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Cover: Nile Crocodile *Crocodylus niloticus* regulating body temperature on a warm day. Digital art on Procreate by © Aakanksha Komanduri.

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ARTICLE

Asiatic Elephant conservation as a driver of forest carbon stock stabilization and avoided degradation in India

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Abstract: The conservation of the Asiatic Elephant *Elephas maximus indicus* serves a dual purpose: it maintains forest integrity and indirectly enhances carbon sequestration in the tropical ecosystems. This study assesses the relationship between elephant conservation efforts, the expansion of elephant reserves (ERs), and wildlife-associated carbon stock enhancement in India from 1992–2025. The present study employed an IPCC Tier-2–aligned model to estimate total carbon stock changes. This model integrated three primary data streams: elephant census data; the temporal expansion of ER surface area; and land use land cover-based carbon densities. Between 1992 and 2025, the ER network expanded from 18,297 km² to 80,777 km². This expansion coincided with a modest 6.7% increase in elephant population. The estimated total carbon stock within ER landscapes increased by 38%; however, this increase primarily reflects enhanced protection and reduced degradation of pre-existing forest carbon stocks, rather than newly generated biomass. Area-based protection accounted for ~95% of the observed change, while direct faunal biomass contribution remained limited. These findings highlight the role of conservation-driven land-use stabilization in supporting climate mitigation, while emphasizing the need for cautious interpretation of wildlife–carbon relationships.

Keywords: Biodiversity, carbon sequestration, climate mitigation, ecosystem engineering, elephant reserves, forest conservation, habitat, population, land-use stabilization, megaherbivores, nature-based solutions, protected areas, redd+, wildlife.

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Author contribution: Tarun kathula conceived the study, collected and analyzed data, and prepared the manuscript and critically revised the manuscript.. Tanu jindal supervised the study, contributed to interpretation of results. final review of manuscript.

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Introduction

Historically, elephants in India were widely distributed across vast Indian landscapes, thriving in harmony with minimal human interference. Their numbers remained stable before the 20th Century, supported by abundant habitats and deep cultural reverence (Chaudhary et al. 2024). However, the early 1900s marked a turning point, as rapid fragmentation of habitats due to developmental activities, agricultural expansion, and large-scale hunting for ivory caused a drastic decline in elephant populations. The growing use of elephants in the timber and military sectors further accelerated this reduction. By the mid-20th century, the decline was evident across many regions, prompting the government to adopt conservation measures in the 1970s and 1980s (Chami et al. 2020). The introduction of wildlife protection laws and the launch of Project Elephant in 1992 marked significant milestones, leading to a gradual recovery in population. Since 2001, elephant reserves have been declared. During the 1990s and 2000s, India's elephant numbers stabilized between 20,000 and 27,000, supported by the creation of elephant reserves (ER) and elephant corridors, even as habitat fragmentation and human-elephant negative interactions continued to rise. The 2017 census estimated 27,312 elephants, indicating stable populations in well-managed protected areas and elephant reserves despite ongoing threats like poaching, habitat loss, and climate change. Over the past decade, India's elephant population has remained relatively stable, about 28,000 to 30,000 individuals spread across 100,000 to 120,000 square kilometers (Baishya et al. 2025). While conservation efforts have mainly been successful in halting declines, challenges such as habitat fragmentation, conflict with humans, and genetic isolation still threaten the species' long-term survival. Project Elephant was launched by the Government of India in 1992 as a centrally sponsored scheme with the objectives of protecting elephants, their habitat and corridors, addressing issues of human-elephant negative interactions (HENI), and ensuring the welfare of captive elephants in India (Sarkar & Mishra 2023). Project Elephant has completed 30 years in 2022. Due to the concerted efforts of the central government, state forest departments, scientific institutions, and civil societies, the pachyderm population in India has significantly increased to around 30,000 (Sarkar & Mishra 2023; Khan et al. 2024). India's Asian Elephant population is estimated to be 22,446, according to the results of the much-delayed Synchronous All India Elephant Estimation (SAIEE) 2021–25. Compared to the last all-India estimate in 2017 (27,312), the population is 4,065 elephants lower, or 17.81%. However, the population report added a caveat that the two figures are not directly comparable due to a change in methodology. It said the latest estimate should be treated as a "new baseline (Sarkar & Mishra 2023). These protected areas not only safeguard the elephants but also maintain the integrity of forest ecosystems, which are crucial carbon sinks. Elephants are ecosystem engineers, and their activities, such as tree uprooting, trampling, and dung deposition, can enhance carbon sequestration and storage. Furthermore, protecting elephant habitats helps conserve diverse tropical forests, which are among the most carbon-dense ecosystems in the world (Baishya et al. 2025). Efforts to conserve Asiatic elephants have the potential to deliver significant co-benefits for climate change. Studies have shown that landscapes in Asia, which are home to the Asiatic Elephant, are not only highly diverse but also among the most carbon-dense in the tropics (Ong et al. 2023). As a result, safeguarding these forests through elephant conservation could contribute to climate change mitigation. Considering the critical role of protected areas in carbon sequestration and the importance of conserving Asiatic elephants, it is evident that exploring the synergies between these two priorities could yield substantial benefits for both biodiversity and climate change mitigation. The diverse array of wildlife species in tropical forests, including gigantic, slow-reproducing animals such as elephants and primates, account for a significant share of total animal biomass. These "large forest architects" play a key role in maintaining the forest's ability to sequester and store carbon, both directly and indirectly (Bangor et al. 2008; Sullivan et al. 2017). It is also evident that elephant population growth would generate a carbon sink of 109 MtC (64-153) across tropical Africa over the next 30 years (Lamba et al. 2023). The present study aimed to quantify changes in total carbon stocks within India's ER network from 1992–2025. Attribute carbon gains to area protection and elephant population changes. Compare findings with similar biodiversity–carbon studies in Asia and globally. Assess implications for India's climate mitigation commitments under REDD+/NDC frameworks.

Materials and Methods

Scope of the study and temporal framework

The analysis encompasses India's network of 33 Elephant Reserves distributed across diverse biogeographical zones, The Asiatic elephant in India occupies a diverse range of landscapes that vary from dense tropical forests to open grasslands and agro-forestry mosaics, reflecting the species' wide ecological adaptability. In the Western Ghats and northeastern states such as Assam, Arunachal Pradesh, and Meghalaya, elephants inhabit tropical moist forests characterized by high rainfall, dense evergreen and semi-evergreen vegetation, and rich biodiversity. In contrast, the central and eastern regions including Odisha, Jharkhand, and Chhattisgarh are dominated by tropical dry deciduous forests with moderate rainfall and fragmented habitats. Along the Himalayan foothills and parts of northeastern India, elephants thrive in tropical moist deciduous forests and riverine grasslands that offer abundant forage and serve as important migration routes. The floodplains of the Ganga and Brahmaputra support extensive grassland–savanna–woodland mosaics, particularly in Kaziranga and Manas, which are vital for seasonal congregation and breeding. In the hilly terrains of the Western and Eastern Ghats and the northeastern hill ranges, elephants occupy montane and semi-evergreen forests with steep slopes and narrow corridors (Baishya et al. 2025). Beyond natural habitats, elephants also traverse human-modified landscapes such as tea gardens, agricultural fields, and plantations that connect fragmented forest patches but often lead to human–elephant conflict. Under Project Elephant, (Chaudhary et al. 2024) the Ministry of Environment, Forest and Climate Change has broadly classified these habitats into ten major elephant landscapes across India, encompassing regions from the Shivalik–Terai belt and North Bengal to the Nilgiri, Anamalai, and Agasthyamalai ranges of the Western and Eastern Ghats (Chaudhary et al. 2024; Rawat & Rawat 2025). The study period covers 1992, 2000s, 2017, and 2025, reflecting key conservation milestones and data availability (Table 1).

Table 1. Asiatic Elephant population over the last five decades in India.

Year	Population estimate	Average Range	Notified ER	Area of ER in sq.km.
1900	100,000	100,000	0	0
1980	14,800–16,455	15,268	0	0
1978–83 (1981)	19,558	19,558	0	0
1989	17,635–24,090	20,863	0	0
1985	16,590–21,361	18,976	0	0
1990	17,310–22,120	19,715	0	0
1992-93	25,604	25,604	3	18,297
1993	22,796–28,346	25,571	3*	18,297*
1997	25,877	25,877	3*	18,297*
2000	28,140–29,190	28,665	3*	18,297*
2002	26,413	26,413	13	30,000
2005	21,200	21,200	32	65,270
2007	27,669–27,719	27,694	29	69,583
2012	29,391–30,711	30,051	32	-65,548
2017	27,312	27,312	33	65,000
2025	22,446	22,446	33	80,777

Note: No data was available, so the earlier year 1992 data is used for 1997 to 2000 with respect to the number of elephant reserves and their areas.

Data sources

The current research work collected the elephant population data from Project Elephant censuses (MoEFCC 1992–2025). The Elephant Reserves data was collected from Elephant Corridors of India 2023 (MoEFCC 2023) and gazette notifications. The Carbon density values derived from the India State of Forest Reports (ISFR 2019, 2021) and FAO Global Forest Resources Assessment 2020, supported by regional studies.

Analytical model

An IPCC Tier-2 carbon accounting framework was applied:

$$C_{total} = (A_f \times C_f) + (A_g \times C_g) + (A_w \times C_w) + (N_e \times BM_e \times CF)$$

Where,

Af, Ag, Aw = area under forest, grassland, and wetlands (ha);

Cf, Cg, Cw = mean carbon density (t C ha^{-1}) of respective land covers.

Ne = number of elephants.

BMe = Mean elephant biomass (kg);

CF = Carbon Fraction (0.5 kg^{-1} dry matter).

The average weight of Asiatic Elephants varies by age, sex, and region. Generally, adult male Asiatic Elephants in India weigh 2,700–4,000 kg (approximately 6,000–8,800 pounds), while females are slightly smaller, typically weighing 2,000–2,700 kg (4,400–6,000 pounds) (Ong et al. 2023; Khan et al. 2024). Therefore, a mean elephant biomass (BMe) of 3,000 kg is considered for the model. With respect to carbon fraction (CF) for practical calculations, of 0.45–0.50 kg C per kg dry matter for an elephant's body (dry weight basis), as a general value used for living biomass (dry matter) is ~50% carbon. For example, the Intergovernmental Panel on Climate Change (IPCC) default carbon fraction for non-tree biomass is 0.50 t C per t dry matter (cdm.unfccc.int+2un-redd.org+2). A conservative average landscape carbon density (C FGW = 215 t C ha^{-1}) was applied, consistent with national estimates for dense tropical forests. Mean elephant biomass was assumed at 3,000 kg per individual (Abernethy et al. 2013), with 50 % carbon content.

Simple linear regression analysis

If the correlation is high, one can create a predictive model to see how many elephants can be expected for every new reserve notified.

The Model:

$$Y = \beta_0 + \beta_1 X + \epsilon$$

Find the Slope β_1 This represents the "impact factor."

$$\beta_1 = \frac{n(\sum XY) - \sum X + \sum Y}{n(\sum X^2) - (\sum X^2)}$$

Find the Intercept (β_0): The estimated population if there were zero reserves.

$$\beta_0 = \overline{Y} - \beta_1 \overline{X}$$

Data interpolation and uncertainty

Where missing, elephant population and area values were linearly interpolated. Sensitivity analysis tested ±20% variation in carbon densities and ±10% in elephant numbers. Uncertainty propagation followed IPCC guidance.

Limitation

The model does not explicitly incorporate ecological variables such as invasive species, forest degradation intensity, or spatial heterogeneity in biomass distribution. Therefore, the results should be interpreted as landscape-level accounting estimates under protection scenarios, rather than precise ecological measurements of carbon gain.

This study has several limitations:

(i) Lack of integration of invasive species and degradation indices.

(ii) Assumption of uniform carbon density across landscapes.

(iii) Use of administrative boundaries rather than ecological units.

(iv) Limited representation of soil carbon pools These factors may influence the accuracy of carbon stock estimation and interpretation.

RESULTS

Elephant population and Elephant Reserve expansion

Between 1992 and 2025, the elephant population increased slightly from 25,604 to 27,312 individuals (+6.7%). In contrast, the total ER area () expanded from 18,297 km² to 80,777 km² (+341 %), reflecting extensive habitat protection initiatives (Table 2). The analysis shows that from 1992 to 2025, the Elephant Reserve area increased by approximately 62,480 km² (a 4.4-fold expansion). The elephant population increased moderately by 1,708 individuals (~6.7% rise), and the total carbon stock increased from 42.34×10^3 t C to 58.34×10^3 t C, representing an overall increase of 16,000 t C (~38% growth in total carbon stock).

Carbon stock enhancement

The estimated increase in total carbon stock from 42.34×10^3 t C in 1992 to 58.34×10^3 t C in 2025 represents a 38% rise within the ER network. However, this increase does not imply that new biomass was generated solely due to conservation interventions. Instead, it reflects the inclusion of additional forest areas under the ER network and the reduced likelihood of deforestation and degradation within these landscapes.

Approximately, 95% of the observed increase is attributable to expansion in protected area coverage, while elephant biomass contributed marginally (~5%). These findings indicate that land-use protection status, rather than faunal population dynamics, is the dominant factor influencing carbon stock estimates in this analysis.

Furthermore, the weak statistical relationship (R^2 = 0.0346) between ER notification and elephant population suggests that administrative expansion alone is insufficient to drive ecological outcomes, highlighting the importance of habitat quality, connectivity, and management effectiveness.

Comparative analysis

The 38% gain aligns with estimates from African forest studies where megafauna protection led to 30–40 % enhancement in vegetation carbon (Berzaghi et al. 2019). Similar relationships were observed in tiger landscapes in India, where protected-area expansion correlated with increased carbon density (Lamba et al. 2023). Globally, tropical forest carbon density averages 150–250 t C ha^{-1} (FAO 2020), consistent with the 215 t C ha^{-1} adopted here, confirming robustness of assumptions. The correlation between faunal integrity and carbon maintenance is supported by Bennett & Robinson (2023), who emphasize wildlife conservation as a direct strategy for avoiding carbon degradation in tropical forests.

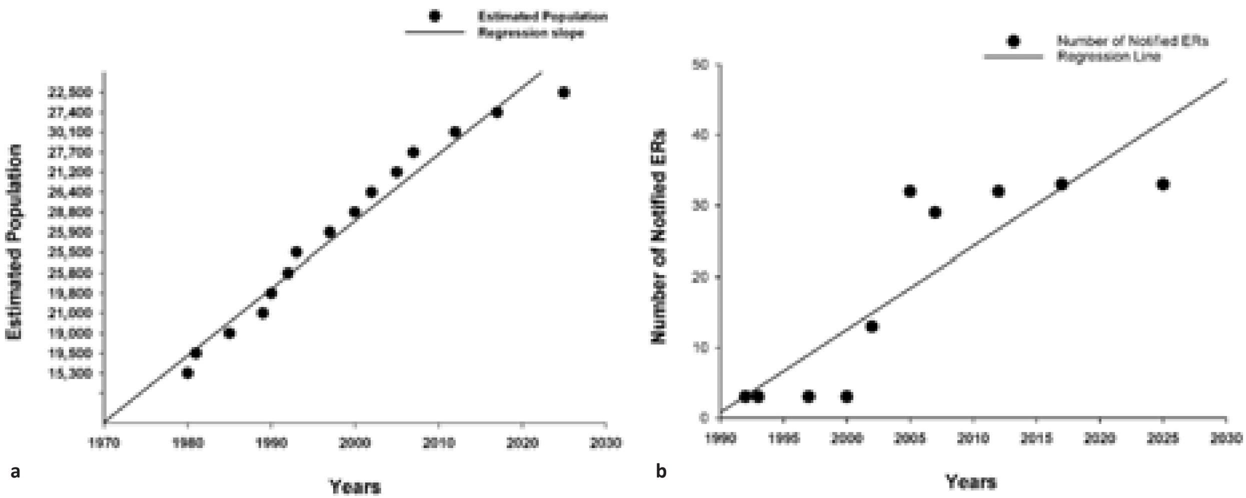

Figure 1. a—Asiatic Elephant population trend in India from 1980 to 2025 | b—Comparative graph of total elephant reserve landscapes in 1992 and 2025.

Table 2. Elephant population and Elephant Reserves trend in India from 1980 to 2025.

Year	Elephant population	No. of ERs	ER area (km²)	Source
1992	25,604	3	18,297	MoEFCC 1992
2017	27,312	33	65,000	MoEFCC 2017
2025	27,312*	33	80,777	MoEFCC 2025

*The population of 2017 is used as the population census of 2025, which used different techniques of census and declared it as the baseline for the upcoming census.

Uncertainty and sensitivity

Sensitivity testing revealed that ±20% variation in carbon density produced 12–15 % variation in total carbon stock. Changes in elephant population had minor effects (< 2%). The largest uncertainty stems from spatial heterogeneity in soil carbon pools and boundary delineation of ERs. The study also utilized a simple linear regression model to quantify the impact of conservation infrastructure (Notified ERs) on the total population of Asiatic Elephants in India. Impact Factor (β_1) The analysis yielded a slope of 30.59. This indicates that for every additional ER notified, the model estimates a marginal increase of approximately 31 elephants in the national population.

Predictive Equation

The relationship can be expressed by the formula:

Estimated population = 25,905 + 30.59 x (Number of ERs)

Model Fit () The coefficient of determination was calculated at 0.0346 (3.46%). This suggests that while there is a positive mathematical relationship between the number of reserves and the population, the quantity of notified areas alone is not a primary predictor of population variance.

DISCUSSION

The strong positive relationship between ER expansion and carbon stock/sink enhancement underscores the importance of protected landscapes in climate mitigation. The results reaffirm global findings that faunal integrity is a critical but often underrepresented factor in carbon accounting (Sullivan et al. 2017; Bennett & Robinson 2023). In India, elephant-driven processes enhance forest resilience: dung enriches soil organic matter, browsing maintains mixed-age canopy structures, and movement facilitates seed dispersal of large-seeded, high-carbon tree species (Berzaghi et al. 2019, 2023; Chami et al. 2020). The modest rise in elephant population, though smaller than area gains, stabilizes ecosystem functioning and ensures long-term carbon retention (Danielsen et al. 2005; Chaudhary et al. 2024). Comparable patterns were observed in African savanna ecosystems where megaherbivore management improved vegetation carbon stocks by 20–30% (Doughty et al. 2013; Rawat & Rawat 2025). Such consistency across regions supports the generalizable ecological principle that megafauna protection complements forest-carbon goals. Policy integration of these results can strengthen India's commitments to achieve 2.5–3 billion t CO_2 equivalent additional carbon sink by 2030 GoI NDC, 2021 (Baasansuren et al. 2019). Incorporating wildlife-inclusive accounting within REDD+ frameworks could generate measurable ecosystem-service credits while ensuring co-benefits for biodiversity.

The success phase (1992–2012)

During the initial two decades of Project Elephant, there was a synchronized rise in both notifications and elephant numbers. This supports the hypothesis that establishing protected areas provides the initial security necessary for megaherbivores to perform their role as ecosystem engineers (Christen et al. 2020; Khan et al. 2024).

The 2025 divergence

A significant finding in this dataset is the population decline recorded in 2025 despite the stabilization of notified reserves at 33. This mathematical "decoupling" suggests that the *quality* of the habitat and the *functional connectivity* of corridors may be more critical for long-term population stability than the mere notification of administrative boundaries.

Implications for carbon enhancement

Since elephants facilitate carbon sequestration by thinning smaller trees and promoting carbon-dense hardwoods, the recent population dip could lead to a reduction in the "carbon driver" efficiency of Indian forests. The data suggests that conservation policy must shift from "Notification of Reserves" to "Habitat Restoration and Corridor Protection" to maintain high carbon sequestration rates.

It is important to note that ERs are not uniformly protected in the same manner as legally designated national parks or tiger reserves. Their effectiveness in conserving biomass and carbon stocks varies depending on enforcement, land-use pressures, and ecological condition. Therefore, attributing carbon stock changes directly to ER designation may lead to overgeneralization unless supported by site-specific ecological assessments.

CONCLUSION

The expansion of India's elephant reserve network between 1992 and 2025 has contributed to the stabilization of forest carbon stocks primarily through reduced deforestation and improved land-use governance. While elephants play an important ecological role, their direct contribution to carbon stock increase is limited within the scope of this model. The findings emphasize that conservation outcomes depend more on habitat quality, connectivity, and effective management than on administrative designation alone. Future studies should integrate ecological variables such as invasive species, forest structure, and soil carbon dynamics to provide a more comprehensive assessment.

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ARTICLE

Genetic polymorphism of Dhofar Toad *Firouzophrynus dhufarensis* (Parker, 1931) (Amphibia: Bufonidae) across central Saudi Arabia

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Abstract: This study investigated the genetic diversity of the Dhofar Toad *Firouzophrynus dhufarensis* in central Saudi Arabia, focusing on three populations: Al-Kharj, Al-Hariq, and Al-Aflaj. Using inter-simple sequence repeat (ISSR) markers, the analysis revealed notable variation in genetic polymorphism among these regions based on 30 individuals (10 per population) selected for genetic analysis from a total of 60 sampled specimens. Al-Kharj demonstrated comparatively higher levels of genetic diversity than the other populations, as reflected by polymorphism rates and diversity indices. In contrast, Al-Hariq and Al-Aflaj exhibited reduced polymorphism, suggesting that isolation may have been caused by habitat fragmentation and limited gene flow. Dendrogram analysis based on Nei's genetic distances indicated a closer relationship between Al-Hariq and Al-Aflaj, while Al-Kharj was more distinct. These findings underscore the conservation significance of Al-Kharj in maintaining amphibian genetic diversity in arid landscapes. Meanwhile, the genetic vulnerability of Al-Hariq and Al-Aflaj emphasizes the need of targeted habitat restoration and improved landscape connectivity. This research also demonstrates the utility of ISSR markers for preliminary genetic assessments in species lacking extensive genomic resources, reinforcing the need for broader geographic and genomic sampling. Future work should incorporate high-resolution markers and expand to populations in regions such as Oman to support transboundary conservation planning.

Keywords: Amphibian conservation, arid landscapes, gene flow, genetic diversity, habitat fragmentation, ISSR markers, landscape connectivity, phylogeography, population differentiation, population structure.

هدفت هذه الدراسة إلى استقصاء التنوع الوراثي لضفدع ظفار في وسط المملكة العربية السعودية، مع التركيز على ثلاث جماعات سكانية توزعت في الخرج والحريق والأفلاج. تم استخدام واسمات التكرارات البسيطة البينية ((ISSR ، حيث أظهرت التحليلات وجود تباين ملحوظ في مستوى التعدد الشكلي الوراثي بين هذه المناطق، وذلك استناداً إلى تحليل 30 عينة (10 عينات من كل جماعة سكانية) تم اختيارها للتحليل الوراثي من أصل 60 عينة جُمعت خلال الدراسة. أظهرت جماعة العينات المجموعة من الخرج مستويات أعلى نسبياً من التنوع الوراثي مقارنة بالعينات من الجماعات الأخرى، كما انعكس ذلك في نسب التعدد الشكلي ومؤشرات التنوع الوراثي. في المقابل، سجلت عينات جماعات مناطق الحريق والأفلاج مستويات أقل من التعدد الشكلي، مما يشير إلى احتمال تأثرها بالعزلة الناتجة عن تجزؤ الموائل الطبيعية ومحدودية تدفق الجينات بينها. كما بيّن تحليل شجرة القرابة الوراثية المعتمد على المسافات الوراثية وفقاً لمعامل ناي وجود علاقة وراثية أوثق بين عينات الجماعات من منطقة الحريق والأفلاج، في حين بدت جماعة الخرج أكثر تميزاً واختلافاً وراثياً وتؤكد هذه النتائج الأهمية المحافظة لجماعة الخرج باعتبارها مخزوناً مهماً للتنوع الوراثي للبرمائيات في البيئات الجافة. وفي الوقت ذاته، فإن الهشاشة الوراثية التي أظهرتها الجماعات التي تم الحصول عليها من مناطق الحريق والأفلاج تبرز الحاجة إلى تنفيذ إجراءات مستهدفة لاستعادة الموائل الطبيعية وتعزيز الترابط البيئي بين المواقع المختلفة لتحسين تدفق الجينات كما تُظهر هذه الدراسة فعالية واسمات التكرارات البسيطة البينية كأداة مناسبة للتقييمات الوراثية الأولية للأنواع التي تفتقر إلى موارد الجينوم المتقدمة، مما يؤكد أهمية توسيع نطاق الدراسات المستقبلية لتشمل عينات جغرافية أوسع وتحليلات للجينوم أكثر شمولاً. ويوصى مستقبلاً باستخدام واسمات وراثية عالية الدقة وتوسيع نطاق الدراسة ليشمل جماعات سكانية من مناطق أخرى، مثل سلطنة عُمان، بما يدعم جهود

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INTRODUCTION

The Dhofar Toad *Firouzophrynus dhufarensis* (Parker, 1931) is distributed across much of the Arabian Peninsula. It is currently classified as 'Least Concern' with a stable population (UAE National Red List Workshop 2022). Its range includes the western mountains near Mecca City and the central regions of Saudi Arabia, such as Ha'il and Riyadh Provinces. It also occurs in peripheral southern Arabia, including Yemen, Oman, and the United Arab Emirates (UAE), typically inhabiting wadis and areas with seasonal water sources (Cunningham & Feulner 2001; Cunningham & Wronski 2010; Soorae et al. 2010; Gardner 2013; Soorae et al. 2013; Alshammari & Ibrahim 2018). The species has also been reported by the IUCN as introduced in parts of the Riyadh region, although its native range boundaries within central Saudi Arabia remain uncertain (UAE National Red List Workshop 2022).

Within Saudi Arabia, *F. dhufarensis* inhabits a range of environments, from the southwestern provinces of Jazan, Asir, and Mecca to the more arid interior regions. It thrives in mountainous areas, valley streams, irrigated farms, and temporary wetlands (Balletto et al. 1985; Soorae et al. 2013; Al-Johany et al. 2014; Al-Qahtani & Al-Johany 2018). Observations from the Ibex Reserve and central provinces, including Al-Kharj, Al-Hariq, and Al-Aflaj, suggest its adaptability to natural and human-altered habitats (Alrefaei et al. 2022). Reports also confirm its presence in Wadi Abather, Al Madinah Province, and across sites with elevations ranging from 55–700 meters (Mashlawi & Masood 2024). While adapted to arid conditions, *F. dhufarensis* may be outcompeted by the Arabian Toad in more mesic environments (Soorae et al. 2013).

Despite its wide distribution, research on the species' genetic structure remains limited. Hafez et al. (2017) conducted a phylogeographic study across the Afro-Arabian regions using mitochondrial DNA (D-loop and 12S rRNA), which indicated low polymorphism and suggested either past population bottlenecks or balancing selection. Alrefaei et al. (2022) further explored the species' 16S rRNA in Riyadh populations, revealing high genetic similarity (99.35%) with Omani populations. These studies provide preliminary insights but highlight the need for further genetic investigation.

Accordingly, this study was designed as an exploratory assessment of genetic variation in *F. dhufarensis* across three populations in central Saudi Arabia. The specific objectives were to (i) quantify levels of genetic polymorphism using ISSR markers, (ii) evaluate whether measurable genetic differentiation exists among geographically proximate populations in an arid landscape, and (iii) provide baseline genetic information to inform future, more comprehensive studies. We hypothesized that populations would exhibit detectable genetic differentiation consistent with localized isolation in arid environments, while recognizing that testing underlying drivers of such patterns requires complementary genetic, ecological, and spatial data.

This study is intended as a preliminary assessment of population-level genetic variation in *F. dhufarensis* within central Saudi Arabia. Given the limited sample size, restricted geographic coverage, and the dominant nature of ISSR markers, the results should be interpreted as exploratory rather than definitive. Nevertheless, in the context of the Arabian Peninsula, where large-scale aridification, habitat fragmentation, and hydrological isolation have shaped amphibian distributions, such baseline genetic data remain valuable. By documenting spatial patterns of genetic polymorphism across arid landscapes, this study provides an initial framework for hypothesis generation and identifies priorities for future, higher-resolution genetic and phylogeographic investigations.

Study Area

This study was conducted in central Saudi Arabia, specifically in the regions of Al-Kharj, Al-Aflaj, and Al-Hariq, which are located south and south-east of Riyadh (Image 1); this description reflects their geographic position rather than a formal ecological or biogeographic division. These sites span approximately 22.15°–24.16° N and 46.50°–47.33° E. The region is characterized by an arid climate with extreme seasonal temperature variation, ranging from highs of 48°C in summer to lows of 3°C in winter. Annual rainfall is typically below 100 mm, while evaporation rates may exceed 2,000 mm. Elevations range from 320–650 m, contributing to local microclimatic and vegetative differences (Sayed & Masrahi 2023). The geology of the study area is predominantly composed of quaternary deposits, featuring significant karstic features, including wadis, sinkholes, and limestone formations. Soils are mainly sandy loam, supporting xerophytic vegetation. The Tuwaiq Mountains to the west create topographic heterogeneity, influencing hydrology and biodiversity. Traditional irrigation systems in Al-Aflaj have historically enabled agriculture in this otherwise arid landscape (Almalki et al. 2022).

Image 1. Sampling locations in Saudi Arabia.

MATERIALS AND METHODS

Sampling

Field sampling was carried out in January 2021. A total of 60 specimens of the Dhofar Toad were collected. Twenty specimens were initially obtained from each site. For genetic analysis, 30 individuals were randomly selected (10 per site) based on the quality of their samples. Specimens were preserved in 96% ethanol immediately upon collection to maintain DNA integrity, following the procedures of Zamani et al. (2011).

DNA Extraction

Genomic DNA was extracted from thigh muscle and skin tissues using a protocol adapted from Kumar et al. (2012). Approximately 5 mg of tissue was homogenized and incubated with 500 µL DNAzol reagent (Molecular Research Center, USA). Following centrifugation at 10,000 g for 2 minutes, the supernatant was transferred, and DNA was precipitated using 100% ethanol. After additional centrifugation and washing with 75% ethanol, the DNA pellet was air-dried and rehydrated in 50 µL nuclease-free water. DNA purity and concentration were assessed using a NanoDrop spectrophotometer, and integrity was confirmed by electrophoresis on a 1.5% SYBR green-stained agarose gel. All samples were diluted to a working concentration of 100 ng/µL.

ISSR-PCR Amplification

ISSR amplification was used to assess genetic diversity. Although ISSR markers are dominant and limited in resolving co-dominant variation, they are effective for detecting genome-wide polymorphism and are widely applied in preliminary population genetic studies, particularly in non-model and conservation-target species with limited genomic resources (Zietkiewicz et al. 1994; Bornet & Branchard 2001; Moradi et al. 2014). Because ISSRs do not allow direct estimation of allele frequencies or fine-scale gene flow, the approach employed here is intended to provide an initial assessment of genetic variability rather than a comprehensive reconstruction of population connectivity or evolutionary history. Nine primers (UBC 813, 814, 816, 817, 819, 821, 822, 825, and 828) were selected based on reproducibility and polymorphic potential (Moradi et al. 2014).

Each PCR reaction consisted of 1 µL of genomic DNA, 0.8 µL of primer, 10 µL of PCR master mix (Solarbio), and 8.2 µL of nuclease-free water (Promega) in a total volume of 20 µL. Amplification was performed using a ProFlex PCR system with an initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 30 seconds, annealing at 46–48°C for 30 seconds, and extension at 72°C for 1 minute. A hold at 4°C followed a final extension at 72°C for 2 minutes. PCR products were separated on 1.5% agarose gels in 0.5× TBE buffer. Gels were stained with SYBR Green, run at 100 V for 1 hour, and visualized under UV light. Molecular weight markers (100–5000 bp) were used to estimate band sizes, and gel images were captured using a BioDocAnalyze system.

To ensure reproducibility and minimize sensitivity to laboratory conditions, all ISSR-PCR reactions were conducted using standardized reagent concentrations, identical thermal cycling parameters, and the same PCR platform throughout the study. Amplifications were repeated independently for a subset of samples to confirm banding consistency, and only clear, reproducible bands observed across replicate reactions were scored. All gels were run under identical electrophoretic conditions and scored conservatively to reduce the inclusion of artefactual fragments. These standardization procedures were applied consistently across all primers and populations to ensure methodological reliability.

Data Analysis

Banding patterns were scored as binary data (1 = presence, 0 = absence). Fragment sizes were calculated using ONE-Dscan software (Scanalytics Inc., USA). Genetic diversity metrics, including percent polymorphic bands (PPB), Nei's genetic diversity (Nei 1987), and the Shannon diversity index (Shannon 1948) were calculated using POPGENE version 1.31 (Yeh & Yang 1999). Nei's unbiased genetic distances among populations were also computed. A dendrogram was constructed based on Nei's genetic distances using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA version 11 (Tamura et al. 2021) to visualize genetic relationships among the three populations of *F. dhufarensis*. Genetic relationships among populations were inferred by constructing a dendrogram based on Nei's unbiased genetic distances using the unweighted pair group method with arithmetic mean (UPGMA).

RESULTS

The analysis of ISSR profiles revealed measurable genetic diversity among *F. dhufarensis* populations from Al-Aflaj, Al-Kharj, and Al-Hariq. Banding patterns generated by nine ISSR primers were scored as binary data (presence = 1, absence = 0) and summarized quantitatively as percent polymorphic bands (PPB), polymorphic loci per primer, and polymorphism information content (PIC) (Table 1 & 2). Primer performance varied among populations, with UBC 813, UBC 819, and UBC 828 consistently yielding the highest numbers of polymorphic loci and higher PIC values, indicating greater discriminatory power (Images 2 & 3).

Nine primers exhibited polymorphism levels that varied across populations. Al-Aflaj exhibited a polymorphism rate of 29% (30 polymorphic loci), Al-Kharj had the highest rate at 41% (24 loci), and Al-Hariq showed a lower polymorphism rate of 25.49% (31 loci). Primer-specific polymorphism ranged from 12% for UBC 817 to 50% for UBC 813 in Al-Aflaj; 15.3% (UBC 822) to 100% (UBC 813 and UBC 814) in Al-Kharj; and 18% (UBC 817 and UBC 819) to 44.44% (UBC 813) in Al-Hariq.

Al-Kharj exhibited the highest allelic richness and genetic variability across the nine ISSR primers, suggesting that this population may retain a broader representation of overall genetic diversity. In contrast, Al-Hariq and Al-Aflaj exhibited lower levels of polymorphism, potentially indicating reduced gene flow or historical isolation. Primer-specific amplification patterns also varied, with UBC 813 consistently generating the highest polymorphic rates in all populations. The dendrogram based on Nei's genetic distances confirmed a closer genetic relationship between Al-Aflaj and Al-Hariq (distance = 0.2). At the same time, Al-Kharj formed a separate cluster, consistent with its elevated intra-population diversity.

The average polymorphic information content (PIC) values also supported these findings, with Al-Aflaj showing the highest average PIC (0.772), followed by Al-Kharj (0.736), and Al-Hariq (0.716). Among all primers, UBC 813 yielded the highest PIC values across populations, establishing its value for future genetic assessments of *D. dhufarensis* (Figure 1).

The dendrogram revealed closer genetic proximity between Al-Aflaj and Al-Hariq (distance = 0.2), while Al-Kharj appeared more genetically distinct, suggesting population-specific divergence potentially driven by ecological or geographic isolation (Figures 2 & 3).

DISCUSSION

The ISSR-based genetic patterns observed among *Firouzophrynus dhufarensis* populations from Al-Kharj, Al-Hariq, and Al-Aflaj should be interpreted within the constraints of a preliminary study. While measurable differences in polymorphism were detected, the limited number of loci, dominance of ISSR markers, and restricted sampling design preclude strong inferences regarding historical demography or evolutionary processes. Nonetheless, the observed population-level differentiation is consistent with expectations for amphibian populations inhabiting arid and semi-arid regions of the Arabian Peninsula, where large-scale aridification, habitat fragmentation, and discontinuous surface water availability can promote isolation and reduced gene flow. These results therefore provide an initial indication of spatial genetic structuring that warrants further investigation using expanded sampling and higher-resolution genomic tools.

These patterns are consistent with ecological observations indicating that *F. dhufarensis* is physiologically tolerant of dry environments and can

Table 1. Genetic diversity parameters of *Firouzophrynus dhufarensis* populations based on ISSR markers.

Population	Total loci	Polymorphic loci	PPB (%)	Nei's genetic diversity (H)	Shannon index (I)
Al-Kharj	58	24	41.0	0.236	0.352
Al-Aflaj	104	30	29.0	0.191	0.281
Al-Hariq	122	31	25.49	0.178	0.263

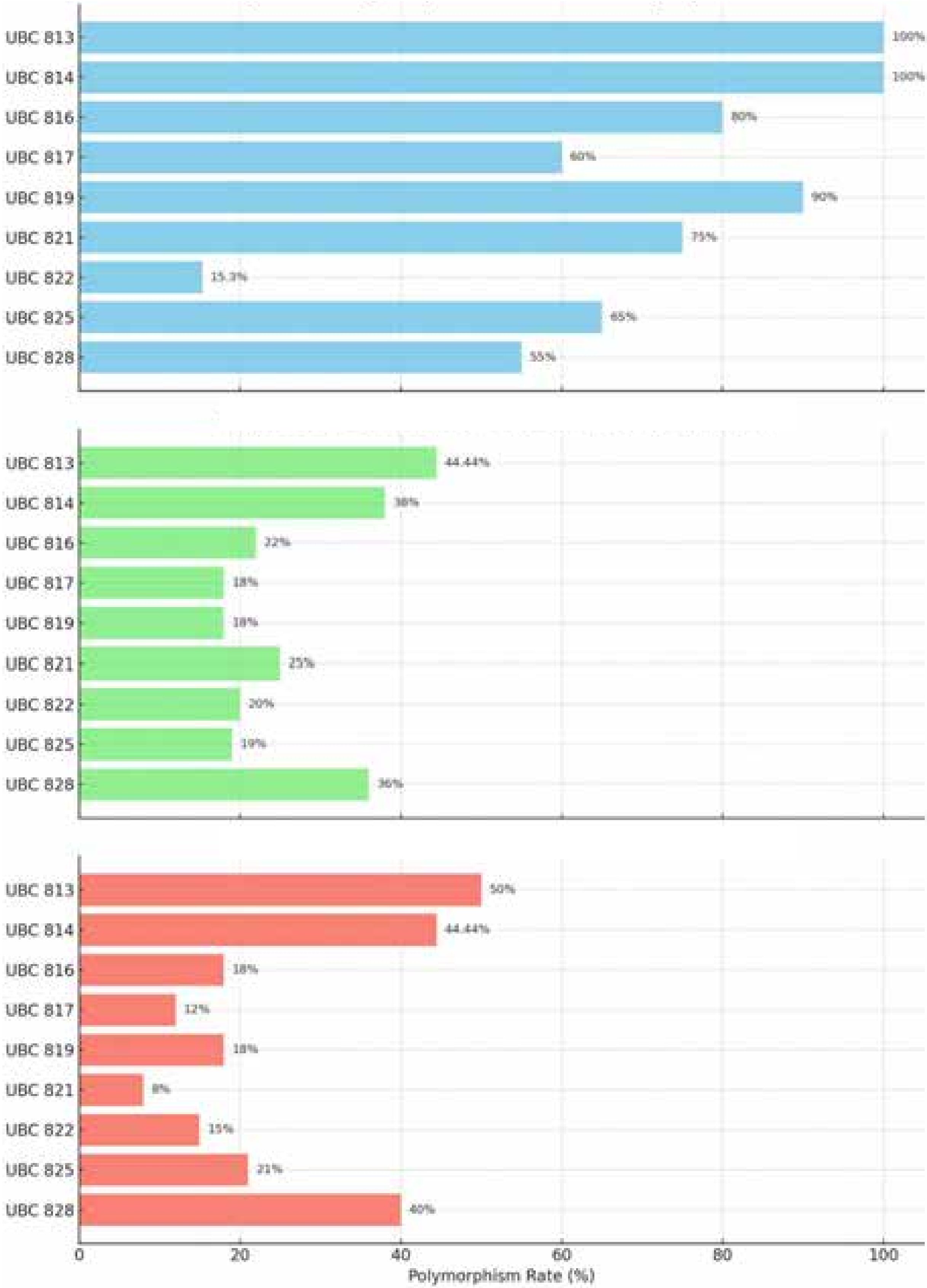

Figure 1. Percentage of polymorphic loci detected by nine ISSR primers in *Firouzophrynus dhufarensis* populations from A—Al-Kharj | (B—Al-Hariq | C—Al-Aflaj, illustrating intra-population genetic variability and differences in primer efficiency.

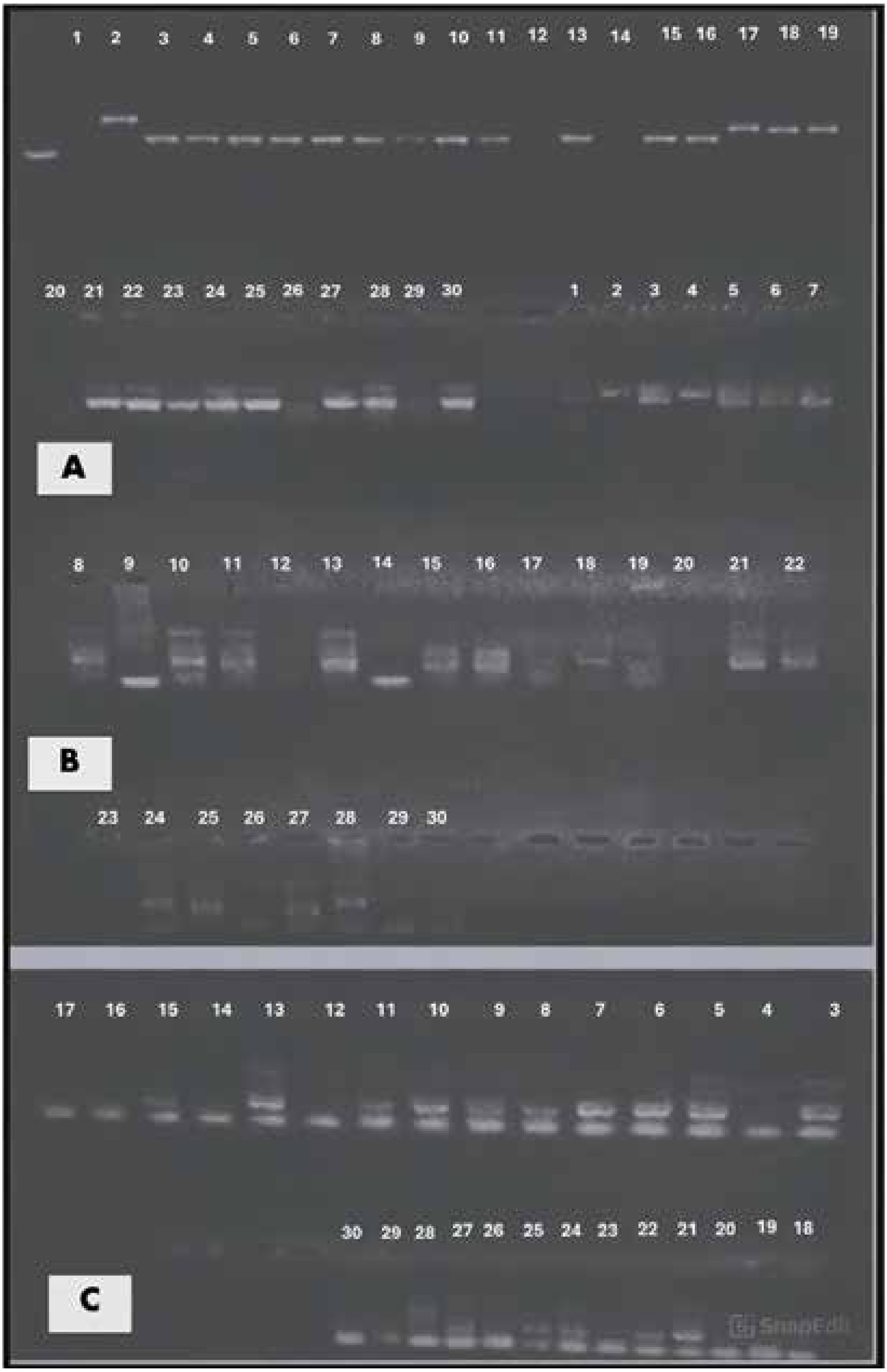

Image 2. Representative ISSR amplification profiles of *Firouzophrynus dhufarensis* from Al-Kharj, Al-Hariq, and Al-Aflaj populations generated using primers UBC 817–UBC 828. Distinct banding patterns illustrate variation in fragment presence and polymorphism among populations.

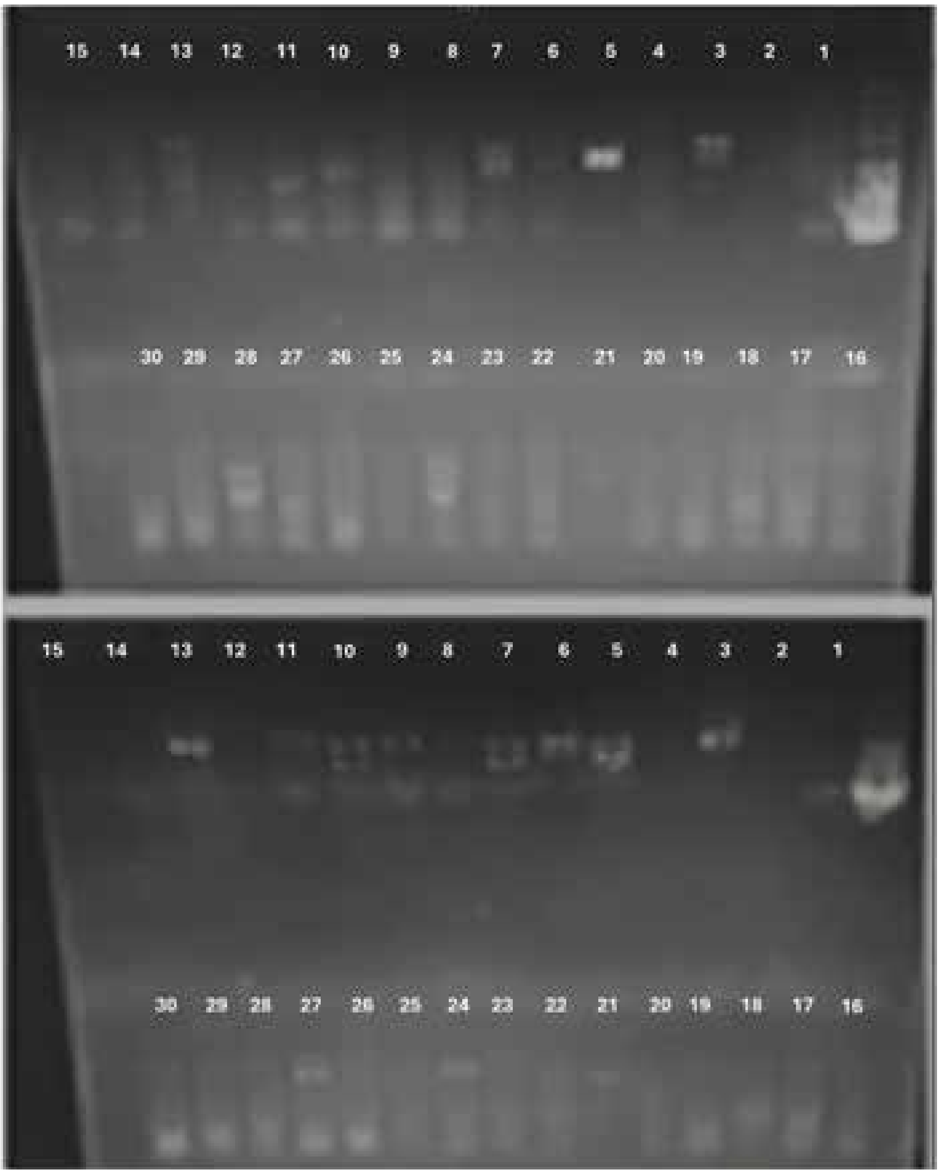

Image 3. ISSR banding profiles of *Firouzophrynus dhufarensis* individuals from Al-Aflaj, Al-Kharj, and Al-Hariq amplified using primers UBC 817 (A), UBC 819 (B), and UBC 825 (C), highlighting primer-specific polymorphism and inter-population genetic variation.

occupy a wide range of habitats, including mountains, wadis, and agricultural areas (Cunningham & Feulner 2001; Al-Johany et al. 2014). Similar trends have been reported for other Bufonidae species in arid landscapes, where habitat quality and landscape connectivity significantly influence genetic structure and long-term population viability (Zeisset & Beebee 2008; Alshammari & Ibrahim 2018; Alrefaei et al. 2022).

Primer performance further supports the genetic differences observed. UBC 813 and UBC 814 consistently yielded high polymorphism across populations, suggesting these primers may target variable genomic regions. In contrast, UBC 817 and UBC 821 produced lower polymorphism, possibly due to amplification of conserved sequences or reduced primer efficiency. These findings underscore the importance of primer selection and support the use of multi-primer ISSR strategies for assessing overall genetic diversity in species lacking extensive genomic resources, as ISSR markers are reproducible, highly polymorphic, and suitable for genome fingerprinting in non-model organisms (Zietkiewicz et al. 1994; Bornet & Branchard 2001).

