsunami records of *B. latro* from the Andaman & Nicobar Islands have indicated the reduction in its distribution range (Table 1). Incidental sightings like this can guide surveys in regions such as Trinket, Kondul, and Chowra, previously thought to be locally extirpated (Sivaperuman et al. 2023; Patankar & D'souza 2012). These regions require additional systematic monitoring of Coconut Crabs to better understand their distribution. Moreover, events like the tsunami may significantly impact its home range, population recovery, genetic diversity, and migration pattern.

For species such as Coconut Crabs, which are rare, elusive, or in challenging environments, incidental sightings provide valuable insights into the species' presence, behaviour, or habitat preferences that might otherwise remain undocumented. This record highlights the importance of surveying interior forests, particularly areas with geological structures like caves, for detecting coconut crabs. This study extends the northernmost recorded distribution of *B. latro* within the Nicobar Archipelago, highlighting the importance of Car Nicobar as a key site for future survey efforts.

Image 2. A—photograph of the Coconut Crab *Birgus latro* | B—Coconut Crab climbing on cave wall | C—pile of sprouting and decaying Coconut *Cocos nucifera* | D—plastic wrapper (packaged food item) found in cave. © Dhanesh Ponnu.

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SHORT COMMUNICATION

Notes on *Garcinia kydia* Roxburghii (Clusiaceae): a lesser known medicinal plant species along the foothills of Arunachal Pradesh, India

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Abstract: *Garcinia kydia* Roxb., a lesser-known medicinal tree species has been documented from the foothills of Arunachal Pradesh, India. Fruit of the species is used against dysentery and diarrhea by the Adi and Mishing tribes of East Siang District. The species has close similarity with *G. cowa* however, can be distinguished from its female inflorescence, and shape of the fruit. In several taxonomic literatures and herbarium collections, the species has been wrongly described. The present study focused on the detailed morphological, taxonomic description, and other relevant information to help apt identification of the species.

Keywords: Distribution, documentation, East Siang, identification, inflorescence, medicine, morphology, northeastern India, taxonomic description.

Garcinia L. is the largest genus of the family Clusiaceae (Guttiferae) (Cox 1976), distributed in the tropical regions of Asia, Africa, and Polynesia (Ridley 1922; Whitmore 1973). A total of 450 species have been reported which are distributed in tropical and southern Africa, Madagascar, tropical Asia, northeastern Australia, western Polynesia, and tropical America (Xiwen et al. 2007). Occurrence of 20 species of the genus has been reported from China having 13 endemic and one introduced species (Xiwen et al. 2007). The distribution of 35 species of the genus has been reported from India, among which 15 species are distributed in northeastern India (Maheshwari 1964).

During field investigations in May 2016 of Pasighat area, East Siang District, Arunachal Pradesh, India, the authors collected specimens of plant with light yellow flowers which differ morphologically from any described *Garcinia* species. The species is distributed along the foothills of the district. After a detailed examination of taxonomic literature and protologue description, the collected specimen was identified as *Garcinia kydia* Roxb. The species was discovered by Colonel Alexander Kyd and had been reported to be native of the Andaman Islands. In 1794, the species was introduced in the Botanic Garden in Calcutta by Colonel Alexander Kyd. The species *Garcinia kydia* has close affinity with *G. cowa* but can be distinguished from its female inflorescence and shape of the fruit (Roxburgh 1824). The species had also been described in the Flora of Assam, however, considered inferior in quality to that of *Garcinia cowa* Roxb. ex Choisy (Kanjilal et al. 1934). The species is fairly distributed in upper Assam up to an altitude of 600 m and often cultivated in homesteads for its acid fruit. The fruit is used to cure dysentery and also applied externally in persistent cases of headache (Kanjilal et al. 1934). The new distribution of the species has been documented

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Acknowledgements: Authors are thankful to the local inhabitants residing in the foothills region of East Siang District (Ruksin Circle) of Arunachal Pradesh and Dhemaji district (Jonai Subdivision) of Assam for sharing their valuable information on the traditional utilization of the species. We acknowledge the Centre with Potential for Excellence in Biodiversity-II (Department of Botany), Rajiv Gandhi University for extending financial support to carry out the research work.

from the foothills of Arunachal Pradesh, eastern Himalaya. Maheshwari (1964) studied the taxonomy of the genus *Garcinia* and described the distinguished character of both the *G. kydia* and *G. cowa*. In the present study, an attempt has been made to describe the species with its detailed taxonomic characterization and compare its distinguishing characters with *G. kydia* reported by Parthasarathy & Nandakishore (2014) (Table 1). The detailed morphological and taxonomic characters with other relevant information have been provided for apt identification of the species. The voucher specimen of the species was deposited in the herbarium of the Botany Department of Rajiv Gandhi University, Arunachal Pradesh, India.