The lower levels of genetic polymorphism observed in the Al-Hariq and Al-Aflaj populations should be interpreted cautiously. Reduced genetic diversity in amphibian populations is often associated with limited

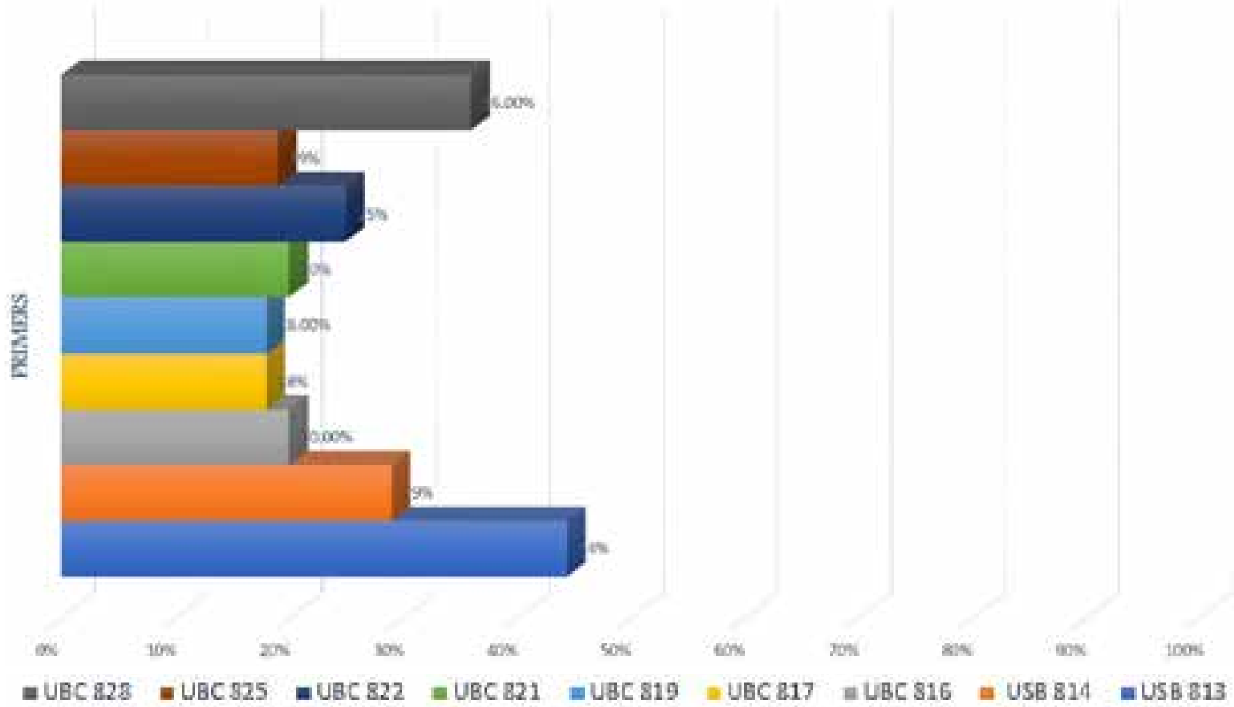

Figure 2. Primer-specific polymorphism rates in the Al-Hariq population of *Firouzophrynus dhufarensis*, expressed as the percentage of polymorphic loci across nine ISSR primers.

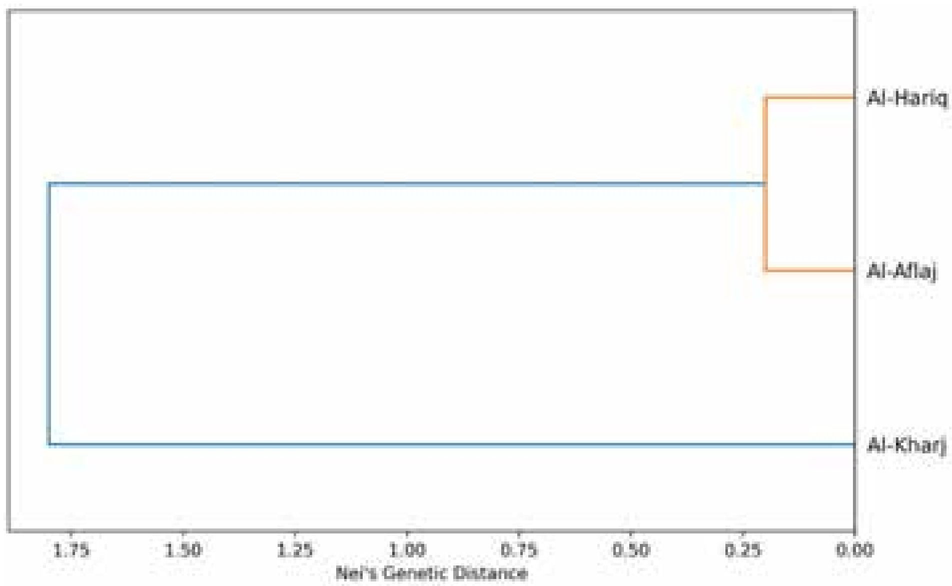

Figure 3. UPGMA dendrogram based on Nei's unbiased genetic distances showing genetic relationships among *Firouzophrynus dhufarensis* populations from Al-Kharj, Al-Hariq, and Al-Aflaj. The scale bar represents Nei's genetic distance, with shorter branch lengths indicating closer genetic similarity.

dispersal, isolation, or small effective population sizes in fragmented or arid landscapes (Frankham 2005; Storfer et al. 2010; Haddad et al. 2015). The present study does not directly test these processes, and no spatial, environmental, or landscape genetic analyses were conducted. Therefore, causal links between genetic variation and habitat fragmentation or environmental heterogeneity cannot be confirmed and should be regarded as hypotheses requiring further investigation.

Recent landscape-scale studies from arid regions

Table 2. Primer-wise ISSR polymorphism and polymorphic information content (PIC) across populations.

Primer	Polymorphism range (%)	PIC (Al-Kharj)	PIC (Al-Hariq)	PIC (Al-Aflaj)
UBC 813	44.44–100	0.812	0.798	0.821
UBC 814	38–100	0.781	0.754	0.768
UBC 816	22–35	0.701	0.689	0.712
UBC 817	12–18	0.665	0.648	0.671
UBC 819	18–42	0.743	0.721	0.758
UBC 821	20–30	0.692	0.676	0.701
UBC 822	15–28	0.684	0.667	0.695
UBC 825	25–40	0.728	0.709	0.736
UBC 828	32–45	0.756	0.732	0.771

of the Arabian Peninsula and adjacent deserts further demonstrate that population connectivity in dryland systems is often shaped by complex interactions among historical aridification, topography, hydrology, and species-specific dispersal capacity. For example, Pola et al. (2024) showed that even broadly distributed desert taxa can exhibit pronounced genetic structuring across environmentally heterogeneous arid landscapes, with connectivity often constrained by discontinuous habitats rather than simple geographic distance. Although such studies typically rely on higher-resolution genomic or spatially explicit approaches, their findings provide an important regional framework for interpreting preliminary genetic patterns observed in arid-zone amphibians such as *F. dhufarensis*. Within this context, the present results should be viewed as an initial indication of localized population differentiation rather than evidence of range-wide isolation or connectivity.

From a conservation perspective, preserving genetic diversity across the species' range is critical, as genetic variation is a key determinant of population resilience in the face of environmental change and stochastic events (Frankham 2005). Al-Kharj may serve as a priority site due to its elevated genetic variability. At the same time, Al-Hariq and Al-Aflaj may benefit from habitat restoration and connectivity-enhancing measures such as ecological corridors. Establishing such linkages could help mitigate the effects of genetic drift, reduce inbreeding, and improve population resilience, especially in fragmented or isolated habitats where landscape-level barriers hinder gene flow (Botstein et al. 1980; Storfer et al. 2010; Haddad et al. 2015; Mashlawi & Masood 2024).

The geographically narrow sampling design represents an important limitation of this study. While the three sampled populations provide insight into local-scale genetic variation within central Saudi Arabia, they do not capture the full extent of genetic diversity across the species' core range in southern Arabia, particularly Oman and Yemen, where *F. dhufarensis* is considered native. As a result, the present data cannot be used to infer species-wide connectivity, historical dispersal routes, or range-wide isolation patterns. Future studies incorporating populations from Oman, Yemen, and southwestern Saudi Arabia are essential to place the central Saudi populations within a broader phylogeographic framework and to robustly evaluate patterns of connectivity, isolation, and potential post-aridification divergence across the Arabian Peninsula.

A key limitation of this study is the exclusive reliance on ISSR markers, which, despite their reproducibility and utility for preliminary assessments, provide lower resolution than co-dominant or sequence-based markers. As dominant markers, ISSRs do not allow direct estimation of heterozygosity, contemporary gene flow, or historical connectivity among populations. Future research should therefore integrate more informative molecular tools, such as microsatellites, mitochondrial DNA sequences, and genome-wide SNP approaches, alongside broader geographic sampling. Such integrative analyses would substantially improve inference regarding population origins, dispersal dynamics, and the evolutionary processes shaping genetic structure across the Arabian Peninsula.

This study provides the first ISSR-based assessment of genetic diversity in central Saudi Arabian populations of the Dhofar Toad. The results reveal clear population-level differences in genetic polymorphism, with Al-Kharj exhibiting comparatively higher genetic diversity, while Al-Hariq and Al-Aflaj show reduced variability, likely reflecting isolation and limited gene flow. These findings highlight the conservation importance of maintaining habitat connectivity and protecting genetically diverse populations in arid landscapes. Although ISSR markers offer valuable baseline insights, future studies employing higher-resolution genomic markers and broader geographic sampling are needed to clarify population origins, connectivity, and conservation units across the species' Arabian range. While the present study is intentionally limited in scope, integrating higher-resolution genetic markers together with morphological, ecological, and spatial data will be essential in future research to rigorously evaluate population connectivity, adaptive variation, and conservation units across the species' Arabian range.

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ARTICLE

Fish diversity in selected urban, suburban, and rural wetlands of Vellore District, Tamil Nadu, India

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Abstract: Fish diversity in relation to macrophyte distribution and physicochemical parameters was studied across six wetlands in Vellore District, Tamil Nadu, India. A total of 20 fish species were recorded, of which 12 are native to India with a higher prevalence in rural wetlands. Urban wetlands exhibit lower relative diversity due to the dominance of *Oreochromis niloticus* and the presence of *Clarias gariepinus* which may pose threats to native fish populations. Macrophytes, which influence fish habitat and growth, are abundant in both urban and rural sites. Of the 20 macrophytes identified, 14 are native to India. Physicochemical parameters show variations across sites, yet Canonical Correspondence Analysis indicates a positive correlation between environmental factors and fish diversity. These findings highlight the importance of habitat conditions in maintaining fish diversity and emphasize the need for conservation strategies to protect declining native fish species in urban wetlands.

Keywords: Catfish, conservation, Cypriniformes, exotic species, fish diversity, invasive species, macrophytes, physicochemical parameters, suburban and rural wetlands, water quality.

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Author contribution: Sampling, species identification, data analysis and manuscript writing was performed by API. SJDhelped in the identification of macrophytes. DSVK helped in guiding, fish species identification and manuscript editing. NMN provided overall guidance and helped in manuscript editing. The final manuscript has been read and approved by all the authors.

INTRODUCTION

Freshwater fish diversity is a vital component of aquatic ecosystems, providing ecological, economic, and social benefits. The composition and abundance of fish species are closely linked to the physical and chemical characteristics of their environment, as well as the structure of macrophyte communities. The study of freshwater fish diversity, particularly in the context of urban, suburban, and rural environments, is crucial for understanding the impact of human activities on aquatic ecosystems.

Fish diversity is greatly impacted by physicochemical parameters such as water temperature, pH, dissolved oxygen levels, conductivity, and nutrient concentrations. These factors have a direct impact on fish metabolism, survival, and reproduction, which changes the distribution and abundance of different species (Karr 1981). According to Carpenter et al. (1998) excessive nutrient levels may lead to eutrophication, which lowers oxygen levels and has a detrimental effect on fish communities. According to Peuranen et al. (1994), fish physiology can be altered by extremely high or low pH levels, which can result in stress or even death.

Macrophytes are essential for maintaining the diversity of freshwater fish. In addition to offering fish habitat, spawning grounds, and food supplies, they also help to stabilize the quality of the water by absorbing nutrients and raising oxygen levels (Chambers et al. 2008). There is a feedback loop between fish diversity, macrophyte presence, and water quality as the physicochemical features of the water body affect the diversity and density of macrophytes.

Fish diversity patterns differ between urban, suburban, and rural areas due to varying levels of anthropogenic influence. Freshwater ecosystems in urban areas are frequently polluted, habitat modified, and hydrologically altered as a result of infrastructure development, industrial discharges, and runoff (Walsh et al. 2005). As urban water bodies frequently experience lower dissolved oxygen levels and increased nutrient loads, these factors can result in degraded water quality and reduced fish diversity (Paul & Meyer 2008). Suburban areas, on the other hand, may have moderate pollution levels, with fewer industrial impacts but ongoing influence from residential development and agriculture. While rural areas are less affected by industrial and urban runoff, agricultural activities, such as pesticide and fertilizer runoff, can still have an impact on water quality and fish habitat (Allan 2004).

The interaction of physicochemical parameters, macrophytes, and fish diversity differs between these environments. In urban areas, water quality degradation frequently leads to a decline in macrophyte cover, which further reduces available habitat and food resources for fish, exacerbating the decline in fish diversity (Seilheimer et al. 2007). Suburban and rural areas may have more diverse macrophyte and fish communities due to better water quality, but agricultural runoff in rural areas can still pose significant risks (Sponseller et al. 2001).

The present study focuses on freshwater fish diversity in relation to physicochemical parameters and macrophytes across urban, suburban, and rural area which sheds light on how human activities can influence the aquatic ecosystems. The present study focuses on the freshwater fish diversity in relation with physicochemical parameter and macrophytes. It emphasizes the importance of effective management strategies addressing pollution, habitat conservation, and water quality maintenance in order to preserve fish diversity in freshwater systems.

MATERIALS AND METHODS

Study Areas

The present study was carried out by categorising water bodies in Vellore District, Tamil Nadu, India into urban (Vellore Fort Moat and Nellorepettai Lake), suburban (Seduvalai and Mel Kavanur Lakes), and rural (Chinna Kesa Kuppam Lake and Mordhana Dam) categories based on population density and their distance from the district headquarters (Image 1). The present study was conducted only during the post-monsoon period. Seasonal sampling was not undertaken due to resource, water and accessibility constraints within the study period. The post-monsoon season was selected for sampling as it typically represents a period of ecological stability in southern Indian wetlands, when water levels are replenished and both native and invasive species are active, providing a representative snapshot of community structure. In total, eight georeferenced sampling points were selected within each wetland to represent the range of available habitat features such as vegetation cover, flow variation, and accessibility. At each point, multiple replicates were obtained by conducting 5–6 standardized net hauls to capture local variability in fish diversity. Guidelines for Implementing Wetlands (Conservation and Management) Rules, 2017 was followed for the present study. ArcGIS software (version 10.8) was used to prepare the maps.

Vellore Fort Moat (VFM) is a water-filled structure

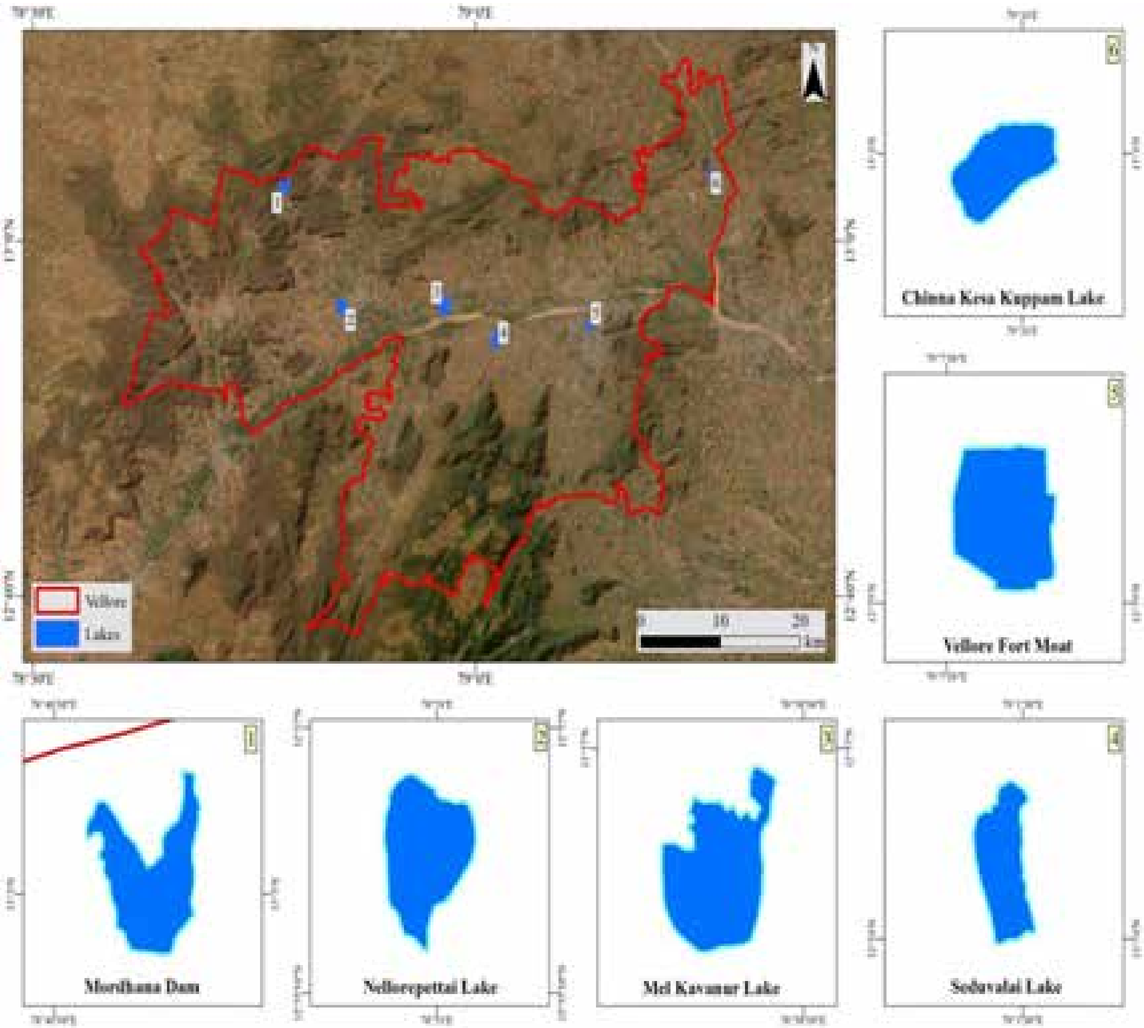

Image 1. Geographic locations of study areas.

that encircles the Vellore Fort, constructed in the 16th century. This moat provides a year-round water supply. VFM covers 50 ha.

Nellorepettai Lake (NEL) is a seasonal lake that captures monsoon runoff from Palar and Kosasthalaiyar River located in Vellore District, Tamil Nadu, India. Approximately, 2,500 urban families live around the lake, and regular fishing supports fishermen's families.

Seduvalai Lake (SEL) is a natural inland water body, which supports around 1,800 suburban families in local irrigation and sustains surrounding vegetation through its seasonal water inflow, primarily from rainfall.

Mel Kavanur Lake (MKL) is a natural inland water body. Around 1,300 suburban families live in MKL, and the lake receives water mainly from local streams and rain. Fishing is done which supports the local fishermen.

Chinna Kesa Kuppam Lake (CKL) supports approximately around 600 rural families by serving as a small reservoir used mainly for agricultural water needs, with seasonal water flow based on rainfall and mountain water nearby.

Mordhana Dam (MOD) is a large dam which stretches across 220 meters and manages a catchment area of 300 ha. It serves the needs of about 480 rural families, providing irrigation, fishing and a recreational space.

Fish Sampling and Identification: Fish were sampled by experienced local fishermen using gill nets and drag nets from eight randomly selected locations across the study areas. These sampling points were strategically distributed to cover the entire area. At the landing site, fish were recorded, photographed, and identified using established taxonomic keys (Day et al. 1875; Talwar & Jhingran 1991; Jayaram 1999; Froese & Pauly 2000). Species were preserved in 10% formalin for laboratory analysis, and their conservation status was assessed based on IUCN criteria (IUCN 2025).

Water Sample Collection and Analysis: Water quality was analysed through samples taken from the study areas, utilizing standard methods, APHA (Standard Methods Committee 2017) to evaluate both physical and chemical parameters, and IS test method to evaluate parameters like pH, total hardness, total alkalinity, chloride, sulphate, and odour.

Macrophyte Assessment: Submerged, free floating, floating-leaved, emergent, and peripheral macrophytes were documented during the survey. Their density was estimated through direct visual assessments, and identification was carried out using established taxonomic references (Cook 1996).

Data Analysis: Diversity and evenness indices were calculated using the Shannon diversity index (Shannon 1948), Pielou evenness index (Pielou 1966), Simpson index (Simpson 1949). Margalef index assessed species richness and species dominance. Environmental variables were log-transformed for normality and variance equality, minimizing the impact of dominant species. Canonical correspondence analysis (CCA) was employed to explore the relationship between fish diversity and physicochemical parameters, utilizing PAST software.

RESULTS

In the present study, a total of 20 fish species were identified from six lakes classified as urban, suburban, and rural lakes. VFM and NEL are situated in urban area, while SEL and MKL are situated in suburban area. CKL and MOD are located in rural area.

At VFM, the species identified were *Catla catla*, *Labeo rohita*, *Channa striata*, and *Oreochromis niloticus.* Among these, *C. catla*, *C. striata*, and *L. rohita* are native to India, while *O. niloticus* is an exotic species. In NEL, the species identified were *C. striata*, *Channa punctata*, *Glossogobius giuris*, *Clarias anguillaris*, *C. catla*, *L. rohita*, and *O. niloticus*. Among these, *C. catla*, *L. rohita*, *G. giuris*, *C. punctata* and *C. striata* are native, while *C. anguillaris* and *O. niloticus* are exotic/invasive (Figure 2; Table 1).

In SEL, the species identified were *O. niloticus*, *O. mossambicus*, *Anabas testudineus*, *C. striata*, and *G. giuris*. Here, both *O. niloticus* and Mozambique tilapia are exotic/invasive, while *A. testudineus*, *C. striata*, and *G. giuris* are native. MKL revealed the presence of *C. catla*, *G. giuris*, *C. striata*, *L. rohita*, *O. niloticus*, Bighead Carp *Hypophthalmichthys nobilis*, and *Clarias gariepinus.* In this lake, *C. catla*, *L. rohita*, *G. giuris*, *C. striata* are native species, and *H. nobilis* is exotic, *O. niloticus* and *C. gariepinus* are exotic/invasive (Image 2; Table 1).

C. catla, *G. giuris*, *C. striata*, *L. rohita*, *O. niloticus*, *Cyprinus carpio*, Orange Common Carp *Cyprinus carpio* var. *auratus* and *Ambasis gymnocephalus* are identified in CKL. Here, *C. catla*, *L. rohita*, *G. giuris*, and *C. striata* are native, while *O. niloticus* is invasive, and both *Cyprinus carpio* and orange common carp are considered exotic. MOD showcased a variety of species such as *Amblypharyngodon mola*, *A. microlepis*, *Ambassis dussumieri*, *O. niloticus*, *C. catla*, *Glossogobius giuris comples*, *L. rohita*, and *Mastacembelus armatus.* In this area, *A. mola*, *A. microlepis*, *A. dussumieri*, *G. giuris complex*, *M. armatus*, *C. catla,* and *L. rohita* are native, while *O. niloticus* is exotic (Image 2; Table 1). The study also, reveals that **Cypriniformes** is the most prevalent order, followed by **Anabantiformes** and **Cichliformes (Figure 1).** Order-wise classification indicates that rural sites host the highest species diversity compared to suburban and urban sites. At the species level**, O. niloticus** dominates with the highest prevalence across most of the sites (Figure 2). This pattern suggests that rural wetlands support greater fish diversity, while certain species, like *O. niloticus*, thrive across different environments, possibly due to their adaptability.

The study underscores the rich diversity of fish species in different habitats, highlighting both native and exotic species across urban, suburban, and rural areas. The findings point to the presence of numerous native species, along with a notable influx of exotic and invasive species that may have implications for local ecosystems.

Fish Diversity

The analysis of fish diversity across six study areas revealed significant differences in biodiversity. MOD exhibited the highest diversity (1.640) (Table 2) according to the Shannon diversity index, followed by CKL (1.612) and MKL (1.347) (Table 2). VFM had the lowest diversity (0.5164) (Table 2), indicating a less varied fish community.

Simpson's diversity index further supported these findings, with higher diversity at MOD (0.7438) and CKL (0.7345), while VFM showed the lowest value (0.2798) (Table 2), suggesting potential ecological imbalances.

The Margalef index indicated that CKL (1.188) and NEL (1.088) had the highest species richness, while VFM (0.4521) showed the lowest. Pielou's evenness index revealed a more equitable distribution of species at MOD (0.6441) and CKL (0.6268) (Table 2), whereas VFM

Table 1. List of recorded Fishes in Vellore Fort Moat, Nellorepettai Lake, Seduvalai Lake, Mel Kavanur Lake, Chinna Kesa Kuppam Lake, and Mordhana Dam.

	Species	Order	Family	Urban		Suburban		Rural		Common name	Native/ Exotic/ Invasive	IUCN
				VFM	NEL	SEL	MKL	CKL	MOD			
1	*Catla catla*	Cypriniformes	Cyprinidae	Y	Y	N	Y	Y	Y	Catla, Indian Carp	Native	LC
2	*Labeo rohita*	Cypriniformes	Cyprinidae	Y	Y	N	Y	Y	Y	Rohu	Native	LC
3	*Channa striata*	Anabantiformes	Channidae	Y	Y	Y	Y	Y	N	Striped Snakehead	Native	LC
4	*Oreochromis niloticus*	Cichliformes	Cichlidae	Y	Y	Y	Y	Y	Y	Nile Tilapia	Exotic/ Invasive	LC
5	*Channa punctata*	Anabantiformes	Channidae	N	Y	N	N	N	N	Spotted Snakehead	Native	LC
6	*Glossogobius giuris*	Gobiiformes	Gobiidae	N	Y	Y	Y	N	N	Giant Goby	Native	LC
7	*Clarias anguillaris*	Siluriformes	Clariidae	N	Y	N	N	N	N	Catfish	Exotic/ Invasive	LC
8	*Oreochromis mossambicus*	Cichliformes	Cichlidae	Y	N	Y	N	N	N	Mozambique	Exotic/ Invasive	VU
9	*Anabas testudineus*	Anabantiformes	Anabantidae	N	N	Y	N	N	N	Climbing Perch	Native	LC
10	*Hypophthalmichthys nobilis*	Cypriniformes	Xenocyprididae	N	N	N	Y	N	N	Bighead Carp	Exotic	LC
11	*Clarias gariepinus*	Siluriformes	Clariidae	N	N	N	Y	N	N	African Catfish	Exotic/ Invasive	LC
12	*Amblypharyngodon mola*	Cypriniformes	Danionidae	N	N	N	N	N	Y	Mola Fish	Native	LC
13	*Amblypharyngodon microlepis*	Cypriniformes	Danionidae	N	N	N	N	N	Y	Small-spotted Mola	Native	LC
14	*Ambassis gymnocephalus*	Perciformes	Ambassidae	N	N	N	N	Y	N	Glassy Perchlet	Native	LC
15	*Ambassis dussumieri*	Perciformes	Ambassidae	N	N	N	N	N	Y	Malabar Glassy	Native	LC
16	*Glossogobius giuris complex*	Gobiiformes	Gobiidae	N	N	N	N	N	Y	Goby complex	Native	LC
17	*Mastacembelus armatus*	Synbranchiformes	Mastacembelidae	N	N	N	N	N	Y	Zig zag Eel	Native	LC
18	*Cyprinus carpio*	Cypriniformes	Cyprinidae	N	N	N	N	Y	N	Common Carp	Exotic/ Invasive	LC
19	*Ctenopharyngodon idella*	Cypriniformes	Xenocyprididae	N	N	N	N	Y	N	Grass Carp	Exotic	LC
20	*Cyprinus carpio var. auratus*	Cypriniformes	Cyprinidae	N	N	N	N	Y	N	Goldfish	Exotic	LC

Y—Present | N—Absent | LC—Least Concern | VU—Vulnerable |Exotic—Introduced species, non-native to India | Invasive—Non-native species that threaten local biodiversity.

(0.419) displayed dominance by a few species.

The dominance index indicated that VFM had the highest dominance (0.7202), suggesting a community structure heavily skewed towards a few dominant fish species. In contrast, MOD (0.2562) and CKL (0.2655) (Table 2) demonstrated lower dominance, suggesting a more balanced and diverse ecosystem.

The present study highlights significant variations in fish diversity and distribution across the different areas, with urban sites like VFM showing lower diversity and higher dominance. In contrast, rural areas like MOD and CKL exhibited greater biodiversity and more balanced community structures. These findings are essential for understanding the ecological dynamics of these aquatic systems and guiding conservation strategies.

Macrophytes

A total of 20 macrophytes were identified across six study areas. Of these, 14 species are native to India, while 6 are exotic or invasive. Among these, six species were 6 submerged (*Hydrilla verticillata*, *Najas marina*, *Ottelia alismoides*, *Blyxa* sp., *Potamogeton nodosus*, and *Vallisneria spiralis*), one is free-floating (*Ipomoea aquatica*), and one species is floating-leaved (*Nymphaea pubescens*), and eight were classified as emergent (*Typha angustifolia*, *Ipomoea carnea*, *Cyperus* sp., *Cyperus difformis*, *Ludwigia grandiflora*, *Ludwigia purslane*, *Scoparia dulcis*, and *Brachiaria* sp.). The remaining four

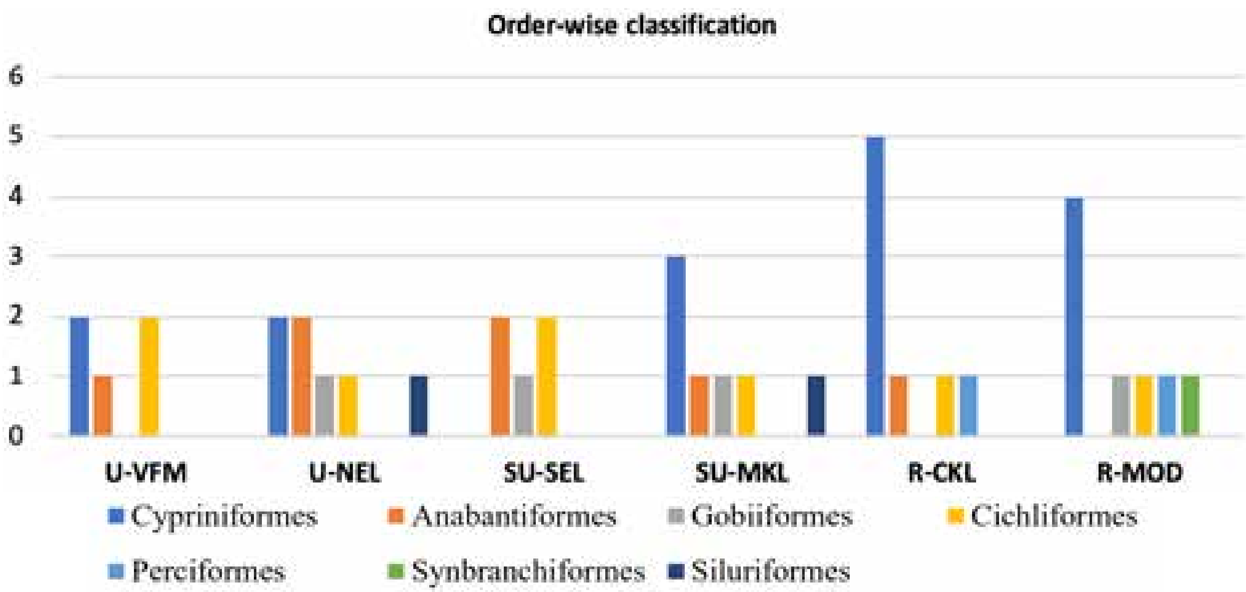

Figure 1. Distribution of fish species by order across different habitat types: Urban – Vellore Fort Moat | Nellorepettai Lake | Sub Urban – Seduvalai Lake Mel Kavanur Lake | Rural – Chinna Kesa Kuppam Lake | Mordhana Dam.

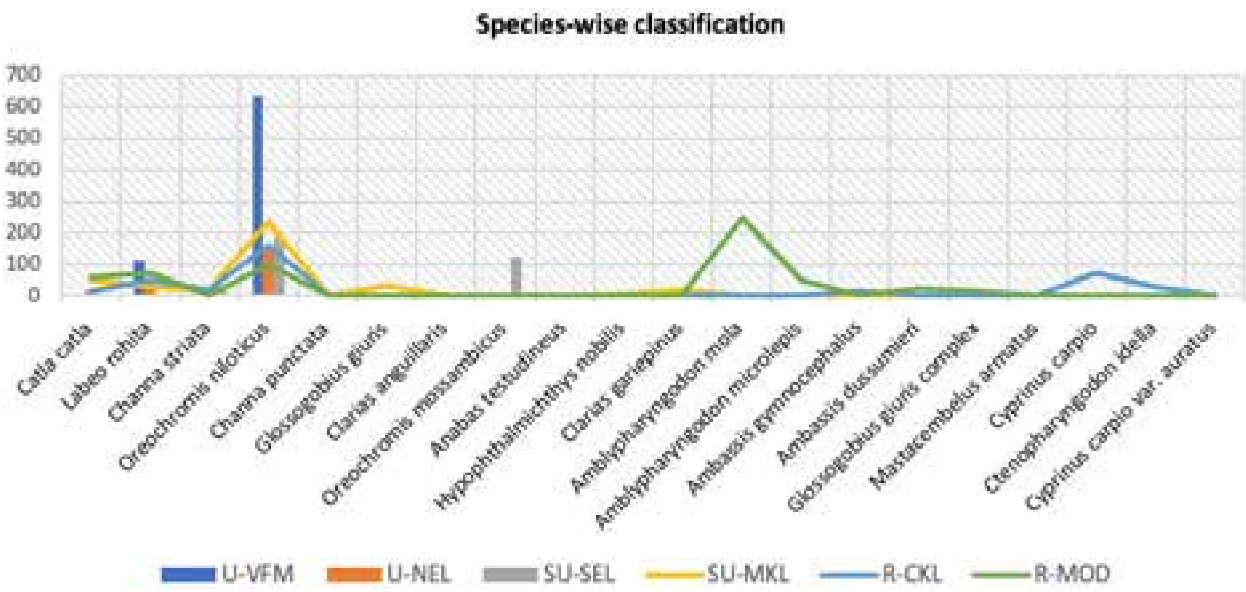

Figure 2. Distribution of fish species by species across different habitat types: Urban – Vellore Fort Moat | Nellorepettai Lake | Sub Urban – Seduvalai Lake Mel Kavanur Lake | Rural – Chinna Kesa Kuppam Lake | Mordhana Dam.

species (*Muntingia calabura*, *Coccinia grandis*, *Borassus flabellifer*, and *Chromolaena odorata*) were identified as terrestrial or marginal plants frequently occurring along the edges of aquatic habitats. Macrophytes were recorded qualitatively through visual observations. Based on these observations, the following species were abundant: *I. aquatica* and *N. pubescens* (VFM), *H. verticillata* and *T. angustifolia* (NEL), and *H. verticillata* and *N. marina* (SEL). In contrast, *N. marina* and *T. angustifolia* (MKL), *I. carnea* and *C. difformis* (CKL), and *O. alismoides* and *V. spiralis* (MOD) were abundant.

In the VFM, the identified macrophytes include *Ipomea aquatica, Nymphaea pubescens*, *M. calabura*, and *C. grandis* (Image 3; Table 3), of which *M. calabura* is exotic. At NEL, *H. verticillata*, *N. marina*, *T. angustifolia*, and *I. carnea* were found. *I. carnea* is exotic and all other species are native and supporting local biodiversity.

In SEL, the recorded species include *I. carnea*,

Table 2. Diversity analysis of Vellore Fort Moat, Nellorepettai Lake, Seduvalai Lake, Mel Kavanur Lake, Chinna Kesa Kuppam Lake, and Mordhana Dam.

	Urban		Suburban		Rural	
	VFM	NEL	SEL	MKL	CKL	MOD
Shannon_H	0.5164	1.276	1.096	1.347	1.612	1.64
Simpson_1-D	0.2798	0.5927	0.6012	0.6094	0.7345	0.7438
Margalef	0.4521	1.088	0.6822	1.007	1.188	1.104
Evenness_e^H/S	0.419	0.512	0.5984	0.5496	0.6268	0.6441
Dominance_D	0.7202	0.4073	0.3988	0.3906	0.2655	0.2562

Cyperus sp., *H. verticillata*, *N. marina*, *L. grandiflora*, and *B. flabellifer* (Image 3; Table 3), among these, *I. carnea* and *L. grandiflora* are exotic/invasive and others are native which further enhancing the area's ecological stability. MKL features *N. marina*, *Chromolaena odorata*, *I. carnea*, *T. angustifolia*, *B. flabellifer*, and *O. alismoides*. Among these, *C. odorata* and *I. carnea* is considered invasive, posing a threat to the local flora.

In CKL, *C. difformis*, *S. dulcis*, *I. carnea*, and *T. angustifolia* (Image 3; Table 3) were recorded, of these *T. angustifolia* being native species. MOD showcases *O. alismoides*, *Blyxa* sp., *L. purslane*, *P. nodosus*, *V. spiralis*, *T. angustifolia* and *Brachiaria* sp., of which *L. purslane* and *Brachiaria* sp. are exotic to India and all others are native, highlighting the rich diversity of aquatic plants in the region.

Overall, the varying levels of fish diversity across these lakes correlate with the presence of native macrophytes, highlighting the crucial role that aquatic plants play in supporting fish populations and maintaining ecological balance.

Physicochemical Parameters

The analysis of 22 physicochemical parameters across the six study areas revealed significant findings. Elevated ammonia levels were recorded in MOD, NEL, CKL, and SEL. High nitrite levels were also observed in MOD, NEL, CKL, and SEL, indicating potential ecological stress (Table 4).

Phosphate concentrations (Table 4) were notably high in MOD, with slight increases in NEL and CKL. Turbidity levels were elevated in MKL, NEL, and CKL. SEL exhibited a very high pH of 12.25 (Table 4), which could indicate alkaline conditions harmful to aquatic life. Additionally, low sulphate levels were detected in CKL and VFM, along with an extremely low magnesium concentration of 1 mg/l (Table 4) in VFM. While other parameters remained normal, the increased levels of ammonia, nitrite, phosphate, turbidity, and extreme pH, alongside low sulphate and magnesium levels, highlight concerns regarding water quality and the health of local aquatic ecosystems.

Relationship between Physicochemical Parameters and Fishes

The CCA results indicate clear relationships between fish species distribution and various environmental factors across the study areas. The first two axes explained 68% of the total variation, with axis 1 accounting for the largest proportion (42.41%) (Table 5). The dominance of a single axis suggests that some of the environmental parameters may be correlated (Table 5).

Certain species, such as *A. mola*, *A. microlepis*, and *A. dussumieri*, show strong positive correlations with elevated levels of ammonia and nitrite (Figure 3), suggesting an ecological preference for conditions characterized by these higher concentrations. Additionally, *O. niloticus* and *O. mossambicus* also exhibit similar trends, indicating their affinity for environments with increased nutrient levels (Figure 3).

O. mossambicus and *A. testudineus* demonstrate a positive relationship with alkaline conditions, particularly where pH levels are elevated (Figure 3), indicating that these species thrive in environments with more alkaline water. *C. catla* also aligns with these alkaline conditions, further reflecting the adaptability of these species (Figure 3).

In addition, *C. striata*, *C. punctata*, and *H. nobilis* are positively correlated with higher concentrations of sulphate and chloride, suggesting that their distribution is influenced by these specific physicochemical parameters. *C. anguillaris* and *C. gariepinus* may also be found in similar conditions, indicating their tolerance for varied water quality (Figure 3).

Conversely, species such as *L. rohita* and *C. carpio* demonstrate lower associations with the environmental gradients (Figure 3), indicating their narrow tolerance to varying physicochemical conditions. Overall, the CCA

Table 3. List of recorded Macrophytes in Vellore Fort Moat, Nellorepettai Lake, Seduvalai Lake, Mel Kavanur Lake, Chinna Kesa Kuppam Lake, and Mordhana Dam.

	Species	Order	Family	Urban		Suburban		Rural		Common name	Native/ Exotic/ Invasive	IUCN Red List
				VFM	NEL	SEL	MKL	CKL	MOD			
1	*Ipomoea aquatica*	*Solanales*	*Convolvulaceae*	Y	N	N	N	N	N	Water Spinach	Native	LC
2	*Nymphaea pubescens*	*Nymphaeales*	*Nymphaeaceae*	Y	N	N	N	N	N	Water Lily	Native	LC
3	*Muntingia calabura*	*Malpighiales*	*Muntingiaceae*	Y	N	N	N	N	N	Jamaican Cherry	Native	LC
4	*Coccinia grandis*	*Cucurbitales*	*Cucurbitaceae*	Y	N	N	N	N	N	Ivy Gourd	Native	LC
5	*Hydrilla verticillata*	*Alismatales*	*Hydrocharitaceae*	N	Y	Y	N	N	N	Water Thyme	Native	LC
6	*Najas marina*	*Najadales*	*Najadaceae*	N	Y	Y	Y	N	N	Horned Pondweed	Native	LC
7	*Typha angustifolia*	*Poales*	*Typhaceae*	N	Y	N	Y	N	N	Cattail	Native	LC
8	*Ipomoea carnea*	*Solanales*	*Convolvulaceae*	N	Y	Y	Y	Y	N	Pink Morning Glory	Exotic/ Invasive	LC
9	*Cyperus sp*	*Poales*	*Cyperaceae*	N	N	Y	N	N	N	Sedge species	Native	LC
10	*Ludwigia grandiflora*	*Myrtales*	*Onagraceae*	N	N	Y	N	N	N	Water Primrose	Exotic/ Invasive	LC
11	*Borassus flabellifer*	*Arecales*	*Arecaceae*	N	N	Y	Y	N	N	Palmyra Palm	Native	LC
12	*Ottelia alismoides*	*Alismatales*	*Hydrocharitaceae*	N	N	N	Y	N	Y	Floating Ottelia	Native	LC
13	*Chromolaena odorata*	*Asterales*	*Asteraceae*	N	N	N	Y	N	N	Siam Weed	Exotic/ Invasive	LC
14	*Cyperus difformis*	*Poales*	*Cyperaceae*	N	N	N	N	Y	N	Creeping Flatsedge	Native	LC
15	*Scoparia dulcis*	*Lamiales*	*Scrophulariaceae*	N	N	N	N	Y	N	Sweet Broomweed	Exotic	LC
16	*Blyxa sp*	*Alismatales*	*Hydrocharitaceae*	N	N	N	N	N	Y	Blyxa	Native	LC
17	*Ludwigia purslane*	*Myrtales*	*Onagraceae*	N	N	N	N	N	Y	Water Purslane	Exotic	LC
18	*Potamogeton nodosus*	*Alismatales*	*Potamogetonaceae*	N	N	N	N	N	Y	Pondweed	Native	LC
19	*Vallisneria spiralis*	*Alismatales*	*Hydrocharitaceae*	N	N	N	N	N	y	Eelgrass	Native	LC
20	*Brachiaria* sp.	*Poales*	*Poaceae*	N	N	N	N	N	y	African Grass	Exotic	LC

Y—Present | N—Absent | LC—Least Concern | VU—Vulnerable |Exotic—Introduced species, non-native to India | Invasive—Non-native species that threaten local biodiversity.

results highlight the distinct preferences of fish species based on environmental factors, illustrating how water quality influences species distribution in the studied areas.

DISCUSSION

Fish Diversity

The diversity of fish across the six study areas reflects the native and exotic species, habitat conditions, and physicochemical characteristics. Shannon diversity index shows low diversity in urban areas (VFM and NEL) when compared to rural areas (CKL and MOD) (Table 2). This may be due to the record of highest percentage (Figure 2) of invasive species *O. niloticus*, known for its presence in degraded water conditions (Figure 2). Martin et al. (2010) documented that *O. niloticus* often outcompetes native species in polluted environment. Similar pattern is observed in the present study, where native fish diversity was comparatively lower in the urban areas. Historically, Nile Tilapia *O. niloticus* was first introduced into Indian freshwater systems during the mid-20th Century, primarily to enhance aquaculture production due to its rapid growth, high reproductive capacity, tolerance to water quality, and strong market demand (Sugunan 1995; De Silva et al. 2004). Over time, accidental and intentional releases from fish farms

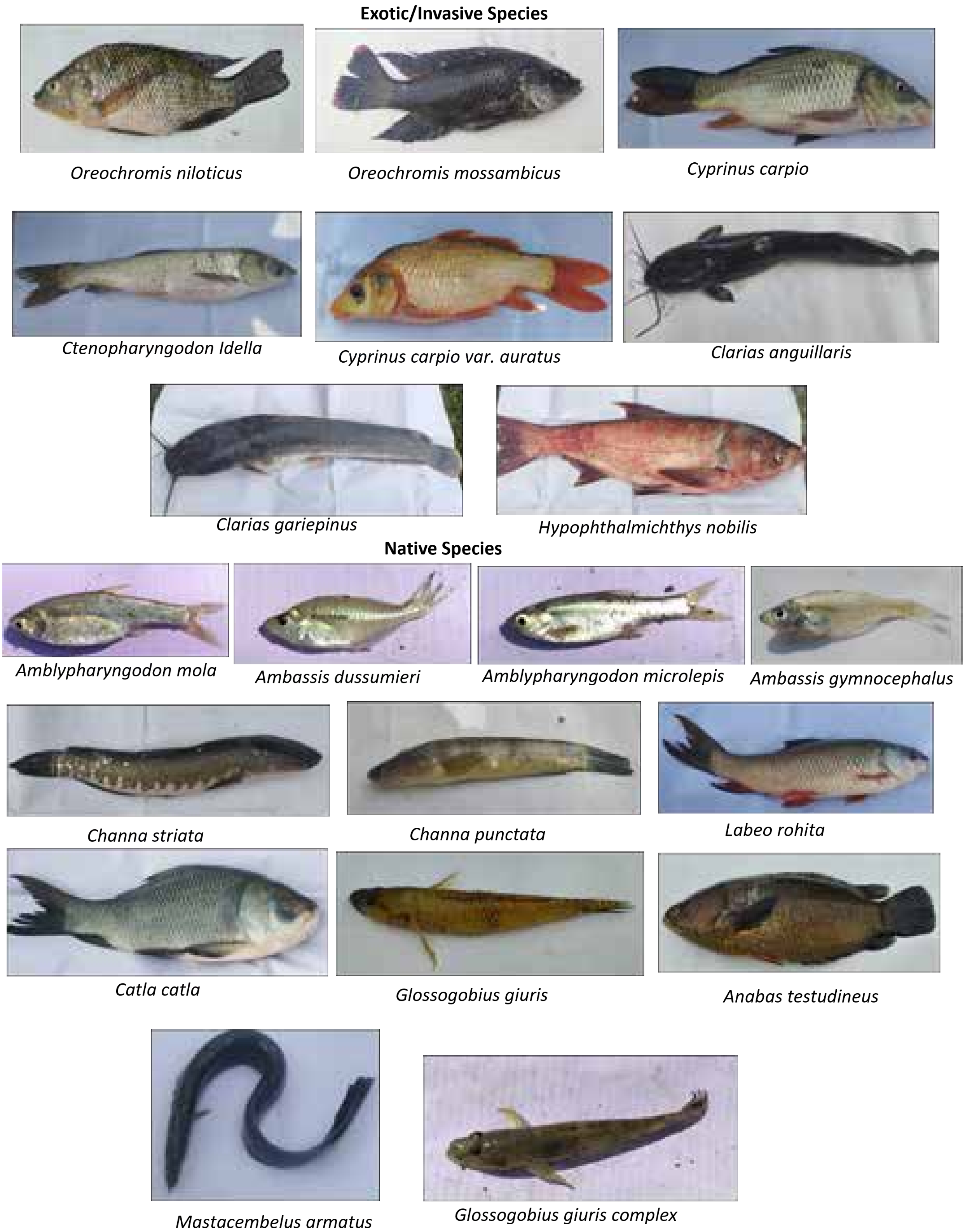

Image 2. Fishes recorded in Vellore Fort Moat, Nellorepettai Lake, Seduvalai Lake, Mel Kavanur Lake, Chinna Kesa Kuppam Lake and Mordhana Dam. © Annie Pushpa Isaac.

Table 4. Physicochemical parameters of water samples collected from Vellore Fort Moat, Nellorepettai Lake, Seduvalai Lake Mel Kavanur Lake, Chinna Kesa Kuppam Lake, and Mordhana Dam.

	Acceptable limit	Maximum permissible limit in the absence of alternative source	Urban		Suburban		Rural	
			VFM	NEL	SEL	MKL	CKL	MOD
I. PHYSICAL PARAMETERS								
1. Appearance	-	-	Slightly turbid	Slightly turbid	clear	Slightly turbid	Slightly turbid	Slightly turbid
2. Colour	-	-	slightly yellowish	slightly yellowish	colourless	slightly yellowish	slightly yellowish	slightly greenish
3. Odour	Agreeable	Agreeable	odour some	odour some	none	odour some	odour some	odour some
4. Turbidity NTU	1	5	2	6	0	6	6	2
5. Total dissolved solids $_{Mg/L}$	500	2000	1396	942	1017	1540	473	446
6. Electrical conductivity micro mho/cm	-	-	1994	1345	1453	2200	675	637
II. CHEMICAL PARAMETERS								
7. pH	6.5–8.5	6.5–8.5	7.1	7.42	12.25	7.54	7.16	7.04
8. Alkalinity pH as $CaCO_{3\ mg}$/L	-	-	-	-	-	-	-	-
9. Alkalinity Total as $CaCO_3$ mg/L	200	600	300	272	228	300	172	148
10. Total Hardness as $CaCO_3$ mg/L	200	600	400	336	244	380	260	22
11. Calcium as Ca mg/L	75	200	70	67	48	76	31	45
12. Magnesium as mg/L	30	100	1	40	30	46	19	26
13. Iron Total as Fe mg/L	0.3	1	0	0	0	0	0	0
14. Manganese as Mn mg/L	0.1	0.3	0	0	0	0	0	0
15. Free Ammonia as NH_3 mg/L	0.5	0.5	0.058	0.67	1.04	0.25	0.7	0.6
16. Nitrite as NO_2 mg/L	0.2	0.2	0.04	0.1	0.11	0.03	0.1	0.25
17. Nitrate as NO_3 mg/L	45	45	20	14	17	23	10	5
18. Chloride as Cl mg/L	250	1,000	442	210	264	470	97	85
19. Fluoride as F mg/L	1	1.5	0.4	0.8	0.6	0.6	0.4	0.4
20. Sulphate as SO_4 mg/L	200	400	79	111	105	160	40	34
21. Phosphate as PO_4 mg/L	-	-	0.13	0.21	0.17	0.06	0.23	0.34
22. Tidys Test 4Hrs.as O_2 mg/L	-	-	0.1	0.1	0.1	0.1	0.1	0

facilitated its spread into rivers, lakes, and reservoirs. These same traits that make it a valuable aquaculture species with broad dietary range, aggressive foraging, and adaptability have also enabled it to dominate wild habitats, often displacing native species (Sugunan 1995; De Silva et al. 2004). Beyond competition with native fish, Nile tilapia are omnivorous fish that feed on both plants and invertebrates. Their feeding behaviour and waste production can modify the nutrient composition of the water, often increasing concentrations of nitrogen and phosphorus (Tesfahun & Temesgen 2018).

In the suburban study areas, SEL and MKL showed an intermediate level of diversity (Table 2). Native species like *C. striata* and *G. giuris* were recorded along with invasive species such as *O. mossambicus* and *C. gariepinus* (Image 2). The African Catfish *C. gariepinus* native to African river systems, was introduced to India in the 1990s to boost aquaculture yields (Singh et at. 2015. However, its ability to survive in low-oxygen waters, tolerate wide temperature fluctuations, and consume a wide variety of prey has contributed to its invasive success. In many cases, its predatory nature has severely impacted small indigenous fish species through both direct predation and competition for resources. Reports from various Indian states have documented significant alterations in aquatic food webs following its establishment (Singh et at. 2015. *C. gariepinus* may also influence macrophyte communities by disturbing sediments during benthic foraging, which can uproot aquatic vegetation and alter habitat structure. This

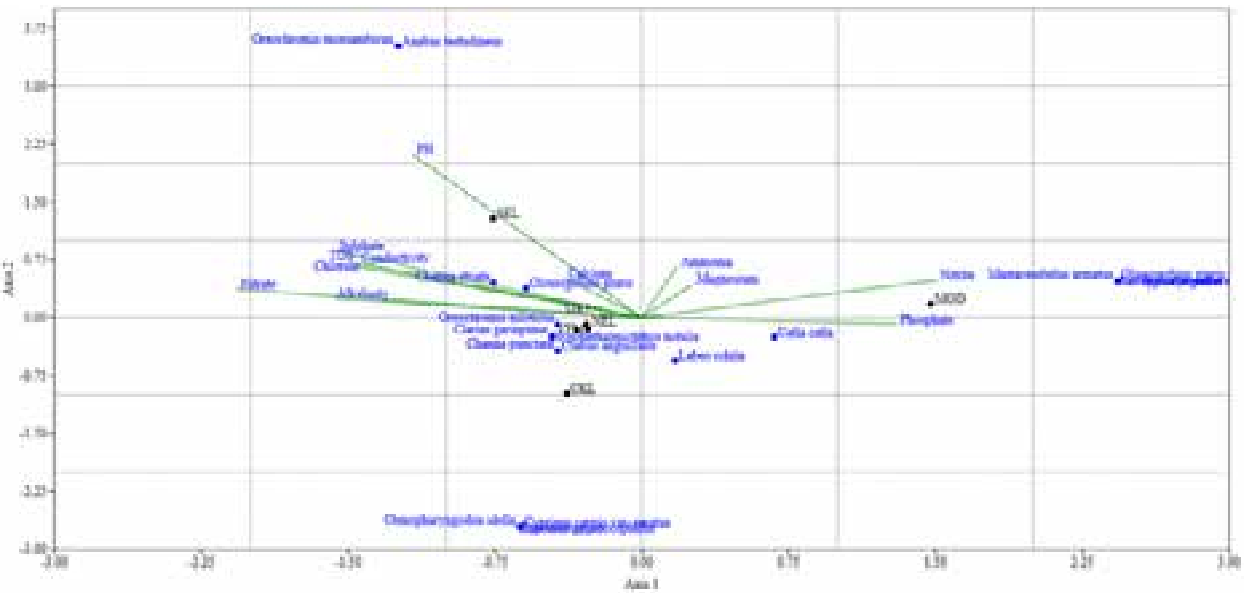

Figure 3. Relationship of fish species with physicochemical parameters using canonical correspondence analysis.

Table 5. Canonical correspondence analysis axis eigenvalues and statistics.

Axis	Eigen value	% of constr. in.	% of total inertia
1	0.6053	42.56	42.41
2	0.3637	25.57	25.48
3	0.2677	18.82	18.76
4	0.1253	8.811	8.78
5	0.06012	4.228	4.213

behaviour, similar to that of the common carp, can lead to a reduction in macrophyte abundance and diversity (Miller & Crowl 2006). The introduction of *C. gariepinus* into non-native ecosystems has led to negative impacts on the small indigenous fish species. Their predatory behaviour can cause declines in native fish populations through direct predation and competition for resources (Kadye & Booth 2012). As per Yam et al. (2015), suburban wetlands often support both native and exotic species due to moderate levels of human activities. According to Leidy et al. (2011) such environments allows both groups to coexist, but invasive species can still impact native fish through competition for various resources, which may explain the intermediate diversity observed in the present studied suburban areas.

In the present study rural areas, CKL and MOD showed the highest diversity (Table 2). Native species like *C. catla, L. rohita*, *A. mola*, *A. dussumieri*, and *M. armatus* were recorded, with minimal invasive fish species (Image 3; Table 3).

I. carnea (NEL, SEL, MKL, CKL), *L. grandiflora* (SEL) and *C. odorata* (MKL) were the most frequent exotic/invasive macrophytes found in the study area (Table 3). Dense *Ludwigia* sps. is reported to form thick surface mats that shade the water column and lower DO. Decomposition of this macrophyte further intensify hypoxia conditions, which affects sensitive native fishes and favour tolerant taxa (Pelella et al. 2023). In southern Asian wetlands, increasing cover of invasive plants, especially *I. carnea* has been linked to significant DO decline and higher free CO_2/alkalinity, with shifts in aquatic biota and reduced fish diversity as coverage increases (Pandey et al. 2020). Terrestrial invasive, *C. odorata* at wetland margins can also alter riparian structure and nutrient/light regimes, indirectly affecting macrophyte mosaics and fish habitat (Rai & Singh 2024). Although *T. angustifolia* is native, extensive *Typha* expansion in many systems similarly reduces nearshore DO and fragments open-water access, constraining fish movements; this helps explain lower diversity where emergent belts dominate littoral zones (Lishawa et al 2023).

In VFM, *M. calabura* occurs along the water edge, with branches overhanging the moat, fruits and leaf litters regularly falling into the water, potentially influencing nutrient cycling and providing organic matter to aquatic biota. Similarly, in MKL, *B. flabellifer* trees were observed growing in the middle of the inundated zone, likely due to seasonal water level changes, thereby contributing shade, structural habitat, and detritus to the aquatic environment. Previous studies have recognized that riparian and emergent vegetation can significantly influence aquatic ecosystem structure and function through shading, organic matter input, and habitat complexity (Gregory et al. 1991; Allan 2004). Thus, their inclusion reflects their ecological role within the aquatic habitats.

In the urban area, VFM, macrophytes such as *I. aquatica*, *N. pubescens*, *M. calabura*, and *C. grandis* are prevalent (Image 3; Table 3). Native fish species like *C. catla* and *L. rohita* were also observed in VFM (Image 2). These macrophytes provide essential cover and help regulate oxygen levels, creating a supportive habitat for these native species (Petr 2005). Though macrophytes are recorded more in VFM the presence of invasive species *O. niloticus*, which outcompete native species. This may be one of the reasons for less fish diversity in VFM. At NEL, macrophytes such as *H. verticillata*, *N. marina*, *T. angustifolia*, and *I. carnea* were recorded (Image 3; Table 3). These plants enhance local biodiversity by supporting fish species. *H. verticillata* and *N. marina*, known for their water oxygenation properties, benefit fish populations by creating breeding-friendly conditions (Durborow 2014).

In the suburban areas, SEL and MKL, a wider range of macrophytes, including *H. verticillata*, *N. marina*, *Cyperus* sp., *T. angustifolia*, and *O. alismoides* were recorded, which supports fish diversity by providing the oxygen-rich environment created by these macrophytes (Image 3; Table 3). However, in MKL, the presence of the invasive macrophyte *C. odorata* is concerning as it can overshadow native vegetation and reduce habitat, potentially affecting native fish species over time.

In the rural sites, CKL and MOD, a diverse macrophytes, such as *C. difformis*, *S. dulcis*, *T. domingensis*, *P. nodosus*, and *V. spiralis* are found, which supports a high level of fish diversity (Image 3; Table 3). These rural sites show a healthier ecosystem, where species like *C. catla*, *L. rohita*, *A. mola* and *M. armatus* are observed (Image 2). Earlier studies confirms that complex habitat and stable water conditions are provided by native macrophytes (*P. nodosus* and *V. spiralis)* which also supports native fishes for spawning (Johnston 1991; Flint & Madsen 1995; Brendonck et al. 2003; Tang et al. 2021). Macrophytes like *Blyxa* spp., *Ludwigia purslane*, and *Brachiaria* spp. recorded in MOD contribute to fish habitat structure, which benefits smaller species, such as *A. mola*, *A. microlepis*, *Ambassis dussumieri*, and larger native species. According to Petr (2005), macrophyte-rich habitats in less-disturbed areas support a wide range of native fish due to the availability of shelter and nutrient cycling provided by these macrophytes.

Overall, the macrophyte diversity at each site appears to impact fish diversity. Urban areas with higher macrophyte diversity and invasive species favour resilient exotic and invasive fish, while suburban and rural areas with richer macrophytes support a higher diversity of native species.

Influence of Physicochemical Parameters on Fish Diversity

The relationship between fish diversity and macrophyte presence in the studied wetlands highlights the crucial role of macrophytes in mitigating the effects of increased physicochemical parameters. Despite increased ammonia concentrations observed at sites such as MOD, NEL, CKL and SEL (Table 4), certain fish species shows resilience to these conditions, which is supported by diverse macrophytes.

In NEL, the coexistence of native species such as *C. striata* and *L. rohita* with the invasive *O. niloticus* demonstrate the complex dynamics which shows positive relation with ammonia (Figure 3). Macrophytes like *H. verticillata* and *T. angustifolia* are providing habitat complexity and enhancing water quality (Longstreth 1989; Zhou et al. 2018). According to Kalengo et al. (2021) the nutrient uptake by these macrophytes can mitigate the impacts of elevated ammonia, thereby supporting fish populations.

MOD also records elevated ammonia levels and decreased chloride, sulphate and nitrate (Table 4) levels but maintains a diverse fish community, including *A. mola* and *M. armatus* (Image 2). CCA shows small native fishes shows positive correlation with these nutrients (Figure 2). The presence of native macrophytes such as *P. nodosus* and *V. spiralis* contributes to ecological stability, offering shelter and breeding grounds for fishes, even in nutrient-stressed environments (Marwat et al. 2011; Tang et al. 2021).

CKL exhibits high fish diversity despite limited aquatic macrophytes and elevated ammonia level decreased calcium, magnesium and other vital nutrients (Table 4). The lake's primary water sources are rainfall, mountain runoff and agricultural runoff. While mountain runoff can introduce both beneficial nutrients and pollutants (Molla et al. 2022), agricultural runoff is a known source of excess nutrients and other contaminants (Rao et al. 2022). Macrophytes, such as *T. domingensis*, typically enhance water quality and provide habitat in aquatic ecosystems (Dhir et al. 2009; Talevska et al. 2009; Kalengo et al. 2021) however, their physicochemical changes in CKL suggests other factors are influencing the fish community. The influx of nutrients and potentially unique thermal conditions from mountain runoff may

Image 3. Macrophytes recorded in Vellore Fort Moat, Nellorepettai Lake, Seduvalai Lake Mel. Chinna Kesa Kuooam Lake and Mordhana Dam. © Annie Pushpa Isaac.

be supporting the diverse fish population, particularly various carp species present in CKL (Table 2). Further research is needed to fully understand the complex interplay of these factors in maintaining the lake's high fish diversity.

SEL shows high pH of 12.25 (Table 4), potentially harmful to aquatic life. *O. mossambicus* and *A. testudineus* demonstrate a positive relationship with alkaline conditions according to CCA (Figure 3) which is found abundant in SEL and it is supported by Thammaratsuntorn et al. (2016) *O. mossambicus* thrive in alkaline conditions, which shows adaptability. Research suggests that such resilience can be linked to the presence of macrophytes that enhance habitats despite challenging water quality (Van der Cruysse et al. 2024).

The interaction between elevated physicochemical parameters and macrophyte presence enhance the importance of macrophytes in maintaining ecosystem. Although these factors cause stress in some locations, native macrophytes improves water quality and add structural complexity, promotes fish diversity and ecological sustainability in various wetland types.

Limitations

While this study calculated a range of physicochemical parameters (such as nitrate, phosphate, ammonia, and conductivity) to assess water quality and infer potential pollution sources, direct measurements of specific pollutant level and agricultural pollutants (pesticide and fertilizer residues) were not conducted. Therefore, the attribution of elevated nutrient levels in urban, suburban and rural wetlands is based on indirect evidence rather than compound-specific analysis. Future studies incorporating targeted chemical analyses would strengthen the ability to link observed nutrient enrichment to specific anthropogenic activities.

CONCLUSION

The study shows that fish diversity is related to the presence of macrophytes and the physicochemical parameters of wetlands. The presence of diverse macrophyte species, along with favourable water quality conditions, promotes increased fish diversity. However, wetlands that are subjected to the dominance of invasive species have lower fish diversity, particularly in urban areas. Based on the findings, an integrated management strategy combining physical removal, biological control (e.g., enhancement of native predators, sterile male release), and habitat restoration is recommended. Removal of *C. gariepinus* and *O. niloticus* is recommended from the study area, as they pose significant predatory, competitive, and reproductive threats to native small indigenous fishes. *O. niloticus*, in particular, can rapidly dominate fish communities due to its high reproductive rate and adaptability, leading to reduced native species abundance. Management of invasive macrophytes, which can alter habitat structure and restrict native macrophyte growth, should also be prioritized through mechanical removal or biological control measures. These findings highlight the importance of habitat restoration and improved management strategies for preserving wetland biodiversity. Additionally, monitoring and mitigating changes in physicochemical parameters, particularly nutrient enrichment, can help to maintain optimal water quality and prevent further ecological disruption.

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ARTICLE

Macrofungal species richness, composition, distribution, and ecological preference along the elevation gradient in Agasthyamala Biosphere Reserve, southern Western Ghats, India

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Abstract: Fungi are a part of the forest ecosystem as saprotrophs, mycorrhizal symbionts, pathogens, and parasites/predators. The health status of an ecosystem is reflected in its macrofungal species richness and diversity. The present study emphasizes the effect of elevation and substrate availability on macrofungal composition, species richness, and distribution in Agasthyamala, a highly endemic, rich tropical wet evergreen forest type. The study was conducted at the forests of Agasthyamala situated in Thiruvananthapuram District, Kerala, part of the Western Ghats, for a period of four years (2021–2024). Macrofungal sampling and quadrats of (10 x 10m) were laid out in 15 different locations under various elevation classes. A record of 1,929 individuals and 112 macrofungal species from 13 orders, 41 families, and 73 genera was documented. Of these, 86% were saprotrophic, 11% were ectomycorrhizal, 2% were pathogenic, and 1% were parasitic. The low- and mid-elevations exhibit high species richness which gradually decrease with increasing elevation. This trend has been explained with the help of substrate availability and other factors. The mid-elevation was supported with more substrate availability and diversity, including dead wood, fallen twigs, litter, soil, and live trees. The presence of an adequate substrate and its diversity, along with other factors including temperature, precipitation, vegetation type, and soil properties influence the macrofungal species richness, diversity, and distribution. Hence, the knowledge of the factors influencing the macrofungal community are very important to study their future species composition and richness under a global climate change scenario.

Keywords: Basidiomyctes, climate change, diversity, ecosystem, ectomycorrhiza, fungi, mushroom, litter, saprotrophs, vegetation.

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Author contribution: KKA has done the survey in Agasthyamala forests of Western Ghats of India and identified the macrofungi. She has written the full part of this manuscript with able guidance of AK & CK. AR & BN revised the manuscript. CK reviewed this manuscript prior to submission.

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INTRODUCTION

Macrofungi are necessary for the ecological functioning of forests. They are involved in different functions including increasing plant-soil connections, improving soil structure, promotion of soil microorganisms, improving organ function, resistance to antagonistic plant root disease pathogens, and the degradation of wood in the forest ecosystem (Huo 2010). Numerous studies on the key elements influencing plant and animal communities were carried out by ecologists. Unfortunately, because of their often concealed nature and frequently short-lived sporocarps, community assembly in macrofungi is rarely researched and understood (Senn-Irlet et al. 2007). Research works have shown that a variety of environmental factors and landscape variability can control the composition of fungal communities (Ferrari et al. 2016). To determine the proportional of environmental variables to fungal diversity and composition in an ecosystem, it is to assess the fungal communities in various ecosystems. Fungal biodiversity displays the variability of habitat and is strongly tied to total site biodiversity; it may be able to give insight into changes in ecosystems (Boddy et al. 2014). The diversity and composition of fungal communities can be influenced by a wide range of biotic and abiotic variables (Tedersoo et al. 2014). According to some research, plant diversity, temperature, and precipitation are the main factors influencing the macrofungal flora (Tedersoo et al. 2014). The development and reproduction of macrofungi are significantly influenced by a variety of factors, including elevation, temperature, light, humidity, soil, surrounding vegetation, and human activities (Tapwal et al. 2013). It is very important to understand the dynamics and composition of the fungal community over place and time may be a helpful tool for assessing the health of forests and setting conservation priorities in various areas. The majority of macromycete studies, however, focus on taxonomy and systematics. Research over an elevation gradient in the Costa area was conducted by Caiafa et al. (2017), who discovered that the functional diversity of macromycetes changes with elevation, that it is more closely associated with microclimatic factors than with vegetation structure, and that heterogeneity of trait abundance and niche complementarity. A few studies have been conducted to study the influence of elevation on macrofungal composition and distribution. Hence this study focuses on the macrofungal species richness, composition, distribution, and ecological preference along the elevation gradient in Agasthyamala Biosphere Reserve, southern Western Ghats, Kerala.

MATERIALS AND METHODS

Study area

Agasthyamala consists of a compact block of hilly range on the southernmost end of Western Ghats, Thiruvananthapuram District of Kerala State. It comprises Neyyar, Peppara, and Schendurney wildlife sanctuaries and the remaining areas of Achenkovil, Thenmala, Konni, Punalur, Thiruvananthapuram Forest Division, and Agasthyavanam Special Division. Agasthyamala was established as a Biosphere Reserve in 2001. The study area has a tropical, humid climate, which is found throughout the western slopes of the Western Ghats (Mohanan & Sivadasan 2002). The temperature of the area ranges from 27–35 ^{o}C with an annual rainfall between 2,400 and 3,500 mm. The soil type is mainly lateritic and red loamy. River Neyyar and Karamanayar drain through the Agasthyamala Biosphere Reserve. The area is well known for its species richness and endemism. The distinguished diversity in ecological conditions and variation in altitude have been accountable for the rich and diverse vegetation in the study area. The tropical wet evergreen forests was one of the major forest types in Agasthyamala distributed in 800–1,400 m elevation range. The second major forest type, southern tropical moist deciduous forests, was seen at lower altitudes from 300–800 m (Mohanan & Sivadasan 2002).

Survey of macrofungi

Macrofungal survey was carried out during monsoon and post-monsoon seasons of 2021–2024 in different forest areas of Agasthyamala Biosphere Reserve, where the sites were selected on the basis of elevation gradient. The macrofungal species from different elevations were documented and these elevations grouped into three elevation classes like low elevation class (300–599 m), mid elevation class (600–899 m), and high elevation class (900–1,199 m). Macrofungal species are arranged according to the elevation classes. During the field survey, macrofungal species were detected by the presence of sporocarps (basidiocarps and ascocarps) that are visible to naked eye (Kirk et al. 2008). In a single field visit, a few macrofungal species were visible hence repeated surveys were done in all the selected sites. Location details of each elevation class were tabulated (Table 1).

Macrofungal sampling

The macrofungal assessment was done using the quadrat method as suggested by Harsh (2021). Fifteen quadrat plots of size (10 x 10m) were laid out across the elevation gradient. These sample plots were categorized into three elevation classes based on the elevation gradient. Fresh samples of macrofungal species collected from each location. The number of sporocarps of each species in each plot was recorded with geographic coordinates and elevation details using a Garmin GPS (*e*trex 30 xs). All the collected macrofungi were photographed within their natural habitat and their ecological characteristics were recorded. Spore prints were made during the study and deposited in the Forest Protection Division Laboratory at ICFRE-IFGTB, Coimbatore (IFGTB/FP- 101 to 108). The collected specimens were kept in a thermocol box and brought into the laboratory for drying. The specimens were dried in a hot air oven at 50 °C for seven hours. These dried specimens were prepared as fungal herbaria for reference work in posterity.

Collection of macrofungi and field observations

Macrofungal fruiting bodies exist on different substrates are separated using various tools like knives, scissors, and forceps. Fresh specimens were collected from each plot with great care to the sporocarps. Soil present on the fruiting bodies was removed using a soft paint brush. The macrofungal fruiting bodies seen on the wood were collected along with the substratum. A small hand lens carried to observe the minor features of the sporocarps. The habitat and morphological features were recorded and the specimens were photographed within their natural habitat before collection using iPhone 6S (Apple iPhone 6s. Released on 25 September 2015, Model number A1688, 143 g, 7.1 mm thickness. iOS 9, up to iOS 15.8.4. 64GB storage, manufacturer: Apple inc. (Designed in Cupertino, California, USA)) to facilitate further identification of the species. The collected specimens were kept in a thermocol box in which dried leaves (dried using hot air oven) of *Casuarina equisetifolia* are arranged as a bed (Akshaya et al. 2023). During collection, the number of sporocarps (fruiting bodies) produced by each species in each plot were counted and recorded in the field book. The field book contains other details such as date of collection, collection number, collector name, locality, habit, habitat, geographic coordinates, elevation, nature of substratum and the forest type. Collected specimens were taken to the field station for further processing.

Table 1. Details of elevation classes, elevation band, locations under each elevation class.

Elevation classes	Elevation band (in m)	Locations
Low-elevation	300–599	Peppara Check Post Vazhukkanpara Kallar Bonacaud picket station Agasthyamala Trekking area
Mid-elevation	600–899	GB Division Top Division Kurushumala Elakkad 36 Mala
High-elevation	900–1,199	Cardamom Estate Kilavanthottam Pandimotta Pandipath Pandipath Top

Drying and preservation of macrofungi

Collected specimens were preserved on open flame at the field station. The tough specimens were kept drying for a longer time (Swapna et al. 2008). The dehydrated specimens were packed in long brown paper covers containing naphthalene balls which prevent the attack of mites and insects. The packed specimens were labelled with date of collection and collection number. After reaching the Forest Protection Division laboratory of ICFRE-Institute of Forest Genetics & Tree Breeding, Coimbatore, the samples were properly dried using a hot air oven at 50 °C for seven hours (Akshaya et al. 2023). Then the dried specimens were labelled with other field details and deposited (IFGTB/FECC- 001 to 112) in the Forest Ecology and Climate Change Division Laboratory, ICFRE- IFGTB for future references.

Examination of spore colour

Spore print is an important character for distinguishing bracket fungi, coral fungi, fleshy gilled fungi, and fleshy pore fungi. Examination of the spore colour of macrofungal species has been done just after the collection and before drying. It is taken by keeping the hymenium surface (spore producing surface) of a fruiting body (with removed stalk) on a glass overnight and covered using a bowl to prevent air currents. Later, this setup gives information on the arrangement of gills and spore colour (Image 1). After recording spore colour and other details, the spore prints were properly tagged and maintained for further microscopic studies (Swapna et al. 2008).

Species identification

The macrofungal species were identified with the help of monographs and the available literature (Christensen 1968; Ryvarden & Johansen 1980). The confirmation of macrofungi at species level was done with the help of Mycologist (Dr. Nirmal S.K. Harsh, former scientist-G & head, Forest Pathology Division & former group coordinator research, Forest Research Institute, Dehradun).

Classification of substrates

Substrates were classified into six categories. These include dead wood, fallen twig, live tree, soil, litter, and dung.

Ecological preference of macrofungi

The nature of substratum was recorded in field as well as from the available literature. The majority of macrofungi associate with forest trees are in obligatory symbiotic ectomycorrhizal (ECM) associations. Saprotrophic fungi are significant group of decomposers, as they grow on a variety of substrates including dead wood, fallen twig, litter, dung, bark and wood of standing trees. In live and dead trees, a number of macrofungi act as pathogens or parasites (Swapna et al. 2008).

Soil sampling and analysis

After removing the debris from the soil surface, composite soil samples were augured from 20 cm depth. The soil samples were collected in zip lock covers and taken to the Soil and Water Testing Laboratory, ICFRE-IFGTB, Coimbatore. Soil samples were air dried and sifted through 2 mm sieve. Standard procedures adopted for analyzing physico-chemical properties are given (Table 2).

Data analysis

Macrofungal species identified from each plot, number of individuals (sporocarps), nature of substratum, soil data, vegetation, GPS coordinates along with the elevation were documented in Microsoft Excel (2007). The data was analyzed using SPSS (version 17.0). One way analysis of variance was done, and the significant difference was determined according to Duncan's Multiple Range Test at significant level of $P < 0.05$.

RESULTS

Macrofungal species composition and distribution

A total of 1,929 individuals were recorded from Agasthyamala, belonging to 112 species, 73 genera, 41 families and 13 orders (Table 3). Most of the macrofungal species come under the division Basidiomycota (92%) and remaining ones were of Ascomycota (8%) (Figure 1a). The dominant order represented in this location is Agaricales (46.43%) and family Polyporaceae (20.54%) is the dominant family (Figure 1b,c). The list of species recorded in this study have been tabulated (Table 3). The low elevation class was recorded with 42 species. Among them 26 species were confined only to low elevation. These include *Auricularia mesenterica*, *Cellulariella acuta*, *Clavaria rosea* var. *subglobosa*, *Clavulinopsis imperata*, *Cotylidia* sp., *Crepidotus variabilis*, *Dacrymyces capitatus*, *Daldinia concentrica*, *Earliella scabrosa*, *Favolaschia calocera*, *Mycena manipularis*, *Fomitopsis quercina*, *Ganoderma lobatum*, *Gymnopus* sp., *Laccaria* sp., *Lycoperdon pyriforme*, *Marasmius guyanensis*, *Marasmius siccus*, *Marasmius* sp. 2, *Microporellus dealbatus, Panaeolus antillarum, Resupinatus tristis, Scleroderma bovista, Stereum* sp., *Tremella fuciformis,* and *Xerotus archeri.*

Areas in mid-elevation revealed the existence of

Table 2. Details of procedures followed for physio-chemical analysis of soil.

Parameters	Methods	Author (s)
Soil pH	pH meter -1:2.5 soil water ratio (Systronics - pH System 361)	Jackson (1973)
Electrical conductivity	Conductometry -1:2.5 soil water ratio (Systronics - Conductivity TDS Meter 308)	Jackson (1973)
Organic carbon	Chromic acid wet digestion	Walkley & Black (1934)
Available nitrogen	Alkaline permanganate method (Kelplus (CLASSIC-DX))	Subbiah & Asija (1956)
Available phosphorus	Neutral / Alkaline soils 0.5 M $NaHCO_3$ extract, Ascorbic acid method (Shimadzu UV 1780 Spectrophotometer)	Olsen et al. (1954)
	Acid soils	Bray & Kurtz (1945)
Available potassium	Flame photometer, Neutral normal Ammonium acetate extraction (Systronics- Flame photometer 128)	Stanford & English (1949)
Calcium & magnesium	Versanate Method	Jackson (1973)
Texture	Hydrometer method	Bouyoucos (1936)

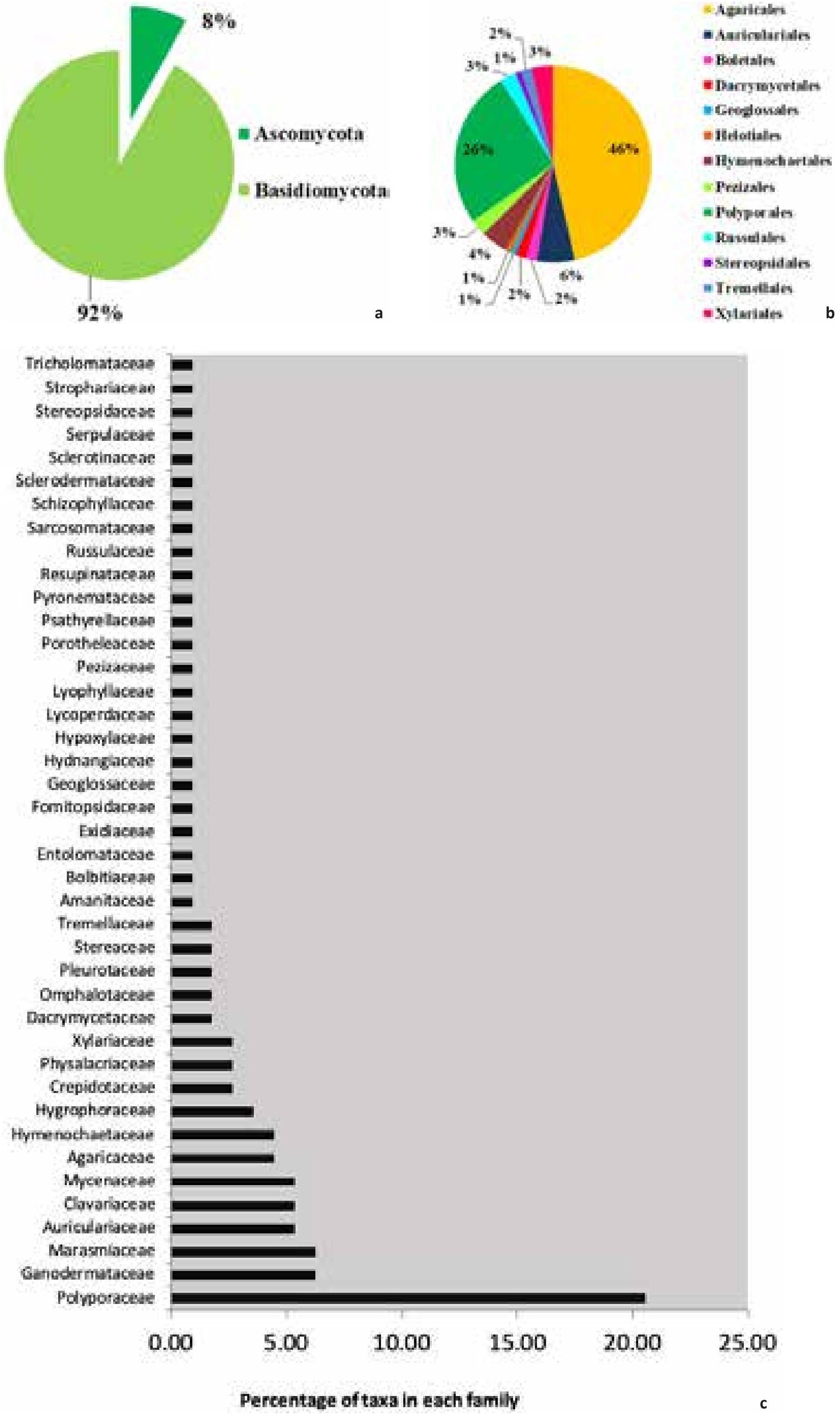

Figure 1. Percentage of distribution of macrofungi: a—different phyla | b—different orders | c—different families.

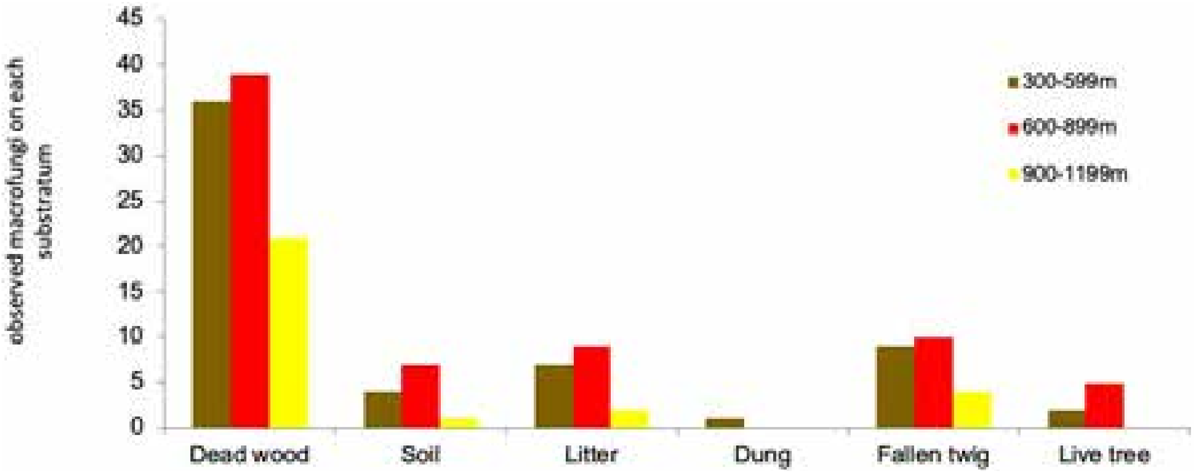

Figure 2. Substrate presence versus observed macrofungi on each substratum in different elevation classes.

71 macrofungal species. Among them, 59 species was restricted to mid elevation class (600–899 m). Species such as *Agaricus* sp., *Amanita vaginata*, *Auricularia fucosuccinea*, *Auricularia nigricans*, *Chlorophyllum molybdites*, *Clavaria miniata*, *Clavaria zollingeri*, *Collybia cookei*, *Crepidotus* sp. 1, *Crepidotus* sp. 2, *Cuphophyllus pratensis*, *Daedaleopsis confragosa*, *Entoloma* sp., *Exidia glandulosa*, *Exidia recisa*, *Favolus grammocephalus, Ganoderma* sp. 1, *Ganoderma tsugae*, *Geoglossum* sp., *Gerronema* sp., *Hygrocybe ceraceae*, *Hygrocybe conica*, *Hymenopellis radicata*, *Hymenopellis* sp., *Lentinus badius*, *Lentinus sajor–caju*, *Lentinus* sp., *Lentinus tigrinus*, *Leucocoprinus fragilissimus*, *Marasmiellus ramealis*, *Marasmius rotula*, *Melanotus* sp., *Mycena adscendens*, *Mycena rhenana*, *Neofavolus alveolaris*, *Panellus pusillus*, *Panus* sp., *Peziza occidentalis*, *Phellinus* sp. 1, *Phellinus* sp. 2, *Phellinus* sp. 3, *Plectania* sp., *Pleurotus* sp., *Pleurotus pulmonaris*, *Polyporus grammocephalus*, *Porodaedalea chrysoloma*, *Poronia nagarholensis*, *Royoporus spathulatus*, *Russula cyanoxantha*, *Schizophyllum commune*, *Scutellina setosa*, *Serpula similis*, *Stereopsis hiscens*, *Tetrapyrgos nigripes*, *Trametes betulina*, *Trame gibbosa, Trametes pubescens, Trametesversicolor, Xerotus nigritum, Xylaria hypoxylon* were restricted to mid elevation class.

There are 18 species of macrofungi identified from the high elevation class (900–1,199 m). Among them eight species such as *Cruentomycena* sp., *Ganoderma* sp. 2, *Ganoderma tropicum*, *Macrolepiota procera*, *Mucronella bresadole*, *Stereum ostrea*, *Termitomyces microcarpus*, and *Tremella mesenterica* were collected only in high elevation.

Two species such as *Dacrymyces spathularia* and *Hexagonia tenuis* were common to all the three elevation classes.

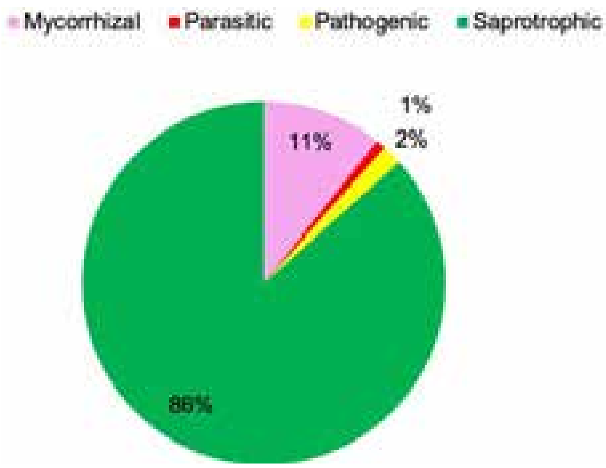

Figure 3. Ecological preference of macrofungi in Agasthyamala.

There are 10 species such as *Clavulinopsis laeticolor*, *Coprinellus disseminatus*, *Cyptotrama asprata*, *Dacrymyces spathularia*, *Dicephalospora rufocornea*, *Fuscoporia gilva*, *Hexagonia tenuis*, *Leucocoprinus rubrotinctus*, *Metacampanella caesia,* and *Xylaria polymorpha* were common to both low and mid elevations.

Three species, *Dacrymyces spathularia*, *Hexagonia tenuis,* and *Microporus vernicipes*, were found in both mid and high elevations.

Macrofungal species such as *Pycnoporus sanguineus*, *Microporus xanthopus*, *Microporus affinis*, *Marasmius haematocephalus*, *Hexagonia tenuis*, *Guepinia helvelloides*, *Ganoderma applanatum*, *Dacrymyces spathularia*, and *Auricularia delicata* were reported from both high and low elevations in the present study.

Table 3. Composition of macrofungi across elevation gradient in Agasthyamala forest.

Species	Identification characters	Division	Order	Low	Mid	High
Agaricus sp.	Fleshy in texture, gills free, annulus present, volva absent	Basidiomycota	Agaricales		1	
Amanita vaginata	Grey in colour, annulus absent, striated cap margin, gills free, volva present	Basidiomycota	Agaricales		2	
Auricularia delicata	Light brown in color, Ear shaped, gelatinous in texture, smooth hymenium	Basidiomycota	Auriculariales	4		1
Auricularia fuscosuccinea	Ear shaped, hymenium smooth, velvety texture on outside	Basidiomycota	Auriculariales		72	
Auricularia mesenterica	Grey in color, hairy surface with concentric grey, white zones, smooth hymenium	Basidiomycota	Auriculariales	26		
Auricularia nigricans	Dark brown in color, ear shaped, hairy outer surface, smooth hymenium and slightly wrinkled	Basidiomycota	Auriculariales		1	
Cellulariella acuta	Greyish in color, tough and leathery in texture, poroid hymenium	Basidiomycota	Polyporales	26		
Chlorophyllum molybdites	Umbrella shaped, white color with brown scales on cap, stipe with annulus	Basidiomycota	Agaricales		3	
Clavaria miniata	Erect, orange in color, soft and fleshy, cylindrical shaped, smooth surface throughout the entire body	Basidiomycota	Agaricales		74	
Clavaria rosea var. *subglobbosa*	Club shaped with rounded or subglobose apex, smooth surface	Basidiomycota	Agaricales	1		
Clavaria zollingeri	Lavender in color, coral like, highly branched with pointed tips, smooth surface, soft and brittle in texture	Basidiomycota	Agaricales		1	
Clavulinopsis imperata	Yellow in color, erect, unbranched with slightly pointed apex, solitary	Basidiomycota	Agaricales	2		
Clavulinopsis laeticolor	Orange in color, unbranched with rounded apex, smooth surface, slender, form small clusters	Basidiomycota	Agaricales	20	4	
Collybia cookei	Pale cream in color, small cap with thin stipe, convex cap, small sclerotium is present.	Basidiomycota	Agaricales		2	
Coprinellus disseminatus	Bell shaped, dense clusters, cap with radial striations, thin fragile stipe, gills turn in color from grey to black with age	Basidiomycota	Agaricales	60	83	
Cotylidia sp.	Fan shaped, leathery in texture, short stipe, smooth surface present on the inner side of the fruiting body	Basidiomycota	Hymenochaetales	42		
Crepidotus sp. 1	Shell shaped, lateral attachment to the substratum, cream in color, sessile, radiating gills	Basidiomycota	Agaricales		57	
Crepidotus sp. 2	Fan shaped, sessile, pale cream in color, smooth surface, slippery in texture	Basidiomycota	Agaricales		175	
Crepidotus variabilis	Shell shaped, white in color, sessile, radiating gills from the point of attachment with the substratum	Basidiomycota	Agaricales	4		
Cruentomycena sp.	Reddish in color, small, convex cap, presence of red exudate, moderately spaced gills	Basidiomycota	Agaricales			1
Cuphophyllus pratensis	Yellow in color, thick widely spaced and decurrent waxy gills, short and thick stipe	Basidiomycota	Agaricales		3	
Cyptotrama asprata	Golden yellow in color, cap is marked with pointed scales, gills yellowish to pale in color and adnate to slightly decurrent, yellow colored small stipe with rough texture	Basidiomycota	Agaricales	3		
Dacrymyces capitatus	Small, gelatinous in texture, globose structure, yellow orange in color, smooth and shiny surface	Basidiomycota	Dacrymycetales	13		
Dacrymyces spathularia	Spatula shaped, gelatinous, yellow orange in color, found in clusters, shrink and dry during dry conditions	Basidiomycota	Dacrymycetales	10	59	56
Daedaleopsis confragosa	Bracket shaped, sessile, presence of concentric zones, maze like pores present on hymenium,	Basidiomycota	Polyporales		1	
Daldinia concentrica	Hard, spherical, dark brown in color, distinct concentric rings visible inside, charcoal like texture	Ascomycota	Xylariales	2		
Dicephalospora rufocornea	Disc shaped, short stalked, yellow in color, very small in size, found in clusters	Ascomycota	Helotiales	14	1	

Species	Identification characters	Division	Order	Low	Mid	High
Earliella scabrosa	Thin bracket shaped, sessile, rough surface with concentric zones, leathery to rough in texture	Basidiomycota	Polyporales	10		
Entoloma sp.	Conical cap with central stipe, silky surface, annulus and volva are absent, crowded gills	Basidiomycota	Agaricales		1	
Exidia glandulosa	Gelatinous, rubbery and soft in texture, possess glandular dots, smooth surface	Basidiomycota	Auriculariales		1	
Exidia recisa	Gelatinous in nature, amber brown in color, smooth surface and shiny with irregular margin	Basidiomycota	Auriculariales		9	
Favolaschia calocera	Small, fan shaped, bright orange in color, distinct honey comb like pores with hexagonal shape, stipe short and lateral, leathery in texture	Basidiomycota	Agaricales	32		
Favolus grammocephalus	Semicircular in shape, yellowish-brown in color, hymenium is poroid with hexagonal shape, short and lateral stipe, leathery texture	Basidiomycota	Polyporales		1	
Fomitopsis quercina	Hard woody bracket, semicircular in shape, rough surface, brown in color, small and densely packed pores on hymenium	Basidiomycota	Polyporales	5		
Fuscoporia gilva	Semicircular in shape, finely hairy, margin with yellow in color, small, round pores on hymenium	Basidiomycota	Agaricales	39	9	
Ganoderma applanatum	Large in size, bracket shaped, hard surface with woody texture, presence of concentric growth zones, pore surface is white when fresh and turns brown when scratched	Basidiomycota	Polyporales	9		5
Ganoderma lobatum	Semicircular in shape with lobed margins, varnished look, dark brown in color, presence of concentric zones, pores small, round and numerous, woody in texture	Basidiomycota	Polyporales	11		
Ganoderma sp.1	Bracket shaped, shiny surface, pores in the underside, stipe absent with woody texture	Basidiomycota	Polyporales		1	
Ganoderma sp. 2	Bracket shaped, numerous small spores on the underside, sessile, woody texture	Basidiomycota	Polyporales			13
Ganoderma tropicum	Semicircular, dark brown in color, hard and woody, poroid hymenium	Basidiomycota	Polyporales			13
Ganoderma tsugae	Bracket shaped, short stipe, shiny and varnished surface, poroid underside, woody texture	Basidiomycota	Polyporales		6	
Geoglossum sp.	Tongue shaped, erect, black in color, upper part of the club is fertile and lower part is sterile stalk	*Ascomycota*	Geoglossales		4	
Gerronema sp.	Convex cap with depressed centre, thin and delicate, smooth surface, gills decurrent, spaced, slender stipe, no ring and volva	Basidiomycota	Agaricales		1	
Guepinia helvelloids	Gelatinous and soft, funnel shaped, orange in color, smooth and shiny	Basidiomycota	Auriculariales	19		68
Gymnopus sp.	Cap convex to flat, thin and dry in texture, smooth surface, gills adnexed to adnate attachment, stipe slender, long and tough	Basidiomycota	Agaricales	1		
Heaxagonia tenuis	Thin, leathery, bracket in shape, sessile, dark brown in color, thin and wavy margin, leathery texture	Basidiomycota	Polyporales	3	6	2
Hygrocybe ceraceae	Yellow in color, smooth surface, gills waxy in texture, adnate to slightly decurrent, slender stipe	Basidiomycota	Agaricales		1	
Hygrocybe conica	Conical to bell shaped, gills waxy and thick, yellow to orange-red in color Cap turns black when bruised	Basidiomycota	Agaricales		1	
Hymenopellis radicata	Convex shaped cap, gills free to adnexed, moderately spaced, slender stipe, greyish-brown in color	Basidiomycota	Agaricales		1	
Hymenopellis sp.	Convex shaped cap, gills free to adnexed, moderately spaced, slender stipe	Basidiomycota	Agaricales		1	
Laccaria sp.	Small, convex to depressed cap, smooth surface, thick widely spaced gills, slender stipe, orange brown in color	Basidiomycota	Agaricales	1		
Lentinus badius	Brown to dark brown in color, surface dry and scaly, decurrent gills, edges serrated, leathery and tough texture	Basidiomycota	Polyporales		1	