Garcinia kydia Roxb., Fl. Ind. 2: 623. 1832; Parkinson, For. Fl. Andamans 90. 1923. *G. cowa* T. Anderson, in Fl. Brit. India 1: 262.1874, p.p. non Roxb. ex DC. 1824. Kanjilal et al. in Fl. of Assam 1(1) 105-106. 1934. Maheshwari in Bull. Bot. Surv. India 6 (2-4). 1964.

Description

Tree, dioecious; 10–18 m tall, elegant with a narrow crown; wood white, bark blackish brown, rough, yellow exudates which hardens into a gum, branchlets glabrous, more or less terete, often drooping, dark coloured when dry. Leaves 8–13 x 3–5 cm, ovate, oblong to lanceolate, acute at base, acuminate at apex, thinly coriaceous, glabrous, shiny, lateral veins, thin, but distinct when dry, slender, rather irregular, ca. 12 pairs with few intermediate ones, all arched to form an intra marginal vein; petiole 1–1.5 cm long, slightly dilated at base. Male flowers not observed. Female flowers: solitary, axillary and terminal, sessile. Sepals 4, 0.15–0.3 cm long, unequal, fleshy, greenish-yellow. Petals 4, light yellow 0.9–1.0 cm long, ovate, concave. Staminodes 4–6, small, alternate with petals. Ovary sessile, 5–6 lobed, 5–6 locular, fleshy; stigma subsessile, style fused. Berries 3.5–4 cm in diameter, globose, apex depressed with a nipple-like protuberance crowned with the stigma, dark green, 5–6 seeded. Seeds slightly curved with protrusion, yellow; 2.4–2.7 cm long; aril soft, acidic, juicy.

Flowering: April–May; Fruiting: May–July.

Ecology: Growing in a humid, shady area within an elevation of 300–600 m.

Distribution: India (Assam, foothills of Arunachal Pradesh, Meghalaya, Andaman & Nicobar Islands), Bangladesh, Myanmar and Malaysia (Sharma & Sanjappa 1993).

Exsiccatae: India, foothills of Arunachal Pradesh between East Siang and Dhemaji District of Assam, 300–600 m, 11.vi.2016, coll. Gaottham Gogoi, #56 (RGU).

Note

Garcinia kydia is a lesser known medicinal tree species distributed in the foothills of East Siang District of Arunachal Pradesh and Dhemaji District of Assam, India. Fruit of the species is used to cure dysentery and diarrhea by the Adi and Mishing tribes residing along the foothills of the East Siang District of Arunachal Pradesh. The taxonomic characterization of the genus *Garcinia* L. was carried out by Parthasarathy & Nandakishore (2014), however, the *Garcinia kydia* was wrongly described. They described the shape of the fruit as ovoid, oblique

Table 1. Morphological comparison of *Garcinia kydia* recorded from the foothills of East Siang District of Arunachal Pradesh.

Characters	***Garcinia kydia*** (as per Parthasarathy & Nandakishore 2014)	***Garcinia kydia*** (present findings)
Bark	Dark-brown	Blackish-brown
Latex	No record	Yellow
Branchlets	Horizontal but usually distally pendulous, slender, striate	Branchlets glabrous, more or less terete
Leaf texture	Coriaceous glabrous	Thinly coriaceous, glabrous, shiny
Leaf shape	Lanceolate or oblong-lanceolate	Ovate, oblong to lanceolate
Leaf size	6–14 × 2–5 cm	8–13 x 3–5 cm
Leaf apex	Acuminate or long acuminate	Acuminate
Female flower	Solitary	Solitary, axillary and terminal, sessile
Ovary	Subglobose; 3–5 celled	Sessile, 5–6 lobed, 5–6 locular
Fruit	Ovoid, oblique	Globose, apex depressed with a nipple like protuberance crowned with the stigma
Fruit colour	Yellow-brown	Shiny, greenish, orange-yellow when ripe
Seeds per fruit	2–4	5–6
Seed shape	Slightly curved with protrusion	Slightly curved with protrusion