Species	Identification characters	Division	Order	Low	Mid	High
Lentinus sajor caju	Fan shaped, white in color, surface smooth, margin inrolled during young stage, gills decurrent, stipe short, leathery to fleshy in texture	Basidiomycota	Polyporales		22	
Lentinus sp.	White in color, dry surface, decurrent gills, stipe tough, leathery to tough in texture	Basidiomycota	Polyporales		5	
Lentinus tigrinus	Whitish to cream in color with brown scales arranged in a tiger like pattern, decurrent gills	Basidiomycota	Polyporales		4	
Leucocoprinus fragilissimus	Cap thin and fragile, pale yellow in color, strongly striated with small central disc, gills free from stipe	Basidiomycota	Agaricales		1	
Leucocoprinus rubrotinctus	Cap whitish to cream in color with pinkish scales, gills free from the stipe, stem slender, cylindrical and fragile with reddish tinge, thin and delicate ring present on the stipe	Basidiomycota	Agaricales	3	1	
Lycoperdon pyriforme	Pyriform shaped, surface covered with small granules on young stage, grows in clusters, yellowish in color	Basidiomycota	Agaricales	1		
Macrolepiota procera	Large cap, umbrella in shape, surface with dark brown scales, central dark umbo present, gills free from the stipe, large thick and movable ring present on the stipe	Basidiomycota	Agaricales			1
Marasmiellus ramealis	Small cap with convex to flat, surface smooth, gills adnate to slightly decurrent, widely spaced, stipe thin, slender and tough, central and smooth	Basidiomycota	Agaricales		51	
Marasmius guyanensis	Small cap. Smooth surface, distant gills, thin wiry and tough dark blackish stipe	Basidiomycota	Agaricales	3		
Marasmius haematocephalus	Cap bright in color, small convex, smooth surface, thin, distant and well-spaced gills, free to adnate, stipe thin, wiry and tough, dark brown to black in color	Basidiomycota	Agaricales	3		1
Marasmius rotula	Small cap, umbilicate, surface deeply pleated, gills widely spaced and free from the stipe	Basidiomycota	Agaricales		1	
Marasmius siccus	Bright orange in color, small convex cap with a depressed centre, surface strongly pleated, distant gills and free from the stipe, long and slender stipe	Basidiomycota	Agaricales	6		
Marasmius sp.	Cap small and convex to flat, pleated, widely spaced gills, free, thin, wiry and tough stipe	Basidiomycota	Agaricales	7		
Melanotus sp.	Small cap with semicircular in shape, surface smooth, gills adnate, moderately spaced	Basidiomycota	Agaricales		17	
Metacampanella caesia	Cap campanulate, surface smooth and thin, gills moderately spaced, slender, long and fragile stipe	Basidiomycota	Agaricales	10	1	
Microporellus dealbatus	Bracket shaped fruiting body, thin and leathery, attached laterally to the substratum, small , round spores on hymenium, white to cream coloured, thin, tough and leathery	Basidiomycota	Polyporales	1		
Microporous affinis	Thin, fan shaped, tough and leathery in texture, cap surface concentrically zoned, color reddish-brown to dark brown, small round pores on the underside with white to cream color	Basidiomycota	Polyporales	12		19
Microporus vernicipes	Bracket shaped, brown to reddish-brown in color, concentrically zoned, smooth surface, small round pores on the underside with white to cream in color, stipe lateral and short with varnished appearence	Basidiomycota	Polyporales	4		4
Microporus xanthopus	Fan shaped, thin tough and leathery, cap brown to dark brown in color with concentric zones, small pores on the hymenium with white to cream in color, distinct lateral stipe with bright yellow in color	Basidiomycota	Polyporales	15		4
Mucronella bresadole	Small coral like fruiting body with pointed teeth, white in color, soft and fragile, found in clusters	Basidiomycota	Agaricales			31
Mycena adscendens	Cap small, bell shaped, white to pale grey in color, surface smooth and translucent, gills adnate to slightly decurrent, moderately spaced, white in color, thin, delicate and translucent stipe with fine hairs at the base	Basidiomycota	Agaricales		1	

Species	Identification characters	Division	Order	Low	Mid	High
Mycena manipularis	Small bell shaped to conical cap, surface smooth, gills adnate to slightly decurrent, moderately spaced, long slender and fragile stipe	Basidiomycota	Agaricales		2	
Mycena rhenana	Cap small, bell shaped to convex, greyish in color, gills adnate to slightly decurrent, moderately spaced, stipe thin, slender and fragile	Basidiomycota	Agaricales		6	
Neofavolus alveolaris	Semicircular bracket shaped, thin, leathery, yellowish in cap color, honey comb like pores on underside, stipe absent	Basidiomycota	Polyporales		1	
Panaeolus antillarum	Cap greyish white in color, surface smooth during young stage, initially gills are in grey color and later black in color, stipe tall, thick and smooth, no ring is present	Basidiomycota	Agaricales	1		
Panellus pusillus	Very small fan shaped fruiting body, gills decurrent, close and narrow, stipe short and laterally attached to the substratum	Basidiomycota	Agaricales		26	
Panus sp	Fan shaped cap, surface hairy, greyish brown in color, decurrent gills, thick and widely spaced, short stem with tough texture	Basidiomycota	Polyporales		5	
Peziza occidentalis	Cup shaped ascocarp, brown color on the inner surface, hymenium smooth, stipe absent	Ascomycota	Pezizales		1	
Phellinus sp.1	Bracket shaped, hard and woody in texture, dark brown to blackish in color, small round pores on the underside	Basidiomycota	Hymenochaetales		1	
Phellinus sp.2	Bracket shaped, hard and woody in texture, dark brown to blackish in color, small round pores on the underside	Basidiomycota	Hymenochaetales		1	
Phellinus sp.3	Bracket shaped, hard and woody in texture, dark brown to blackish in color, small round pores on the underside	Basidiomycota	Hymenochaetales		4	
Plectania sp.	Cup shaped, black in color, smooth inner surface, shiny black in color, stipe short	*Ascomycota*	Pezizales		10	
Pleurotus pulmonaris	Fan shaped, pale cream in color, smooth surface, decurrent gills, stipe short, soft and fleshy in texture	Basidiomycota	Agaricales		7	
Pleurotus sp.	Fan shaped, white in color, smooth surface, decurrent gills, soft and fleshy in texture	Basidiomycota	Agaricales		4	
Porodaedalea chrysoloma	Hoof shaped, thick, woody, dark brown in color, rough surface, small pores on the underside, color yellowish brown to brown	Basidiomycota	Hymenochaetales		14	
Poronia nagaraholensis	Disc like stroma, black ostioles on surface, perithecia embedded in stroma	*Ascomycota*	Xylariales		2	
Pycnoporus sanguineus	Bright orange in color, bracket shaped, thin, tough and leathery, small round pores on the underside, thin and corky type of flesh	Basidiomycota	Polyporales	16		10
Resupinatus tristis	Small, shell shaped, short stipe lateral in position, cap grey in color, gills radiating from the point of attachment	Basidiomycota	Agaricales	8		
Royoporus spathulatus	Fan shaped, grows in clusters, white in color, color changes with age, small round pores on the underside which is white in color	Basidiomycota	Polyporales		55	
Russula cyanoxantha	Cap convex to flat, smooth surface, gills adnate and white to cream in color, stipe white, cylindrical and brittle, central in position and smooth	Basidiomycota	Russulales		1	
Sanguinoderma rugosum	Bracket shaped, thick, hard and woody, dark brown in color, surface rough, small round pores on the underside, pore surface white to cream in color during initial stage later becoming brown with age	Basidiomycota	Polyporales		1	
Schizophyllum commune	Fan shaped fruiting body, greyish in color, sessile, distinctively split gills, white in color	Basidiomycota	Agaricales		10	
Scleroderma bovista	Globose shaped, partly buried in soil, thick and tough, peridium yellowish to brown in color, mature fruiting body breaks irregularly to release spores	Basidiomycota	Boletales	1		

Species	Identification characters	Division	Order	Low	Mid	High
Scutellina setosa	Small cup shaped, inner surface bright orange in color, margin surrounded with dark brown bristles, inner surface smooth and outer surface slightly hairy	Ascomycota	Pezizales		2	
Serpula similis	Yellowish in color, leathery texture, sessile, hymenium turmeric yellow daedaloid pores	Basidiomycota	Boletales		11	
Stereopsis hiscens	Small, fan shaped, upper surface brown in color, hymenium smooth surface, thin and leathery texture	Basidiomycota	Stereopsidales		8	
Stereum ostrea	Thin, bracket shape, concentric bands are present, smooth hymenium	Basidiomycota	Russulales			27
Stereum sp.	Bracket shaped, smooth hymenium, concentric zones are present, tough and leathery in texture	Basidiomycota	Russulales	14		
Termitomyces microcarpus	Cap umbonate, gills free to slightly adnate, stipe cylindrical, whitish and bulbous at base, found in clusters	Basidiomycota	Agaricales			80
Tetrapyrgos nigripes	Small, convex, gills adnate to adnexed, widely spaced, white to cream in color, slender stipe, black in color	Basidiomycota	Agaricales		1	
Trametes betulina	Bracket shaped, upper surface with concentric zones, wavy margin, porous hymenium, leathery texture during fresh, later hard and woody on drying	Basidiomycota	Polyporales		1	
Trametes gibbosa	Bracket shaped, upper surface furrowed, greyish in color, margin wavy, porous hymenium, thick, tough and woody on drying	Basidiomycota	Polyporales		1	
Trametes pubescence	Thin, bracket shaped, upper surface pubescent, wavy margin, porous hymenium	Basidiomycota	Polyporales		25	
Trametes versicolor	Thin, fan shaped, upper surface with zones of concentric bands of varying colors – brown, cream, porous hymenium with white to cream in color, stipe absent	Basidiomycota	Polyporales		18	
Tremella fuciformis	Gelatinous, translucent and forms irregular lobes, white in color, soft, slippery in texture and fragile in nature	Basidiomycota	Tremellales	2		
Tremella mesenterica	Gelatinous, lobed, irregular in shape, bright yellow in color, soft, jelly and slippery in texture, smooth surface	Basidiomycota	Tremellales			48
Xylaria hypoxylon	Erect, black base with white tips on young stage, black portion rough and white portion powdery, hard and woody when mature and soft and brittle when young	*Ascomycota*	Xylariales		9	
Xylaria polymorpha	Erect, club shaped, black in color, hard and woody on mature and brittle when dry	*Ascomycota*	Xylariales	14	16	
Xerotus archeri	Rust orange in color, small fan shaped, widely spaced radiating gills with orange to brown in color, sessile	Basidiomycota	Polyporales	85		
Xerotus nigritum	Small, fan shaped, black in color, widely spaced radiating gills, balck in color, sessile	Basidiomycota	Polyporales		37	

Macrofungal species richness along the elevation gradient

Among the different elevation classes, low elevation and mid elevation classes were observed with more number of macrofungi compared to high elevation class (Table 4). The low elevation class recorded 42 macrofungal species with species richness (11.8 ± 2.8). Mid elevation class recorded 71 species with species richness (14 ± 2.9). High elevation class recorded 18 species with species richness (5.4 ± 2.30) In Wet evergreen forests of Agasthyamala, low elevation and mid elevation possess high species richness and found to be gradually decreased with increasing elevation.

Substrate availability along elevation gradient

The presence of substrates up on which macrofungi exist in different elevation classes were diverse in nature (Figure 2). Each elevation class had more number of macrofungi found on dead wood compared to other types (Low elevation (39% of dead wood); mid elevation (45% of dead wood) and high elevation (16% of dead wood). The mid elevation class (600–899 m) was recorded with more availability and diversity of substrates *viz*., dead wood (45%), fallen twig (69%), live tree (72%), soil (52%), and litter (45%) in contrast to low and high elevation classes (Image 2). Presence of dung (cow) is noted in a low elevation area (Figure 2; Image 2e).

Ecological preference of macrofungi

The current study shows that 86% of macrofungal species are saprotrophic, 11% of species were mycorrhizal, 2% were pathogenic, and remaining 1% is parasitic in nature. Higher percentage of saprotrophic fungi (86%) are observed in the study (Figure 3). Many of the polypores are saprotrophic which depend up on dead and decaying wood, fallen twig and litter. Species like *Microporus xanthopus*, *Cyptotrama asprata*, and *Auricularia delicata* were saprophytic in nature.

Certain species such as *Amanita vaginata*, *Cuphophyllus pratensis, Macrolepiota procera, Leucocoprinus rubrotinctus Russula cyanoxantha,* and *Termitomyces microcarpus* were reported as mycorrhizal. The study revealed the existence of parasitic and pathogenic fungi also. *Ganoderma applanatum* is a parasitic fungus reported from the study. Species such as *Fuscoporia gilva* and *Ganoderma lobatum* are pathogenic fungi. The low elevation class were recorded with saprotrophic fungi (91%), mycorrhizal fungi (2%), pathogenic fungi (5%) and parasitic fungi (2%). The mid elevation class shows that 93% of fungi were saprotrophic, followed by 6% of mycorrhizal fungi, and 1% of pathogenic fungi. The high elevation class were noted with 83% of saprotrophic fungi followed by 11% of mycorrhizal fungi and 6% of parasitic fungi.

Edaphic properties

The edaphic variables play an essential role on determining the fungal communities in an ecosystem. pH is an important factor that controls the macrofungal species richness. Electrical conductivity, Organic matter, base cations, Nitrogen, etc are important for determining the species composition of macrofungi. Selected edaphic properties are represented (Table 5). pH ranged from 3.86 to 4.58, which shows that soil pH increases slightly with elevation. Electrical conductivity shows very low in overall. Organic carbon shows highest at low elevation (1.97%) and decreases with elevation. The available Nitrogen peaks at low elevation (386.62kg/ha), drops sharply at higher elevations. The availability of Nitrogen reduces significantly at high elevation. There is no significant elevation-related trend were found among the edaphic parameters including available phosphorus and available potassium. calcium and magnesium were decreasing with increasing elevation.

Table 4. Macrofungal species richness across elevation gradient.

Elevation Classes	Elevation band (in m)	Species richness
High-elevation	900–1,199	5.4 ± 2.30a
Mid-elevation	600–899	14 ± 2.91b
Low-elevation	300–599	11.8 ± 2.86b

Values shown are means; standard deviations of the means. Values with different lowercase letters (a, b) are significantly different at $P < 0.05$.

The present study noted that there is a slight increase of bulk density with elevation. The texture of soil samples across all elevations shows loam sandy.

DISCUSSION

Fungi are the most species rich taxa in the terrestrial ecosystem (Wang et al. 2020) after flowering plants. Among the fungal group, macrofungi are highly economic important and play an inevitable role in the forest ecosystem including material cycling, energy flow and plant community succession. Many of the macrofungi are becoming extinct or are in danger due to loss of habitat and hosts, over exploitation, climate change, developmental activities, and pollution (Harsh 2021). Several researches have been conducted to explain the factors influencing the macrofungal species composition and distribution (Kujawska et al. 2021). The studies on the macrofungal diversity along the elevation gradient in Agasthyamala Biosphere Reserve, southern Western Ghats showed that elevation is a factor for macrofungal growth and distribution along with other biotic and abiotic variables.

Akshaya et al. (2023) conducted a study on the status of macrofungal diversity in wet evergreen forests of Agasthyamala biosphere reserve, Southern Western Ghats that form the foundational data of the area. The study revealed the existence of 62 macrofungal species in Agasthyamala Biopshere Reserve. This study revealed the existence of 112 macrofungal species and most of the species belonged to the division Basidiomycota (92%).The macrofungi belong to Basidiomycota are omnipresent in forest soils (Cairney 2005) and play an important role in nutrient cycling. Agaricales (46.43%) was the dominant order, similar to the study by Tapwal et al. (2013). More recently, Gogoi et al. (2024) also had the similar result of dominance of the order Agaricales. Polyporaceae is the dominant family having highest number of macrofungal species. The dominance of polyporaceae family has been reported in the earlier

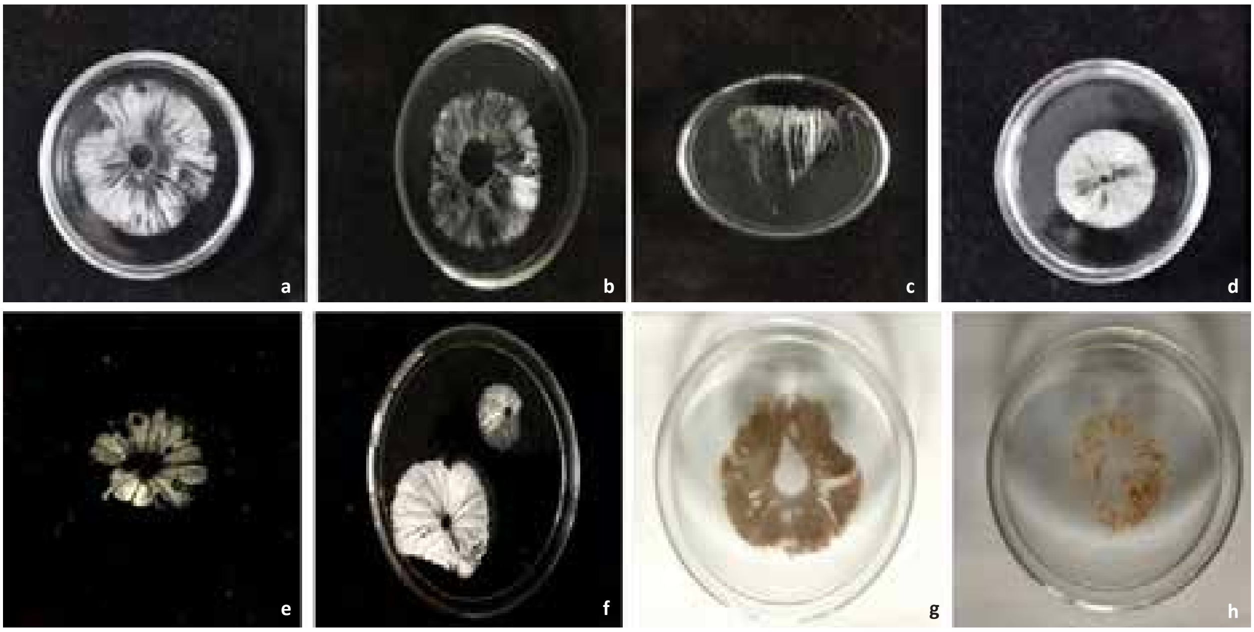

Image 1. Spore print images of macrofungal species collected from Agasthyamala forest: a—*Russula cyanoxantha* (Mycorrhizal) | b—*Amanita vaginata* (Mycorrhizal) | c—*Macrolepiota procera* (Mycorrhizal) | d—*Leucoagaricus rubrotinctus* (Mycorrhizal) | e—*Marasmius haematocephalus* (Saprotrophic) | f—*Cyptotrama asprata* (Saprotrophic) | g—*Chlorophyllum molybdites* (Saprotrophic) | h—*Cuphophyllous pratensis* (Mycorrhizal). © K. K. Akshaya.

Image 2. Different types of habitat of macrofungi identified from wet evergreen forests of Agasthyamala Biosphere Reserve, Kerala: a—soil (*Clavulinopsis laeticolor*) | b—dead wood (*Pleurotus ostreatus*) | c—fallen twig (*Favolus grammocephalus*) | d—live tree (*Ganoderma* sp.) | e—dung (*Paneolus anticillarum*) | f—litter (*Marasmius haematocephalus*). © K. K. Akshaya.

Table 5. The selected edaphic properties across the elevation gradient.

Soil parameters	Low-elevation (Mean ± SD)	Mid-elevation (Mean ± SD)	High-elevation (Mean ± SD)
pH	3.86 ± 0.05a	4.13 ± 0.02b	4.58 ± 0.26c
Electrical conductivity (dS/mm)	0.03 ± 0.01a	0.09 ± 0.04a	0.17 ± 0.18a
Organic carbon (%)	0.76 ± 0.38a	1.97 ± 1.25a	1.33 ± 1.14a
Available nitrogen (kg/ha)	315.84 ± 14.60a	386.26 ± 46.61a	380.94 ± 69.28a
Available phosphorus (kg/ha)	9.98 ± 6.90a	15.23 ± 3.33a	15.27 ± 8.92a
Available potassium (kg/ha)	121.44 ± 40.46a	165.58 ± 22.85ab	213.44 ± 73.36b
Calcium (meq/100 g)	3.2 ± 1.33a	3.72 ± 0.58a	3.5 ± 1.10a
Magnesium (meq/100 g)	1.41 ± 0.70a	1.5 ± 1.58a	0.80 ± 0.55a
Bulk density (gm/cc)	1.09 ± 0.04a	1.13 ± 0.07ab	1.23 ± 0.10b
Texture	Loam sandy	Loam sandy	Loam sandy

Values with different lowercase letters (a, b) are significantly different ($p < 0.05$).

studies conducted by Mohammad et al. (2019); Kumar & Gogoi (2024). The study stated that the abundance of this family in an area is due to the availability of substrates such as dead and decayed wood, fallen twigs, and others. Each elevation class reported a larger number of saprotrophic fungi belonging to the Polyporaceae family (low-elevation 91%, mid-elevation 93%, and high-elevation 83%).

The present study shows that low elevation (300–599 m) and mid elevation (600–899 m) classes were observed with more number of macrofungal species compared to high elevation class (900–1,199 m). According to Li et al. (2018b) more macrofungal species were recorded in regions with optimum conditions depending on the season, temperature and amount of rainfall. Some studies showed that temperature, precipitation and plant diversity are the main drivers of macrofungal flora (Tedersoo et al. 2014). Moore (2008) studied that the composition and diversity of macrofungi were different which may due to the difference in vegetation types along the elevation. Chen et al. (2018) stated that the growth of sporocarps of macrofungi is depending up on light. The macrofungal species show a positive correlation with low light habitat. The availability of strong light inhibits mycelia growth (Miles & Chang 2004). The suitable light will help macrofungal sporocarps to grow (Miles & Chang 2004; Chen et al. 2018). In high altitude area, the forest canopy was large and causes high light level, high temperature and low humidity that promote the low sporocarp production (Jayaseelan et al. 2014). Moreover, the variation in the sporocarp structures that increases the degree of dispersal of fungal spores which contributes to the abundance of macrofungal species in an area (Mohammad et al. 2019).

According to Cozzolino et al. (2016), edaphic variables play an essential role on determining the fungal communities. The present study shows that low pH values were associated with low and mid elevations. This lower pH supports more number of macrofungal species. The high pH decreases the macrofungal species by negatively influencing the expansion of fungi and the production of sporocarps. This is similar to those studies by Puangsombat et al. (2010). The effect of electrical conductivity on shaping the fungal community is ignorant. Here, the occurrence of more macrofungal species were directed towards the low electrical conductivity plots. High elevation areas were recorded with high electrical conductivity compared to low and mid elevation areas. This result is in accordance with the study by Alem et al. (2020). Base cations like Ca^{2+}, Mg^{2+}, K^{+} are essential in plant photosynthesis, that can affect the amount of carbon, which is needed for fungi in the soil (He et al. 2017).

Organic matter is inevitable for mycelia growth and network formation of fungi. This is because of the fact that organic matter has strong water holding capacity and nutrient availability. High level of organic carbon supports high level of macrofungi especially the saprophytic species. Some cases, ectomycorrhizal fungi may also attract organic matter rich sites (Lindahl & Tunlid 2015). Nitrogen is vital factor for the composition of fungi. Nitrogen helps in the mycelium and sporocarp formation (Trudell & Edmonds 2004).

Topography is an indirect environmental variable. This variable serves as an important driver of microhabitat in forest ecosystems. This is because different topographic conditions results in various microhabitats. Different microhabitats results in the composition and distribution of variety of macrofungi (Chen et al. 2018).

The diversity in macrofungal species are based on habitat. The fungi growing on various substrates may exhibit distinct growth and dispersion features (Senn-Irlet et al. 2007). The study revealed the presence of different types of substrate for macrofungi including soil, dead wood, fallen twig, live tree, animal dung and litter. Saprotrophic fungi are important for cycling of soil nutrients because they are one of the most active degraders of forest ecosystem. According to Li et al. (2018a) saprotrophic macrofungi are dominant and diverse fungal group in tropical forest. The dominance of this type of macrofungi is seen in this study. The dead wood dependent saprophytes were seen more in each elevation class. Saprotrophic macrofungi include *Sanguinoderma rugosum*, *Auricularia delicata*, *Dacrymyces spathularia*, and *Daldinia concentrica*. The present study noted with ectomycorrhizal fungi such as *Cuphophyllus pratensis*, *Leucocoprinus rubrotinctus*, and *Russula cyanoxantha*. The symbiotic mycorrhizal association enhances the overall well-being of the ecosystem by making an efficient nutrient uptake system in nature. Macrofungi that grow on woody substrate may be saprophytic or pathogenic as stated by Mueller et al. (2007). The human settlements in Agasthyamala have the practise of cattle farming and poultry farming. Most of the people in Agasthyamala are settled in the low elevation areas. Coprophilous macrofungi '*Panaeolus antillarum*' was reported from low elevation area only. This may be due to high grazing by cow in that area.

CONCLUSION

Elevation plays an important role in contributing macrofungal diversity, composition and distribution. The low and mid elevation areas showing high number of macrofungal species compared to high elevation area. There are a lot of factors playing important role in determining growth and distribution of macrofungi such as soil properties, temperature, precipitation, vegetation type, forest canopy, substrate availability. The variation in macrofungal composition and distribution is due to difference in vegetation type along with altitude that causes differences in the availability of substrate. The high elevation areas are dominated by mosses, liverworts, lichens. The vegetation composition affects the availability of substrate and hence contributed to variation in composition of macrofungi in high elevation area compared to low and mid elevation areas. In addition, the forest canopy gap was huge at high altitudes resulting high intensity of light, higher temperatures and low humidity causes low production of sporocarps. Edaphic variables like soil pH, organic carbon, base cations, Nitrogen have important role on shaping fungal communities in an ecosystem. The information on mycodiversity and substrate relationship is important for conservation and utilisation as well as for the sustainable forest ecosystem management. Understanding the factors tailoring the macrofungal communities in an ecosystem is very tectonic to predict future species composition and richness under global climate change scenario.

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ARTICLE

Efficacy of 5% neem seed kernel extract against ectoparasites in six captive wildlife species at Rajiv Gandhi Zoological Park, India

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Abstract: This wildlife study aimed to assess the prevalence of ectoparasites and evaluate the in vivo efficacy of a 5% Neem Seed Kernel Extract (NSKE) insecticide in captive wildlife species at Rajiv Gandhi Zoological Park and Wildlife Research Centre (RGZPWRC), Katraj, Pune, Maharashtra, India. Ectoparasites were collected non-invasively from six wildlife species, namely, Black Buck *Antelope cervicapra*, Spotted Deer *Axis axis*, Leopard *Panthera pardus*, Tiger *Panthera tigris*, Elephant *Elephas maximus*, and Sloth Bear *Melursus ursinus* using insect traps in animal shelters and night houses, alongside combing and visual inspection. A total of 865 ectoparasites were documented in the entire study duration of 19 days, of which, 662 ectoparasites were documented before and 203 after the application of 5% NSKE, each over a nine-day period. Flies comprised 93.99% of the total ectoparasites found (865) during the entire study, followed by lice at 5.55% and fleas at 0.46%. The overall relative prevalence was 76.53% pre-treatment and 23.47% post-treatment. By species, Black Buck showed the lowest relative prevalence at 6.24%, and Elephants the highest at 21.73%, with an average relative prevalence of 16.67%. Leopards, Tigers, and Elephants had values of 21.62%, 19.66%, and 21.73%, respectively. Taxonomically, flies represented 96.30% in Black Buck, 63.91% in Spotted Deer, 98.93% in Leopards, and 100% in both Elephants and Sloth Bears. The estimated prevalence exceeded 100% in some species due to multiple parasites per host based on average parasitic load calculations. During treatment, shelters and night houses were washed and sprayed with 5% NSKE. Re-sampling after nine days showed a marked reduction in ectoparasite counts. Efficacy of 5% NSKE against flies, fleas, and lice was 69.29%, 100%, and 66.67%, respectively, with an overall efficacy of 69.33% ($P < 0.001$) with a 95% confidence interval ranging from 65.70% to 72.90%. The highest efficacy was observed in Elephants at 81.76%. Use of 5% NSKE is hence strongly recommended as an insecticide or repellent in captive wild animal housing systems. These findings support the integration of botanical insecticides into integrated pest management programs for captive wildlife.

Keywords: Acaricidal activity, alternative pest management, animal welfare, *Azadirachta indica*, Azadirachtin, botanical biopesticides, Katraj Zoo, parasite control in zoos, Pune, veterinary parasitology.

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INTRODUCTION

India's tropical climate creates favourable conditions for the growth and survival of parasites and vector populations, which increases the risk of ectoparasitic infestations in both humans and animals. There are only limited or sporadic studies available on the prevalence of ectoparasites in captive and wild animals in Indian zoological parks and wildlife sanctuaries (Moudgil & Singla 2021). As noted by Moudgil et al. (2015), the understanding of parasitic diseases in wild animals is still developing. Difficulties in tracking and sampling wildlife, especially in their natural habitats, have made it challenging to generate reliable data on host distribution and the spread of zoonotic parasites (McCallum & Dobson 1995). Ectoparasites found in wild mammals include lice (Phthiraptera), ticks (Order: Acarina), fleas, biting flies (Diptera), mites, and bot flies. Among these, species like *Haemaphysalis cuspidata*, *H. kinneari*, and *Amblyomma hebraeum* have been reported from big cats like leopards and tigers in various zoo-based studies. Infestations with *Sarcoptes scabiei*, *Ctenocephalides felis*, and the dog chewing louse *Trichodectes canis* have also been recorded in zoo and wild animal species (Gaurava & Singh 1999; Nashiruddullah & Chakraborty 2001; Islam 2010; Moudgil et al. 2015). These ectoparasites are known to cause significant effects such as blood loss, skin irritation, hypersensitivity, transmission of diseases, and behavioural disturbances. However, the impact and control of these parasites in captive zoo animals, especially in Indian zoological parks, remains a poorly researched area (Samuel et al. 2001). Without sufficient data on ectoparasite prevalence, it becomes difficult to implement focused health management, vector control, and conservation strategies in zoo settings. Recently, there has been an increasing focus on using safer, eco-friendly alternatives to chemical insecticides for controlling ectoparasites. Among these, botanical insecticides such as neem seed kernel extract (NSKE) have shown promising results. Given the growing concerns around pesticide resistance and chemical residues, NSKE is being considered as a potential alternative for use in zoological environments, where animal safety and environmental sustainability are key concerns. Among various botanical formulations explored for ectoparasite control, NSKE has emerged as a widely studied and promising alternative. It is known for being biodegradable, non-toxic to non-target organisms, and free from harmful residues, making it suitable for application in sensitive environments such as zoological parks. NSKE contains bioactive compounds like azadirachtin, which have shown broad-spectrum efficacy against multiple ectoparasitic arthropods, particularly ticks and fleas. Previous studies have reported a significant reduction in ectoparasite load following its application, highlighting its potential in practical field use (Webb & David 2002; Albarrán-Rodríguez et al. 2019). In addition to its ectoparasitic properties, factors such as local availability, cost-effectiveness, and alignment with animal welfare practices contribute to its increasing relevance in current ectoparasite control strategies, especially in captive wildlife settings. The objectives of this study were:

1. To determine the prevalent ectoparasites in the six wildlife species, namely, Black Buck, Spotted Deer, Leopard, Tiger, Elephant, and Sloth Bear housed at the Rajiv Gandhi Zoological Park and Wildlife Research Center (RGZPWRC), Katraj, India.
2. To calculate the efficacy of 5% NSKE as a botanical insecticide.

This study tested the efficacy of 5% NSKE as a botanical insecticide.

MATERIALS AND METHODS

Site and duration of the research: The research work was conducted from January–March 2025. The ectoparasites were collected from six wildlife species namely, Black Buck *Antelope cervicapra*, Spotted Deer *Axis axis*, Leopard *Panthera pardus*, Tiger *Panthera tigris*, Elephant *Elephas maximus*, and Sloth bear *Melursus ursinus*, housed at RGZPWRC, Katraj (Table 6). All the necessary permissions and approvals were procured from the principal chief conservator of forests (PCCF) wildlife, Maharashtra State Biodiversity Board and RGZPWRC, Pune. Also, the ethics committee for animal experiments from KNP College of Veterinary Science, Shirwal approved the research vide minutes of the 24th Institutional Animal Ethics Committee (IAEC) meeting, Item No. 3, Sr. No. 08, protocol number: IAEC/08/24/KNPCVS/2024.

Collection of ecto-parasites: A pre and post-treatment design was used for evaluating the efficacy of 5% NSKE as an insecticide used in vivo strictly in the animal shelters (not topically) and to study the relative prevalence of ectoparasites. Non-invasive sampling methods such as light traps (Image 1), glue-based traps (Image 2 & 3), Chemical cue-based (slow CO_2-releasing) insect luring traps (Image 4) (Smallegange et al. 2010; Schoenthal 2015; Aldridge et al. 2016; Sukumaran et al. 2016; Gibb & Oseto 2019; Schilling et al. 2022)

Image 1. Light based insect luring trap (Flea trap).

Image 2. Glue based insect traps (rolls).

Image 3. Glue based insect traps (boards).

Image 4. Carbon dioxide based insect traps.

were implied for the collection of ectoparasites from the animal shelters, along with noninvasive physical screening methods. Animal handling was strictly avoided in this study, and the ectoparasite samples were collected only using the traps. These samples were then assessed and considered indicative of the parasitic load in the given wildlife species.

Those ectoparasites that could not be separated from the insect traps due to their strong adhesion with the glued insect traps or due to the chances of disruption of the parasite morphology were digitally collected by photographically capturing them using 10x and 20x optical lenses mounted on a mobile phone camera lens for enhanced magnification and a clearer image capture (Image 5 & 9). The screening and collection of ectoparasites were carried out for nine days each in the pre-treatment and post-treatment phases. A nine-day sampling was conducted to minimize the study duration so as to assess the single use efficacy of NSKE. Samples were to be collected from various wild animal species, including herbivores and carnivores, herd animals, as well as individual animals. Hence, all the animals housed in every species were included in the study.

Condition of the animal housing studied: All six species of animals studied had separate night houses

Image 5. Digital collection of fragile ectoparasites.

Image 6. Commercially available neem seed kernel extract.

Image 7. Preparation of 5% NSKE solution.

Image 8. Washing of animal housings with 5% NSKE.

and enclosures. This study focused on setting up traps in the night houses at a fixed position and particular distance so as to avoid harm to the animals while resting and ensure the closest proximity for luring ectoparasites into the traps. The night houses had assigned caretakers who ensured regular washings and periodic disinfection to avoid infections and repel insects while the animals rested at night. The commonly used practices at every night house included foot baths of potassium permanganate, washings of the floor with salt solution, cleaning of biowastes and drying with the help of fans.

Application of 5% NSKE in animal shelters of the zoo: On the 10th day of the study, a 5% solution of NSKE was prepared using tap water and commercially available concentrate of NSKE, as per the manufacturer's guidelines and applied once in all the six wild animal shelters by spraying, washing and avoiding a direct contact of the solution with the animals in the enclosures (Image 6, 7, & 8). A single application of NSKE ensured that the efficacy is calculated with respect to the minimal use of NSKE, and the frequency of application could therefore be increased in further studies and on field applications as per the requirements.

Processing, identification and ectoparasite prevalence calculation: The collected samples were preserved using 5% glycerinated 70% alcohol solution until processing and identification based on morphological features (Soulsby 1982). The ectoparasite specimens were classified accordingly into the five major ectoparasite categories (and sub-categories wherever required) of interest which are; flies, fleas, ticks, mites and lice. The processed flies were identified to genus level using keys mentioned in (Soulsby 1982) (Image 10, 11, 12, 13, & 14). Mosquitoes were identified on the basis of morphology and mouth parts (Image 15 & 16). Fleas were processed to observe the comb and classified to species level on the basis of morphological characteristics. Similarly, lice were processed, identified, recorded and classified accordingly (Image 17 & 18). The data obtained from the processing and identification was recorded for calculation of species specific prevalence, relative prevalence and estimated prevalence of ectoparasites in the six selected wildlife species at the zoo.

Formulae used for calculations: The values of 'average parasitic load per infested host animal' ($\overline{Z}$) were compiled (Table 1) from various studies which recorded and calculated them as the mean / average count of an ectoparasite usually occurring in a given species of infested host (Griffiths 1978; Lehmann 1994; Rózsa et al. 2000; Krasnov 2008; Sarkar et al. 2012; Eads et al. 2015; Razali et al. 2018; Zajac et al. 2021; Oliver & Eckerlin 2022). These values were then used for calculation of the estimated number of infested hosts in the given population of wildlife species by using the formula:

Estimated number of infested hosts (H_{inf}) = Total ectoparasite count ÷ $\overline{Z}$

Further, the H_{inf} value was used for the calculation of the estimated prevalence of an ectoparasite in a given wildlife species by using the formula:

$$\text{Estimated Prevalence (\%)} = \text{Estimated no. of infested hosts } (H_{inf}) \div \text{Total number of animals (N)} \times 100$$

The estimated prevalence itself defines the estimation of prevalence in scenarios where the calculation of actual prevalence is not possible or may result in false positives. In this study, using non-invasive sampling for ectoparasite collection in the captive wildlife species and calculation of actual prevalence values was impossible as observation and allotting of procured ectoparasites to a part of the population studied becomes impossible. So, as an equally efficient alternative, the relative prevalences were calculated, which gave an idea about the proportion of one type or species of ectoparasite among the total ectoparasites collected. As a part of this, the estimated prevalence gave an idea about the probability of the number of animals infested with a given ectoparasite within the studied population. Also, the values of the estimated prevalence should not be solely interpreted or treated as actual prevalence values.

Calculations for the efficacy of 5% NSKE as an insecticide: The efficacy of 5% NSKE was calculated on the basis of reduction in the ectoparasite count, by comparing the mean ectoparasite count before and after the application of 5% NSKE, by using modified Abbott's Formula (Webb & David 2002; Tabassam et al. 2008; Narladkar 2018) which is:

$$\text{Efficacy (\%)} = (C_{pre} - T_{post}) \div C_{pre} \times 100$$

Where, C_{pre}: Mean ectoparasite count before treatment of 5% NSKE.

T_{post}: Mean ectoparasite count after treatment of 5% NSKE.

Statistical analysis of the data: Ectoparasite counts recorded before and after treatment were summarized as frequencies and percentages. Treatment efficacy was calculated as the percentage reduction in ectoparasite counts following application of the insecticidal formulation. The recorded data was analyzed with the help of the Statistical Package for Social Sciences (SPSS-20). Descriptive statistics that include frequency and means were used for the analysis. Non-parametric statistical test; chi-square test (χ^2) was used with one degree of freedom. The 95% confidence intervals (CI) for efficacy estimates were calculated using the Wilson score method, which provides robust interval estimation for binomial proportions. A p-value < 0.05 was considered statistically significant. All statistical interpretations were based on standard biostatistical methods.

RESULTS

During the nine-day pre-spraying phase, a total of 662 ectoparasites were collected from animal shelters before the application of 5% NSKE. Before treatment, flies constituted the majority at 93.95% (622/662), followed by lice at 5.45% (36/662), and fleas at 0.60% (4/662), making them the least prevalent. In the following nine days after treatment, a total of 203 ectoparasites were collected, with flies making up 94.09% (191/203), followed by lice at 5.91% (12/203), which were the least observed during this phase (Figure 1). Overall, 865 ectoparasites were recorded collectively during the pre and post-spraying phases across various wild animal species. The overall relative prevalence of ectoparasites was lowest in Black Buck (6.24%) and highest in Elephants (21.73%), followed by Leopard (21.62%), Tiger (19.66%), Sloth Bear (15.38%), and Spotted Deer (15.37%). The average relative prevalence of ectoparasites was 16.67%, indicating higher ectoparasite burden in Leopards, Tigers, and Elephants compared to the overall average (Table 2) (Figure 2). Out of the total 865 ectoparasites collected during the study, 76.53% were recorded before and 23.47% after the application of 5% NSKE. This reflects a highly significant difference in the distribution of the ectoparasite among the six wildlife species studied, with chi-square test

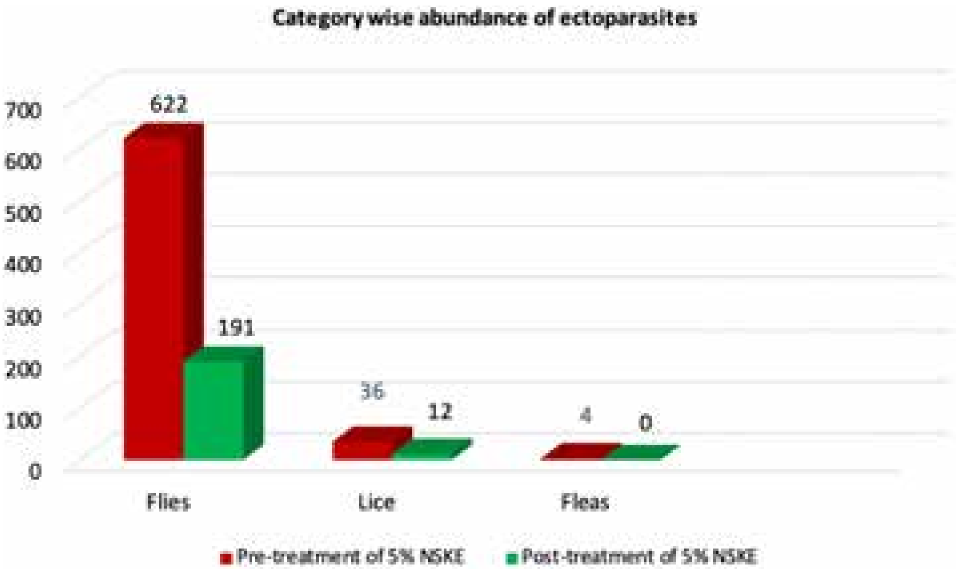

Figure 1. Category wise abundance of ectoparasites.

Table 1. Values of 'average parasitic load per infested host animal ($\overline{Z}$)' in various wildlife host species (in captivity).

Wildlife species (Binomial name)	$\overline{Z}$ values for the ecto-parasite species/category					
	Muscid flies	Midges	Mosquitoes	Lice	Fruit flies	Fleas
Black Buck *Antelope cervicapra*	3–4	10	8	15–20	5	7
Spotted Deer *Axis axis*	4	12	10	20	6	8
Leopard *Panthera pardus*	3	6	5	10	3	4
Tiger *Panthera tigris*	2	7	6	10	4	5
Elephant *Elephas maximus*	5	15	12	1	4	4
Sloth Bear *Melursus ursinus*	2	5	4	4	3	3

values of 78.628 and 28.340 before treatment and after treatment, total ectoparasite counts with P<0.001 (Table 2). Among the six wild animal species studied, the highest estimated prevalence of flies was observed in Sloth Bear (475.00%), followed by the Leopard (362.66%), Elephant (261.11%), Tiger (223.68%), Black Buck (4.26%), and Spotted Deer (2.55%). For a better understanding here, the estimated prevalence simply gives an idea about the probability of the number of animals infested with a given ectoparasite within the studied population. (Note: estimated prevalence values > 100% indicates that the ectoparasite population exceeds the capacity of individual hosts based on average parasitic load values, suggesting environmental accumulation in housing structures). For example, the estimated prevalence reported as 475% in sloth bear signifies that the current prevalence of flies in the animal housings of sloth bear is 4.75 times 100% or 4.75 times greater than the average number of flies a single infested Sloth Bear can harbor. Bovicola spp. lice were found only in Spotted Deer (2.78%). Fleas (*Ctenocephalides* spp.) showed the highest prevalence in Leopard (16.66%) and a low level in Black Buck (0.63%), while no fleas were detected in the other species (Table 2). The present study finds the highest estimated prevalence of ectoparasites in Sloth Bears (475.00%) and the lowest estimated prevalence of ectoparasites in Black Buck (4.89%), the most prevalent taxa being the flies, followed by the fleas, and lastly the lice as per the estimated prevalence values in various wildlife species studied at RGZPWRC (Table 3). Also, no ticks and mites were observed and recorded from the wildlife host species studied at the zoo. Estimated prevalence values exceeding 100% also indicate that the calculated number of infested hosts was too low to account for the total ectoparasites found. This is calculated using the average parasitic load values for each category of ectoparasite in different hosts, from previous studies. Therefore, estimated prevalence alone

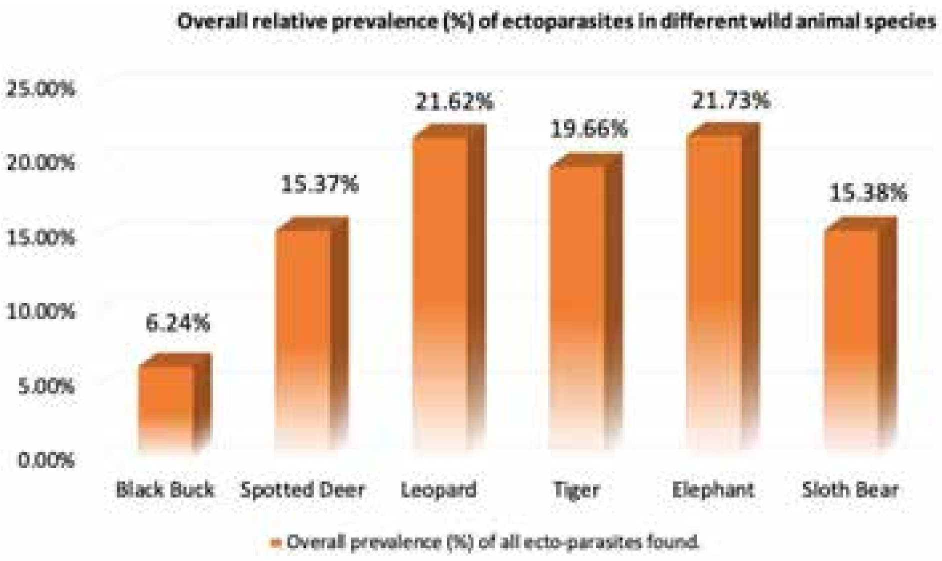

Figure 2. Overall relative prevalence (%) of ectoparasites in different wild animal species.

Table 2. Host species wise prevalence of ectoparasites observed in the wildlife species at Katraj Zoo:

	Wildlife species (Binomial name)	Total no. of wild animals observed	Total no. of ectoparasites observed w.r.t the application of 5% NSKE (Prevalence % in the host species)		Total no. of ectoparasites observed [A+B] (Overall prevalence %)
			Before [A]	After [B]	
1.	Black Buck *Antelope cervicapra*	45	43 (79.63)	11 (20.37)	54 (6.24)
2.	Spotted Deer *Axis axis*	104	93 (69.93)	40 (30.07)	133 (15.37)
3.	Leopard *Panthera pardus*	03	147 (78.61)	40 (21.39)	187 (21.62)
4.	Tiger *Panthera tigris*	04	118 (69.41)	52 (30.59)	170 (19.66)
5.	Elephant *Elephas maximus*	02	159 (84.57)	29 (15.42)	188 (21.73)
6.	Sloth Bear *Melursus ursinus*	02	102 (76.69)	31 (23.30)	133 (15.38)
Total		**160**	**662**	**203**	**865**
χ^2 value:			78.628**	28.340**	

*—Significant at P < 0.05 | **—Highly significant at P < 0.01 | NS—Non-significant.

should not be interpreted to assess the prevalence and should always be interpreted alongside relative or actual prevalence data (Eads et al. 2015; Klepeckienė et al. 2020; Smith et al. 2023). But it surely does give us an idea about the existing condition of parasitic infestations in situations where individual host monitoring is difficult or impossible, and hence, actual prevalence cannot be calculated.

Efficacy of 5% NSKE as an insecticide/insect repellent: It was observed that the reduction in the prevalence of the ectoparasites in various wild animal species was highly significant (Table 4). The efficacy was calculated to be as high as 81.76% in the Elephant. The average efficacy of 5% NSKE was calculated to be 69.33% with a 95% confidence interval that ranged 65.70–72.90%. (Table 4, Figure 3), which indicates a highly significant decrease in the overall ectoparasite counts in all six wildlife species studied. This is supported by highly significant decreases in flies (χ^2 = 228.49, df = 1, P < 0.001) and lice (χ^2 = 12.00, df = 1, P < 0.001), complete elimination of fleas (given that sample size was very small), and an overall significant reduction in total ectoparasites (χ^2 = 243.562, df = 1, P < 0.001) (Table 5). The efficacy of 5% NSKE was noted as the most effective against the fleas, which was found to be 100%, followed by the efficacies against flies, which was found to be 69.29% and lice, which was found to be 66.67% (Table 5). In the species-wise analysis, the efficacy of the treatment ranged from 55.93% in tigers to 81.76% in elephants. This large variation in efficacy is mainly due to differences in the animal housing

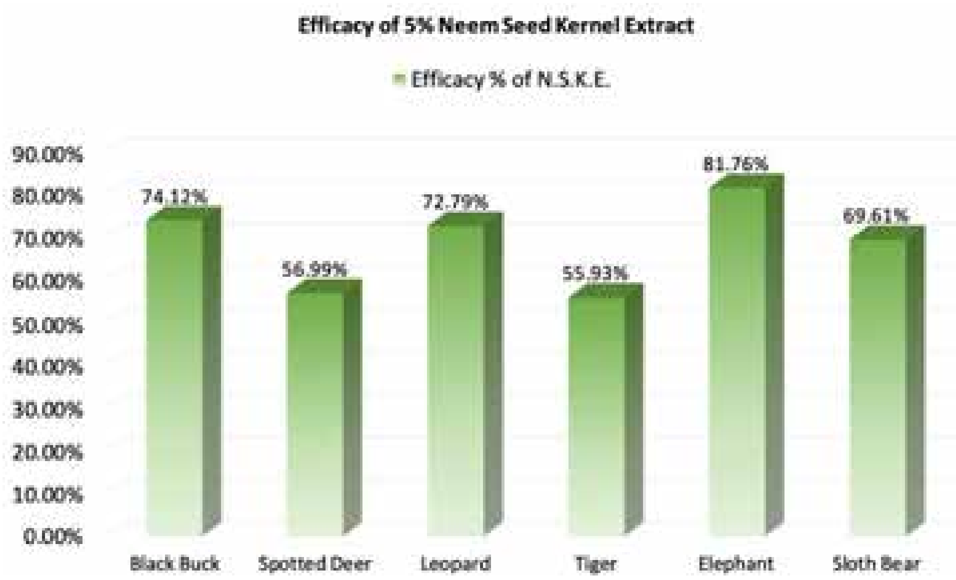

Figure 3. Efficacy of 5% neem seed kernel extract.

Image 9. Digitally collected midge.

Image 10. Processed *Drosophila* spp. fly (male) (4X).

conditions, sample size and micro-environmental factors. All the species showed statistically significant reductions in parasite counts, with chi-square values above the critical limit at P < 0.01. The 95% confidence intervals showed that elephants (74.86–87.43 %) and leopards (64.84–79.79 %) had the most consistent results with narrower ranges. Overall, the species-wise analysis revealed a statistically significant reduction in ectoparasite infestation following application of 5% NSKE across all species, including Black Buck (χ^2 = 18.96, df = 1, P < 0.001), Spotted Deer (χ^2 = 21.12, df = 1, P < 0.001), Leopard (χ^2 = 61.23, df = 1, P < 0.001), Tiger (χ^2 = 25.62, df = 1, P < 0.001), Elephant (χ^2 = 89.89, df = 1, P < 0.001), and Sloth Bear (χ^2 = 37.90, df = 1, P < 0.001), with an overall highly significant reduction in total ectoparasite load (χ^2 = 243.562, df = 1, p < 0.001) (Table 4). The treatment also showed different levels of effectiveness against the observed ectoparasites. For flies, the count reduced from 622 before treatment to 191 after, giving an efficacy of 69.29% with a 95% confidence interval between 65.67% and 72.92%, indicating a consistently high reduction rate. In case of fleas, the count dropped from four before treatment to zero after, showing 100% efficacy with a 95% confidence interval of 39.80% to 100%, thus achieving complete control in the study animals. For lice, the count came down from 36 before

Image 11. Processed *Drosophila* spp. fly (female) (4X).

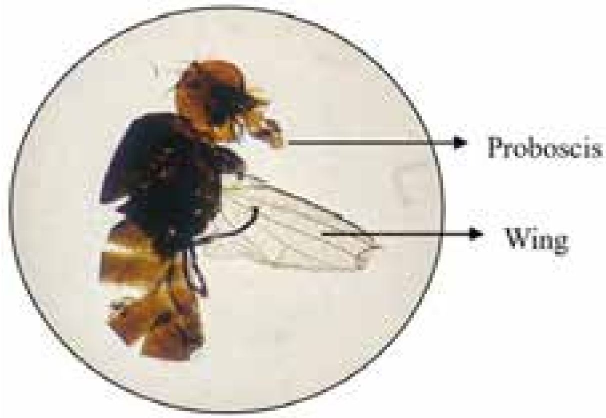

Image 12. Processed *Musca* spp. fly (male) (4X).

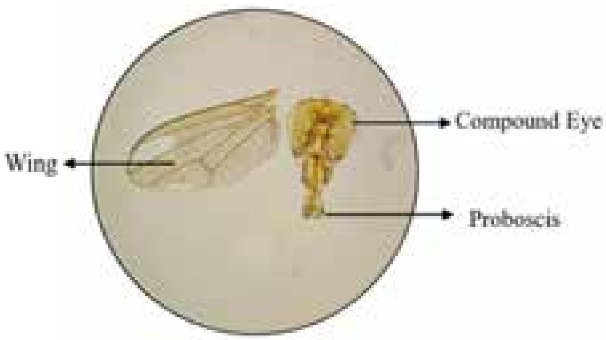

Image 13. Wing and mouth parts of *Drosophila* spp. fly (4X).

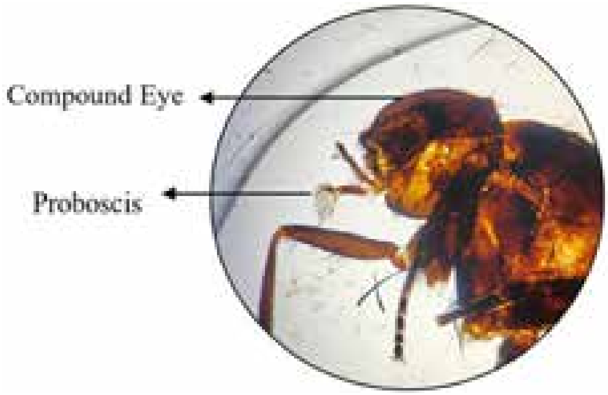

Image 14. Mouthparts of *Musca* spp. fly (4X).

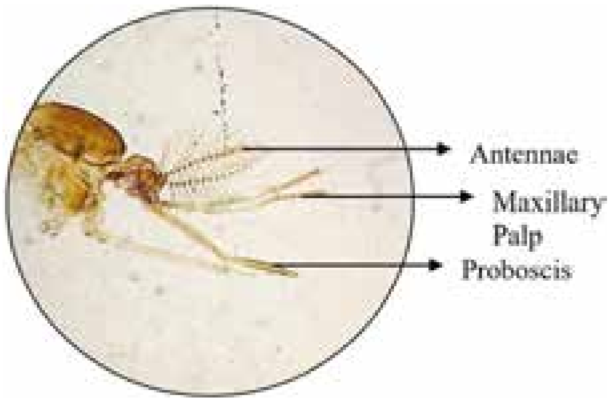

Image 15. Mouthparts of *Anopheles* spp. mosquito (10X).

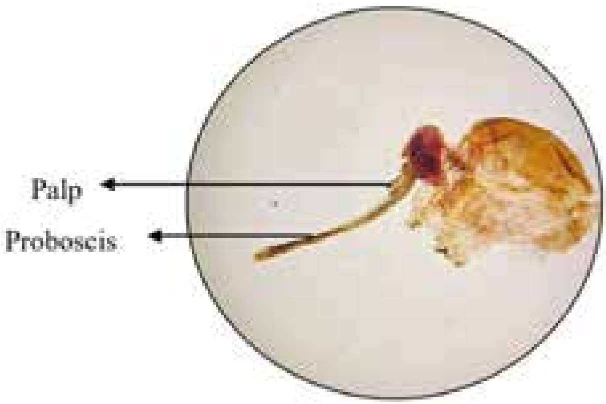

Image 16. Mouthparts of *Culex* spp. mosquito (10X).

treatment to 12 after, giving an efficacy of 66.67% with a 95% confidence interval ranged from 51.27% to 82.07%. Though the reduction was considerable, the wider confidence interval indicates some variation, possibly due to the smaller number of lice cases observed. Overall, the treatment was found to be most effective against fleas (mathematically), followed by flies and lice (Table 5).

Table 3. Overall estimated prevalence of various ectoparasites in wildlife species at Katraj Zoo.

Species	Flies		Fleas		Lice	
	H_{inf}	P_{est} (%)	H_{inf}	P_{est} (%)	H_{inf}	P_{est} (%)
Black Buck	1.92	4.26	0.28	0.63	0	0.00
Spotted Deer	2.65	2.55	0.00	0.00	2.9	2.78
Leopard	10.88	362.66	0.50	16.66	0	0.00
Tiger	8.94	223.68	0.00	0.00	0	0.00
Elephant	5.22	261.11	0.00	0.00	0	0.00
Sloth Bear	9.5	475.00	0.00	0.00	0	0.00

*—The H_{inf} values are mathematical estimates and not actual host count.
**—The estimated prevalence values > 100% indicates the ectoparasite population exceeds the capacity of individual host.
H_{inf}—Estimated number of infested hosts | P_{est}—Estimated prevalence value.

Table 4. Overall reduction of ectoparasite count and efficacy of 5% neem seed kernel extract against ecto-parasites in various wild animals at Katraj Zoo.

Species	Ecto-parasites observed w.r.t. application of 5% NSKE		Reduction of ecto-parasites (A-B)	Efficacy (A-B / A) × 100 (%)	Total no. of ecto-parasites observed in the study	χ^2 value	CI (%) range
	Before (A)	After (B)					
Black Buck	43	11	32	74.42	54	18.963**	58.82 – 86.48
Spotted Deer	93	40	53	56.99	133	21.120**	46.30 – 67.21
Leopard	147	40	107	72.79	187	61.225**	64.84 – 79.79
Tiger	118	52	66	55.93	170	25.624**	46.49 – 65.06
Elephant	159	29	130	81.76	188	89.894**	74.86 – 87.43
Sloth Bear	102	31	71	69.61	133	37.902**	59.71 – 78.32
Total:	**662**	**203**	**459**	**69.33**	**865**	**243.562****	**65.70 – 72.90**

*—Significant at P < 0.05 | **—Highly significant at P < 0.01 | NS—Non-significant.

Table 5. Overall efficacy of 5% neem seed kernel extract in various taxa of ectoparasites.

Taxa	Ectoparasites observed w.r.t. application of 5% NSKE		Reduction of ectoparasites [A-B]	Efficacy [A-B / A] × 100 (%)	Total no. of ectoparasites observed in the study	χ^2 value	CI (%) range
	Before [A]	After [B]					
Flies	622	191	431	69.29	813	228.488**	65.67 – 72.92
Fleas	4	0	4	100.00	4	--	39.80 – 100.0
Lice	36	12	24	66.67	48	12.000**	51.27 – 82.07
Total:	**662**	**203**	**459**	**69.33**	**865**	**243.562****	**65.70 – 72.90**

*—Significant at P < 0.05 | **—Highly significant at P < 0.01 | NS—Non-significant.
Note: The 100% efficacy against fleas is mathematical result of small sample size.

DISCUSSION

Safety data of NSKE used as an insecticide

Comparisons show organic concentrates of NSKE are more potent and present higher host risk, whereas aqueous extracts and Neem seed oil formulations are generally less hazardous (Isman 2006). The reported systemic toxicity in lab mammals mainly follows oral or chronic exposure, not single topical use. The current literature has limits; most of the 'no adverse effects' reports are observational, without haematology, biochemistry, or histopathology, so subclinical effects

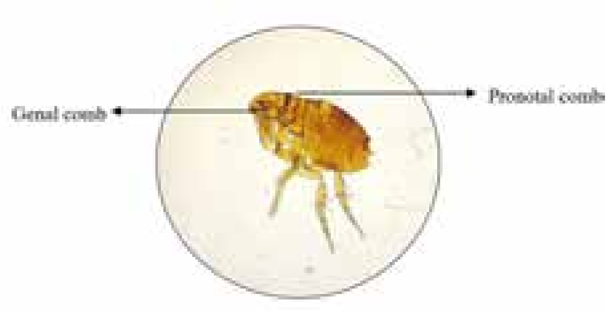

Image 17. *Ctenocephalides* spp. flea (10X).

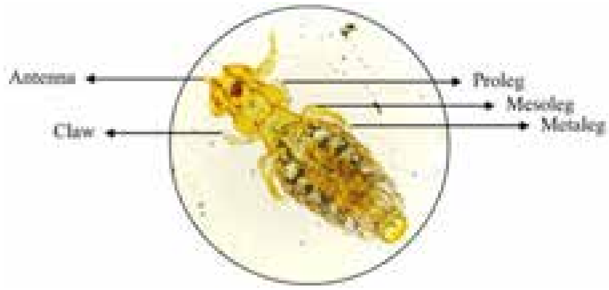

Image 18. *Damalinia* (*Bovicola*) spp. louse (10X).

Table 6. Details of animals studied and their categorization.

Wild animal	No. of animals	Category	Year
Black Buck *Antelope cervicapra*	45	Least Concern (LC)	IUCN 2016
Spotted Deer *Axis axis*	104	Least Concern (LC)	IUCN 2016
Leopard *Panthera pardus*	03	Near Threatened (NT)	IUCN 2023
Tiger *Panthera tigris*	04	Endangered (EN)	IUCN 2022
Elephant *Elephas maximus*	02	Endangered (EN)	IUCN 2024
Sloth Bear *Melursus ursinus*	02	Vulnerable (VU)	IUCN 2023

Note: Conservation status was assigned according to the IUCN Red List assessments (IUCN 2016, 2022, 2023, 2024). (Duckworth et al. 2015; Williams et al. 2020; Dharaiya et al. 2020; Goodrich et al. 2022; Steinmetz et al. 2023; Shivakumar et al. 2025; Stein et al. 2025)

cannot be excluded (Cotticelli et al. 2023). As controlled or published trials do not exist for large zoo species such as tigers, leopards, elephants, sloth bears, etc. so extrapolation requires caution. Available evidence supports topical 5% aqueous NSKE as a safe zoo-use candidate when: (a) only aqueous preparations are used, (b) treatment frequency is minimised, (c) pregnant or neonatal animals are treated cautiously, and (d) clinical and laboratory safety monitoring is included in protocols (Boeke et al. 2004; Isman 2006).

Taxa-wise dominance of ectoparasites

The present study demonstrated a pronounced taxa-wise dominance of flies, which constituted 93.99% of the total ectoparasite count (865), while lice and fleas occurred in comparatively lower proportions. This dominance may be attributed to the high reproductive rate and mobility of flies, along with favourable enclosure conditions such as the presence of organic matter and moist substrates. The smart trapping approach employed, particularly CO_2-based, light, and glued traps, was more efficient in capturing volant ectoparasites, thereby enhancing fly detection relative to host-dependent parasites like lice. Additionally, the study period from January–March, corresponding to late winter and early summer, provided optimal conditions for fly activity, while being less conducive for flea and lice proliferation. Seasonal variation is likely to influence taxa-wise patterns, with monsoon conditions potentially increasing ticks, fleas and lice prevalence due to higher humidity, and summer months further intensifying fly dominance, whereas cooler periods may result in reduced overall ectoparasite abundance.

Calculation of estimated prevalence using average ectoparasite load values

Average ectoparasite load values ($\overline{L}$) used in this study were synthesized from published reports describing parasite counts or mean intensity in the same or ecologically comparable wildlife species and were applied as standardized reference estimates for analytical calculations. These values were not intended to represent exact infestation levels under the present zoo conditions, but to provide a pragmatic proxy in the absence of site-specific quantitative ectoparasite enumeration, which is often constrained in large captive wildlife. Although ectoparasite loads may vary with environmental conditions, host management, and season, the uniform application of literature-derived values across species ensures internal consistency, and such variability is unlikely to affect the comparative outcomes or validity of the calculations.

Efficacy rates of commonly used synthetic insecticides as compared with NSKE

In wildlife ectoparasite management, synthetic insecticides such as deltamethrin, cypermethrin, permethrin, and amitraz have demonstrated high efficacy rates, often exceeding 90% against a wide range of ectoparasites, including ticks, fleas, and flies. In contrast, 5% NSKE generally provides moderate but meaningful efficacy. While synthetic compounds offer rapid knockdown and extended residual action, NSKE's slower action is counterbalanced by its excellent safety profile, biodegradability, and lower risk of resistance development, making it a suitable eco-friendly alternative in zoo and conservation programmes where chemical load reduction is critical (Isman 2006). Although synthetic insecticides such as pyrethroids and amitraz exhibit higher immediate efficacy, their use in zoological settings is constrained by concerns related to animal safety, environmental contamination, residue persistence, and resistance development. In contrast, 5% NSKE, despite its comparatively moderate efficacy, offers a favourable cost–benefit balance by providing adequate ectoparasite control while ensuring low toxicity, biodegradability, and minimal ecological impact. NSKE is economically viable, locally available, and suitable for repeated environmental application without imposing chemical stress on captive wildlife or their surroundings. Esspecially in zoo and conservation programmes where long-term sustainability, safety of non-target organisms, and reduction of chemical load are priorities, NSKE represents a pragmatic and responsible alternative to high-efficacy synthetic insecticides rather than a direct replacement.

Environmental impact of NSKE

Due to azadirachtin, NSKE has a substantially lower ecological impact compared to many synthetic insecticides. Studies have shown it to be far less toxic to fish and aquatic invertebrates than pyrethroids such as deltamethrin, and its biodegradability limits long-term persistence in the environment (Stark 2001). While effects on non-target organisms can occur at high concentrations, mesocosm studies indicate minimal disruption to aquatic communities at realistic exposure levels, with most toxicity linked to formulation additives rather than azadirachtin itself (Kreutzweiser et al. 2004). This positions NSKE as a more eco-compatible option for ectoparasite management in wildlife habitats.

Overall outcome of the study and economic analysis of NSKE used in the study

The prevalent ectoparasites recorded during the study at RGZPWRC, Katraj, Pune, were flies, fleas, and lice. The overall efficacy of 5% NSKE applied in animal shelters and night houses across the studied wildlife species was 69.33%, showing a highly significant reduction in ectoparasites ($P < 0.001$). Hence, the use of 5% NSKE as an insecticide/insect repellent was seen to be highly effective especially in wildlife settings where veterinarians are restricted to use chemical or synthetic insecticides. Also, the total quantity of concentrated solution of 5% NSKE used for a single washing of all the animal housings was 1600 millilitres, which cost INR 400.00 (at discounted rate of INR 250 per L on wholesale purchase) against INR 1200.00 (at INR 750 per L at retail price). The total area of animal housing washed was 1000 m^2. Hence, a single washing or application of 5% NSKE in all the animal housings costed between INR 0.4 (per m^2 of area) to INR 1.2 (per m^2 of area) if purchased at retail price. Hence, NSKE proved to be a cost-efficient and safer alternative to chemical insecticides for its preventive use in captive wildlife settings.

Limitations of the study

This study was conducted as a post-graduation dissertation work with limited permissions and a restricted environment with respect to time and technical clearances. Also, the short duration of this study allowed it to merely test the effect of the use of NSKE in wildlife as an ectoparasiticide rather than a comparative study of different treatments of ectoparasiticides. Although being one of the very few directly conducted studies on the efficacy of NSKE in captive wildlife, it was limited only to using NSKE in the animal housing structures as an in vivo assessment and not as a direct treatment to the captive wild animal species, which limited the assessment of NSKE a preventive insecticide rather than as a treatment option. A better study design could not be implemented due to the inclusion of wild animals with contrasting food (carnivores, herbivores and omnivores) and living habits (herd, pair or solitary living) in the study. This is a major factor for ectoparasite management and also affects prevalence calculation and estimation due to differences in study designs. Implementation of different types of designs for different categories of wild animals could have been highly time-consuming and may have resulted in errors while comparing efficacy calculated through different study designs.

Comparison with previous studies

The results of the present study are in agreement with earlier findings that report moderate but consistent efficacy of neem-based formulations against ectoparasites. Studies in livestock have shown that NSKE achieves a 65–72% reduction in ectoparasite burden, values comparable to the 69.33% overall efficacy observed in the present investigation (Akhtar & Isman 2013; George et al. 2014). In contrast, synthetic insecticides such as deltamethrin, cypermethrin, and amitraz frequently demonstrate > 90% efficacy, but their repeated use is associated with toxicity concerns, environmental persistence, and resistance development, particularly in sensitive settings like zoological parks (Isman 2006). Wildlife ectoparasite studies have also reported flies as the dominant taxa, largely influenced by environmental conditions and sampling techniques, supporting the taxa-wise dominance recorded in this study (Miller et al. 2014). Compared to earlier works, the present study is distinctive in adopting a smart, non-invasive digital trapping approach, providing ethically sound surveillance while reinforcing the practical applicability of 5% NSKE as an eco-friendly ectoparasite control option in captive wildlife.

Recommendations for future studies

a. Tailored study designs should be planned for different categories of wildlife, taking into account their food habits, living habits, and other characteristic features.

b. Species-specific studies should be conducted, with one study focusing exclusively on a single animal species to obtain the most accurate results. This approach would provide detailed, species-wise variations in efficacy, aiding further analysis.

c. Comparative studies between captive and free-ranging wildlife should be undertaken to determine if natural behaviours or environmental factors in free-ranging animals protect them from ectoparasitic infestations. Any such factors identified could be adapted for use in captive management.

d. Optimised study designs should be developed for postgraduate research and short-duration studies to save time and minimise trial-and-error approaches.

e. In vitro investigations on ectoparasites and their life cycle stages should focus specifically on parasites collected from the host species being studied.

f. Pharmacokinetic and safety studies on biological insecticides should be conducted to assess absorption, metabolism, and elimination in host species. This would help detect and prevent subclinical effects before any serious issues arise from long-term use.

g. Long-term efficacy trials of 5% NSKE, in comparison with synthetic and other botanical insecticides, should be carried out to identify the most effective and sustainable ectoparasite control measures.

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Author contribution: Dr. S.B. Kendre has designed the study of this research such that the objectives are accomplished by noninvasive sampling and with minimal animal handling. He designed the parasite traps exclusively. All the photographs and images for this research were captured by him. Field and laboratory work involved in this study was conducted as well as monitored by him. Dr. P.D. Pawar is the major advisor of this research. He planned, monitored and executed this entire research. He is the main coordinator of this study. Dr. R.V. Jadhav is the advisory committee member and Director of the institute / research center where the research was conducted. He co-ordinated the on field research work and supervised the wild animal handling, parasite collection and application of the 5% NSKE in animal enclosures. He actively secured the necessary permissions from the Maharashtra State Zoo Authority for commencement of this research study. Dr. U.M. Tumlam is the advisory committee member and contributed in the research with her expertise and guidance in research management and execution. Dr. A.Y. Doiphode is the advisory committee member in this research. He actively developed a special study design that favored sample collection in wildlife conditions, with minimal biases. Dr. V.G. Nimbalkar handled the raw data sets of this study and carried out all the statistical analysis. With her expertise in statistics, she converged all the raw numerical observations of this study into meaningful and statistically sound conclusions. Dr. P.K. Bhangale helped in the field execution of this research, monitored sample collections, insecticide application and collected photographic evidences at every step of the research. Dr. V. C. Priyal obtained all the prerequisite permissions and necessary authorizations required to conduct the study smoothly. With her knowledge in wildlife, she closely monitored samples, study results and data sets for corrections if any. Dr. S.M. Meshram contributed in conduction of the study by collecting, preserving, processing and storing the parasite samples. She kept the phase wise records of all the samples, animal enclosures and animal species studied in this research.

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ARTICLE

A comparative web-traffic analysis of three renowned wildlife conservation organisations - International Union for Conservation of Nature (IUCN), Wildlife Conservation Society (WCS) and World Wide Fund for Nature (WWF)

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Abstract: The International Union for Conservation of Nature (IUCN), Wildlife Conservation Society (WCS), and World Wide Fund for Nature (WWF) are among the leading wildlife organisations that provide significant scientific information. These organisations, through their highly data-centric and interactive websites, communicate conservation and have been accessed by an audience base across the globe. Each part of the world encounters a diverse range of conservation-related issues. Analysing the communication strategies of each of these websites can provide key insights into the domain of wildlife conservation pedagogy. This study is based on quantitative web analytics utilising web tools and software that provide insights such as attributes and visual portraits on the engagement of the audience of these websites, trending search topics, audience engagement, learning behaviours, website performance, user behaviour analysis, and communication strategies. WWF recorded the highest monthly visits (≈ 1.56 million) but the highest bounce rate (68.63%), whereas IUCN recorded the longest mean visit duration (2 min 21 s). The findings describe how the three organisations' publicly reported web-traffic profiles differ in audience geography, search-term composition, social-referral mix and engagement metrics and discuss the limits of inferring audience intent from aggregate analytics.

Keywords: Audience engagement, communication strategy, International Union for Conservation of Nature, web analytics, websites, wildlife conservation, Wildlife Conservation Society, World Wide Fund for Nature.

ସାରାଂଶ: ଇଣ୍ଟରନ୍ୟାସନାଲ ୟୁନିଅନ୍ ଫର୍ କଞ୍ଜରଭସେନ୍ ଅଫ୍ ନଚେର୍(IUCN), ୱାଇଲ୍ଡଲାଇଫ୍ କନଜର୍ଭସେନ୍ ସୋସାଇଟି (WCS) ଏବଂ ୱାର୍ଲ୍ଡ ୱାଇଡ୍ ଫଣ୍ଡ ଫର୍ ନଚେର (WWF) ହେଉଛନ୍ତି କେତେକ ଅଗ୍ରଣୀ ବନ୍ୟପ୍ରାଣୀ ସଂଗଠନ, ଯେଉଁମାନେ ଗୁରୁତ୍ୱପୂର୍ଣ୍ଣ ବୈଜ୍ଞାନିକ ତଥ୍ୟ ପ୍ରଦାନ କରନ୍ତି। ଏହି ସଂଗଠନଗୁଡ଼ିକ ସେମାନଙ୍କର ତଥ୍ୟ-କେନ୍ଦ୍ରିକ(data-centric) ତଥା ଇଣ୍ଟରାକ୍ଟିଭ୍ ୱେବସାଇଟ୍ ମାଧ୍ୟମରେ ସଂରକ୍ଷଣ ସମ୍ବନ୍ଧୀୟ ବାର୍ତ୍ତା ପ୍ରସାରଣ କରନ୍ତି ଏବଂ ବିଶ୍ୱବ୍ୟାପୀ ଏକ ବିସ୍ତୃତ ଦର୍ଶକ ଶ୍ରେଣୀ(audience base) ଦ୍ୱାରା ଏହା ବ୍ୟବହାର କରାଯାଇଛି। ବିଶ୍ୱର ପ୍ରତ୍ୟେକ ଅଞ୍ଚଳ ବିଭିନ୍ନ ସଂରକ୍ଷଣ-ସମ୍ବନ୍ଧୀୟ ସମସ୍ୟାର ସମ୍ମୁଖୀନ ହୋଇଥାଏ। ଏହି ପ୍ରତ୍ୟେକ ୱେବସାଇଟ୍ର ଯୋଗାଯୋଗ ରଣନୀତି (communication strategies) ବିଶ୍ଳେଷଣ କରିବା ଦ୍ୱାରା ବନ୍ୟପ୍ରାଣୀ ସଂରକ୍ଷଣ ଶିକ୍ଷାଦାନ (pedagogy) କ୍ଷେତ୍ରରେ ଗୁରୁତ୍ୱପୂର୍ଣ୍ଣ ଧାରଣା ମିଳିପାରିବ। ଏହି ଅଧ୍ୟୟନଟି ପରିମାଣାତ୍ମକ ୱେବ ଆନାଲିଟିକ୍ସ (quantitative web analytics) ଉପରେ ଆଧାରିତ, ଯେଉଁଥିରେ ୱେବ ଟୁଲ୍ସ ଓ ସଫ୍ଟୱେର ବ୍ୟବହାର କରାଯାଇଛି। ଏଗୁଡ଼ିକ ଏହି ୱେବସାଇଟଗୁଡ଼ିକର ଉପଭୋକ୍ତା/ଦର୍ଶକଙ୍କ ସମ୍ପୃକ୍ତି (audience engagement), ଟ୍ରେଣ୍ଡିଂ ସର୍ଚ୍ଚ ବିଷୟ (trending search topics), ଶିକ୍ଷଣ ଆଚରଣ (learning behaviours), ୱେବସାଇଟର କାର୍ଯ୍ୟଦକ୍ଷତା (website performance), ବ୍ୟବହାରକାରୀଙ୍କ ଆଚରଣ ବିଶ୍ଳେଷଣ (user behaviour analysis) ଏବଂ ଯୋଗାଯୋଗ ରଣନୀତି ଭଳି ବୈଶିଷ୍ଟ୍ୟ ଏବଂ ଭିଜୁଆଲ୍ ଚିତ୍ର ପ୍ରଦାନ କରିଥାଏ। WWF ସର୍ବାଧିକ ମାସିକ ପରିଦର୍ଶନ (≈୧.୫୬ ନିୟୁତ) ରେକର୍ଡ କରିଥିଲା କିନ୍ତୁ ସର୍ବାଧିକ ବାଉନ୍ସ ରେଟ୍ (୬୮.୬୩%) ମଧ୍ୟ ରେକର୍ଡ କରିଥିଲା, ଅନ୍ୟପକ୍ଷରେ ଆଇୟୁସିଏନ୍ ସର୍ବାଧିକ ହାରାହାରି ପରିଦର୍ଶନ ଅବଧି (୨ ମିନିଟ୍ ୨୧ ସେକେଣ୍ଡ) ରେକର୍ଡ କରିଥିଲା। ଏହି ଅନୁସନ୍ଧାନରୁ ଜଣାପଡ଼ିଛି ଯେ କିପରି ଏହି ତିନୋଟି ସଂଗଠନର ସାର୍ବଜନୀନ ଭାବରେ ପ୍ରକାଶିତ ୱେବ୍-ଟ୍ରାଫିକ୍ ପ୍ରୋଫାଇଲ୍ ସେମାନଙ୍କର ଦର୍ଶକଙ୍କ ଭୌଗୋଳିକ ସ୍ଥିତି (audience geography), ସର୍ଚ୍ଚ-ଟର୍ମ ଗଠନ (search-term composition), ସୋସିଆଲ୍-ରେଫେରାଲ୍ ମିଶ୍ରଣ (social-referral mix) ଏବଂ ସମ୍ପୃକ୍ତି ମେଟ୍ରିକ୍ସ (engagement metrics) କ୍ଷେତ୍ରରେ ଭିନ୍ନ ଅଟେ ଏବଂ ଏହା ସହିତ ଏକତ୍ରିତ ବିଶ୍ଳେଷଣ(aggregate analytics) ରୁ ଦର୍ଶକଙ୍କ ପ୍ରକୃତ ଉଦ୍ଦେଶ୍ୟ ଅନୁମାନ କରିବାର ସୀମା ବିଷୟରେ ଆଲୋଚନା କରେ।

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INTRODUCTION

In the world wide web ecosystem, search engines facilitate information-seeking behaviour for day-to-day activities, data, and knowledge. Global internet usage has grown substantially in recent decades, intensifying both the volume of online conservation information and the challenge of identifying credible sources. Search engines return large volumes of information of varying quality, creating challenges for users seeking reliable scientific data on wildlife and conservation (Cuan-Baltazar et al. 2020). Credible websites characterized by institutional affiliation, evidence-based content, and transparent governance represent a small but disproportionately influential subset of this online information environment (Guess et al. 2020). Few websites of each industry are referred to as the most reliable source with negligible misinformation (Guess et al. 2020). The online information disseminated through some of the leading organizations is backed up by its content, website design, navigation, algorithmic architecture, and user engagement (Anwyl-Irvine et al. 2021). It becomes much more intricate when the community tries to look for scientific information about wildlife (Cuan-Baltazar et al. 2020). Conservation and protection are the key measures to help nature grow and recover from habitat degradation. There are various stakeholders, including government and Non-Governmental Organisations (NGOs), who work toward conservation and communicate conservation (Pati & Kumar 2023). These three organizations were therefore selected as case studies for the present analysis (see Methods for full selection criteria).

Despite the growing use of digital platforms in conservation communication, limited peer-reviewed research has systematically evaluated the online audience engagement strategies of major wildlife NGOs using web analytics data. It remains unclear how these organizations differ in terms of digital reach, audience demographics, and content emphasis, information that could help conservation practitioners optimize online communication. This study addresses that gap by applying SimilarWeb web analytics to compare IUCN, WCS, and WWF across quantitative metrics of audience engagement, geographic distribution, and search behaviour.

The IUCN was established in 1948 and as of 2024, it is one of the leading wildlife organizations in the world, collaborating with governments, NGOs, environmental networks, researchers, and scientists to promote conservation and sustainable development. IUCN focuses on biodiversity conservation, climate change mitigation, and habitat protection. It is internationally recognized for the IUCN Red List of Threatened Species, a globally authoritative index of extinction risk. On the other hand, WCS strikes a balance between community needs and conservation objectives. It aims to conserve 14 priority (large-scale) wildlife ecosystems in the world, which represent half of the world's biodiversity. It works closely with tourists, travelers, and activists to achieve conservation-related goals. They invest in zoological gardens, central parks, and movies to promote conservation. With various on-ground activities and online initiatives, they create engaging conservation approaches for the users. Similarly, WWF is a pioneer in six global environmental concerns, namely forest, marine, freshwater, wildlife, food, and climate. They work across 200 ecologically valuable regions and are famous for using modern communication strategies (Martin 2005). WWF reports working with more than six million supporters across 100+ countries on its mission to conserve nature and reduce threats to biodiversity.

Accordingly, among various organisations, the three highest-traffic wildlife conservation websites IUCN, WCS and WWF selected based on global web traffic rankings from SimilarWeb (see Methods) are taken into consideration to understand and analyze the audience engagement, demographics, geography and various wildlife-related trending topics based upon the metadata analysis conducted in SimilarWeb software. The three websites were selected based on the following objective criteria: (1) global organizational scope; (2) ranking among the top three wildlife-focused domains by monthly visitor count as recorded by SimilarWeb during the study period; and (3) availability of complete web analytics data for the study timeframe. Web traffic statistics are one of the most widely accepted methods for shortlisting a website. The study refers to the average number of posts or metrics of popularity (Stringham et al. 2021).

The website's selection also varies depending on the desired information required. Leads of websites are generated by social media that promote wildlife-related topics and increase website traffic. Each of these websites has various attributes that differ, and the community refers to one of these for a specific requirement. The relevant keywords then help the websites generate a larger audience and maintain their hold among the huge set of information floating across the search engine.

Although conservation organizations increasingly rely on websites as primary communication channels, few studies compare how leading global organizations differ in the audiences they reach and the topics those audiences search for. This study addresses that gap by comparing the publicly reported web-traffic profiles of IUCN, WCS, and WWF, asking whether organizations with different missions show measurably different audience geographies, search-term compositions, and engagement metrics. Characterizing these differences offers conservation practitioners a baseline for understanding which audiences each organization currently reaches online, and where outreach gaps may exist.

MATERIALS AND METHODS

Data were extracted from SimilarWeb on 30 April 2024. For each of the three websites (iucn.org, wcs.org, worldwildlife.org), the following metrics were recorded for 01 February–30 April 2024: total monthly visits, average visit duration, pages per visit, bounce rate, total page views, audience geographic distribution (top five countries by traffic share), social traffic sources (percentage by platform), and industry distribution categories. Values represent estimated monthly averages as reported by SimilarWeb. Top search terms were identified using the SimilarWeb 'Search' module and visualized as word clouds using Mentimeter online platform. No inferential statistical analyses were performed; all results are reported descriptively.

The research begins with data acquisition using SimilarWeb, a commercial web analytics platform that generates estimated traffic data based on a proprietary panel and algorithmic modelling. It is important to note that SimilarWeb data were estimates rather than direct measurements; the platform does not publish confidence intervals for individual site estimates, which is acknowledged as a limitation of the present study. SimilarWeb was selected because it provides standardized, multi-metric web traffic data across multiple organizations simultaneously, enabling direct comparison. Its use in web analytics and digital communication research is well established (Weischedel & Huizingh 2006; Tarafdar & Zhang 2008). However, it is important to recognise that the initial phase of this study involves extensive data cleaning and preparing datasets for more efficient analysis. Given the wide variety of formats used by different organizational websites, manually cleaning and formatting the data is crucial for eliminating inconsistencies and ensuring a uniform set of information for analysis. All traffic metrics were exported directly from SimilarWeb's web interface; no web scraping was performed. Exported fields were checked manually for completeness and formatting consistency before analysis.

With the rise of dynamic websites replacing static ones, it is essential to understand how these advancements impact communication strategies in wildlife organizations. These organizations utilize websites as their main platform to inform the public, raise awareness, and foster engagement with conservation efforts (Weinreich et al. 2008). Through web optimization strategies, organizations can enhance user experience, increase their reach, and convey information more effectively (Weischedel & Huizingh 2006). Websites play a critical role in attracting visitors, informing them about conservation projects, and encouraging support for wildlife protection through various channels (Tarafdar & Zhang 2008).

The three organizations were selected because each has a global remit, an English language site, and a comparable conservation mission. Data source used was SimilarWeb, covering February–April 2024. Metrics extracted were monthly visits, mean visit duration, pages per visit, bounce rate, page views, gender split, country traffic share, social-referral share, industry/ category distribution, and top search terms. Search terminology word clouds were generated from SimilarWeb's reported search terms.

Scope and Limitation

Search-term data indicate the terms users searched for before or after visiting these websites, but cannot reliably establish user intent, motivation or organizational communication strategy. This limitation impacts the ability to ensure that the analysis of wildlife communication is based solely on credible sources. The presence of misinformation can skew understanding and may affect the validity of the findings. The selection of websites for analysis was based on subjective criteria, such as metadata from SimilarWeb and general popularity metrics. Manual data collection is time-intensive and prone to human error, which can impact the reliability of the data. The challenge of integrating data from different website formats and ensuring its accuracy further complicates the analysis. These methods often operate in a legal grey area and may breach privacy or data protection regulations (Zimmer 2010). Ethical constraints around acquiring sensitive or unauthorized data, especially from the dark web, pose additional challenges (Tai 2012). Ensuring ethical compliance while collecting and analyzing data remains a critical concern.

Ethical Considerations and Legal Framework

SimilarWeb provides aggregated, anonymized, publicly available estimates of website traffic. No personal data were collected, no human participants were involved, and no web scraping was performed; therefore, no ethics approval was required (Sula 2016).

Research Questions

The central aim of this research is to understand how key wildlife conservation websites engage their audiences and disseminate information.

These research questions are grounded in a web analytics framework for evaluating organizational digital communication effectiveness (Tarafdar & Zhang 2008; Weinreich et al. 2008), in which audience demographics, geographic reach, content discovery terms, and social traffic serve as proxy indicators of communication performance. Together, they reflect the breadth, depth, and thematic orientation of audience engagement across the three organizations. To achieve this, the following research questions are posed:

R1: What are the primary demographics of users accessing these websites, and how do they engage with the content?

R2: Which countries around the world follow these websites for seeking information?

R3: What conservation-related topics are most frequently searched on these websites?

R4: How does social traffic influence engagement on these wildlife conservation platforms?

Data Collection Process

This is a descriptive quantitative web-analytics study. Differences between the three organisations are reported descriptively; no inferential statistics were applied because the data are population-level estimates for single websites. Using SimilarWeb, the researcher gains access to valuable data on web traffic, audience behaviour, and geographical distribution of users. The tool was selected for its robust features, which provide comprehensive insights into the reach and effectiveness of the websites being studied. Quantitative analysis focuses on measuring metrics like visitor count, engagement rates, and the geographic location of the audience, which allows for a comparative study of web traffic and audience demographics. On the qualitative side, the content quality of each website is assessed based on credibility, alignment with scientific research, and clarity in communication. The comparative analysis of these three wildlife websites (WWF, WCS, IUCN) allows the researcher to identify patterns in how different organizations communicate their conservation efforts and engage their online audiences.

Timeframe and Research Duration

This study spans a period of three months, from February to April 2024, to ensure that a representative sample of data is collected. This three-month window provides a snapshot of website engagement during this specific period. It is acknowledged that a three-month duration is insufficient to fully capture annual seasonal variation in web traffic; findings should therefore be interpreted as representative of this particular period rather than as annual trends. It also allows sufficient time to conduct both quantitative and qualitative analyses, ensuring that the study's findings are both reliable and actionable.

RESULTS

The following interpretations are descriptive inferences from observed traffic patterns. SimilarWeb data reflect user behaviour metrics and cannot directly establish organizational strategy, user intentions, or communication effectiveness. The IUCN had significant searches for research, species, biology, social networks, conservation, books, references, and publications (refer to Figure 1). The commonly searched words among the other two websites (WCS & WWF) are news, jobs, publication, and community. The most frequent search terms associated with the IUCN site were research, species, biology, conservation, books, and publications. IUCN's traffic patterns suggest a predominantly science-oriented audience. IUCN maintains multiple organizations, such as the IUCN Red List, Green List, and many more that provide universally accepted information about species and nature.

The analysis of search terms for IUCN compared to WCS and WWF reveals a distinct focus of user interest. IUCN's search terms, such as research, species, biology, and conservation indicate a strong inclination towards in-depth scientific research and authoritative studies on biodiversity and sustainability. This contrasts with the WCS and WWF, where commonly searched terms include news, jobs, and community, reflecting a broader interest in current studies, career opportunities, and engagement with conservation communities.

These differences indicate that the search terms associated with IUCN were more research oriented

Figure 1. Word cloud of trending searches and key words on the IUCN website.

than those associated with WCS & WWF. Its resources, including the IUCN Red List and Green List, are globally recognized for their rigorous standards and comprehensive data on species and ecosystems.

The WWF had audiences who searched for education, magazines, nature, shopping, science, environment news, sustainability, activism, charity, blogs, and wildlife mostly (refer to Figure 2). This organization is charitable and works for various communities across 100+ countries to generate funds and provide a deep immersion experience through adaptation schemes. WWF provides a deep understanding of various charismatic species and tries to conserve wildlife using these species as umbrella species. The organization works mostly on content creation and distribution, resulting in trending searches such as shopping, magazines, journals, and books. The website contains a designated active link to help viewers sponsor or donate to a cause. The website also offers a variety of artefacts such as wildlife magnets, badges, books, and magazines for sale. They cover mostly charismatic species.

The search terms associated with the WWF such as education, magazine, nature, shopping, and activism highlight its focus on engaging the public through diverse content and fundraising efforts. WWF's strategy involves leveraging charismatic species to drive awareness and support for conservation. The frequent searches related to shopping, magazines, and blogs suggest that WWF actively promotes merchandise and educational materials as part of its outreach. This aligns with its mission to generate funds and foster deeper public engagement with conservation issues. The presence of links for donations and a range of merchandise for sale indicates WWF's dual approach: advancing conservation through direct funding and enhancing public interest via engaging content and products. Search terms and on-site content for WWF frequently referenced charismatic species, such as the Tiger and Snow Leopard, alongside merchandise and donation pages.

WCS delivered the most different trending searches word cloud with searches such as movies, zoo, credit cards, shopping, travel, news, and retail stores (refer to Figure 3). This website had a significantly low conservation-based search, with 'environment activism action' being the only trending search. This website has a lot of active links for wildlife bookings and participation, resulting in searches based on travel, credit cards, movies, zoos, and shopping. Active links of different offers and ads lead to the search of topics such as English news, online US news, email news online, Google Sign, search engines, web search live, and social networks to a large extent. WCS looks for individuals who participate in fieldwork and experience conservation, wildlife or nature-based issues in a practical environment.

WCS's trending searches predominantly commercial and recreational terms such as zoos, travel, movies, and shopping suggest an audience oriented toward experiential wildlife engagement rather than

Figure 2. Word cloud of trending searches and key words on the WWF website.

Figure 3. Word cloud of trending searches and key words on the WCS website.

conservation-focused information-seeking. The low incidence of conservation-related searches, with 'environment activism action' being the sole exception, underscores WCS's strategy of engaging audiences through experiential and commercial avenues rather than through activism or scientific discourse.

The presence of numerous active links for bookings and participation in wildlife experiences suggests that WCS targets travelers seeking immersive encounters with nature. This approach is reflected in the popular searches related to travel, credit cards, and retail stores. WCS's search patterns are consistent with a tourism- and experience-oriented audience, though the available traffic data were unavailable to draw conclusions about whether this reflects deliberate organizational strategy or user self-selection. The emphasis on offers, ads, and experiential content aligns with its goal of engaging a broader audience through appealing,

Table 1. Top five industry distribution of data of IUCN.

Industry Distribution	IUCN.org
Computers Electronics and Technology> Social Network	12.40%
Law and Government> Government	8.23%
News and Media	8.18%
Science and Education> Science and Education - Other	7.08%
Science and Education> Environmental Science	6.56%
Others	57.55%

Table 2. Top five industry distribution of data of WWF.

Industry Distribution	WWF.org
Science and Education > Education	11.02%
News and Media	10.15%
Science and Education > Environmental Science	7.13%
Computers Electronics and Technology	5.20%
Law and Government > Government	4.99%
Others	61.50%

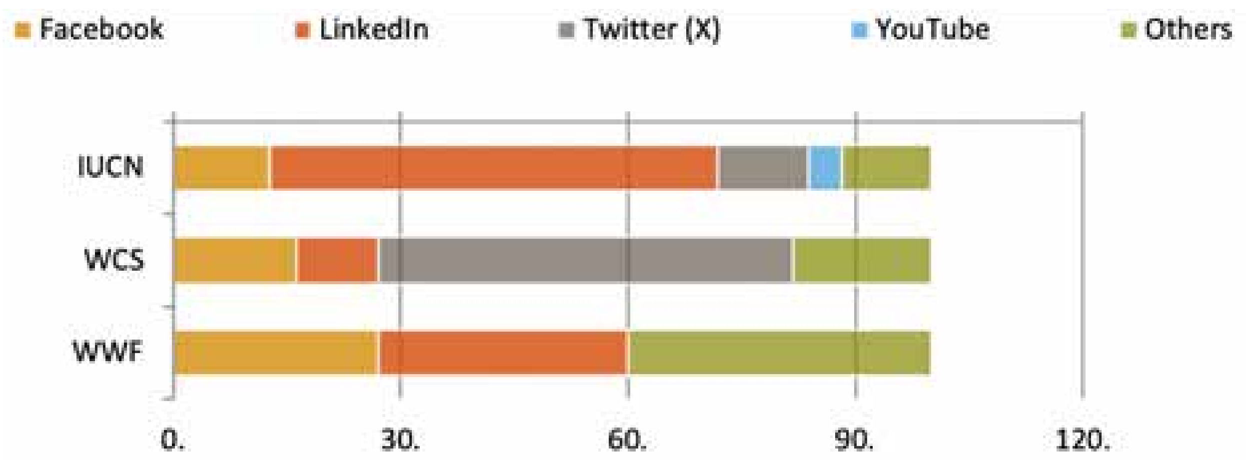

Figure 4. Social traffic of IUCN, WCS, & WWF.

practical experiences rather than through traditional conservation messaging.

Industry Distribution Analysis

The data distribution analysis for the IUCN reveals a significant emphasis on social networking platforms, which account for 12.40% of the data distribution. This prominence in the social networking category indicates IUCN's strategic focus on leveraging digital and social media channels to reach and engage with a broad audience. Given that IUCN is heavily involved in policymaking and governance, this high percentage underscores the organization's commitment to using these platforms for advocacy, information dissemination, and stakeholder engagement (refer to Table 1). The substantial allocation of data to social networking reflects IUCN's recognition of the importance of digital communication in modern conservation efforts. By prioritizing social media, IUCN can effectively communicate its standards, policies, and conservation messages to a global audience, enhancing its visibility and influence.

Following social networking, the data distribution in 'Law and Government' at 8.23% highlights IUCN's role in shaping conservation policies and its reliance on official channels for policy-related information. The third-highest data distribution in 'News and Media' (8.18%) further emphasizes the importance of media in amplifying IUCN's messages and updates. The comparatively lower percentage for each Science and Education sub-category (7.08% and 6.56%) is individually lower than 'Social Networking' (12.40%), and 'Law and Government' (8.23%), indicates that within IUCN's observed industry distribution, traffic is proportionally more concentrated in social and policy-related categories than in educational content categories during the study period. This approach aligns with IUCN's mission to impact policy and public awareness, leveraging its substantial presence on social platforms to drive engagement and influence in conservation matters.

The WWF demonstrates a notable emphasis on science and education, with 11.02% of its content allocated to this category, and an additional 7.13%

Figure 5. Audience engagement – top countries.

dedicated specifically to Environmental Science, totaling 18.15%. This substantial focus highlights WWF's commitment to disseminating scientific knowledge and educational resources related to environmental conservation. Despite the IUCN having a more prominent role in scientific research, WWF's considerable investment in Science and Education underscores its strategic objective to educate the public and stakeholders about conservation issues through accessible and impactful content.

The significant 10.15% allocation of data to the news and media further illustrates WWF's strategy of using media to amplify its research findings and educational initiatives. This focus on media outreach complements their educational efforts by ensuring that conservation messages reach a broad audience through various channels, enhancing public engagement and awareness.

In contrast, the relatively lower percentages for computers, electronics, technology (5.20%), and Law & Government (4.99%) suggest that while WWF does address technological and policy aspects, these are less central to their primary mission compared to their educational and media efforts. This distribution indicates WWF's strategic prioritization of science-based content and public education, reflecting its commitment to fostering informed environmental stewardship and leveraging media to broaden its reach and impact in the conservation arena (refer to Table 2).

The WCS shows a distinctive approach in its data distribution across various sectors, with a primary focus on News and Media, which accounts for 12.78% of their content. This indicates a strategic emphasis on leveraging media platforms to engage the public and disseminate information about wildlife conservation. The substantial investment in News and Media reflects WCS's commitment to keeping its audience informed about current events, updates, and conservation efforts through widely consumed channels. In addition to its media focus, WCS allocates 6.95% of its data to Law and Government, highlighting its involvement in policy-related matters and its role in shaping and advocating for environmental legislation. This is complemented by a 5.94% allocation to Computers, Electronics, and Technology, which suggests that WCS also engages with digital tools and platforms, albeit to a lesser extent compared to News and Media.

One notable aspect of WCS's data distribution is its 4.58% allocation to Finance. This is unique among the three organizations analyzed, indicating that WCS integrates financial aspects into its outreach strategy. This allocation suggests a focus on financial engagement, possibly through fundraising or financial support mechanisms. The presence of such a significant percentage in Finance underscores WCS's approach to driving financial contributions and support through its media and social networking efforts (refer to Table 3). Overall, the data distribution reflects WCS's strategy of combining media outreach with financial engagement. By prioritizing News and Media and leveraging social networking platforms, WCS effectively communicates its conservation messages and engages with its audience. This approach likely enhances public awareness and support while facilitating financial contributions to further its conservation goals. The emphasis on media and finance illustrates a unique strategy for balancing information dissemination with financial sustainability.

Social Media Traffic

The social traffic of these three websites depicts very different strategies followed by each of them (refer to Table 4 and Figure 4). IUCN uses LinkedIn the most for social presence, whereas WCS and WWF use Twitter (X) and other platforms more, respectively. IUCN communicated through almost all social platforms:

Metric	iucn.org	wcs.org	worldwildlife.org
Monthly visits	509,306	467,649	**1.555M**
Visit duration	**00:02:21**	00:01:07	00:01:37
Pages per visit	**2.64**	2.06	2.51
Bounce rate	**58.32%**	62.46%	68.63%
Page Views	1.344M	961,436	**3.910M**

Figure 6. A comparative website audience engagement of IUCN, WCS, & WWF.

59.02% via LinkedIn, 12.76% Facebook, 11.96% Twitter (X), and 4.46% YouTube, with the remaining 11.8% spread across other minor platforms such as WhatsApp. (February-April 2024).