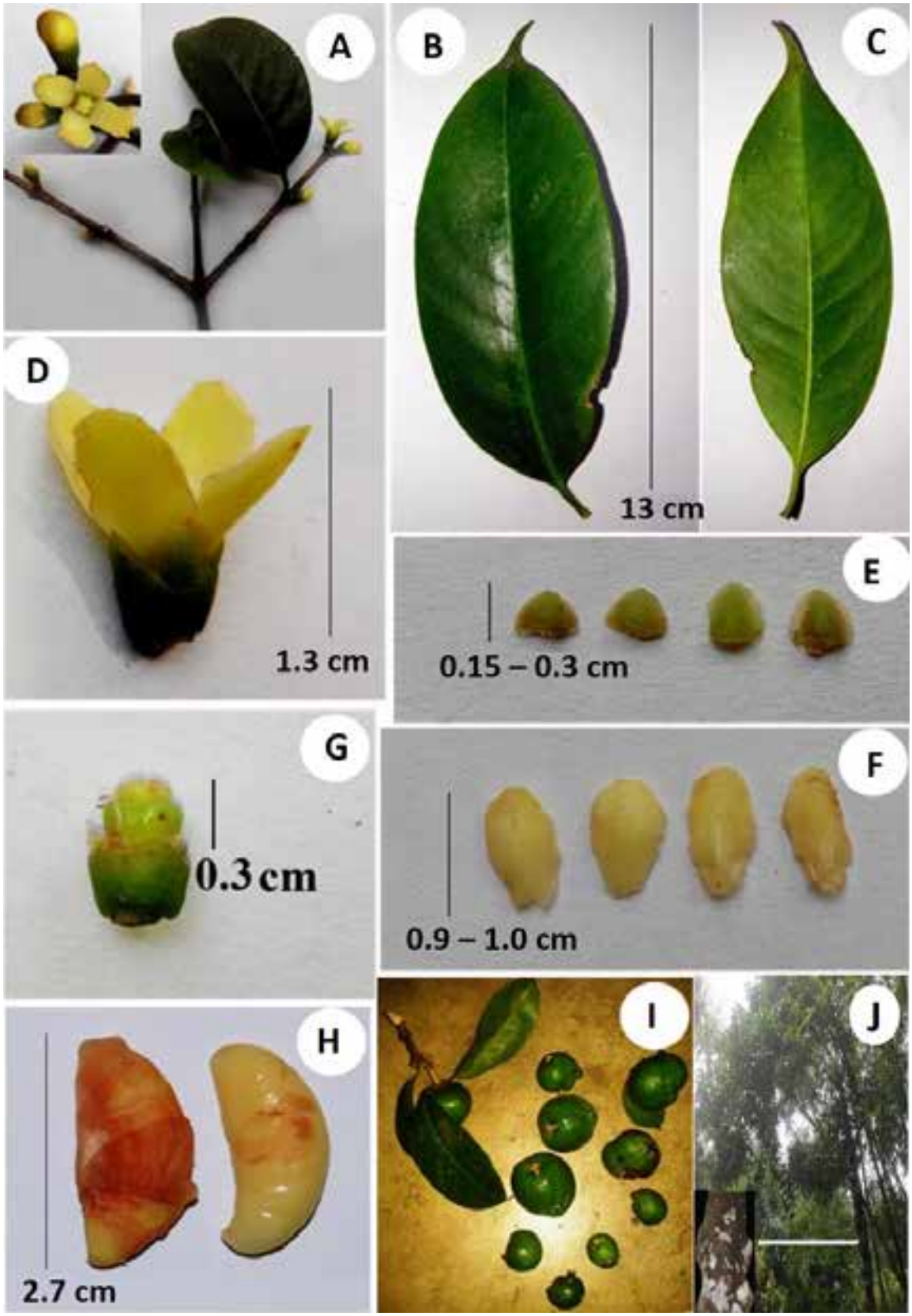

Image 1. Taxonomic characteristics of *Garcinia kydia*: A—Twig with female flower | B—Adaxial side of leaf | C—Abaxial side of leaf | D—A complete female flower | E—Sepals | F—Petals | G—Ovary | H—Seeds | I—Mature fruits | J—Mature tree (arrow showing close view of bark). © Gaottham Gogoi.