WCS's communication strategy is to reach 54.75% of the audience through Twitter. Unlike IUCN, the organization uses LinkedIn for 10.83% and Facebook for 16.19% of its social traffic to communicate with their audience. No measurable YouTube referral traffic was recorded for WCS between February and April 2024. This organization targets consumers who are active on Twitter to showcase their conservation-related matters and targets financial support more than the IUCN and WWF.

Social traffic to WWF.org was primarily driven by LinkedIn (32.89%) and Facebook (27.06%). Twitter data for WWF were unavailable in SimilarWeb during the study period. The large proportion attributed to other platforms (40.05%) limits precise interpretation of WWF's social media engagement profile. No measurable YouTube social traffic was recorded for WWF.org during this period.

Each of the organizations used different strategies and platforms to communicate with their target audience and was successful enough to have a steady growth in social traffic through the months.

The top five countries are the United States with 38.97% of traffic share, followed by India with 6.07%, United Kingdom with 3.87%, Canada with 2.88% and Mexico with 2.81% (refer to Figure 5). The United States accounted for the highest combined traffic share (38.97%) across the three websites. The reasons for this geographic distribution were not assessed in the present study and may reflect multiple factors beyond the scope of this dataset. WWF has the most traffic share split among the three in all five top countries. It is surprising to witness the massive traffic share WWF has and the impact it must have created over time. WWF has 74.4% of the traffic share in Mexico, followed by the United States with 67.9%, 65.8% traffic share in Canada, 60.2% in the United Kingdom and 57.9% in India. WWF demonstrated the highest traffic share in all five top countries during the study period, indicating broader geographic reach relative to IUCN and WCS based on SimilarWeb data. IUCN holds 33.0% of the traffic share in India, followed by the United Kingdom at 25.8%, 24.0% in Mexico, 18.9% in Canada and a 5.4% traffic share in the United States.

Table 3. Top five industry distribution of data of WCS.

Industry Distribution	WCS.org
News and Media	12.78%
Law and Government > Government	6.95%
Computers Electronics and Technology > Social Network	5.94%
Computers Electronics and Technology > Programmed Content	5.11%
Finance > Banking Credit and Lending	4.58%
Others	64.65%

WCS has the least traffic share in these five top countries, with 26.7% being the highest in the United States. WCS has a traffic share of 15.3% in Canada, followed by 13.9% in the United Kingdom, 9.1% in India and the least in Mexico with a 1.6% traffic share. This comparative study would provide overall website engagement over three months (February–April 2024). The measure criteria are monthly visits, average visit duration, pages per visit, bounce rate, and page views (refer to Figure 6).

IUCN provides their audience with informative and engaging content, helping them get the best visit duration

Figure 7. Demographic analysis of audience engagement (IUCN, WCS, & WWF).

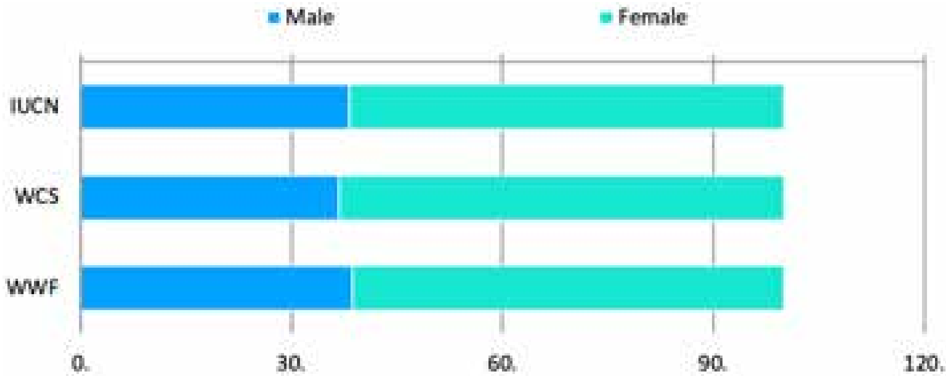

Table 4. Social traffic data of IUCN, WCS, & WWF.

Organizations	Facebook	LinkedIn	Twitter (X)	YouTube	Others
IUCN	12.76%	59.02%	11.96%	4.46%	11.80%
WCS	16.19%	10.83%	54.75%	0	18.23%
WWF	27.06%	32.89%	NA	0	40.05%

of 2 minutes and 21 seconds over 2.64 pages surfed in a single visit. IUCN manages to hold the audience's attention and provides the necessary information. The bounce rate is low compared to the other two organizations. IUCN recorded 509,306 monthly visits and 1.344 million page views over the study period. It indicates very high audience engagement relative to a smaller number of monthly visits.

WCS website lags in all categories, except bounce rate is slightly better than WWF. WCS recorded 467,649 monthly visits and the mean visit duration of 1 minute 07 seconds. WCS recorded the fewest pages per visit (2.06) and page views of 961,436 of the three sites.

WWF has the highest number of monthly visits at 1.56 million and the highest number of page views with 3.910 million in three months. WWF recorded the highest bounce rate (68.63%) and an average visit duration of 1 minute 37 seconds. While these metrics indicate that a large proportion of visitors exited after viewing a single page, bounce rate alone cannot support conclusions about content quality. High bounce rates may also reflect direct-access behaviour or single-page goal completion.

The three websites project a very similar gender-based engagement, where females make up more than 60% of each of the websites. IUCN has a 38.26% male audience. WCS has 36.82% males, followed by WWF with 38.61% males as their website audience. Female audiences were recorded more in the three months, with 61.74% with IUCN, 63.18% with WCS, and 61.39% in WWF (refer to Figure 7).

DISCUSSION

The results reveal a trade-off between audience reach and engagement depth. IUCN's higher engagement-quality metrics despite a lower monthly visit volume, and WWF's high traffic volume alongside a relatively higher bounce rate, exemplify this inverse relationship. WWF demonstrates a contrasting approach with a heavy emphasis on Science and Education (18.15%), indicating a robust commitment to educational outreach and scientific knowledge dissemination. The organization's focus on charismatic species and the substantial allocation of resources to News and Media (10.15%) reflects its strategy of using compelling narratives and media to drive public engagement and fundraising efforts. WWF's integration of shopping and charitable initiatives into its website, alongside educational content, reveals a dual approach: advancing conservation through public engagement and leveraging merchandise sales to fund its activities. This strategy underscores WWF's emphasis on blending activism with commercial outreach to foster deeper connections with a broader audience. WWF's integration of merchandise, activism, and education is consistent with the dual-mode conservation communication strategy (Martin 2005).

WCS exhibits a unique strategy by focusing predominantly on News and Media (12.78%) and integrating financial aspects into its outreach (4.58%). The heavy emphasis on media and commercial elements such as travel and retail indicates WCS's strategy to attract tourists and generate revenue through immersive wildlife experiences. This approach contrasts with the more traditional conservation messaging of IUCN and WWF. WCS's relatively low focus on conservation-specific content and higher engagement with tourism and retail suggest a model that combines conservation with consumerism. The inclusion of finance and travel-related content highlights WCS's strategic focus on engaging a financially contributing audience through direct interactions with wildlife. WCS's tourism-oriented engagement model is consistent with experiential conservation communication approaches documented by Weinreich et al. (2008).

The comparative analysis of these organizations reveals divergent strategies in digital engagement. IUCN's strength lies in leveraging social media for policy advocacy and broad outreach. WWF excels in combining scientific education with engaging media and fundraising efforts, while WCS integrates tourism and financial engagement into its conservation strategy. Each organization's approach reflects its broader mission and audience engagement goals, offering insights into how digital platforms are used to advance conservation objectives. The data distribution also highlights regional differences in audience engagement, with WWF showing a strong presence in Mexico and the United States. This geographic disparity underscores the varied impacts of these organizations' digital strategies across different regions. The substantial traffic share for WWF in Mexico suggests successful localized outreach, while WCS's lower traffic in these regions reflects a more niche audience. The analysis underscores the distinct website communication strategies of IUCN, WWF, and WCS. While IUCN emphasizes scientific and policy-driven engagement, WWF integrates educational content with consumer-driven outreach, and WCS focuses on experiential and financial engagement. These approaches reflect each organization's strategic priorities and offer valuable insights into how digital platforms can be utilized to achieve diverse conservation goals.

CONCLUSION

The study showcased various strategies for promoting and communicating conservation across different platforms. The researcher considered various key factors to find out how web metrics can differ significantly and still help organizations get the desired reach. The top three leading wildlife organizations (IUCN, WCS, & WWF) were taken into consideration to prove the same. SimilarWeb data for February–April 2024 reveals distinct digital engagement profiles for each organization. IUCN's traffic was characterized by higher per-visit engagement (longest visit duration, lowest bounce rate) and science-oriented search terms. WWF recorded the highest overall traffic volume, the greatest geographic reach, and the strongest emphasis on Science and Education in its industry distribution. WCS showed the lowest combined traffic share and a predominance of tourism and retail-related search activity.

Industry distribution of IUCN indicated that the website is very much inclined towards social network promotion and has most of the information disseminated through social network platforms. The organization also worked on government policies and research-based activities, making it prominently visible in the science and education industry. WWF also followed a similar approach and has good visibility in Science and Education. WCS had a simpler strategy and targeted a niche audience through Twitter. They projected a variety of ads and promotions of fun-filled conservation-related experiences. They had 4.58% of industry distribution under finance, making it inclined towards direct payment from the audience.

The findings pointed strongly towards the social media platforms. To get a much more informative insight into the communication strategies, Facebook, LinkedIn, Twitter, YouTube, and other platforms were analyzed. The majority of the organizations used LinkedIn and Twitter to keep the audience informed. WWF used Facebook as well for communication.

To understand the audience and their origins, the researcher analyzed audience engagement across the top five countries. WWF had the highest engagement throughout the five countries, ranging from 57.9% in India to 74.4% in Mexico. WCS has the least traffic share in four of the five top countries; the exception is the United States, where IUCN holds the smallest share. Another important question was to understand the number of people being involved in three months and their particulars to understand the audience's engagement in detail. A comparative website study was done to understand the audience's engagement in the top three wildlife organizations. WWF recorded the highest visit volume, whereas IUCN recorded the longest visit duration, the most pages per visit and the lowest bounce rate. Most of the audience were females, with more than 60% for each of the organizational websites.

Future Research Directions

Future research could benefit from incorporating additional data sources, such as user feedback, surveys, and qualitative assessments of website content. This would provide a more holistic view of how information is perceived and utilized by different audiences. Analyzing the quality and impact of the content disseminated by these organizations could offer deeper insights. As web technologies evolve, future research should consider the impact of new digital tools and platforms on information dissemination. Exploring the use of emerging technologies, such as artificial intelligence and interactive media, could offer insights into how these innovations shape audience engagement and conservation efforts.

Developing a robust ethical framework for web scraping and data collection in research could address current challenges. Establishing clear guidelines for handling sensitive data and ensuring compliance with legal and ethical standards would enhance the integrity of future studies. Future research should focus on assessing the real-world impact of online conservation efforts. Measuring how digital engagement translates into tangible conservation outcomes, such as increased funding, policy changes, or improved conservation practices, would provide valuable insights into the effectiveness of different communication strategies.

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Author contribution: Saswat Pati & V. Vijay Kumar: Data curation, investigation, methodology, original draft preparation, writing-review & editing. Saswat Pati: Formal analysis, software, visualization. V. Vijay Kumar: Project administration, resources, supervision, validation.

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COMMUNICATION

First photographic record of the Himalayan Red Panda *Ailurus fulgens* (Mammalia: Carnivora: Ailuridae) in Yordi Rabe Supse Wildlife Sanctuary, Arunachal Pradesh, India

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Abstract: The Himalayan Red Panda *Ailurus fulgens*, an endangered species of the eastern Himalaya, is threatened by habitat loss, fragmentation, and limited ecological data across parts of its range. In Arunachal Pradesh, despite extensive suitable habitat, confirmed records from several protected areas remain scarce. Here we present the first confirmed photographic evidence of the Red Panda *Ailurus fulgens* from the Yordi Rabe Supse Wildlife Sanctuary (YRSWS) using camera traps. The species was recorded at elevations of 2,409 m and 2,848 m in high altitude temperate forest habitats characterized by dense multi-layered vegetation with a moist understory, providing important field-based confirmation of the species' presence in a previously undocumented area. These records highlight the conservation significance of YRSWS and emphasize the need for further systematic monitoring and strengthened protection of its habitats in central Arunachal Pradesh.

Keywords: Biodiversity documentation, camera trapping, Eastern Himalaya, forest connectivity, mammalian conservation, protected area, range extension, species occurrence, temperate forest, wildlife monitoring.

सारांश: रेड पांडा *Ailurus fulgens*, जो पूर्वी हिमालय का एक संकटग्रस्त प्रजाति है, आवास क्षरण, खंडन तथा इसके वितरण क्षेत्र के कई हिस्सों में सीमित पारिस्थितिकीय आंकड़ों के कारण गंभीर खतरे में है। अरुणाचल प्रदेश में, व्यापक रूप से उपयुक्त आवास होने के बावजूद, कई संरक्षित क्षेत्रों से इसके पुष्ट अभिलेख अभी भी दुर्लभ हैं। यहाँ हम योरडी राबे सुपसे वन्यजीव अभयारण्य (YRS WLS) से कैमरा ट्रैप के माध्यम से रेड पांडा *Ailurus fulgens* का प्रथम पुष्ट फोटोग्राफिक साक्ष्य प्रस्तुत करते हैं। यह प्रजाति 2,409 मीटर तथा 2,848 मीटर की ऊँचाई पर निम्न समशीतोष्ण वनों में दर्ज की गई, जो घनी बहु-स्तरीय वनस्पति तथा आर्द्र अधस्तलीय परत से युक्त हैं। यह अध्ययन एक पूर्व में अप्रमाणित क्षेत्र में इस प्रजाति की उपस्थिति का महत्वपूर्ण क्षेत्र-आधारित प्रमाण प्रदान करता है। ये अभिलेख YRS WLS के संरक्षणात्मक महत्व को रेखांकित करते हैं तथा मध्य अरुणाचल प्रदेश में इसके आवासों की सुदृढ़ सुरक्षा और व्यवस्थित निगरानी की आवश्यकता पर बल देते हैं।

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Author contribution: YM conceived the study, conducted field surveys, deployed and monitored camera traps, analysed the data, and prepared the first draft of the manuscript. SDG contributed to study design, data interpretation, manuscript review, and overall supervision. SL contributed to study conceptualization, data interpretation, manuscript review, and provided critical inputs that improved the final version of the manuscript. All authors read and approved the final manuscript.

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INTRODUCTION

The Red Panda *Ailurus fulgens*, an elusive species found in the eastern Himalaya and southwestern China, is currently listed as 'Endangered' on the IUCN Red List due to a continued decline in its habitat quality, population size and geographic range (Glatston et al. 2015). It primarily inhabits temperate forests with dense bamboo at elevations ranging between 2,200–4,800 m. Red Pandas feed primarily on bamboo (Choudhury 2001). Traditionally, Red Pandas have been classified into two subspecies, *Ailurus fulgens fulgens* and *Ailurus fulgens styani*, which differ subtly in morphology (e.g., pelage colouration and skull characteristics) and geographic range. However, recent genomic evidence suggests that the two subspecies may actually be two distinct phylogenetic species: the Himalayan Red Panda *Ailurus fulgens* and the Chinese Red Panda *Ailurus styani* (Hu et al. 2020; Dalui et al. 2025). Further, genetic evidence suggests that the Siang River in Arunachal Pradesh separates both the species, with *Ailurus fulgens* occurring west of the river and *Ailurus styani* occurring to the east (Joshi et al. 2021). This highlights the importance of implementing conservation strategies that are both region-specific and transboundary in nature.

In India, the Himalayan Red Panda is primarily distributed across Sikkim, northern West Bengal (including Darjeeling and Kalimpong districts) and Arunachal Pradesh (Bhutia et al. 2023). Of these, Arunachal Pradesh is ecologically important, accounting for over 90% of the species' estimated 12,500 km² potential habitat in India (Ghose & Dutta 2010). Yet, the species' actual area of occupancy within the country is likely much lower due to fragmentation and patchy distribution (Choudhury 2001). Despite its rich biodiversity, Arunachal Pradesh remains under-explored, with confirmed records of red panda presence still limited.

Within Arunachal Pradesh, photographic evidence has confirmed the presence of red pandas in select locations including Karle Village, Shi-Yomi and Dirang in West Kameng District (Megha et al. 2021). More recently, sightings have also been confirmed in Namdapha National Park (Yomcha et al. 2025). However, many lesser-known sanctuaries in the central part of the state lack records, indicating the need for comprehensive field surveys.

Yordi Rabe Supse Wildlife Sanctuary (YRSWS) spans the West Siang and Siang districts. Notified in 2007, the Sanctuary remains unexplored due to its challenging terrain, limited access and lack of ecological assessments. While reports suggest the presence of several rare species, including the Tiger *Panthera tigris*, the scientific literature on the sanctuary is sparse (Ete 2014). Since YRSWS lies on the western side of the Siang River, it falls within the predicted range of the Himalayan Red Panda *Ailurus fulgens*—a species characterised by lighter fur and more prominent tail rings compared to *Ailurus styani* (Hu et al. 2020). However, despite this expected distribution, no prior direct or photographic evidence has confirmed its occurrence in this protected area. The present study, therefore, provides the first photographic documentation of the species from this under-explored and scientifically unreported landscape, highlighting the requirement for systematic surveys and targeted conservation efforts in Arunachal Pradesh.

Study Area

The Yordi Rabe Supse Wildlife Sanctuary, located in the West Siang and Siang districts of Arunachal Pradesh (Image 1), is one of the most remote and unexplored protected areas in the state. The name Yordi Rabe translates locally to "a distant and faraway hill," while Supse refers to one of the highest mountain peaks within the sanctuary. The landscape is dominated by steep slopes, rugged hills and gorges, with elevations ranging 771–3,929 m, as derived from the ALOS PALSAR RTC DEM (Japan Aerospace Exploration Agency 2015).

The sanctuary spans a total area of 397 km², located between 94.367°–94.583° E and 28.133°–28.417° N (Ete 2014). It has also been recognised as an Important Bird and Biodiversity Area (IBA) under criterion A1 (2004) with the IBA code: IN-AR-27, as designated by BirdLife International (2025).

Despite its ecological significance, YRSWS remains poorly documented, with no scientific research having been conducted to date. The region encompasses a diverse range of forest types – tropical and semi-evergreen forests, subtropical broad-leaved forests, and sub-temperate broad-leaved forests (MoEFCC 2016). This creates a rich mosaic of habitats—from temperate forests and bamboo thickets to patches of subalpine zones—providing a complex ecological environment.

Four major rivers, namely Yogong, Shichuk, Sikka, and Tagurshit flow through the Sanctuary. The Yogong and Shichuk rivers flow into the Hirik, a tributary of the Yomgo/Siyom River, while the Sikka and Tagurshit join the Subansiri River (Ete 2014).

The combination of dense canopy, moist undergrowth, and river systems contributes to the high habitat complexity and ecological integrity of the sanctuary.

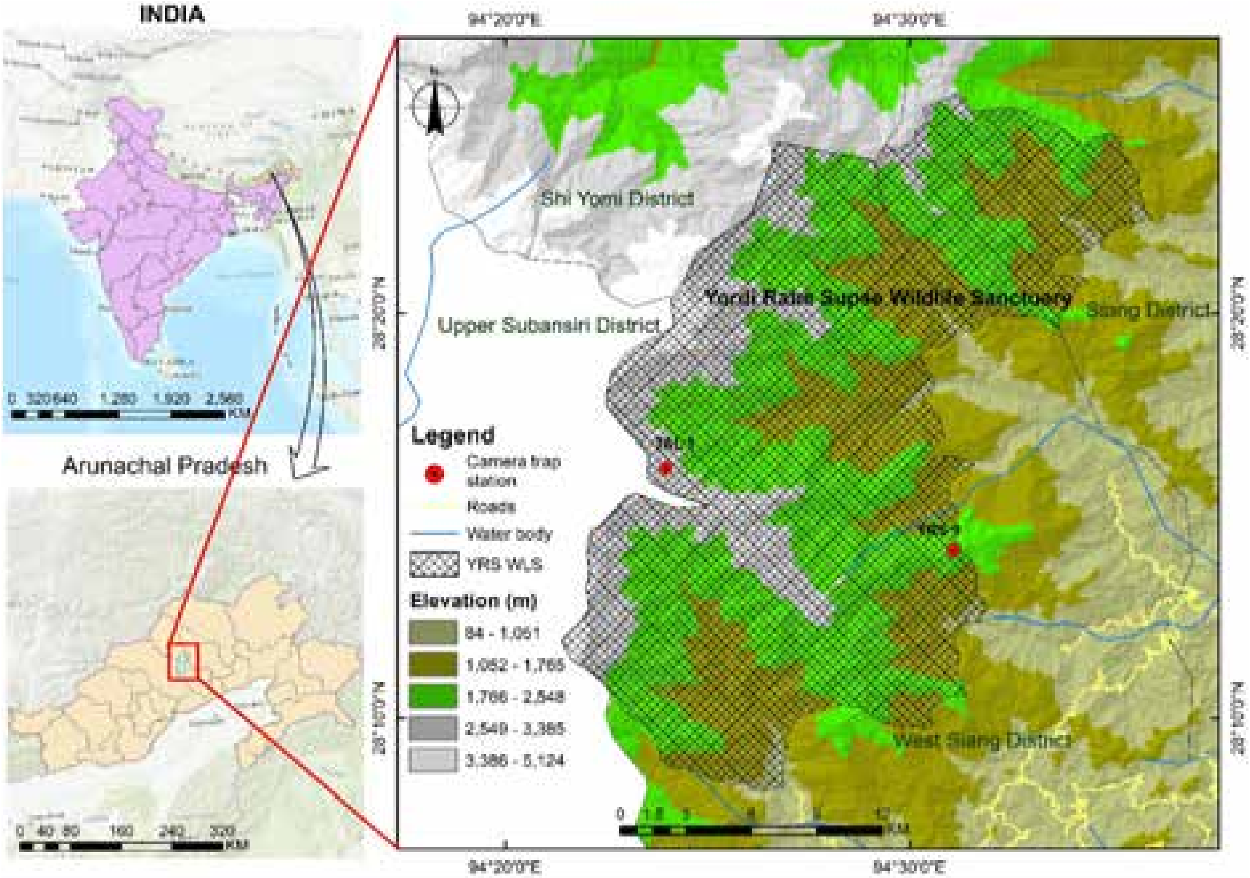

Image 1. Yordi Rabe Supse Wildlife Sanctuary and camera trap station.

METHODS

As part of a systematic ecological study on Mishmi Takin conducted in December 2024, we installed motion-triggered camera traps at various suitable locations within the sanctuary. These cameras were kept running continuously, day and night, until their batteries were fully drained.

We used two models of Browning Trail cameras. The first unit (TAL 1) was a 20-megapixel Dark OPS MAX HD PLUS, set in photo mode with a 1-second picture delay. It had a motion detection range of ~24 m (80 ft), with a fast trigger speed and a long-range IR flash. The second unit (YRS 9) was a 24-megapixel Dark OPS PRO X 1080, set in both photo and video mode. It was programmed to record 30-second videos with a 1-second capture delay, and both night exposure and motion detection were set to ~27 m (90 ft) long range.

Each camera was mounted around 1 m above the ground, which is close to the shoulder height of adult takins (~1.5 m). We placed the cameras approximately 4 m from animal trails, as recommended for large-bodied mammals, to capture full-body images without distortion or overexposure (O'Connell et al. 2011; Rovero et al. 2013). Cameras were installed either facing straight across the trail or at a 20–30 ° angle to help reduce motion blur and improve detection accuracy. No bait or attractants were used at any of the camera trap stations to avoid altering animal movement patterns. TAL 1 was installed on 12 December 2024 and remained active for 132 days, while YRS 9 operated from 08 September 2024 to 06 May 2025 (241 days).

We identified the species captured by comparing visible morphological features from the camera trap images with standard descriptions (Hu et al. 2020). We also considered elevation and the known distribution of species to further confirm the identification.

RESULTS

The Himalayan Red Panda was recorded from two separate camera trap stations, roughly 12 km apart in aerial distance. These detections confirm the presence

Image 2. Camera trap image of Ailurus fulgens recorded at 2,848 m elevation, Liromoba range on the western side of the Yordi Rabe Supse Wildlife Sanctuary.

of the species in two different forest ranges within the YRSWS.

The first record came from camera unit TAL 1,where a single Himalayan Red Panda was photographed on 14 December 2024 at 09.20 h (Image 2), just two days after installation. The animal was photographed at an elevation of 2,848 m in a dense, moss-covered temperate forest within the Liromoba range on the western side of the sanctuary. The undergrowth consisted of ferns, leaf litter and small bamboo patches, indicating an undisturbed habitat. Light snowfall was occasionally observed, including on 07 March 2025, which left a patchy snow cover. No signs of human activity were recorded throughout the deployment period. The species detection occurred in broad daylight hours under cold environment conditions.

The second detection came from camera unit YRS 9 on 31 October 2024, at 1050 h (Image 3), shortly after a rain event; there was still slow dripping water in the area. The animal was photographed at an elevation of 2,409 m on a mountain ridge slope with tree roots and nearby rock cliffs, within a temperate rainforest. This unit was programmed to capture both photos and videos. The animal was recorded in both formats once. This site also experienced minimal disturbance, with only two visits by the field guide during the whole period.

The individuals photographed at both sites exhibited characteristic light reddish-brown fur and distinct tail rings, unique to the species. These elevation ranges (2,409–2,848 m) match previous photographic evidence from Karle Village, Shi-Yomi District and other parts of central and western Arunachal Pradesh (Megha et al. 2021), suggesting a broader, but fragmented distribution of the species across these higher temperate forest zones.

DISCUSSION

Based on morphological characteristics visible in the camera trap images—the lighter, reddish-brown fur and distinct tail rings—the individuals closely resemble the Himalayan Red Panda. These traits are consistent with features that distinguish it from the Chinese Red Panda, which typically has darker fur and fainter tail rings (Hu et al. 2020). However, such identification based solely on external morphology may be prone to error and, therefore, requires confirmation through genetic analysis for accurate subspecies delineation.

Since the sanctuary is located west of the Siang River, it falls within the expected range of the Himalayan Red Panda. Genetic studies indicate that the Siang River serves as a barrier between the two distinct species (Joshi et al. 2021). Photographic records provide

Image 3. Camera trap image of *Ailurus fulgens* recorded at 2,409 m elevation, Darak Range on the eastern side of the Yordi Rabe Supse Wildlife Sanctuary.

evidence from a remote, under-surveyed protected area in Arunachal Pradesh, offering vital support for species distribution models that often overestimate actual occupancy. Recent studies, such as those by Megha et al. (2021), have helped fill the gap by confirming the presence of red pandas in previously unconfirmed areas and highlighting the importance of intact temperate forests with bamboo cover and minimal human impact. Within this wider conservation framework, our records from YRSWS fill an important spatial gap in the species' known distribution. The occurrence of red panda in this landscape suggests habitat continuity and indicates that YRSWS may act as a corridor linking populations in western Arunachal Pradesh (Tawang, West Kameng, Shi-Yomi) with those in central regions such as Mouling National Park.

However, infrastructure projects, including roads and hydropower, pose growing threats by fragmenting forest habitats and creating barriers to movement (Srivastava & Dutta 2010). For example, the third package of the Taliha–Tato stretch of NH-913 (Frontier Highway), covering km 87.13 to km 121.541, has been approved by the MoRTH (2024), highlighting the increasing developmental pressure in northern part of YRSWS. Such linear infrastructures may disrupt forest connectivity between the sanctuary and adjoining high-elevation forests extending towards southern Tibet, potentially affecting wildlife movement and habitat continuity. Maintaining forest connectivity is crucial for reducing the risks of inbreeding and local extinction, a trend already observed in parts of the Khangchendzonga landscape (Ghose & Dutta 2010). Other recent field efforts have reported red pandas in non-protected and community-managed landscapes, such as Karle Village and Shi-Yomi (Megha et al. 2021), highlighting the complementary role of local leadership. The conservation project led by Rimung Tasso in Mouling National Park and Monigong demonstrates how grassroots level action can protect Red Panda habitats. The Padmaja Naidu Himalayan Zoo's breeding and biobanking program, and its contribution to successful Red Panda releases in Singalila National Park highlight the importance of integrating ex situ conservation with habitat-based recovery (MoEFCC 2024). Together, these efforts illustrate the value of strengthening local conservation models and expanding systematic surveys in lesser-known sanctuaries, such as Yordi Rabe Supse. The two detections in this study, despite being only 12 km apart, provide baseline evidence of Himalayan Red Panda presence across distinct forest ranges and emphasise the need for ongoing monitoring in Arunachal's fragile mountain ecosystems.

CONCLUSION

The first photographic record of the Himalayan Red Panda in Yordi Rabe Supse Wildlife Sanctuary adds significant new information on the species' distribution in Arunachal Pradesh. This record, from one of the region's most remote and under-surveyed protected areas, highlights the vital role of intact high-altitude temperate forests as refuges for this endangered species. The finding supports existing evidence that much of Arunachal's mountain habitats remain unexplored yet ecologically important. Strengthening systematic surveys and expanding community-led conservation models, which have already shown promise in other parts of the state, will be critical to securing the long-term survival of the red panda in India's eastern Himalaya. Future research should prioritise landscape-level monitoring, participatory habitat management, and the integration of camera trapping with genetic validation to better understand population connectivity across central Arunachal Pradesh.

Ethical Standards

All fieldwork was carried out with prior approval from the Department of Environment and Forests, Government of Arunachal Pradesh (Permit No. CWL/Gen/996/2023/Pt-II/191-93). The study employed non-invasive camera trap methods and did not involve any direct handling of wildlife. The authors affirm that all relevant institutional and national ethical guidelines for wildlife research were followed.

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OPEN ACCESS

COMMUNICATION

Lotus *Nelumbo* cultivations of Beehama Ganderbal offer novel habitats for diversity and seasonal variation of wetland birds

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Abstract: Conversion of marshy paddy fields into *Nelumbo* (lotus) gardens by the farmers of Beehama Ganderbal, Jammu & Kashmir has not only increased their livelihood and economy but also started yielding ecological dividends although unintentionally, because these *Nelumbo* gardens, along with adjoining *Salix* cultivations are now serving as alternative and rich habitats for the thriving of wetland birds. The present study was designed to assess the wetland bird assemblages in these artificial *Nelumbo* gardens to understand the species composition and richness along with their seasonal variation from March 2023 to February 2025. The study revealed that these artificial *Nelumbo* gardens can act as potential alternative feeding and breeding grounds for the diversity of wetland birds when main wetlands are shrinking due to anthropogenic activities.

Keywords: Alternate breeding grounds, anthropogenic activities, artificial garden, bird assemblages, lotus cultivation, marshy paddy field, species composition, wetland bird diversity.

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Author contribution: STS is the main author of the paper who collected the entire data and clicked all the photographs. FAA did the statistical analysis of the paper including applying Shannon Weiner index and Simpson index and SAW accompanied the author for carrying out census of birds in different seasons.

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INTRODUCTION

Wetlands are transitional lands between terrestrial and aquatic ecosystems where the water table is usually at or near the surface or the land is covered by shallow water (Mitsch & Gosselink 1986). Wetlands provide a home for a diversity of wildlife such as birds, mammals, fish, frogs, insects, and plants (Buckton 2007). Thus, wetlands help maintain biodiversity of flora and fauna. Wetlands are complex and productive ecosystems that occupy about six per cent of the Earth's land surface (Maltby & Turner 1983; Maltby 1986; Unni 2002; Thomas et al. 2026). Hence, wetlands are known as "biological supermarkets" because of the extensive food chains and rich biodiversity that they support, providing unique habitats for a wide range of flora and fauna (Mitsch & Gosselink 2000). They are also important habitats for water birds, which use them for feeding, roosting, nesting and rearing young. In addition to being the most productive ecosystems, wetlands play a vital role in flood control, aquifer recharge, nutrient absorption and erosion control.

Lotus or golden lotus is a perennial plant that grows in lakes in many parts of the world, including Kashmir. In Kashmir, it naturally grows in Dal, Wular, Manasbal, and Anchar lakes. As the rhizome of the plant is used as a delicacy and food by the people of the valley, it is an economically important plant. So, people have also started cultivating it for their livelihood, and the land near lakes and wetlands is being brought under *Nelumbo* cultivation. In summer, these cultivations provide cover, food, and nesting habitat for birds, while in winter they provide food and open water areas. Hence, these cultivations attract birds in the summer as well as the winter. These man-made *Nelumbo* gardens not only provide alternatives for the cultivation of *Nelumbo*, reducing the anthropogenic pressure on the wetlands for harvesting *Nelumbo*, but also act as alternative safe feeding and breeding grounds for migratory and resident aquatic birds. As no study has yet been conducted on the avian diversity of these habitats, in this present study, an attempt has been made to document the species richness and diversity of the avifauna of these habitats.

Study area

The present study was carried out in the lotus (*Nelumbo*) cultivations of Beehama Ganderbal (34.207° N, 74.78° E). The study site is located about 18 km north of the capital city Srinagar on the west side of the Srinagar-Leh Highway at an altitude of approximately 1,585–1,619 m. The site used to be marshy paddy fields connected to the Pandach Wetland Reserve, which were converted into *Nelumbo* cultivations by the locals for better economic returns by selling *Nelumbo* rhizomes on the National Highway. These cultivations are not only yielding economic benefits to local farmers but also starting to yield ecological dividends by acting as alternative feeding and breeding habitats for resident and migratory wetland birds.

METHODS

The study was carried out on these newly emerging habitats for two years, from May 2023 to April 2025. The study site is a continuous *Nelumbo* cultivation of about 6.89 lac sq ft in area with some patches filled for residential houses and some patches of deep open waters. The study area was visited weekly, and the birds were counted in all four seasons, viz., Spring (March–May), Summer (June–August), Autumn (September–November), and Winter (December–February). Therefore, the study site was visited 12 times in each season. Counting of birds was done using the Nikon binocular (22 x 50) and employing the point count, flock count, and group count method (Choudhary & Soni 2023), considering the landscape of the study site. We selected six vantage points in different cardinal directions of the study site, spending 5–10 minutes at each point, as this duration maximizes detection efficiency while minimizing bias (Bonthoux & Balent 2012) for bird counts. Species were identified using eBird/Clements Checklist (Clements et al. 2025), and data were collected species-wise. Surveys were abandoned on days with rain, snowfall and strong wind (Verner 1985; Bibby et al. 2000). Surveys were also halted during paddy agricultural disturbance periods, like one week in April during nursery preparation, one week in June during planting of saplings and one week in October during harvest. Double-counting was avoided by recording the birds that flew into and out of the plots during the census. Birds with fewer than 10 records were categorized as rare, those with 10–50 records as common, and those with more than 50 records as abundant (Bibby et al. 2000). Total abundance (number of birds observed from different counting points) and mean total abundance (total number of birds counted divided by total number of counting points) were also recorded. A checklist of species was also prepared. Birds were photographed using a Nikon D5600 camera with a 200–500 mm Nikkor lens. The data collected was compiled in Microsoft Excel. Shannon-Weiner index (H) and Simpson diversity index (D) were used to evaluate

Image 1. Study site.

Image 2. Study site during summer season. © Sheikh Tanveer Salam.

Image 3. Study site during winter season. © Sheikh Tanveer Salam.

species diversity during different seasons of the study.

Shannon-Weiner index

$$H = - \Sigma\, p_i \, ln(p_i)$$

where: Σ: A Greek symbol that means "sum", *ln*: Natural log, p_i: The proportion of the entire community made up of species *i*

Simpson index

$$D = 1 - \frac{\Sigma n\,(n-1)}{N\,(N-1)}$$

Where n = number of individuals of each species, N = total number of individuals of all species

RESULTS AND DISCUSSION

A total of 71 species of birds belonging to 12 orders and 29 families were recorded in the present study (Table 1). Passerines, represented by 35 species, contribute about 49.29% of the bird diversity of the study area. Of the total diversity, 43.66% (31 species) were summer migrants (SM), followed by 42.25% (30 species) of residents (R), 11.26% (8 species) of winter migrants (WM) and only 2.81% (2 species) of local altitudinal migrants (LAM). No passage migrant was observed. Among these, some like Mallard *Anas platyrhynchos*, Eurasian Teal *Anas crecca*, Northern Shoveller *Anas clypeata*, Common Moorhen *Gallinula chloropus*, Grey-headed Swamphen *Porphyrio poliocephalus* and Little Grebe *Tachybaptus ruficollis* are typical marsh-dwelling species, while some upland birds like Indian Pond Heron *Ardeola grayii*, Black-Crowned Night Heron *Nycticorax nycticorax*, Little Bittern *Ixobrychus minutus*, Eurasian Collared Dove *Streptopelia decaocto*, European Roller *Coracias garrulus*, etc., spend a great deal of their time in the lake for feeding but nest on adjoining trees and shrubs. A total of 592 species of resident and non-resident birds have been reported from Jammu & Kashmir (Kichloo et al. 2024). About 92 species have been recorded from the wetlands of Kashmir (Holmes & Parr 1988; Ahangar 2008). The present study clearly revealed that a good number of species are visiting these newly created habitats, thereby providing a ray of hope that these *Nelumbo* gardens can act as alternative feeding and breeding sites for different types of birds, besides providing economic benefits to the locals. The areas with floating vegetation like *Trapa*

Table 1. Checklist of birds identified in the study.

Order	Family	Common name	Scientific name	Status*	Abundance
Podicipediformes	Podicipedidae	Little Grebe	*Tachybaptus ruficollis* Pallas, 1764	R	Common
Ciconiiformes	Ardeidae	Little Egret	*Egretta garzetta* Linnaeus, 1766	R	Common
		Indian Pond Heron	*Ardeola grayii* Sykes, 1832	R	Abundant
		Black-crowned Night Heron	*Nycticorax nycticorax* Linnaeus, 1758	R	Rare
		Little Bittern	*Ixobrychus minutus* Linnaeus, 1766	SM	Rare
Anseriformes	Anatidae	Mallard	*Anas platyrhynchos* Linnaeus, 1758	WM	Abundant
		Northern Shoveler	*Anas clypeata* Linnaeus, 1758	WM	Abundant
		Eurasian Teal	*Anas crecca* Linnaeus, 1758	WM	Abundant
Falconiformes	Accipitridae	Black Kite	*Milvus migrans* Boddaert, 1783	R	Abundant
		Common Buzzard	*Buteo buteo* Linnaeus, 1758	WM	Rare
		Long-legged Buzzard	*Buteo rufinus* Cretzschmar, 1827	WM	Rare
		Eurasian Sparrowhawk	*Accipiter nisus* Linnaeus, 1758	WM	Rare
		Western Marsh Harrier	*Circus aeruginosus (Linnaeus, 1758)*	WM	Rare
Gruiformes	Rallidae	Ruddy-breasted Crake	*Porzana fusca* Linnaeus, 1766	SM	Rare
		Grey-headed Swamphen	*Porphyrio poliocephalus* Linnaeus, 1758	R	Abundant
		Common Moorhen	*Gallinula chloropus* Linnaeus, 1758	R	Abundant
		Common Coot	*Fulica atra* Linnaeus, 1758	WM	Abundant
Charadriiformes	Scolopacidae	Common Sandpiper	*Actitis hypoleucos* Linnaeus, 1758	R	Common
		Green Sandpiper	*Tringa ochropus* Linnaeus, 1758	R	Common
	Laridae	Whiskered Tern	*Chlidonias hybrida* Pallas, 1811	SM	Rare
	Charadriidae	Red wattled Lapwing	*Vanellus indicus (Boddaert, 1783)*	SM	Rare
		Northern Lapwing	*Vanellus vanellus (Linnaeus, 1758)*	SM	Rare
Columbiformes	Columbidae	Blue Rock Pigeon	*Columba livia* Gmelin, 1789	R	Abundant
		Eurasian Collared-Dove	*Streptopelia decaocto* Frivaldszky, 1838	SM	Common
		Oriental Turtle-Dove	*Streptopelia orientalis* (Latham, 1790)	SM	Rare
Psittaciformes	Psittacidae	Rose-ringed Parakeet	*Psittacula krameri* Scopoli, 1769	R	Rare
Cuculiformes	Cuculidae	Common Cuckoo	*Cuculus canorus* Linnaeus, 1758	SM	Rare
		Pied Crested Cuckoo	*Clamator jacobinus* Boddaert, 1783	SM	Rare
Coraciiformes	Alcedinidae	Common Kingfisher	*Alcedo atthis* Linnaeus, 1758	R	Abundant
		White-breasted Kingfisher	*Halcyon smyrnensis* Linnaeus, 1758	R	Abundant
		Lesser Pied Kingfisher	*Ceryle rudis* Linnaeus, 1758	R	Rare
	Coraciidae	European Roller	*Coracias garrulus* Linnaeus, 1758	SM	Rare
	Upupidae	Common Hoopoe	*Upupa epops* Linnaeus, 1758	SM	Common
Piciformes	Picidae	Himalayan Pied Woodpecker	*Dendrocopos himalayensis* Jardine & Selby, 1831	R	Common
		Brown-fronted Pied Woodpecker	*Dendrocopos auriceps* Vigors, 1831	R	Rare
		Large Scaly-bellied Green Woodpecker	*Picus squamatus* Vigors, 1831	R	Rare
Passeriformes	Passeridae	House Sparrow	*Passer domesticus* Linnaeus, 1758	R	Abundant
	Hirundinidae	Common Swallow	*Hirundo rustica* Linnaeus, 1758	SM	Common
	Motacillidae	White Wagtail	*Motacilla alba* Linnaeus, 1758	R	Common
		Citrine Wagtail	*Motacilla citreola* Pallas, 1776	R	Common
		Grey Wagtail	*Motacilla cinerea* Tunstall, 1771	R	Common
		Rosy Pipit	*Anthus roseatus* Blyth, 1847	SM	Rare
		Water Pipit	*Anthus spinoletta* Linnaeus, 1758	SM	Rare
		Oriental Tree Pipit	*Anthus hodgsoni* Richmond, 1907	SM	Rare
	Campephagidae	Long-tailed Minivet	*Pericrocotus ethologus* Bangs & Phillips, 1914	SM	Rare

Passeriformes	Pycnonotidae	Himalayan Bulbul	*Pycnonotus leucogenys* Gray, 1835	R	Abundant
		Black Bulbul	*Hypsipetes leucocephalus* P.L.S. Muller, 1776	LAM	Common
	Laniidae	Rufous-backed Shrike	*Lanius schach* Linnaeus, 1758	SM	Common
	Troglodytidae	Winter Wren	*Troglodytes troglodytes* Linnaeus, 1758	LAM	Rare
	Muscicapidae	Blue Whistling-Thrush	*Myiophonus caeruleus* Scopoli, 1786	R	Abundant
		Tickell's Thrush	*Turdus unicolor* Tickell, 1833	SM	Rare
		Spotted Forktail	*Enicurus maculatus* Vigors, 1831	SM	Rare
		Streaked Laughingthrush	*Garrulax lineatus* Vigors, 1831	SM	Common
		Indian Great Reed-Warbler	*Acrocephalus stentoreus* Hemprich & Ehrenberg, 1833	SM	Common
		Indian Paradise Flycatcher	*Terpsiphone paradisi* Linnaeus, 1758	SM	Rare
		Siberian Stonechat	*Saxicola maurus* Linnaeus, 1766	SM	Common
		Common Chiffchaff	*Phylloscopus collybita* Vieillot, 1817	SM	Common
		Lemon-rumped Warbler	*Phylloscopus chloronotus* G.R. Gray & J.E. Gray, 1846	SM	Rare
		Hume's Warbler	Phylloscopus humei (Brooks, 1878)	SM	Rare
	Paridae	Coal Tit	*Parus ater* Linnaeus, 1758	SM	Rare
		Great Tit	*Parus major* Linnaeus, 1758	R	Common
	Certhiidae	Bar-tailed Tree-Creeper	*Certhia himalayana* Vigors, 1832	R	Rare
	Sturnidae	Common Starling	*Sturnus vulgaris* Linnaeus, 1758	SM	Common
		Common Myna	*Acridotheres tristis* Linnaeus, 1766	R	Abundant
	Oriolidae	Indian Golden Oriole	*Oriolus oriolus* Linnaeus, 1758	SM	Rare
	Dicruridae	Black Drongo	*Dicrurus macrocercus* Vieillot, 1817	SM	Common
		Ashy Drongo	*Dicrurus leucophaeus* Vieillot, 1817	SM	Common
	Corvidae	Yellow-billed Blue Magpie	*Urocissa flavirostris* (Blyth, 1846)	R	Abundant
		Eurasian Jackdaw	*Corvus monedula* Linnaeus, 1758	R	Abundant
		House Crow	*Corvus splendens* Vieillot, 1817	R	Abundant
		Jungle Crow	*Corvus macrorhynchos* Wagler, 1827	R	Common

*—Each species was classified according to its residential status, R—Residents | SM—Summer migrants | WM—Winter migrants | LAM—Local altitudinal migrants, based on field evidence and corroborated with authentic online datasets (Bates & Lowther 1952; Swati & Swati 2026).

natans, *Nymphoides* sp., and *Potomogeton* were used for nesting by birds like Little Grebe and Whiskered Tern *Chlidonias hybrida*. The emergent macrophytic vegetation, *Phragmites communis*, *Typha angustata*, *Scirpus palustis*, *Sparganium ramosum*, and *Butomus umbellatus* were used for nesting by birds like Mallard, Little Bittern, Indian Great Reed-Warbler *Acrocephalus stentoreus*, Common Moorhen and Wagtails, while the peripheral trees served as the nesting sites for a number of upland species like Herons, Doves, Rollers, Indian Golden Oriole *Oriolus oriolus*, Rufous-Backed Shrike *Lanius schach*, and Indian Paradise Flycatcher *Terpsiphone paradisi*. These findings are consistent with earlier studies from the region (Bates & Lowther 1952; Ali 2002; Ahanger 2008; Fazili et al. 2017).

The diversity of birds assembling at the *Nelumbo* gardens varied among different seasons. Diversity was found to be relatively higher during early Spring (H = 3.961; D = 0.9586) and late Winter (H = 3.811; D = 0.9572), followed by Summer (H = 3.376; D = 0.8706), and the least diversity was reported in Autumn (H = 3.267; D = 0.8281). The highest diversity during spring can be attributed to the assemblage of early summer migrants and already staying late winter migrants, besides the resident species, increasing the diversity of avifauna during Spring. The reason for the lowest diversity during autumn can be due to the presence of only residents in these sites during this period (Table 2) because during Autumn, summer migrants migrate to their feeding grounds, and most of the resident birds also move

Table 2. Avian diversity in different seasons of the study.

Diversity index	Spring (Mar–May)	Summer (Jun–Aug)	Autumn (Sep–Nov)	Winter (Dec–Feb)
Shannon-Weiner index (H)	3.961	3.376	3.267	3.811
Simpson index (D)	0.9586	0.8706	0.8281	0.9572

Image 4. Northern Shoveler pair.

Image 5. Grey-headed Swamphen.

Image 6. Mallard pair.

Image 7. Common Moorhen.

Image 8. Ashy Drongo.

Image 9. Ruddy-breasted Crake.

Image 10. Little Bittern.

Image 11. Little Egret.

Image 12. Common Teal pair.

Image 13. Reed Warbler.

Image 14. Indian Pond Heron.

Image 15. White-throated Kingfisher.

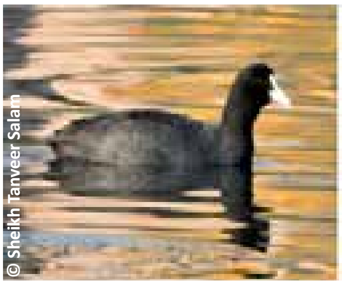

Image 16. Common Coot.

Image 17. Common Kingfisher.

Image 18. Whiskered Tern.

Image 19. Common Cuckoo.

towards residential areas and nearby paddy fields (Byju et al. 2023). Besides this, there is a huge disturbance to birds during late autumn and early winter due to intensive harvesting of *Nelumbo* stems locally called *Nadru* for selling them on small stalls at the adjoining Srinagar-Leh National Highway. The observations in the present study are in line with earlier observations from the wetlands of the adjoining states (Choudhary & Soni 2023).

With respect to abundance, 18 species (25.35%) were abundant, 22 species (30.98%) were common, and 31 species were rare (43.66%). The maximum total abundance (1908 individuals) was recorded in the winter season, with a mean abundance of 477 individuals. The minimum total abundance (184 individuals) was recorded in the autumn season with a mean abundance of 46 individuals.

These newly emerging habitats are facing serious anthropogenic threats due to the filling of these wetlands for making residential plots. Besides this, demarcations of the different plots by the owners using nets threaten and repel the birds. Harvesting of grass along footpaths within these *Nelumbo* gardens by the local women for cattle fodder may significantly disturb avifaunal communities inhabiting these wetlands and can lead to degradation of nesting habitats.

CONCLUSION

Conversion of less productive full-time water-logged paddy fields into productive *Nelumbo* (lotus) gardens, as revealed from the survey of the households involved in this venture, represents a sustainable land-use alternative with significant economic and ecological advantages. Economically, this practice offers higher and more stable returns compared to conventional paddy cultivation under water-logged conditions, thereby improving livelihood opportunities for local farmers by selling the rhizomes of the lotus on the adjacent Srinagar-Leh National Highway. Ecologically, these sites are emerging as structurally diverse wetland habitats that support rich biodiversity by attracting a wide range of resident and migratory bird species for feeding, breeding and shelter. A good avifaunal diversity observed at the study site underscores the role of *Nelumbo* gardens in enhancing ecosystem services, and this land use-transformation aligns agricultural productivity with wetland conservation goals, suggesting that *Nelumbo-based* systems can serve as a viable mode for sustainable management of water-logged agricultural landscapes. These systems can be managed to optimize both livelihood benefits and biodiversity outcomes through an integrated, eco-sensitive approach by engaging local communities, particularly women, for promoting sustainable harvesting by providing incentives for conservation-friendly practices. Identify and mapping of key nesting and roosting sites to establish temporary exclusion zones during breeding periods. Maintain optimal and stable water levels to support both *Nelumbo* growth and avifaunal requirements, and retain patches of open water interspersed with *Nelumbo* to support diverse bird guilds like waders, dabblers and diving birds. Encourage a mosaic of native macrophytes alongside *Nelumbo* to increase structural complexity and food availability for sustaining higher bird diversity. There is an immediate need to integrate these systems into wetland conservation policies and rural development schemes to ensure institutional backing for sustainable management.

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COMMUNICATION

Mating behavior of the Oaxacan Oak Anole *Anolis quercorum* (Squamata: Sauria: Anolidae) on a shade coffee plantation in Sierra Madre del Sur of Oaxaca, Mexico

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Abstract: Courtship and mating events in Mexican *Anolis* species have been reported mainly in captive conditions. Herein, we report a field observation of the mating behaviour of *A. quercorum* on a coffee plant, in the Sierra Madre del Sur, Oaxaca. Neck biting by the male, positioning on the female, head waving, copulation/intromission, as well as dislodging and retreat behaviours were observed in the wild. The mating duration was 25 minutes, which is within the reported period in the related *A. punctatus*. Future studies should improve and build on courting and mating observations to clarify if the observation duration reported here is part of the typical behavioural repertoire of *A. quercorum*.

Keywords: Anoles, behavioral signals, courtship, hemipenis, mating observation, reproductive.

Resumen: El cortejo y apareamiento en las especies de *Anolis* no ha sido reportada, excepto en experimentos de laboratorio. Aquí, reportamos una observación de campo del comportamiento de apareamiento de *A. quercorum* sobre una planta de café, en la Sierra Madre del Sur, Oaxaca, México. La duración del apareamiento fue de 25 minutos, lo cual se encuentra dentro del intervalo de tiempo reportado en otras especies como *A. punctatus*. Es importante reconocer que los futuros estudios incluyan más observaciones de cortejo y apareamiento para esclarecer si la observación reportada aquí es parte del repertorio típico de *A. quercorum*.

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Author contribution: JGG and GAAL contributes to data collection. Study conception, preparation, data collection and review were performed by JGG, ABS, GAAL, DANR and IPI. The first draft of the manuscript was written by JGG and all authors contributed to refining and revising the manuscript.

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SECRETARÍA DE CIENCIA, HUMANIDADES, TECNOLOGÍA E INNOVACIÓN

INTRODUCTION

Under natural conditions, courtship and mating of anole lizards are rare to observe and document because most species usually mate perched on trees (Ribeiro et al. 2011; Oliveira & Moraes 2021). Particularly, this genus displays a variety of actions performed by males and females prior to copulation (Carpenter 1978). For example, diverse array of visual displays such as head bobbing, pushups, tail lifting, throat dewlap extension, and changing colour are performed by adult male anoles during nuptial season (Losos 2009; Driessens et al. 2014; Steffen & Guyer 2014; Beltrán et al. 2016), though these visual displays are also exhibited during territorial interactions (Losos 2009; Reedy et al. 2017; Horr 2019).

To date, studies on the ecology and life history of *Anolis* species have mainly focused on Caribbean islands (e.g., Losos 1996; Gianissi et al. 1997; Howard et al. 1999; Creer et al. 2001), and the North American mainland (e.g., Guyer 1988 a,b; Vitt et al. 1995). In Mexico, ecological studies of anoles are scarce compared to their diversity (e.g., Ramírez-Bautista et al. 2002), and most studies have been focused on taxonomy and systematics (e.g., Guyer & Savage 1986; Campbell et al. 1989; Nieto-Montes de Oca 1994, 1996; Köhler et al. 2014, 2019; Gray et al. 2016). Among Mexican anoles, behavioural data about mating duration has not been reported; however, information on these events is also of special interest because mating duration can differ among breeding events within a single anole species (Losos 2009) or in response to the presence of a predator or an observer (Beltrán et al. 2016). Most of the knowledge associated with reproductive events of anoles comes from laboratory experiments or captive animals (e.g., Stamps 1975; Lima & Souza 2006; Pandav et al. 2007, 2010; Driessens et al. 2014).

Some information has been documented for *Anolis quercorum*. For example, Ramírez-Bautista et al. (2002) conducted observations in laboratory conditions on egg development, size and volume, and relative clutch mass, and reproductive condition. However, the complete description of the duration of courtship and mating events in this species in the wild remains unknown. Herein, we report a field observation of mating behavior of *A. quercorum* on a coffee plant, in the Sierra Madre del Sur, Oaxaca, Mexico.

MATERIALS AND METHODS

We observed the reproductive event of the Oaxacan Oak Anole *Anolis quercorum* while assessing the biodiversity in a shade-grown coffee in Las Nieves farm, located in the municipality of San Juan Lachao (16.193° N, 97.058° W; elevation 1,450 m), in the state of Oaxaca, Mexico. The observation occurred in Finca Cafetalera Las Nieves of the Sierra Sur de Oaxaca; this is a ranch with rustic shade plantations that grow under the canopy of humid mountain forests. The climate in this region is subhumid temperate with annual air temperatures ranging from 12–20 °C and annual precipitation ranging from 1,000–3,500 mm (De Santis et al. 2018).

To avoid interfering with the displayed behavioural signals, we remained silent and at least 2 m from the individual during the entire reproductive event. Digital images and video were obtained using an iPhone (XR Model, 12 MP resolution) and a digital camera (Canon Eos Rebel) with a telephoto lens (Canon EF 70–300 mm). We recorded the start and end time of the event, as well as notes on the deployment of behaviours.

OBSERVATIONS

On 15 June 2024, at 1248 h we observed a female *A. quercorum* perched horizontally on top of the leaves of a coffee plant (*Coffea arabica*, Image 1A) at approximately 95 cm from the ground. She was constantly moving her head laterally to the left, and then returning it to its initial position. Five minutes later (1252 h), from a nearby tree, a male jumped over the female's dorsum and immediately positioned himself on her body and started biting the back of the neck (Image 1B). Because the male was outside our initial range of vision, we were unable to obtain information on the entirety of the displays exhibited by the male prior to mating or on the behaviours that triggered the observed mating position.

During the next 10 minutes, the male remained in the same position, taking quick bites over the back and side of the neck (Image 1C). Both right extremities (front and back) of the male were propped on the trunk, giving him support. At 1303 h, the male began to shift his hip to the left side of the female with the intention of joining the cloacas, while she began to raise the base of the tail (Image 1D).

Assuming his position, the male was most likely inserting its right hemipenes into the female (13.04 h). Both individuals remained almost immobile during the entire mating event (1304–1312 h), suggesting insertion

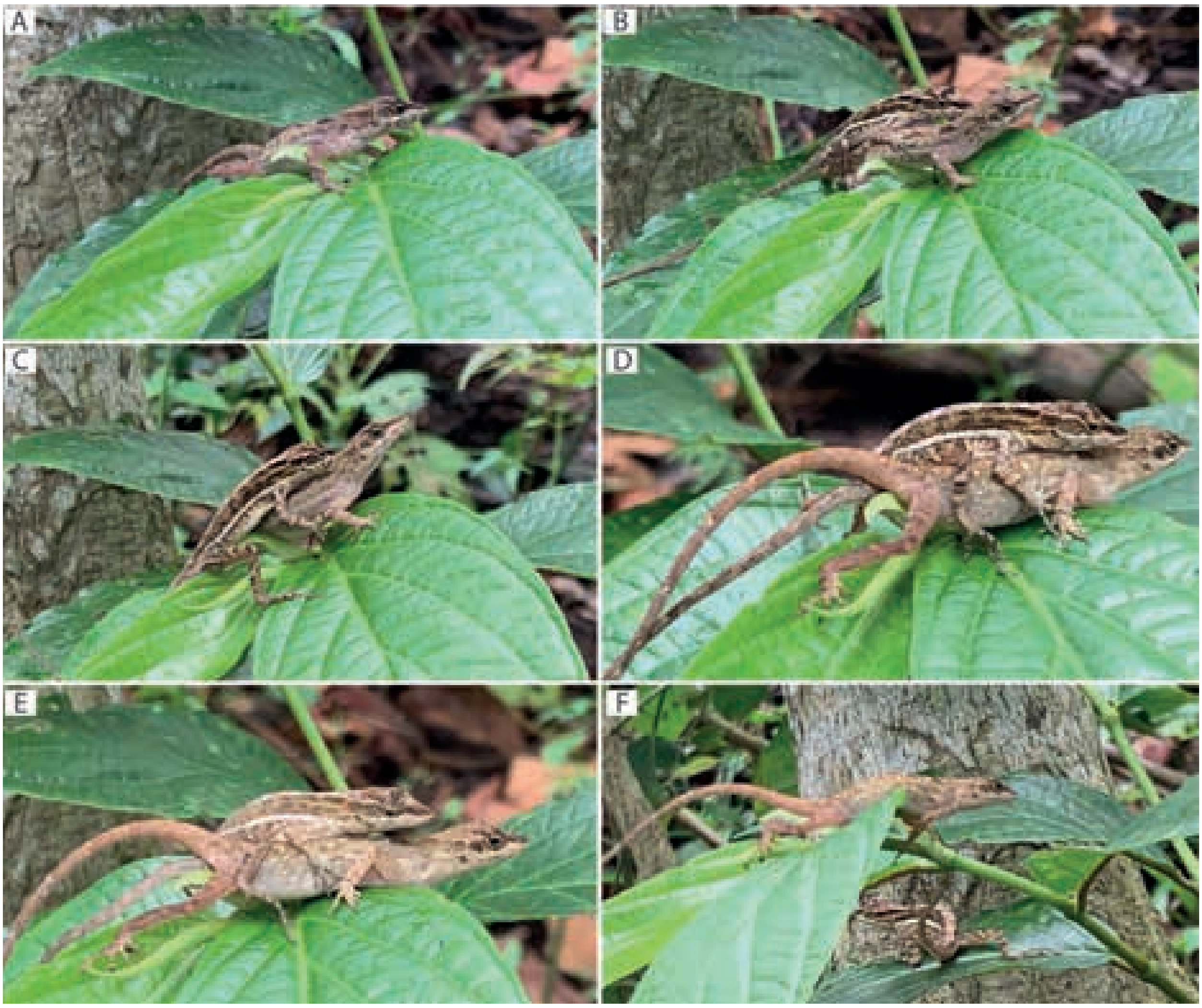

Image 1. Mating behavior of Oaxacan Oak Anole *Anolis quercorum* in a shade coffee plantation of Sierra Sur de Oaxaca, Mexico: A—Female perched horizontally on top of the leaves of a coffee plant | B—Male over the female back and biting the neck | C—Male taking short-lasting bites over the back of the neck | D—Male bringing the cloacas together for copulatory phase | E—Copulatory phase | F—End of the mating. © Jesús García Grajales.

of the hemipenis and insemination (copulatory phase), except for subtle movement of the female's head. During that time, the male stopped biting the female (Image 1E). After about eight minutes, the female became agitated and displayed side movements of the body and head to the left, apparently trying to disengage herself from the male. A few seconds later, both individuals moved separately for a short distance, then the male jumped to the nearest leaves, and the female stayed in the same location (Image 1F), thus ending the mating event at 1313 h.

After disengaging, the male returned to the same tree from which he jumped at the beginning of the event. Both individuals remained immobile (1313–1337 h) until the end of the observation period. During the mating event, both the anoles remained in the same constant body colour without any change.

DISCUSSION

Our present report is the first mating event observation of these anoles horizontally on coffee plant leaves in Mexico, as there are no such earlier reports. According to literature, the behaviour and positioning as well as the chosen substrate, of the mating individuals varied among anole species (Losos 2009; Oliveira & Moraes 2021). Silva-Neto et al. (2019) reported courtship behaviour of the Spotted Amazonian Green Anole *Dactyloa philopunctata* taking place on the vertical trunk of an introduced tree species (*Syzygium cumini*) in the central Brazilian Amazonian. Oliveira & Moraes (2021) reported a courtship event on a vertical tree of Southern Amazonia, Brazil. During our observations, we did not record evasion by the female, but we documented nape biting by the male on the female. In a similar way, Silva-Neto et al. (2019) and Oliveira & Moraes (2021) reported these aggressive behaviours prior to the hemipenial insertion (copulatory phase). Unfortunately, we were unable to observe the nuptial display behaviour of the male (head bobbing, push-ups, and extending dewlaps) prior to mating, because it was not sighted until it approached the female.

We did not observe a colour change during this mating event. In fact, Silva-Neto et al. (2019) also did not report colour change during mating in *D. philopunctata*. Although Olivera & Moraes (2021) hypothesized that the colour change in males of *Anolis* may be result of combined effect of its excitement during copulation and insemination that progressively intensifies during mating. However, this hypothesis must be evaluated in greater detail in future studies.

Due to the potential exposure of the breeding pair to greater predation risk, mating events of anole species range from 10 minutes (Losos 2009; Beltrán et al. 2016) to up to 64 minutes (Alfonso et al. 2014). Here, the mating duration was 25 minutes, which is within the reported duration for other similar species such as *A. punctatus* (Silva-Neto et al. 2019; Moraes & Oliveira 2021). This study broadens our knowledge on mating behaviour in another anole species. Future studies should improve documenting the courting and mating observations to clarify if the mating duration reported here is part of the typical behavioural repertoire of *A. quercorum*.

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COMMUNICATION

Evidence for the local extirpation of the Dehradun Stream Frog *Amolops chakrataensis* Ray, 1992 from the type locality, Chakrata in western Himalaya, India, and associated threats: a call for urgent conservation action

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Abstract: The current occurrence status of the 'Critically Endangered' Dehradun Stream Frog *Amolops chakrataensis* was assessed at its type locality in the Chakrata landscape in Dehradun District, Uttarakhand in the western Himalaya, India. Daytime and nighttime visual encounter and aural surveys were conducted for 39 survey days between 2023 and 2024, across multiple stream systems and seasons. Despite extensive effort, no individuals of *A. chakrataensis* were detected. This study identifies two additional ongoing threats under the IUCN Red List Threats Classification Scheme: pollution and residential & commercial development. Discrepancies in the current IUCN Red List distribution map were also detected, and an updated preliminary estimate of the indigenous range (sensu IUCN) was provided prior to putative local extirpation. These findings suggest a putative local extirpation at the type locality and highlight the urgent need for targeted monitoring and habitat conservation.

Keywords: Anthropogenic pressure, conservation monitoring, Critically Endangered amphibian, habitat degradation, Himalayan biodiversity, local extinction, stream habitat, pollution, type locality reassessment, unsustainable tourism.

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INTRODUCTION

Dehradun Stream Frog *Amolops chakrataensis*, Ray, 1992, is a CR amphibian found in Uttarakhand, in the western Indian Himalaya (IUCN 2023; Image 1). This status is primarily due to its extremely limited extent of occurrence (EOO) and the severe decline in both the quality and extent of its habitat at its type locality in the Chakrata Hills (Ray 1992). According to the IUCN Red List assessment, habitat degradation and modifications of the flow of streams caused by the construction of dams are the major threats to this species (IUCN 2023). This species was collected by Dr. Ray of Zoological Survey of India, Dehradun, from a stream along the Tuni road, approximately 14 km north-west of Chakrata Town, in 1985. Since then, there have not been confirmed records of this species.

Amolops chakrataensis can be easily distinguished from its congeners by distinct yellowish dorsolateral glandular folds. It is a medium-sized frog with females having a snout-vent length of approximately 55 mm. Its head is wider than long. The posterior of its thighs is blotched dark brown and creamy yellow. The dorsum is ashy brown, with the lateral sides below the dorsolateral glandular folds appearing dark green. The dorsolateral glandular folds exhibit a golden yellowish color (Ray 1999). The ecology, breeding, and behaviour of the species are also poorly known except for depositing eggs under boulders (IUCN 2023).

To determine the current status and to identify the key threats to *A. chakrataensis* at the type locality and surrounding area, the research team, including the first five authors, conducted multiple daytime and night-time visual encounter surveys and acoustic surveys (Borzée et al. 2016; Prasad et al. 2020; Nowakowski et al. 2024) across streams in the Chakrata landscape, including the type locality of *A. chakrataensis*. Surveys were conducted by a team of experienced field herpetologists with prior expertise in Himalayan amphibian identification for a total of 39 days across different seasons between 2023 and 2024 (Table 1), with efforts including two researchers for seven days in February 2023, three researchers for six days in April 2023, three researchers for nine days in June 2024, three researchers for six days in September 2024, and two researchers for 11 days in October 2024. A standard search effort across sites was applied, spending 2 h surveying each approximately 500 m stream and riparian section to allow careful inspection of available microhabitats. On each survey day or night, 5h and 30 min of active surveying were conducted. The surveys were conducted in Tuna Forest, Masak, Jadi, Kanaser, Lokhandi, Moila, Gvasa, Tiger waterfall, and Dava, at altitudes ranging from approximately 1,050–2,700 m in the Chakrata landscape in Dehradun District of Uttarakhand (Image 2). Each site was surveyed at least three times to maximize the probability of detection. Sampling was conducted during night-time (1800–2330 h) using headlamps and torches and daytime (1030–1600 h), searching through stream banks, pools, leaf litter, roadside moist habitats, and surrounding vegetation within the stream channel and immediate riparian zone, corresponding to the expected detection distance for stream-dwelling anurans. To detect the presence of any calling males of *A. chakrataensis*, a 5 min assessment of calling activity at each site prior to initiating visual encounter surveys during night-time visits (Borzee et al. 2016), with observers actively listening for calls while slowly walking along stream sections.

In the absence of finding adult individuals of *A. chakrataensis*, tadpoles were sampled to confirm its presence. Based on 16S mtDNA barcoding, the six sampled tadpoles were identified as belonging to the *A. formosus* (n = 1), *A. jaunsari* (n = 1), *Nanorana vicina* (n = 2), and *N. minica* (n = 2), with no evidence of *A. chakrataensis.* DNA was extracted from the clipped tail tissue of tadpoles using the DNeasy Blood & Tissue Kit (QIAGEN), following the manufacturer's protocol. A fragment of the mitochondrial 16S rRNA gene was amplified with universal primers under standard PCR conditions. Amplicons were purified, sequenced bidirectionally, and the resulting sequences were edited and assembled in Geneious. Bayesian phylogenetic analyses were conducted to confirm species identity, following established protocols (Wang et al. 2023). Detailed laboratory protocols and full barcoding analyses will be presented in a separate manuscript. Despite the extensive efforts, no individuals of *A. chakrataensis* were recorded during the surveys.

In addition, data on the threats to *A. chakrataensis* were collected during the field surveys using visual observations of habitat, photographic documentation and taking field notes on mobile devices. Information on local land-use practices and perceived threats was further gathered through informal interviews with local villagers and tribal communities. During the surveys, it was observed that the streams in the type locality of *A. chakrataensis* were polluted with plastic waste, directly disposed into the streams. Items such as food wrappers, plastic bottles, bottle caps, grocery bags, straws, and stirrers stemming from excessive tourism are regularly thrown into the streams by tourists and hotel owners. During informal interviews in Jadi, local villagers told us that this plastic waste is increasing primarily due to unsustainable tourism (Luo et al. 2018; Ziegler et al. 2023) in the Chakrata landscape. It was also observed that some parts of the streams were affected by algal bloom, most likely caused due to agricultural pesticide runoff from surrounding fields in nearby villages in the Chakrata landscape. Such pollution is known to alter water quality and microhabitat structure and affect the functioning of Himalayan Freshwater ecosystems (Peng 2019; Talukdar et al. 2023), which may have negative consequences for stream-dependent amphibians, particularly during aquatic life stages (Gill et al. 2025). This study identifies two additional threats to *A. chakrataensis* under the IUCN Red List Threats Classification Scheme (Version 3.3): pollution (sub-categories–garbage and solid waste, and agricultural and forestry effluents) and residential and commercial development (sub-category–tourism and recreation areas), alongside the previously recognized threats of natural system modifications and invasive and other problematic species, genes, and diseases. These threats are ongoing and are most likely to impact all populations of *A. chakrataensis* in Chakrata.

Waste dumping in streams presents a significant threat to the amphibians (Rahman et al. 2024), including *A. chakrataensis* and other sympatric anurans such as *Amolops formosus*, *A. jaunsari*¸ *Duttaphrynus himalayanus*, *Nanorana minica* and *N. vicina*. While garbage dumps may provide a constant source of food to animals, they also expose wildlife to risks of pathogen infection and toxic substances, which can have dire consequences for wildlife health and population viability (Azevedo-Santos et al. 2021). Garbage dumps act as ecological hotspots that attract invasive and toxic species, posing serious risks to environmental health (Sangkachai et al. 2024). Invasive amphibians, such as *Duttaphrynus melanostictus*, can exploit these sites for abundant food resources and potential breeding habitats, thereby enabling their establishment and spread into native ecosystems (Penerbit 2019; Dufresnes et al. 2025). This process can intensify competition and toxicity pressures, further threatening native amphibian populations (Plaza & Lambertucci 2017).

Plastic pollution poses a lethal threat to amphibians in freshwater ecosystems, affecting several species of amphibians (Azevedo-Santos et al. 2021). Plastic pollution poses significant risks to amphibians as the microplastics accumulate in the organs, causing external morphological changes, mutagenic effects, and

Image 1. Holotype of *Amolops chakrataensis* in the Museum of Zoological Survey of India, Dehradun, collected in 1985. The registration number 'A.197' was noted on the holotype during our visit, whereas Ray (1999) referred to it as 'NRS/ZSI A-25'. © Vishal Kumar Prasad.

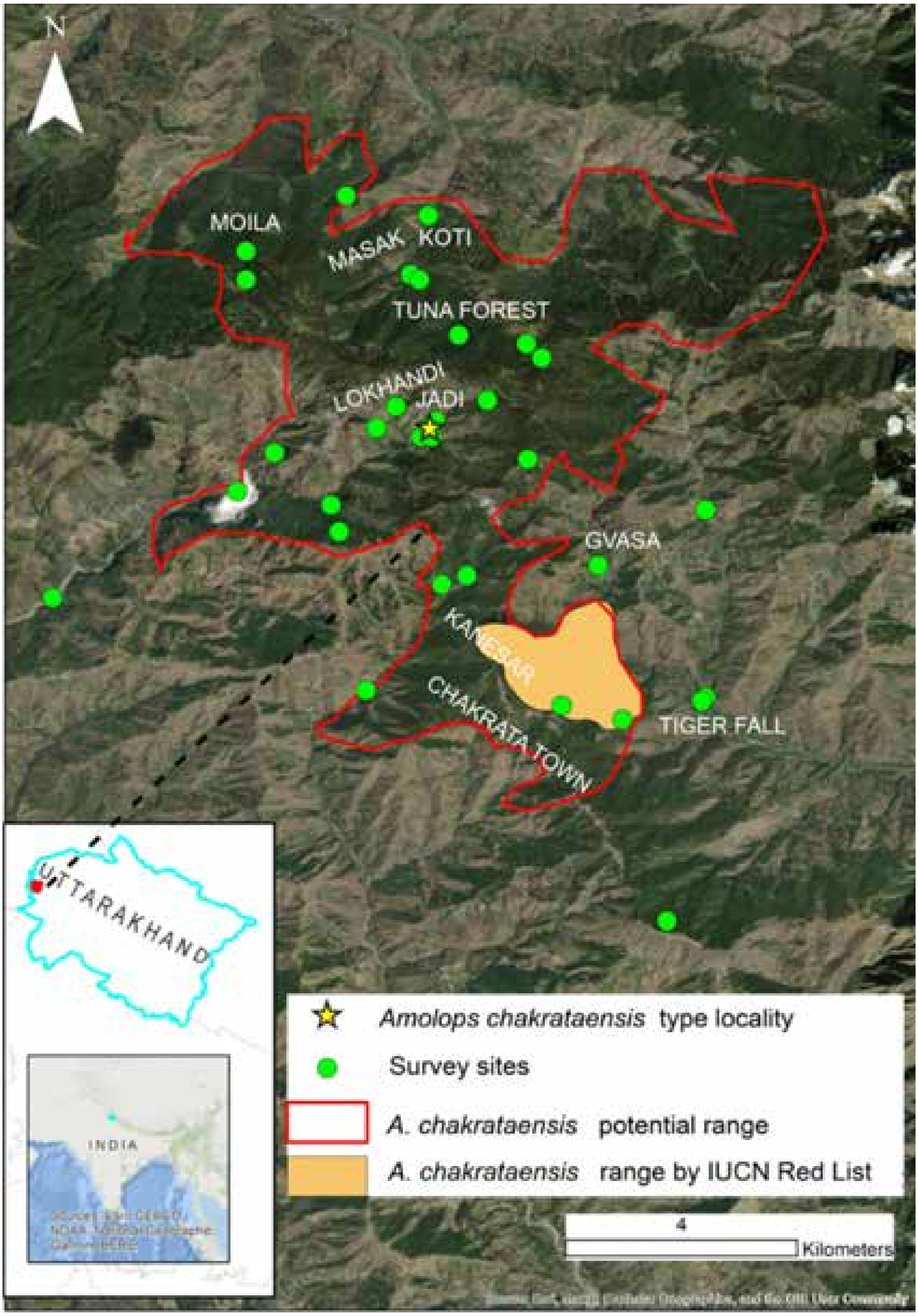

Image 2. Map of the survey area in the Chakrata landscape explored for *Amolops chakrataensis* in in this study. The yellow star marks the type locality of *A. chakrataensis*. Green dots represent the survey sites within streams where visual encounter and aural surveys were conducted in the Chakrata landscape, Dehradun District (Uttarakhand, India). The red outline represents a potential distribution range of *A. chakrataensis* in the Chakrata landscape. The orange polygon indicates the range of *A. chakrataensis* as shown in the IUCN Red List of Threatened Species that requires an update.

Table 1. Details of sampling sites surveyed for *Amolops chakrataensis* in the Chakrata landscape (Dehradun District) and the Bhagirathi River basin (Uttarkashi District), Uttarakhand, India, between 2023–2025. The table summarises geographic location, elevation, survey effort, seasonal coverage, habitat characteristics, observed associated threats, and other amphibian species detected at each site. Coordinates are provided in decimal degrees (WGS84), and altitude is in metres. Sampling seasons are as I = Spring (February–March), II = Summer (April–May), III = Pre-monsoon (June), and IV = Post-monsoon (September–October).

Sites	Latitude	Longitude	Altitude (m)	Number of observers	Sampling season	Target species	Other amphibians	Habitat type	Observed associated threats
Tuna Forest	30.7825	77.8375	1,936	2	I, II, III, IV	Absent	*Duttaphrynus himalayanus, Nanorana minica, N. vicina*	Deodar–pine forest streams	Plastic pollution
Masak	30.7698	77.8223	2,330	2	I, II, III	Absent	*D. himalayanus, N. minica, N. vicina*	Deodar forest streams	Plastic pollution
Jadi village	30.7465	77.8505	2,246	3	I, II, III, IV	Absent	*N. minica, N. vicina*	Mixed forest–urban interface	Road widening, urbanization, plastic pollution, algal blooms
Kanaser	30.7127	77.8662	2,029	3	I, II, IV	Absent	*N. minica, N. vicina*	Deodar forest streams	Plastic pollution
Lokhandi village	30.7586	77.8109	2,415	3	I, II, IV	Absent	*N. minica, N. vicina*	Deodar forest streams	Road widening, urbanization, tourism pressure
Moila forest	30.7765	77.7883	n/a	2	II, III, IV	Absent	*N. minica, N. vicina*	Deodar–pine forest streams	Household wastewater, plastic pollution
Gvasa forest	30.7229	77.8786	1,819	3	I, II, III	Absent	*D. himalayanus, Amolops formosus, N. minica*	Forest streams near settlements	Plastic pollution, tourism pressure
Tiger waterfall	30.7046	77.894	1,524	2	II, III, IV	Absent	*A. formosus, A. jaunsari, D. himalayanus, N. minica*	Open stream, semi-urban	Tourism pressure, urbanization
Dava	30.7336	77.8933	1,776	2	I, II, III	Absent	*D. himalayanus, N. minica*	Agricultural land with oak patches	Agricultural disturbance
Dabri Khadd	30.7701	77.8475	1,882	2	I, II, III	Absent	*D. himalayanus*	Agricultural land with oak patches	Agricultural disturbance
Sahiya	30.6241	77.8663	1,096	3	I, II, IV	Absent	*A. jaunsari, D. himalayanus, D. melanosticus, N. minica, Minervarya* sp.	Wide stream/ river system	Urbanization, tourism pressure
Bhagirathi River basin (Maneri) 1	30.7283	78.5314	1,321	2	IV	Absent	*A. formosus, A. jaunsari, N. minica*	Stream bordered by forest and paddy fields	Agriculture, dam influence
Bhagirathi River basin (Maneri) 2	30.7668	78.591	1,409	2	IV	Absent	*A. formosus, A. jaunsari, N. minica*	Stream bordered by forest and paddy fields	Agriculture, dam influence

cytotoxic damage, which severely affect the health and development of amphibians (da Costa Araújo et al. 2020). Large freshwater amphibians also face entanglement in fishing nets (Azevedo-Santos et al. 2021). The trend of threat from plastic waste to amphibians in Chakrata is most likely similar; this needs a detailed assessment (Image 3).

Despite extensive surveys between 2024 and 2025, no individuals of *Amolops chakrataensis* were detected, which is concerning. Since its original description, there have been no confirmed records of this elusive and rare species. Non-detection does not necessarily indicate extirpation and may reflect low detectability associated with rarity, small population size, cryptic behaviour, seasonal variability or sampling efforts (Button & Borzée 2024). This absence may also partly reflect historical limitations, including a lack of targeted surveys, limited scientific exploration, and the absence of long-term monitoring. Therefore, a putative extirpation was referred to the type locality, pending further targeted assessments. Additionally, the IUCN Red List reports the presence of *A. chakrataensis* in the Bhagirathi River basin (IUCN 2023); this record is not supported by published literature and is also not represented in the IUCN Red List range map. Hence, it warrants verification.

This site was sampled in October 2024, but did not detect the species. Moreover, this locality lies approximately 65 km (straight-line distance) from Chakrata. In addition, the current IUCN Red List distribution map appears inaccurate, as it excludes the

Image 3. Plastic waste disposed and thrown in the stream habitats in the type locality of *Amolops chakrataensis*. © A—Vishal Kumar Prasad, B,C,D—Devendra Singh.

type locality and places the range approximately 5 km away from the locality reported by Ray (1992, 1999). Based on the field surveys, an updated preliminary range map for *A. chakrataensis* (Image 2) is provided, estimating an extent of occurrence of 52.79 km², compared to the 4 km² currently depicted in the IUCN Red List range polygon. The area was calculated in ArcGIS Pro (version 3.1.5) using the calculate geometry attributes tool. This revised range map can serve as a baseline for future targeted surveys, resampling efforts, and conservation assessments.

It is recommended to conduct targeted seasonal surveys during peak breeding periods to improve detectability. The collection of live individuals as vouchers should be strictly prohibited for this species to prevent further population decline (Minteer et al. 2014). Environmental DNA (eDNA) surveys may serve as a sensitive, non-invasive tool for assessing the presence of species in low-density populations. Surveys should also extend beyond the type locality to evaluate the potential persistence of the species in adjacent stream systems. Habitat restoration measures, including removing accumulated plastic waste, reducing agricultural runoff, and maintaining natural stream flow regimes, should be implemented to enhance microhabitat quality for this stream-dependent amphibian. On a broader scale, engaging with district-level policy frameworks, along with actively involving local communities in amphibian conservation initiatives in Uttarakhand, is essential to integrate freshwater biodiversity considerations into tourism regulation, waste management planning, and watershed conservation strategies. Implementing these measures would establish baseline ecological conditions necessary for future monitoring, reassessment, and potential recovery initiatives for *A. chakrataensis*.

It is concluded that the 'Critically Endangered' *A. chakrataensis* has become an extremely rare amphibian and is likely on the verge of extinction, given the absence of confirmed sightings or collections since its original description based on a single holotype. This indicates a high risk of range-wide extinction and highlights the urgent need for immediate conservation action. The putative local extirpation of *A. chakrataensis* from its type locality, Chakrata, highlights the critical necessity of prioritizing amphibian conservation in the western Himalaya (Lötters et al. 2023; Luedtke et al. 2023; Wren et al. 2024). Consistent with recent global amphibian synthesis, targeted habitat protection, improved monitoring including eDNA sampling, and integration of site-based conservation frameworks are essential to halt further declines and prevent irreversible biodiversity loss (Borzée et al. 2025). Rapid and coordinated interventions can help ensure that other amphibian species in this fragile Himalayan ecosystem do not follow similar extirpation trajectories.

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COMMUNICATION

First record of the genus *Berlandina* Dalmas, 1922 (Araneae: Gnaphosidae) from India, with notes on *B. plumalis* (O. Pickard-Cambridge, 1872) and its synonymy

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Abstract: The ground spider genus *Berlandina* Dalmas, 1922 is reported for the first time from India with the record of *B. plumalis* (O. Pickard-Cambridge, 1872) in the Kachchh region of Gujarat. Previously known from western Africa through the Mediterranean to central Asia, including Iran and Afghanistan, *B. plumalis* is here recorded for the first time from India, extending its known distribution eastwards into the Indian subcontinent. Also, *B. afghana* Denis, 1958 syn. rest. is once again proposed as a junior synonym of *B. plumalis*. A detailed diagnosis is provided to distinguish *B. plumalis* from other congeners, supported by comprehensive illustrations of the male and female copulatory organs.

Keywords: Arachnida, arid habitat, biodiversity, distribution, ground spider, Gujarat, Kachchh, morphology, range extension, taxonomy.

સારાંશ: આ અભ્યાસમાં ગુજરાતના કચ્છ પ્રદેશમાંથી *Berlandina plumalis* (O. Pickard-Cambridge, 1872) પ્રજાતિની નોંધ સાથે *Berlandina* Dalmas, 1922 જાતિનો ભારતમાંથી પ્રથમ અહેવાલ રજૂ કરવામાં આવે છે. આ પ્રજાતિ અગાઉ પશ્ચિમ આફ્રિકા, ભૂમધ્યસાગરીય પ્રદેશ, ઈરાન, અફઘાનિસ્તાન તથા મધ્ય એશિયાના અન્ય વિસ્તારોમાંથી જાણીતી હતી. ભારતમાંથી થયેલી આ નવી નોંધ તેના જાણીતા વિતરણક્ષેત્રને ભારતીય ઉપખંડ સુધી વિસ્તારે છે. ઉપરાંત, *B. afghana* Denis, 1958 syn. rest. ને પુનઃ *B. plumalis* નો Junior synonym તરીકે પ્રસ્તાવિત કરવામાં આવે છે. *B. plumalis* ને સમાન જાતિની અન્ય પ્રજાતિઓથી અલગ ઓળખી શકાય તે માટે તેના નિદાનાત્મક લક્ષણોનું વિગતવાર વર્ણન આપવામાં આવ્યું છે, જેને નર અને માદા જનન અંગોની વિસ્તૃત આકૃતિઓ દ્વારા સમર્થન આપવામાં આવ્યું છે.

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INTRODUCTION

The spider family Gnaphosidae Banks, 1892 comprises 2,498 species across 154 genera globally (WSC 2026), with 28 genera and 140 species known from India (Caleb & Sankaran 2026). Despite extensive taxonomic and distributional studies on this family, continued research regularly yields new species descriptions and additional records, contributing to a more comprehensive understanding of its global and regional diversity (Sankaran & Caleb 2021, 2025; Zakharov & Ovtsharenko 2022; Lin & Li 2023; Wunderlich 2023; Esyunin et al. 2024; Liu & Zhang 2024; Shodmonov & Fomichev 2025).

The genus *Berlandina* Dalmas, 1922, a member of subfamily Gnaphosinae Banks, 1892, consists of 43 species distributed across various biogeographical regions. The genus is widespread across the Palaearctic region and the northern part of Afrotropical region (Shodmonov & Fomichev 2025; WSC 2026). In this study, we document the first record of the genus *Berlandina* from India, marked by the occurrence of *B. plumalis* (O. Pickard-Cambridge, 1872) in the arid region of Kachchh, Gujarat.

Berlandina afghana Denis, 1958 was originally described from Afghanistan with two subspecies, *B. a. afghana* and *B. a. spinitarsis* (Denis, 1958). It was later synonymised with *B. plumalis* by Levy (1995) based on variation in epigynal structures, but was subsequently revalidated by Marusik et al. (2014b) based on differences in receptacle size. Based on observed intraspecific variation in female copulatory morphology, the present study again treats *B. afghana* as a junior synonym of *B. plumalis*.

MATERIALS AND METHODS

The specimens were hand–collected, preserved in 70% ethanol and studied under a Zeiss SteREO Discovery.V20 stereomicroscope. Microphotographic images were taken by a Axiocam 208 color digital camera attached to the stereomicroscope and enabled with the software Zeiss ZEN 3.3. The left male palp was removed, studied and photographed by placing it in a cavity block filled with 70% ethanol. The epigynes were dissected, cleared of soft tissues using 10% KOH, and studied and photographed by placing them in a cavity block filled with 70% ethanol. Drawings were hand made by examining the microscopic images. All measurements are in millimeters (mm). The species was identified based on Levy (1995), Shodmonov & Fomichev (2025) and Marusik et al. (2014b), and the terminology follows the former. The examined specimens have been deposited in the reference collection of Zoology Research Lab at the Department of Zoology, R.R. Lalan College, Bhuj, Kachchh.

The following abbreviations are used: AH—anterior hood | AME—anterior median eye | ALE—anterior lateral eye | CD—copulatory duct | CO—copulatory opening | E—embolus | F—fovea | MA—median apophysis | PME—posterior median eye | PLE—posterior lateral eye | PR—primary receptacle | RTA—retrolateral tibial apophysi | S—septum | SD—sperm duct | SR—secondary receptacle | ST—subtegulum.

TAXONOMY

Family Gnaphosidae Banks, 1892
Genus *Berlandina* Dalmas, 1922
Type species: *Gnaphosa plumalis* O. Pickard-Cambridge, 1872
Diagnosis: For detailed diagnostic features of the genus see (Koch 1839; Dalmas 1921; Levy 1995; Marusik et al. 2014a).

***Berlandina plumalis* (O. Pickard-Cambridge, 1872)**
(Images 1–25)

Gnaphosa plumalis O. Pickard-Cambridge, 1872: 225, pl. 15, fig. 3 (male)

Berlandia plumalis Dalmas, 1921: 268, figure 45, 52–53 (male, female, S of *Callilepis passerina* (Simon, 1884), *Gnaphosa cinereoplumosa* (Simon, 1878) and *G. rhodopis* L. Koch, 1875); Levy, 1995: 940, figure 53–56; Marusik et al. 2014b: 418, figure 7a–b, 8; Shodmonov & Fomichev 2025: 514, figure 10–23, 43

Berlandina afghana Denis, 1958: 92, figure 14 (female) syn. rest.

Berlandina afghana Marusik et al. 2014b: 416, figure 1, 3a–b, 4, 5a–b, 6 (female)

For a complete list of taxonomic references, see the WSC (2026).

Materials examined

RRLC–ZC/SP–10, 16.vii.2024, 1 female, India, Gujarat, Kachchh District, Bhuj, from R.R. Lalan College Campus (23.238° N, 69.658° E), 107 m, leg. S. Parmar; RRLC–ZC/SP–11, 3.ix.2024, 2 female, India, Gujarat, Kachchh District, from Kodki Village (23.246° N, 69.597° E), 638 m, leg. S. Parmar; RRLC–ZC/SP–12, 2.i.2025, 1 male and 2 female, India, Gujarat, Kachchh District, from Kukma Village (23.217° N, 69.753° E), 190 m, leg. S. Parmar.

From arid habitat with sparse vegetation.

Diagnosis

The male of *B. plumalis* is most similar to that of *B. nabozhenkoi* Ponomarev & Tsvetkov, 2006 by having a hook–like retrolateral tibial apophysis (RTA) bent dorsally and a long membranous embolus (E), but can be easily distinguished by following combination of characters: thinner apical part of RTA in *B. plumalis* (vs. thick apical part of RTA in *B. nabozhenkoi*; figure 106 in Marusik et al. (2014a)) and straight E in *B. plumalis* (Image 4, 6–8, 18) (vs. strongly curved in *B. nabozhenkoi*; figure 105 & 108 in Marusik et al. (2014a)). The female of *B. plumalis* is also very close to that of *B. nabozhenkoi* by having a well–developed elongated septum (S) and large arcuate copulatory ducts (CD), but can be easily distinguished by following combination of characters: epigynal receptacles, secondary receptacles (SR) placed entally to CD in *B. plumalis* (vs. SR placed posteriorly in *B. nabozhenkoi*; figure 2 in Ponomarev et al (2018)) and a circular fovea (F) in *B. plumalis* (vs. trapezoidal fovea in *B. nabozhenkoi*; figure 1 in Ponomarev et al (2018)).

Description

Male (in alcohol, from Kukma Village) (Image 1–8, 18–19): Body length 5.98. Carapace length 2.72, width 2.35, abdomen length 3.26, width 2.52. Eye inter–distances: AME 0.13, ALE 0.11, PME 0.10, PLE 0.10, AME–AME 0.08, AME–ALE 0.03, ALE–ALE 0.27, ALE–PME 0.17, PLE–PLE 0.38, PME–PME 0.09, PME–PLE 0.07, AME–PME 0.15, ALE–PLE 0.14. Measurements of legs: I 7.2 [2, 1.1, 1.7, 1.3, 1.1], II 6.5 [1.9, 1, 1.2, 1.4, 1], III 6 [1.7, 0.7, 1, 1.6, 1], IV missing. Leg formula 1234. Spination (dprv): I [Fe d1–1–0 v0–0–1; Ti r0–1–1; Mt r2–0–2], II [Fe d1–1–0 v0–0–1; Ti r2–0–2 v0–1–0; Mt r1–1–2 v0–0–1], III [Fe d2–2–2 v0–0–1; Pa d1–0–1 p1 v1; Ti d1–1–1 p1–0–0 v2–2–2; Mt d2–2–2 r2–2–2 v0–0–1]. Carapace yellowish-brown with slightly darker median groove and dark setae patches on lateral sides. Labium, endites, and sternum yellowish-brown. Chelicerae brownish. Legs the same colour as carapace, without annulations. Abdomen beige, without any clear pattern, although some brown markings visible. Spinnerets uniformly light brown. Palp (Image 4–8, 18–19): RTA wide, as long as tibia, broad at the base with hook–like pointed tip, curved dorsally; cymbium yellowish-brown, twice as long as tibia,

Images 1–8. Male of *Berlandina plumalis* (O. Pickard-Cambridge, 1872): 1 –General appearance, dorsal view l 2—Same, ventral view l 3—Cephalothorax, antero–dorsal view l 4—Left palp, ventral view l 5—Same, retrolateral view l 6—bulb, ventro–prolateral view l 7—same, dorsal view l 8—Same, retrolateral view. Abbreviations used in the images: E—embolus l MA—median apophysis. Scale bars: 1 mm (1–2) l 0.2 mm (3) l 0.1 mm (4–8). © Subhash Parmar.

Images 9–10. Live female of *Berlandina plumalis* (O. Pickard-Cambridge, 1872): 9—dorsal view l 10—anterodorsal view. © Subhash Parmar.

dorsally covered with dense scopula; cymbial furrow elongated; subtegulum (ST) placed postero-ventrally; median apophysis (MA) long, bent ventrally, smaller than E and partly hidden by E; E long, spirally twisted basally, straight apically with arrow–like tip.

Female (Image 9–10, 11–17, 20–21, 23, 23; from Kukma Village): body length 6.65. Carapace length 3.00, width 2.20, abdomen length 3.61, width 2.30. Eye sizes and inter–distances: AME 0.12, ALE0.12, PME 0.13, PLE 0.12, AME–AME 0.11, AME–ALE 0.03, ALE–ALE 0.26, ALE–PME 0.17, PLE–PLE 0.29, PME–PME 0.09, PME–PLE 0.07, AME–PME 0.15, ALE–PLE 0.16. Measurements of legs. I 6.91 [2.09, 1.17, 1.51, 1.20, 0.92], II 6.0 [1.80, 1.07, 1.10, 1.14, 0.89], III 6.13 [1.7, 0.94, 1.00, 1.49, 1.00], IV 8.87 [2.34, 1.23, 1.62, 2.40, 1.28]. Leg formula 4132. Leg supination (dprv): I [Fe d1–1–0 p0–0–1; Ti v2–1–1; Mt v3–0–2], II [Fe d1–1–0 p1–0–1; Ti p0–0–1 v1–1–2; Mt v2–1–2], III [Fe d1–2–1 p1–1–1 r0–2–1; Pa p1 r1; Ti d1–0–0 p1–0–1 r0–1–1 v1–2–2; Mt d1–2–0 p1–1–0 r0–1–0 v2–1–0], IV [Fe d1–1–1 p0–0–1 r0–1–0; Pa r1; Ti d1–0–1 p1–1–1 r0–1–2 v2–2–2; Mt d1–2–0 p1–1–0 r1–0–0 v2–1–2]. Carapace yellowish-brown, with dark edges and three pairs of dark spots laterally. Chelicerae brownish. Labium, endites and sternum light brown. Coxae yellow. Abdomen yellowish-grey with herringbone pattern; yellow colored ventrally. Spinnerets light brown. Legs yellowish-brown. Epigyne (Image 15–17, 20–21, 23): epigynal plate wider than long; fovea (F) circular; anterior hood (AH) arcuate with horizontal "3" shaped marking on margin; copulatory openings (CO) large, placed laterally; copulatory ducts (CD) large and arcuate; primary receptacles (PR) and secondary receptacles (SR) oval shaped and subequal in size.

Variations in male palpal morphology

In the present study the male palp has long spirally twisted E with arrow-like tip (Image 4, 6, Figure 2) which is similar to those from Uzbekistan (Shodmonov & Fomichev 2025, figure 13–14,16–18) but minor different from Israel (E situated within a long sheath; Levy 1995, figure 53–54) and Crete (well sclerotized with a distal curl; Chatzaki et al. 2002, figure 13–14); tegulum flat (Image 4–5, 18–19), which is similar to those from Uzbekistan (Shodmonov & Fomichev 2025, figure 13–18) but minor different from Crete (voluminous tegulum, Chatzaki et al. 2002, figure 13–14); RTA as long as tibia (Image 4–5, 18–19) in present specimen which is similar to those from Uzbekistan (Shodmonov & Fomichev 2025, figure

Images 11–17. Female of *Berlandina plumalis* (O. Pickard-Cambridge, 1872): 11—general appearance, dorsal view l 12—Same, ventral view l 13—cephalothorax, anterodorsal view l 14—Spinnerets, ventral view l 15—Intact epigyne, ventral view l 16—macerated epigyne, ventral view l 17—Same, dorsal view. Abbreviations used in the images: AH—anterior hood l CD—copulatory ducts l CO—copulatory openings l F—fovea l PR—primary receptacles l S—septum l SR—secondary receptacles. Scale bars: 1 mm (11–12) l 0.5 mm (13–15) l 0.1 mm (16–17). © Subhash Parmar.

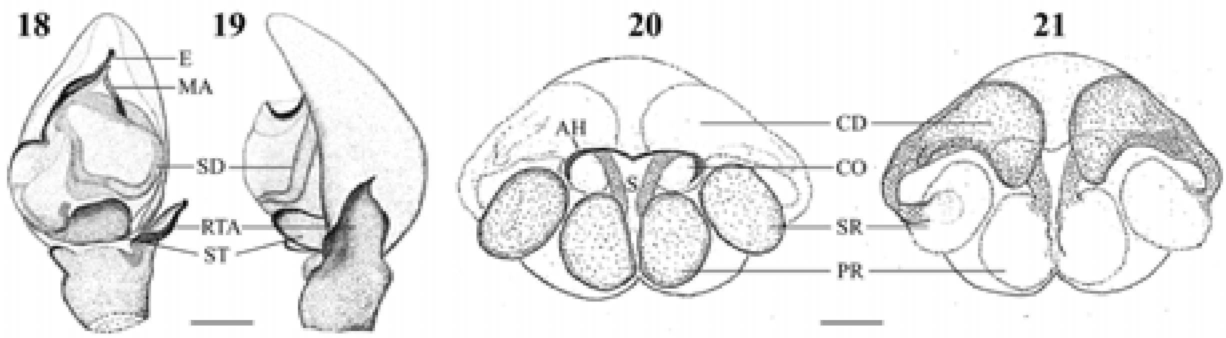
13–18) but differ from that of Israel (RTA longer than tibia, Levy 1995, figure 53–54).