whereas in the present study, globose shaped fruit with depressed apex and a nipple like protuberance crowned with the stigma has been observed. The present description corresponds to that described by Roxburgh (1824) (Image 1). The detailed distinguishing morphological characters of *G. kydia* (Parthasarathy & Nandakishore 2014) and *G. kydia* (present findings) are presented in Table 1.

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NOTE

First photographic evidence and record of the Indian Pangolin *Manis crassicaudata* (Mammalia: Pholidota: Manidae) from Rajkot, Gujarat, India

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The eight species of pangolins distributed across Africa and Asia constitute the order Pholidota and the single family, Manidae. All pangolin species have experienced a drastic decline in populations, primarily because of hunting and illegal international trade in wild caught pangolins and are at the edge of extinction in Asia (Heinrich et al. 2016). In India, there are two species: the Indian Pangolin *Manis crassicaudata*, which is relatively widely distributed throughout much of the country, extending from the southern part of the Himalaya and into southern Nepal, Bangladesh, Pakistan, and Sri Lanka (Tikader 1983; Srinivasulu & Srinivasulu 2012; Mahmood et al. 2020; Aditya et al. 2021) and the Chinese Pangolin *Manis pentadactylus*, primarily found in the northeastern states of India and in Nepal (Srinivasulu & Srinivasulu 2012; Challender et al. 2019). Although these two pangolin species appear similar, they can be distinguished by their scale characteristics. The Indian Pangolin has larger scales and 11–13 rows of scales along its back, while the Chinese Pangolin has smaller scales and 15–18 rows across its back (Pocock 1924).

Indian Pangolins occur in a wide range of habitats across the Indian subcontinent, including both forested and non-forested areas (Roberts 1977; Mahmood et al. 2020). The Indian Pangolin is protected under Appendix I of the Convention on International Trade in Endangered Species (CITES) and Schedule I in the Wildlife (Protection) Act 1972; it is currently classified as 'Endangered' on the IUCN Red List of Threatened Species due to a rapid decline in its population because of hunting & wildlife trade, habitat loss, mortalities from electric fences, and human wildlife interactions (Mahmood et al. 2020). Pangolins exist in very low densities; few published studies measuring densities for different species of pangolins indicate densities of 0.0001 individuals per km^2 for the Indian Pangolin, and 0.001 individuals per km^2 for the Chinese Pangolin. The Indian Pangolin is nocturnal, sleeping in burrows throughout the day.

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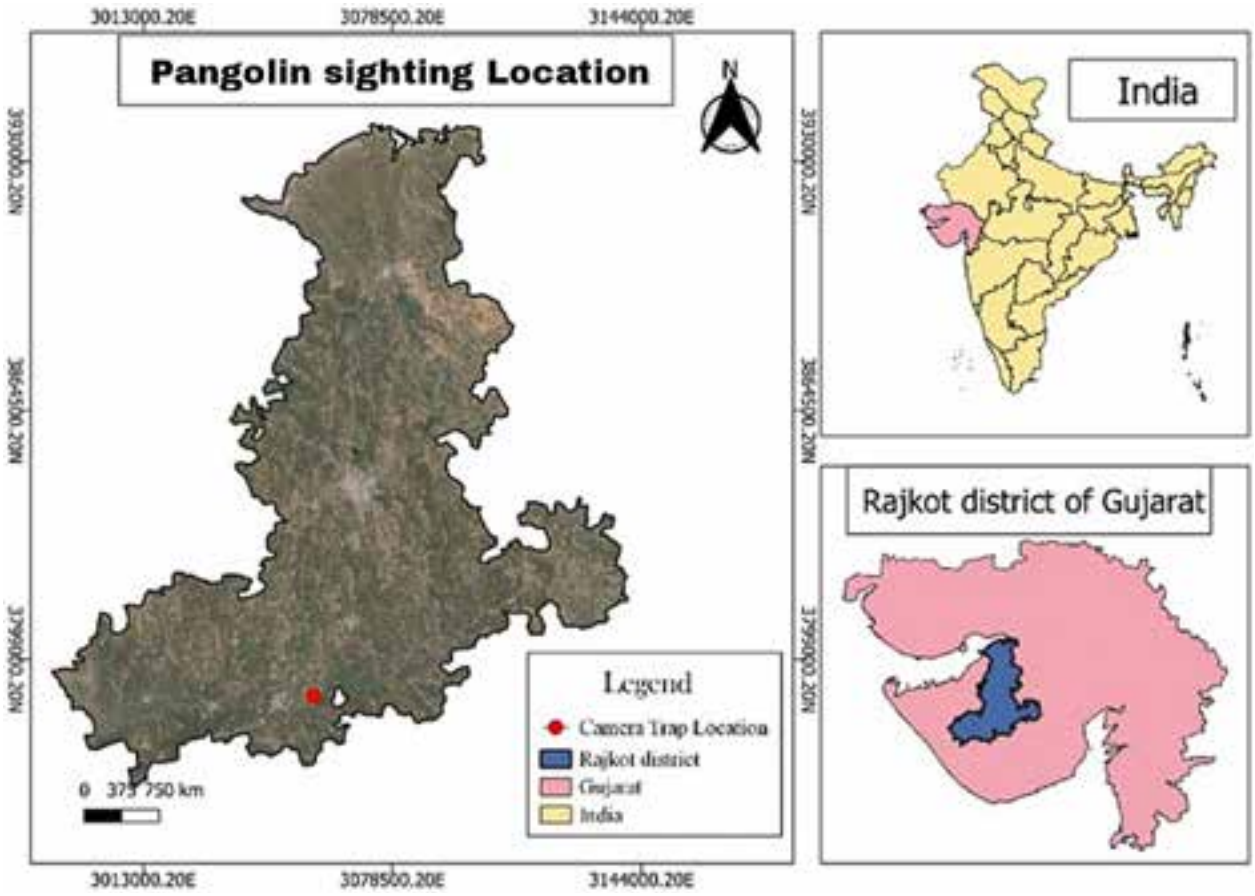

Image 1. Location of the Indian Pangolin recorded from Rajkot District, Gujarat, India.

Image 2. Camera trap record of Indian Pangolin *Manis crassicaudata* from Rajkot District, India.

Indian Pangolins have been seen to burrow in two types: feeding burrows and living burrows (Mahmood et al. 2020).

Pangolin scales are used as a whole, or in powdered form in the preparation of traditional medicines in east Asia, mainly China and Vietnam (Baillie et al. 2014; Mohapatra et al. 2015; Challender & Waterman 2017 Mahmood et al. 2018). Indian Pangolins in their habitat are often killed due to the belief that they dig up graves and pull out the buried dead bodies. In addition, farmers kill the animals allegedly for damaging their crops and agricultural lands by digging burrows (Mahmod et al. 2018). Two camera traps were strategically deployed at random locations informed by local knowledge provided by shepherds who routinely bring their livestock to a nearby water source. The selected area also comprised agricultural land in proximity to hill slopes. The camera traps were installed approximately six meters apart. Notably, one of the camera traps successfully documented the presence of a pangolin (*Manis* sp.), indicating its occurrence in the study area. A record from one of the camera traps of the pangolin was obtained as part of a camera trapping study that is being undertaken currently by the authors to inventory the mammal community and its diversity patterns across the Rajkot District of Gujarat (22.303^{0}N, 70.802^{0}E) (Images 1 & 2). The dominant forest type in this area is tropical dry deciduous and scrub forests (Champion & Seth 1968). Passive digital infra-red camera traps (Trail Cam and Bushnell 8 mp, Scout Guard 20 mp) were set across habitat types in the study area. The species was identified using a field identification guide for mammals (Menon & Daniel 2003).

The presence of the Indian Pangolin indicates the possibility of the persistence of the population in the region. Pangolins rely on relatively undisturbed natural habitats for food, shelter, and breeding grounds. Further research into their distribution could help reveal more information about their habitat selection and ecology in this region.

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NOTE

A rare Long-eared Owl *Asio otus* sighted after a gap of 22 years in Al Wathba Wetland Reserve in Abu Dhabi, UAE

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A total of nine species of owl have been recorded in the UAE, out of which three are migrants (Majeed & Al Hamoudi 2021). An adult Long-eared Owl was found and photographed on 1 January 2022 in Al Wathba Wetland Reserve (AWWR) where it was roosting on a branch of *Casuarina* tree (Image 1) on the northern side of the Reserve. The bird was recorded early morning at around 0630 h. The previous day (31 December 2021) areas in and around AWWR experienced very high winds early morning and heavy rains throughout the day. The photograph was taken from 10 m distance from the ground and the bird flew away instantly. The previous record of the species was reported on 14 October 1999 from the Reserve (Table 1).

Long-eared Owl is a highly migratory species with an extremely wide distribution range in Eurasia. Outside Europe, they are found as breeding species in Turkey, northernmost Syria, Israel, and Lebanon (Jiguet & Audevard 2017; Birdlife International 2018). Breeding has also been confirmed from northwestern part of Iran (Tohidifar et al. 2011). In UAE, the species is a vagrant and extremely rare. Between 1971 to 2013, nearly 20 individuals of Long-eared Owl have been sighted in different years from the parks, inland wetlands, golf courses, and some offshore islands of UAE (Pedersen et al. 2021). Global population trend of the species is decreasing; however, it is listed as 'Least Concern' (LC) by the IUCN Red List of Threatened Species. Since 2013, the species has not been documented from the entire country of UAE (Pedersen et al. 2021). Most of the individuals were recorded from the Emirate of Abu Dhabi, Dubai, and Sharjah during the winter months between October and March (Table 1).

About Al Wathba Wetland Reserve

Al Wathba Wetland Reserve is located 40 km south-east of Abu Dhabi city and covers an area of 5 km². It is the first declared protected area in the Emirate of Abu Dhabi, and this site has been under the management of the Environment Agency—Abu Dhabi (EAD) since 1998. In 2013, it was recognised as a Wetland of International Importance under the Ramsar Convention. Furthermore, in 2018, the wetland was listed in the IUCN Green List of Protected and Conserved Areas by UNEP.

AWWR is the most important site for migratory waterfowls, waders, diurnal birds of prey and owls, the higher number and diversity of birds are recorded from late August–Early May, whereas the peak numbers occur in mid-winter (Campbell et al. 2019; Soorae et al. 2019). EAD has been regularly monitoring this site for bird diversity for nearly 18 years, and to date 262 species of birds have been recorded in the reserve, which makes up about 61% of the total bird species documented from the entire Emirate of Abu Dhabi. Other owl species recorded from the reserve include Little Owl *Athene noctua* and Western Barn Owl *Tyto alba* (Soorae et al. 2019).

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Table 1. Previous sighting of Long-eared Owl *Asio otus* in UAE (Pedersen et al. 2021).

Sighting records	Year	Month	No. individuals	Location	Emirate
1	1971	Jan	1	Sharjah City	Sharjah
2	1978	Mar	1	Khor Fakkan	Sharjah
3	1979	Oct	1	Port Rashid	Dubai
4	1991	Dec	1	Das Island	Abu Dhabi
5	1994	Nov	1	Das Island	Abu Dhabi
6	1996	Nov	1	Jebel Ali Hotel and Golf Resort	Dubai
7	1998	Dec	1	Das Island (Dead one)	Abu Dhabi
8	1999	Oct	1	Al Wathba Wetland Reserve	Abu Dhabi
9	2001	Apr	1	Das Island	Abu Dhabi
10	2003	Feb–Mar	4	Mushrif National Park	Dubai
11	2003–2004	Dec–Mar	2	Mushrif National Park	Dubai
12	2004–2005	Dec–Jan	2	Mushrif National Park	Dubai
13	2006	Nov	1	Khalidia*	
14	2011–2012	Dec–Jan	1	Mushrif National Park	Dubai
15	2013	Jan	1	Mushrif National Park	Umm al-Qaiwain
16	2022	Jan	1	Al Wathba Wetland Reserve	Abu Dhabi

*—Exact emirate of sighting cannot be determined.

Image 1. An adult Long-eared Owl *Asio otus* roosting on a tree in Al Wathba Wetland Reserve. © Muhammad Maqsood.

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