Variations in female epigynal morphology

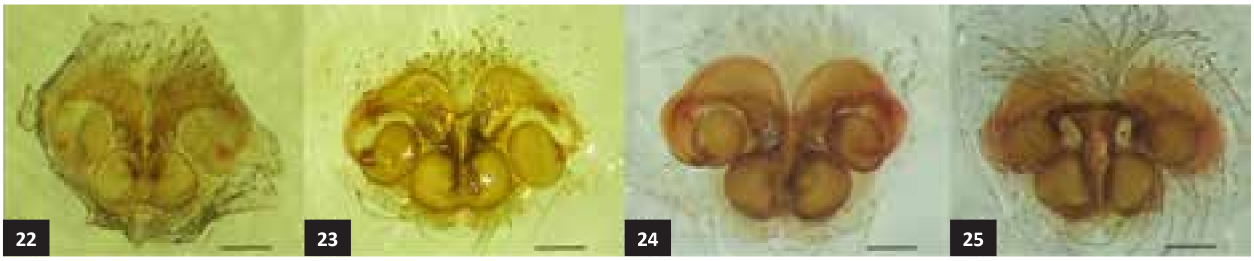
In the present study we have collected a total of five female specimens, which show noticeable variation in the size of receptacles. One female (from Kukma Village) has unequal size of receptacles, larger PR and smaller SR (Image 24), which is similar to those from Uzbekistan (Shodmonov & Fomichev 2025, figure 21–23) and Israel (SR much smaller than in our specimens) (Marusik et al. 2014b, figure 7–8) and Crete (Chatzaki et al. 2002, figure 15–16), but female from Israel collected by Levy (1995) had smaller PR and larger SR (see figure 55–56 in Levy, 1995); three females (from Bhuj, Kodki and Kukma villages) have subequal PR and SR (Image 19), similar to that of *B. afghana* from Pakistan (Marusik et al. 2014b, figure 3–6) and the other female (from Kodki village) has subequal PR and SR but SR constricted (Image 22). The female from Kukma Village has arcuate AH (Image 25), which is similar to those of from Uzbekistan (Shodmonov & Fomichev 2025, figure 21–23), Israel (Marusik et al. 2014b, figure 7–8) and Iran (Zamani et al. 2015, figure 2c); other specimens have AH arcuate with horizontal "3" shaped marking on margin (Image 15–16, 20), which is similar to that from Crete (Chatzaki et al. 2002, figure 15–16).

Justification of synonymy

The male and female specimens were collected together from the same localities (Kukma Village (1 male, 2 female) and Kodki village (2 female), Gujarat, India). An



Images 18–21. Copulatory organs of *Berlandina plumalis* (O. Pickard-Cambridge, 1872): 18—Left palp, ventral view l 19—same, retrolateral view l 20—Epigyne, ventral view l 21—same, dorsal view. Abbreviations used in the images: AH—anterior hood l CD—copulatory ducts l CO—copulatory openings l E—embolus l MA—median apophysis l PR—primary receptacles l RTA—retrolateral tibial apophysis l S—septum l SD—sperm duct l SR—secondary receptacles l ST– subtegulum. Scale bars: 0.2 mm (18–21). © Subhash Parmar



Images 22–25. Intraspecific variation in epigyne of *Berlandina plumalis* (O. Pickard-Cambridge, 1872) collected from different localities: 22—from Kodki Village l 23—from Bhuj l 24—from Kukma Village, dorsal view l 25—same, ventral view. Scale bars: 0.2 mm (22–24). © Subhash Parmar.

examination of these specimens revealed that the male fully corresponds in palpal morphology to *B. plumalis* as described by Levy (1995) and redescribed by Shodmonov & Fomichev (2025). However, the associated females exhibit noticeable variation in epigynal morphology. Some specimens closely match the epigyne of *B. plumalis* (Image 24), whereas others resemble that described for *B. afghana* (Image 16–17; 22–23), particularly in the size and shape of the receptacles and the configuration of the copulatory ducts. The differences in size of epigynal receptacles, can be considered as intraspecific variation (Image 22–25). Previously, *B. afghana* was synonymised with *B. plumalis* by Levy (1995) based on variation in external and internal parts of epigynum. Later, Marusik et al. (2014b) rejected this synonymy based on the size of primary and secondary receptacles (observed in single specimen), however, they could not assess variability. Based on these observations we consider *B. afghana* as a junior synonym of *B. plumalis* and this synonymy is reinstated in the present study.

Remarks

The species was collected near the burrows of the Indian spiny–tailed lizard (*Saara hardwickii* (Gray, 1827)) (Image 27). Previously, this species had been reported from rodent burrows (Denis 1958).

Habitat and Vegetation (Image 26–27)

The specimens were collected from Kachchh District, an arid region in the westernmost part of Gujarat, characterized by xerophytic vegetation. The habitat consists mainly of thorny shrubs, grasses, and herbs, including *Neltuma juliflora*, *Launaea procumbens*, *Tridax procumbens*, *Xenostegia tridentata*, *Evolvulus alsinoides*, *Alysicarpus* sp., and *Echinochloa colonum*. The species' presence near the burrows of the Indian spiny–tailed lizard indicates an ecological association with arid environments. Previously, it was observed that the species is more abundant in the semi–arid and arid habitats (Levy 1995; Marusik et al. 2014b; Shodmonov & Fomichev 2025).

Distribution: West Africa, Mediterranean to Central Asia, Afghanistan, Iran, Pakistan (WSC 2026) and India (new record, Figure 1).

Distribution in India: Gujarat (new record, Figure 1).

Images 26–27. Habitat of *Berlandina plumalis* (O. Pickard-Cambridge, 1872): 26—arid habitat with sparse vegetation in Kachchh, Gujarat l 27—spiny–tailed lizard's burrow (marked by yellow arrow). © Subhash Parmar.

Figure 1. Distribution of *Berlandina plumalis* (O. Pickard-Cambridge, 1872) in Gujarat, India (red star).

DISCUSSION

The present record of *B. plumalis* from Gujarat represents a significant eastward extension of the genus into the Indian subcontinent. Comparative analysis with previously published accounts from the Palaearctic and adjacent regions reveals considerable variation in copulatory structures. In females, variation is evident in the relative size and shape of primary and secondary receptacles, as well as in the morphology of the epigynal hood. Minor variability is also observed in male palpal morphology, particularly in the length of the retrolateral tibial apophysis, the degree of embolar curvature and sclerotization. Although these differences are notable, no consistent, discrete characters were identified that would allow separation of the Indian material from *B. plumalis* as currently understood. The general morphology of genital structures in both sexes agrees well with published diagnoses of the species. The observed variation is therefore interpreted as intraspecific, possibly reflecting geographic differentiation across its wide distribution, as well as inconsistencies in earlier illustrations and descriptions. The extent of morphological variability documented across populations from different regions raises the possibility that *B. plumalis* represents a species complex. In this context, the species described from Afghanistan may prove to be conspecific.

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COMMUNICATION

First record of leucosiid crab *Lyphira perplexa* Galil, 2009 (Decapoda: Brachyura: Leucosiidae) from the eastern coast of India in West Bengal

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Abstract: This study documents the occurrence of the leucosiid crab, *Lyphira perplexa* Galil, 2009, along the West Bengal coast of India. Specimens were collected from the Petuaghat seabeach and adjacent fishing harbor in Purba Medinipur District. The findings contribute to the limited understanding of the diversity and distribution of leucosiid crabs in Indian waters, specifically in the Bay of Bengal region. The observations include detailed morphological descriptions, habitat preferences, and geographic distribution. This contribution highlights the need for more extensive research on marine biodiversity along the Indian coast, emphasizing its ecological and conservation significance.

Keywords: Bay of Bengal, Crustacea, geographic distribution, Indian coast, marine biodiversity, morphological descriptions, pebble crab, Petuaghat seabeach, purba medinipur, taxonomy.

সারসংক্ষেপ: এই গবেষণায় ভারতের পশ্চিমবঙ্গ উপকূলে লিউকোসিডি (Leucosiidae) গোত্রভুক্ত কাঁকড়া *Lyphira perplexa* Galil, 2009-এর উপস্থিতি নথিভুক্ত করা হয়েছে। নমুনাগুলি পূর্ব মেদিনীপুর জেলার পেটুয়াঘাট সমুদ্রসৈকত এবং সংলগ্ন মৎস্য অবতরণ কেন্দ্র (ফিশিং হারবার) থেকে সংগ্রহ করা হয়। এই অনুসন্ধান ভারতীয় জলসীমায়, বিশেষত বঙ্গোপসাগরীয় অঞ্চলে, লিউকোসিডি কাঁকড়ার বৈচিত্র্য ও বিস্তার সম্পর্কে সীমিত জ্ঞানের পরিধি বৃদ্ধিতে সহায়ক হবে। পর্যবেক্ষণের অন্তর্ভুক্ত ছিল প্রজাতিটির বিস্তারিত আকৃতিগত (morphological) বৈশিষ্ট্য, আবাসস্থল পছন্দ এবং ভৌগোলিক বিস্তার। ভারতীয় উপকূলীয় সামুদ্রিক জীববৈচিত্র্য সম্পর্কে আরও বিস্তারিত গবেষণার প্রয়োজনীয়তা এই গবেষণার মাধ্যমে তুলে ধরা হয়েছে, যা এর পরিবেশগত ও সংরক্ষণগত গুরুত্বকে বিশেষভাবে গুরুত্ব দেয়।

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Author contribution: Prabir Sahoo: Resources, Investigation; Sagar Samanta: Resources, Validation; Avik Bhanja: Writing- Original draft preparation, Formal analysis; Final manuscript preparation; Manas Das: Software, Validation; Pijush Payra: Conceptualization, Methodology

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INTRODUCTION

Leucosiidae, commonly referred to as nut crabs or pebble crabs, is a distinctive family of small brachyuran crabs widely distributed on soft-bottom substrates and coral rubble habitats from the intertidal zone to continental slope depths (Alcock 1896). The family is particularly diverse in the Indo-West Pacific region (Tan 1995; Poore & Ahyong 2023), 765 leucosiid crabs (Brachyura: Leucosiidae) are considered one of the major brachyuran families worldwide (Ng et al. 2008; Beleem et al. 2017; Mohanty et al. 2019; WoRMS 2024). The crabs belong to leucosiid group are popularly known as nut and pebble crabs (Lee et al. 2009; Beleem et al. 2017). A checklist of marine brachyuran crabs from the Western Coast of India documented 84 species of the family Leucosiidae, out of which 22 species were reported from the western coast, whereas 52 species were recorded from the eastern coast of India (Dev-Roy 2013). Leucosiids are commonly associated with shallow seagrass meadows and, less frequently, rocky reef habitats. On tropical coral reefs, certain species possess highly eroded and flattened carapaces that closely resemble broken coral fragments or pieces of the calcareous alga Halimeda, providing effective camouflage within reef environments (Man 1888; Beleem et al. 2017). A higher diversity of the leucosiid group of the crabs is found along the Indian coast of the Bay of Bengal (Chopra 1933; Sarkar et al. 2024).

The genus *Lyphira* currently comprises 10 recognised species worldwide, including three species reported from Indian waters: *L. georgei* Trivedi et al., 2016; *L. heterograna* (Ortmann, 1892); and *L. perplexa* Galil, 2009. Members of the genus can be readily distinguished by a combination of morphological characters, including the ovate exopod of the third maxilliped, a transversely narrow first male pleonal somite, fused male pleonal somites 2–6 bearing a subterminal denticle, and the presence of a short apical process on the first male gonopod (Mohapatra et al. 2026).

This report represents the first documented occurrence of *Lyphira perplexa* along the West Bengal coast of India.

MATERIALS AND METHODS

Two male *L. perplexa* samples were collected from the Petuaghat seabeach area (21.7868° N, 87.8897° E) and near the Petuaghat fishing harbour (21.7947° N, 87.8833° E) in the Purba Medinipur District (Figure 1).

The specimens were measured with precision using Vernier calipers to the nearest 0.1 mm and a plastic ruler graduated to the nearest 0.1 cm. Specimens were identified, measured, and subsequently preserved in 10% formalin. Taxonomic identification was confirmed at the species level according to standard taxonomic keys (Galil 2009; Sudharma et al. 2014; Ebadi et al. 2018; Beleem et al. 2019; AL-Maliky 2020). Diagnostic assessments of the specimens were conducted in the Aquaculture Lab of the Department of Biological Sciences, Midnapore City College, Paschim Medinipur, West Bengal, India, and the Crustacea Division laboratory of the Zoological Survey of India, Prani Vigyan Bhawan, M-Block, New Alipore, Kolkata, India. One representative specimen was registered (Registration No. CR501) and deposited in the Crustacea Division of the Zoological Survey of India, Prani Vigyan Bhawan, M-Block, New Alipore, Kolkata, India for further reference and study.

RESULTS AND DISCUSSION

Systematic accounts
Class: Malacostraca Latreille, 1802
Order: Decapoda Latreille, 1802
Infraorder: Brachyura Latreille, 1802
Family: Leucosiidae Samouelle, 1819
Genus: *Lyphira* Galil, 2009

***Lyphira perplexa* Galil, 2009** (Image 1)

Materials examined: Two males, Petuaghat seabeach (Bay of Bengal), India, coll. Prabir Saho. 16.i.2024.

Description: Carapace is broadly oval and smooth. The posterior region of the carapace exhibits a prominent dome-shaped elevation, contributing to a trilobate appearance that is more pronounced compared to the anterior region. Lack of spines or sharp ridges, dorsal surface of the carapace exhibits a dense arrangement of closely spaced granules. Larger granules are prominently distributed in the hepatic, branchial, and intestinal regions. Along the posterior margin of the carapace, granules of varying sizes are organised in series, with finer granulation observed along the frontal margin and external maxillipeds. The pterygostomial region is distinctly adorned with prominent granules.

Anterior edge of the epistome displays a central indentation bordered by two acute inner angles located adjacent to the afferent branchial canals. The anterior margin of the abdominal sulcus is densely granulated, contributing to its distinct texture. Granulation on the cheliped's merus is notably coarse on the anterior-

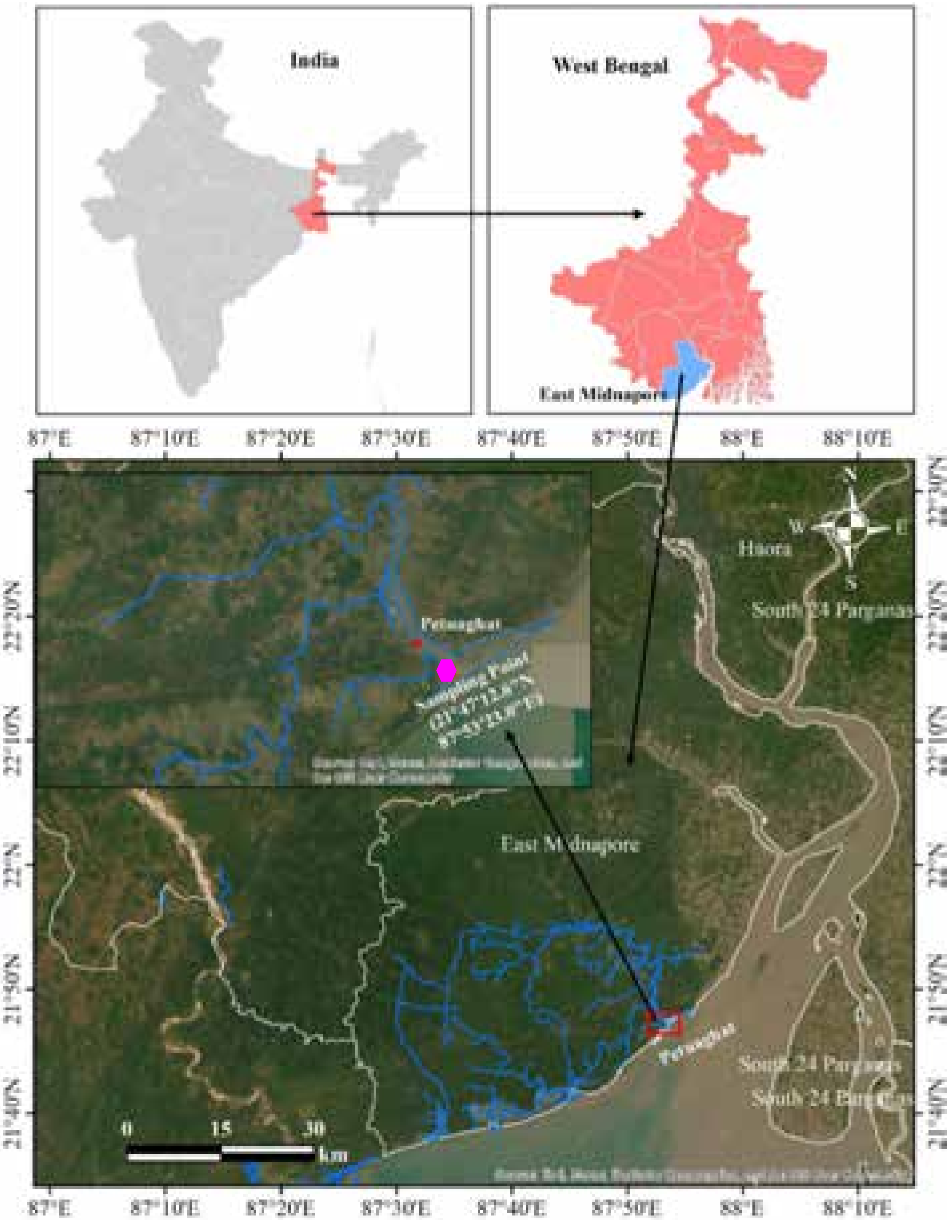

Image 1. Collection sites of *Lyphira perplexa*: ⬢—Petuaghat seabeach area sampling site | *—Petuaghat fishing harbour sampling site.

posterior surface and the proximal region, gradually diminishing in size and prominence towards the distal region. The dorsal and ventral surfaces of the propodus, as well as the ventral inner surface, exhibit a pattern of conical granules (Image 2).

In the case of pereiopods, merus of the first pereiopod is characterized by a ventral row of spherical granules, whereas the meri of the second to fourth pereiopods possess elongated clusters of finer granules along their ventral margins. The thoracic sterna exhibit fine granulation, particularly in the anterior segments, with an absence of granules noted on the dorsal aspect of the apron. The gonopod is elongated, with its apical region covered in dense, hair-like structures. The tip of the gonopod is smooth and devoid of setae, culminating in a prominent, pointed aperture (Image 3).

Fresh Colouration: The carapace exhibits a rosy-brown hue. The merus of the anterior cheliped transitions from pinkish-brown at the proximal region to a lighter brown towards the propodus and dactylus. The distal portion of the chelae is whitish, with the tips of the fingers distinctly white. The carpus, thoracic sternum, and abdominal regions are uniformly creamy white, providing a distinct contrast to the darker appendages.

Habitat: Inhabits sandy or muddy seabed in shallow marine environments, such as intertidal zones, estuaries,

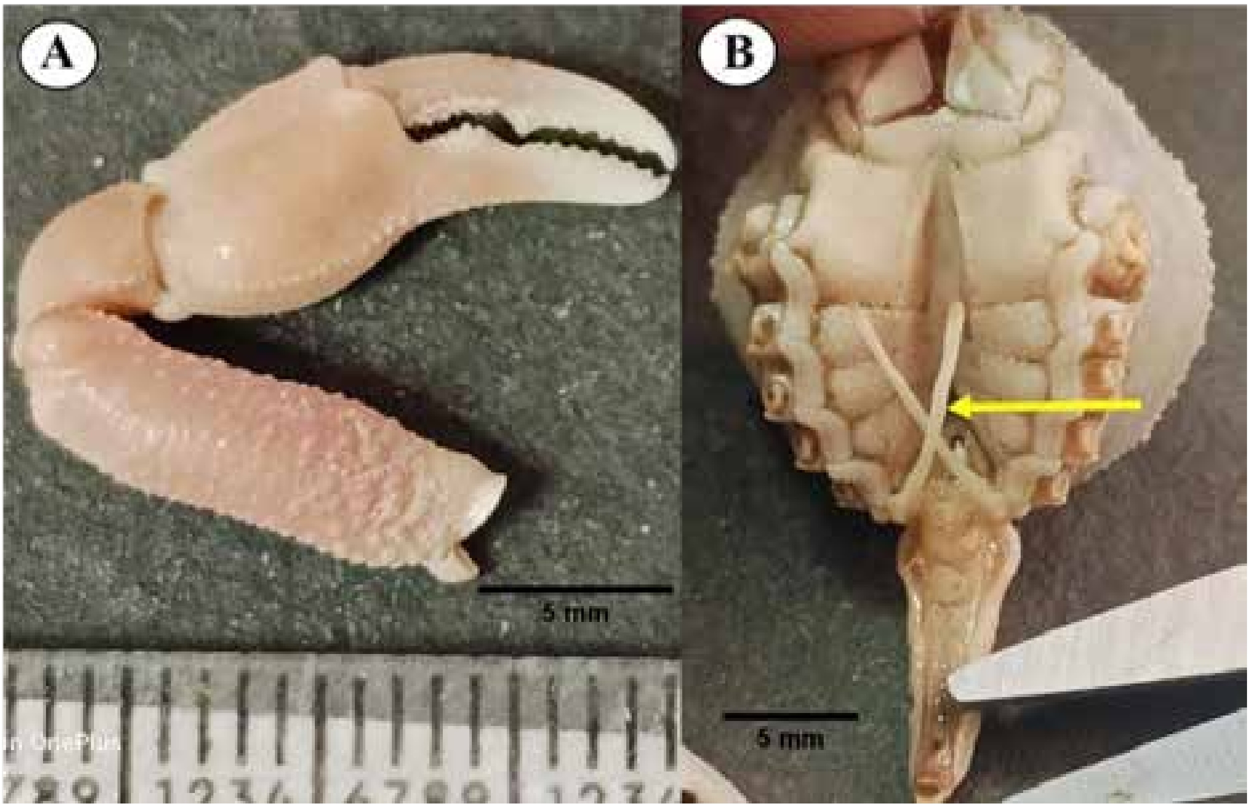

Image 2. *Lyphira perplexa* (male): A—Dorsal view | B—Ventral view. © Authors.

and coastal areas.

Distribution: This species is primarily distributed across various regions in the Indo-West Pacific including the Gujarat coast, Kerala coast, Andaman Islands, Persian Gulf, Gulf of Oman, Kuwait, Iranian coasts, Arabian Gulf, and in the present study on the West Bengal coast of India (Galil 2009; Sudharma et al. 2014; Trivedi et al. 2016; AL-Maliky 2020).

Remarks: The species *L. perplexa* Galil, 2009 was first recorded from off the coast of Calicut, Kerala, by Sudharma et al. (2014) based on a male specimen, and they conducted the first DNA barcoding of the species in Indian waters. In 2013, Trivedi & Arya collected one male and one female specimen of *L. perplexa* from the Cochin Fishing Harbour, Kerala, India. Subsequently, they conducted a comparative analysis between *L. perplexa* and *L. georgei* to identify distinguishing morphological characteristics (Trivedi et al. 2016). The genus *Lyphira* differs from *Philyra* Leach, 1817 in several morphological features: it has an ovate exopod on the external maxilliped, a transversely narrow first abdominal segment in males, fused second to sixth abdominal segments, and lacks the subterminal denticle typically found on the fused male abdominal segments.

Table 1. Measurements of two *Lyphira perplexa* Galil, 2009 (male).

Parameters	Sample 1	Sample 2
Carapace		
Carapace Width (CW)	18.90 mm	17.67 mm
Carapace Length (CL)	18.68 mm	17.42 mm
Carapace Depth (CD)	3.04 mm	2.67 mm
Abdomen		
Abdomen Width (AW)	4.74 mm	4.50 mm
Total Abdomen Length (AL)	13.03 mm	12.3 mm
Telson Width (TW)	1.78 mm	1.45 mm
Penultimate Segment Length (PSL)	3.44 mm	3.22 mm
Penultimate Segment Width (PSW)	2.59 mm	2.38 mm
Abdomen Area (AA)	39.39 mm^2	37.02 mm^2
Appendage		
Cheliped's Dactylus Length (CDL)	8.83 mm	8.23 mm
Cheliped's Propodus Length (CPL)	13.55 mm	12.59 mm
Cheliped's Propodus Depth (CPD)	4.84 mm	4.51 mm
Cheliped's Carpus length (CCL)	5.86 mm	5.47 mm
Cheliped's Merus Length (CMEL)	13.02 mm	12.15 mm
Cheliped's Merus Width (CMEW)	3.82 mm	3.47 mm
4^{th} Pereiopod Merus Length (4PML)	5.60 mm	5.18 mm
4^{th} Pereiopod Merus Width (4PMW)	1.25 mm	1.13 mm

Image 3. A—Right cheliped of *Lyphira perplexa* (male) | B—The sternopleonal cavity of *Lyphira perplexa* (male) showing G1 (first gonopod). © Authors.

L. perplexa can be distinguished from *L. heterograna* by the finer granule size on the surface and margins of the carapace (AL-Maliky 2020). While *L. georgei* is morphologically closest to *L. perplexa*, the proximal margin of *L. georgei* is gently concave, whereas in *L. perplexa*, it is deeply concave. Additionally, the male first left gonopod of *L. georgei* has a medial angular turn, whereas in *L. perplexa*, it is nearly straight (Trivedi et al. 2016).

CONCLUSION AND FUTURE DIRECTIONS

This study documents the first observation of the brachyuran crab *L. perplexa* Galil, 2009, from the West Bengal coast of India. Previously unreported along the eastern coast of India, this record extends the known distribution range of the species. Such findings enhance our understanding of regional biodiversity and contribute valuable data toward the effective management and conservation of marine ecosystems (Silambarasan et al. 2015). In order to improve these results, future studies should focus on molecular confirmation of the species' identity to strengthen taxonomic accuracy, conduct population ecology studies to assess habitat preferences and conservation requirements, and map out comparative distributions in other coastal areas of India. Through these efforts, we will be able to better understand the ecological significance of *L. perplexa* and develop strategies for managing and protecting it in rapidly changing marine environments.

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COMMUNICATION

An evasive naticid surfaces in India: first confirmed report of *Gennaeosinum perobliquum* (Dautzenberg & Fischer, 1907) (Gastropoda: Naticidae)

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Abstract: This study provides the first record of the naticid species *Gennaeosinum perobliquum* in Indian waters. Previously documented from Vietnam, Indonesia, and South Africa, *G. perobliquum* was collected during a benthic survey, from the intertidal zone of Chandipur, Balasore, Odisha, on the eastern coast of India. A clear description of the shell morphology is provided, along with detailed morphometric and imaging analyses. By comparing *G. perobliquum* with related naticid species found in India, this work aims to refine taxonomic understanding and contribute to knowledge of naticid biodiversity in the region. This newly documented record not only expands the known geographic distribution of *G. perobliquum* but also underscores the importance of continued benthic faunal surveys for further exploration of molluscan biodiversity in the Bay of Bengal and adjacent regions.

Keywords: Bay of Bengal, benthos, Chandipur, first record, geographic distribution, Indian waters, intertidal, mollusc biodiversity, moon shells, Odisha.

Abbreviation: TSL—Total Shell Length | WB—Width of the last whorl | HB—Height of the last whorl | SL—Spire Length | CL—Columella Length | UL—Length of the Umbilical callus | $(AW)_a$—Aperture Width towards anterior side | $(AW)_b$—Aperture Width towards posterior side | dd—Dead specimen | RZEVB—Ravenshaw University Zoology Environmental Science Budhabalanga Estuary | ZSI M—Zoological Survey of India Mollusca.

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Competing interests: The authors declare no competing interests.

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Author contribution: Conceptualization: Dipti Raut, N. V. Subba Rao; Methodology: Dipti Raut, Aparna Mishra, Sanjaya Dalai; Data Collection: Sanjaya Dalai, Aparna Mishra, Roberto Ardovini; Data Analysis: Aparna Mishra, Sanjaya Dalai, Roberto Ardovini; Writing Original Draft: Aparna Mishra, Sanjaya Dalai; Writing Review and Editing: Dipti Raut, N. V. Subba Rao, Roberto Ardovini; Supervision: Dipti Raut

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INTRODUCTION

Members of the cosmopolitan family Naticidae within the phylum Mollusca and class Gastropoda inhabit a wide range of environments, from intertidal to the deep sea. Commonly called moon shells, these oval-shaped predatory gastropods are known for their distinctive drilling behavior, which they use to prey on various bivalves and gastropods. They typically dwell in sandy and muddy substrates, where they remain hidden (Huelsken et al. 2008). The Naticidae family includes four subfamilies, 38 genera, and about 260–270 species (Kabat 1996; WoRMS 2025).

The genus *Gennaeosinum* Iredale, 1929, comprises three valid species. Kilburn (1976) classified it as a subgenus of *Eunaticina*; *Gennaeosinum* is consistently characterized by a thick funicle, a feature absent in all *Eunaticina* species. Although 36 Naticidae species across 12 genera have been documented in India, no *Gennaeosinum* species has been reported from the region (Tripathy et al. 2024). This study documents the first record of *Gennaeosinum perobliquum* (Dautzenberg & Fischer, 1907) in the Bay of Bengal, expanding its known distribution. *Gennaeosinum perobliquum* was described initially from "Ben-Son" (probably Binh Son, south of Da Nang) in Vietnam (Dautzenberg & Fischer 1907) and later recorded from Durban, Kwazulu-Natal, South Africa (Kilburn 1976) and Indonesia (Bakker et al. 2026). This species is often misidentified as *Eunaticina papilla* (Gmelin, 1791) due to similar spiral lirae patterns. As detailed here, *G. perobliquum* can be reliably distinguished from *Eunaticina* species occurring in India.

A recent study (Mishra et al. 2024) identified the naticid *Sinum laevigatum* (Lamarck, 1822) along Odisha's coast, underscoring the need for further research on Naticidae in this region. Consequently, extensive surveys were conducted along Odisha's entire coastline, with particular focus on the intertidal zone of Chandipur, where specimens were collected.

This study not only adds *G. perobliquum* to the list of species known in Indian waters but also highlights the rich malacofaunal diversity present along the largely unexplored eastern coast of India. It provides a thorough examination of the specimen, including the holotype description and comparative analyses with closely related species, aiming to mitigate future taxonomic ambiguities. Enhanced photographic documentation and detailed morphometric measurements are included to enrich the understanding of shell sculpture and address existing gaps in the literature.

MATERIALS AND METHODS

A survey was conducted in the Chandipur intertidal region (21.455° N, 87.045° E) (Figure 1) during September 2025. Samples were randomly collected during low tide by hand-picking and stored; dead specimens were placed in ziplock pouches, and live specimens were preserved in 4% formalin. Dead specimens were carefully washed, air-dried, and sorted. Sediment samples were obtained through manual excavation using a hand shovel for the purpose of analyzing soil texture. Morphometric measurements were obtained using a dial caliper (Safeseed, China; resolution 0.1 mm, accuracy ± 0.2 mm), while shell surface structures were examined under a stereo microscope (Leica E24W, Germany). Specimen photographs were captured with a Nikon Z 30 camera (MC 50 mm / 2.8 f). Following vouchering, specimens were deposited in the Environmental Science Laboratory, Department of Zoology, Ravenshaw University, Cuttack, Odisha, India.

Morphological identification was performed based on the holotype description (Dautzenberg & Fischer 1907), supplemented by additional information from Kilburn (1976). A critical comparison was made with other naticids reported from India, as documented by Rao (2003). The taxonomic status was verified through the World Register of Marine Species (WoRMS 2025).

RESULT

Systematics

Family: Naticidae Guilding, 1834
Subfamily: Sininae Woodring, 1928
Genus: *Gennaeosinum* Iredale, 1929
Type species: *Gennaeosinum peleum* Iredale, 1929 (type by original designation)

***Gennaeosinum perobliquum* (Dautzenberg & H. Fischer, 1907) (Image 1A–H)**

Chresonomy

Sigaretus (Eunaticina) perobliquus Dautzenberg & H. Fischer, 1907: p. 178, pl. 5, figures 4–5.

Eunaticina papilla (non Gmelin); Kensley, 1973: 93, fig. 323.

Eunaticina (Gennaeosinum) perobliqua (Dautzenberg & Fischer, 1907) - Kilburn 1976; p. 872, figure. 22 (c).

Eunaticina (Gennaeosinum) perobliqua (Dautzenberg & Fischer, 1907) - Bakker H, Gill A, Creuwels J (2026). Naturalis Biodiversity Center (NL) - Mollusca. Naturalis Biodiversity Center. Occurrence dataset https://doi.

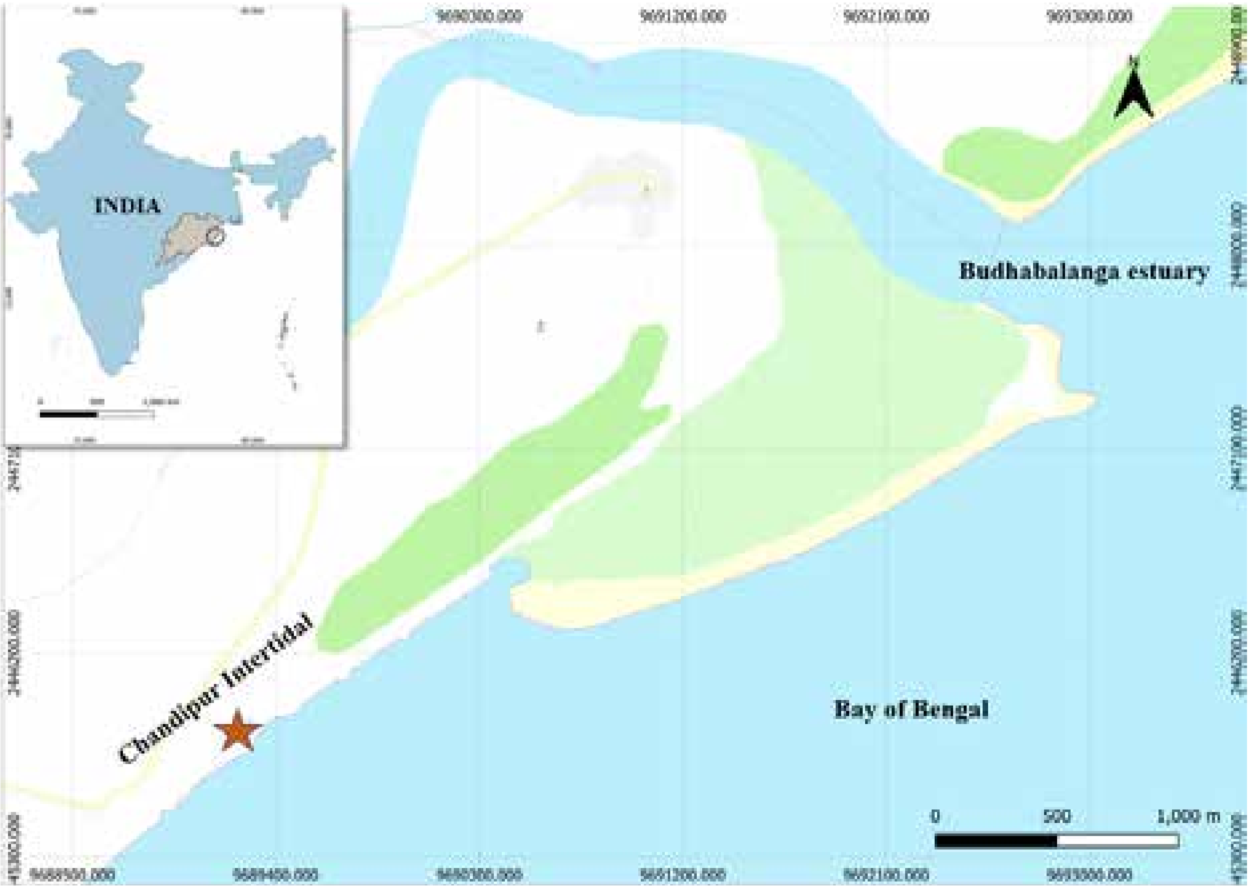

Figure 1. Map showing the sampling location of the Chandipur intertidal region, Odisha, eastern coast of India.

org/10.15468/yefvnk accessed via GBIF.org on 2026-05-05. https://www.gbif.org/occurrence/2444340122.

Type Locality: "Ben-Son" (probably Binh Son, south of Da Nang) in northern Vietnam

IUCN status: Not Evaluated.

Material examined: Two specimens (dd), Accession number: RZEVB18 (TSL 13.5 mm, WB 10.2 mm); RZEVB19 (TSL 13.9 mm, WB 10.5 mm) were examined from the Chandipur intertidal, Balasore, Odisha, eastern coast of India (21.455° N, 87.045° E), coll. Sanjaya Dalai & Aparna Mishra.

Additional material studied: Figured type material of *Sigaretus* (*Eunaticina*) *perobliquus* Dautzenberg & Fischer, 1907 (images examined from Dautzenberg & Fischer 1907; p. 178, pl. 5, figure 4–5).

Topotype of *Eunaticina papilla* (Gmelin, 1791) (Zoological Survey of India, Registration number ZSI M 22150/4).

Specimen of *Eunaticina linnaeana* (Récluz, 1843) (Zoological Survey of India, Kolkata, Registration number M25925/5).

Description: Shell solid, white, moderately elongated oval; aperture strongly prosocline. Low, depressed spire with blunt tip; deep suture. Protoconch slightly convex, smooth, glossy white with brown tinge; two whorls. Transition to teleoconch not well defined; feeble spiral ribs present. Teleoconch with two whorls, last whorl highly convex much larger than previous whorls. Teleoconch surface initially smooth, later marked by strong arc-like growth lines; faint spiral ribs becoming distinct and unevenly distributed on last whorl. Around 9–10 spiral ribs on penultimate whorl, separated by shallow, broad interspaces. Ribs more elevated below suture, flattening with lower interspaces on last whorl. Spiral ribs become denser towards base; growth lines become stronger towards outer lip. Spiral threads continue into inner side of umbilical groove. Aperture semicircular, wider anteriorly. Inner lip almost straight, slight concavity towards parietal region; anterior region curved. Thick parietal callus extends over umbilicus, forming a funicle that covers umbilicus backwardly. Funicle divided by shallow groove. Wide open anteriorly, deep umbilical groove. Operculum corneous.

Remarks: A thick callus pad covers the umbilical

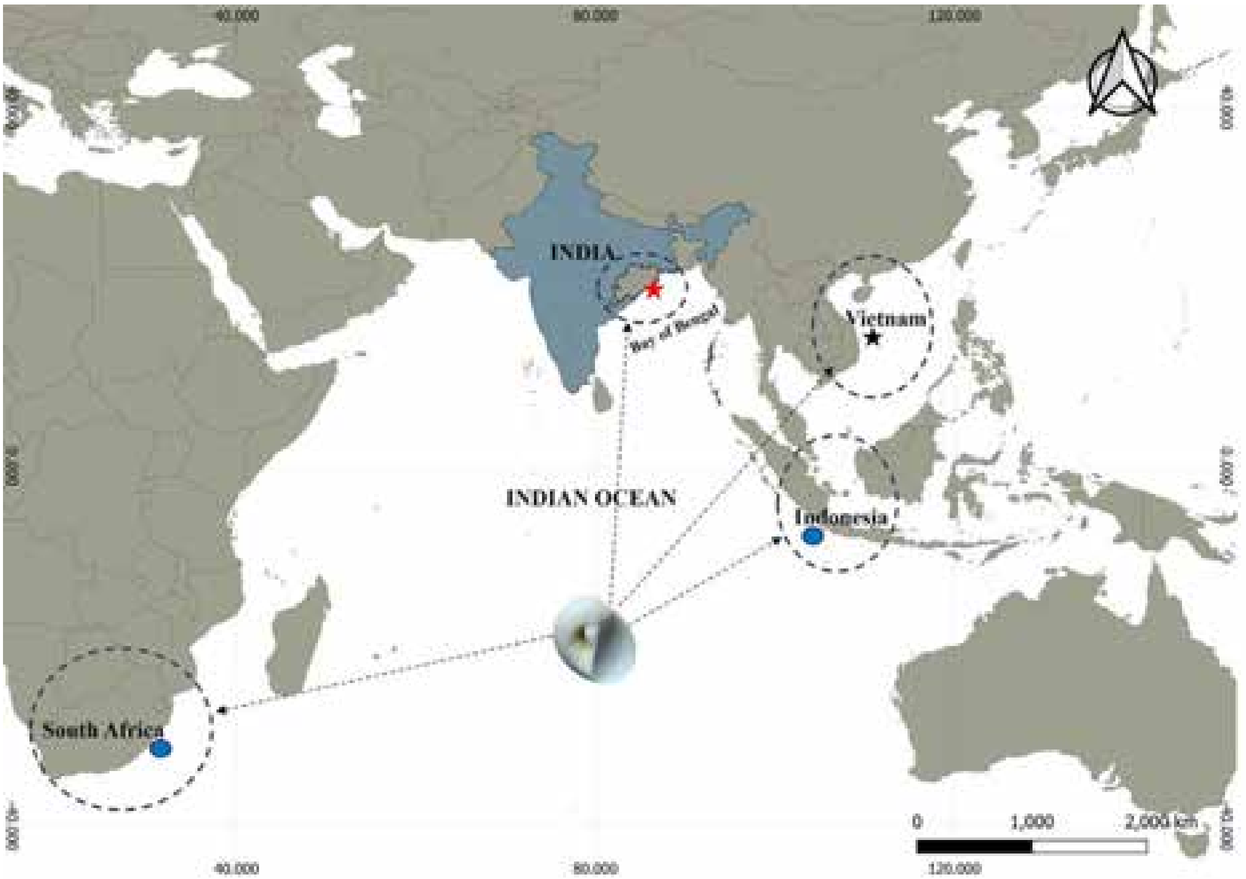

Figure 2. Distribution of *Gennaeosinum perobliquum* in the Indo-West Pacific region (Black star: type locality; Red star: Present report; Blue circles: Natal and Indonesia).

Table 1. Morphometrics of *Gennaeosinum perobliquum* collected from Odisha.

Specimen	TSL	WB	HB	SL	CL	UL	$(AW)_a$	$(AW)_p$
RZEVB18	13.5	10.2	7.8	3.8	11.3	4.0	6.8	3.9
RZEVB19	13.9	10.5	8.9	3.9	11.0	3.8	7.4	4.3

groove and the funicle, which has a shallow groove that divides it into two. These are the key identification features that confirm the first report of the genus *Gennaeosinum* and the species *G. perobliquum* from India. The multi-spiral protoconch with two whorls signifies a planktonic veliger larval stage (Vendetti, 2007). This developmental form implies improved dispersal abilities; therefore a large distribution area is to be expected.

Distribution: From Vietnam (Holotype) through Indonesia (GBIF) and India to South Africa (Kilburn, 1976) (Figure 2).

Locality within India: Chandipur Intertidal, Balasore, Odisha, east coast of India, present study (Figure 2).

Habitat: The species has repeatedly been recorded from shallow water. Chandipur's intertidal stretch shows diverse sedimentary conditions, with a silty mudflat in the middle region. The upper intertidal region has a coarse, sandy substratum, encompassing high diversity.

DISCUSSION

Identification of Naticidae can be challenging because of their highly cryptic morphology and very minor interspecific variation (Sharma et al. 2021). In India, three genera of the Sininae subfamily are represented: *Eunaticina* P. Fischer, 1885, *Sigatica O.* Meyer & Aldrich, 1886, *Sinum* Röding, 1798. The genus *Gennaeosinum* herein reported is the fourth,

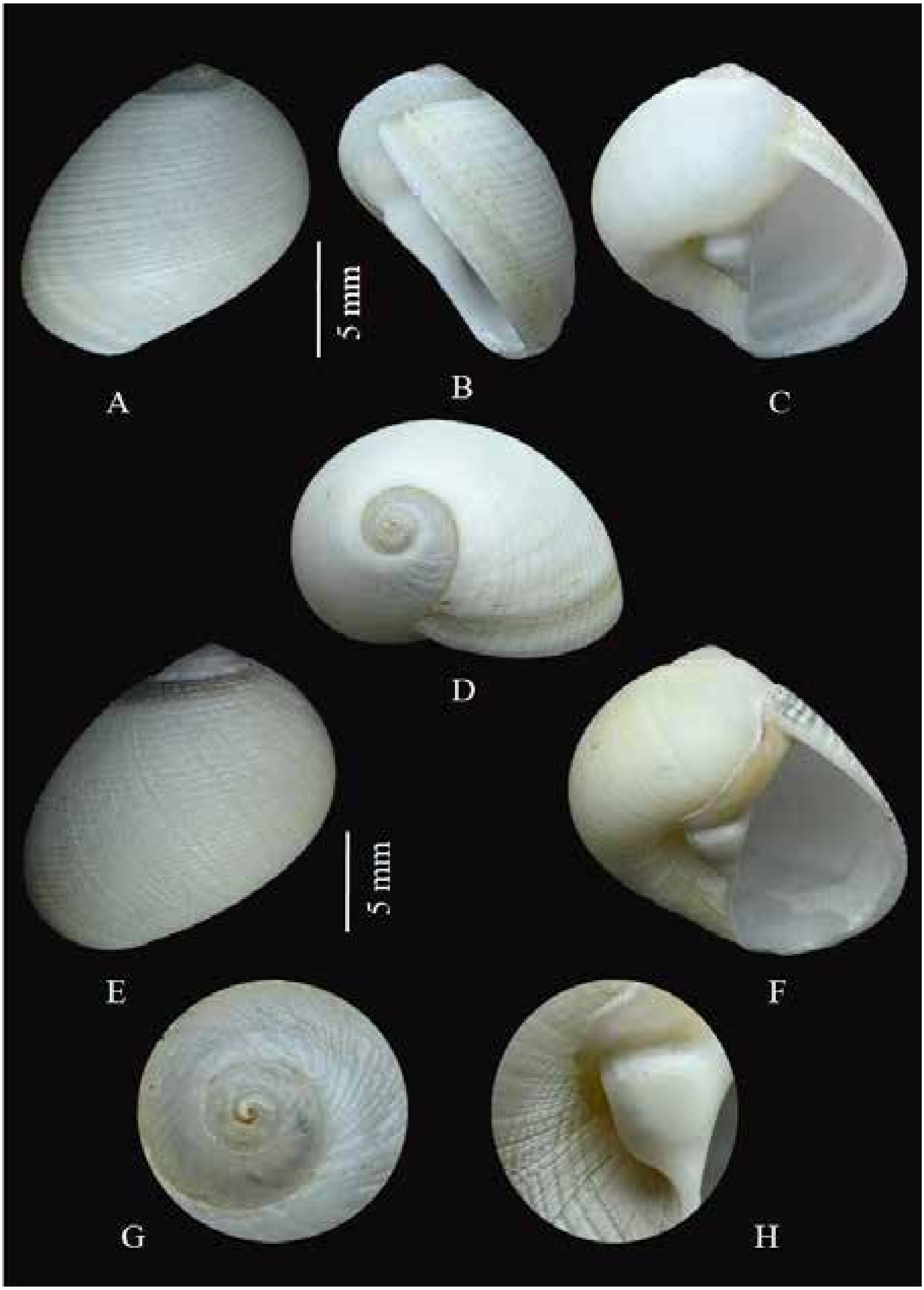

Image 1. *Gennaeosinum perobliquum*. RZEVB18 (A–D): A—dorsal view | B—side view | C—ventral view | D—apical view. RZEVB19 (E–H): E—dorsal view | F—ventral view | G—protoconch | H—callus pad. © Aparna Mishra and Sanjaya Dalai.

represented by a single species. The aforementioned three genera are unique in several ways. *Eunaticina* differs from *Sinum* by its auricular, more depressed shape and wider aperture. *Gennaeosinum* is similar to *Eunaticina* and was treated as its subgenus by Kilburn (1976). *Gennaeosinum* is differentiated from *Eunaticina* by a thick callus pad in the parietal region, which is absent in *Eunaticina*. *Gennaeosinum* includes three species: *G. intercisum* Iredale, 1931, *G. peleum* Iredale, 1929, and *G. perobliquum* (Dautzenberg & Fischer, 1907). Kilburn included several other species, namely *G. kraussi* (E.A. Smith, 1902) and *G. linguifera* Thiele, 1925, but now *G. kraussi* is accepted as *Eunaticina kraussi* whereas *G. linguifera* is accepted as *Natica Linguifera*. This work confirmed the first report of the *Gennaeosinum* genus and the species *G. perobliquum* from Indian marine waters. Previously, this species was found in the western and central Indo-Pacific regions. It now extends its range into the Bay of Bengal. There are few descriptions or images of the species besides the original description, which has excellent figures. Kilburn (1976) did not provide an illustration. The Indian specimens align with the original description and Kilburn's description of the features of the South African specimen.

The species can be confused with two *Eunaticina* species well known from the Indo-West Pacific: *E. papilla* (Gmelin, 1791) and *E. linnaeana* (Récluz, 1843). Kilburn (1976) compared *G. perobliquum* with *E. linnaeana*, but the specimens are more similar *to E. papilla. Eunaticina linnaeana* is larger and broadly ovate, while *G. perobliquum* is smaller and moderately ovate. Both have low spires, but the conical shape distinguishes the latter. *Eunaticina linnaeana* has fine spiral grooves, whereas *G. perobliquum* is ornamented with well-developed, flat spiral ribs. *Eunaticina linnaeana* has an aperture that is wider, elongated, and larger, giving it a distinct look compared to the semicircular opening in *G. perobliquum. Eunaticina linnaeana* has frequently been misidentified as *E. papilla*, leading to similar confusion with *G. perobliquum. E. papilla* has an exserted spire, shallow suture, and ovate aperture. *G. perobliquum* resembles *E. papilla* in size, spiral ribs, and moderately large aperture. Distinctively, *G. perobliquum* stands out from both *Eunaticina* species because it features a thick callus pad in the parietal region, divided into two by a shallow groove, a feature always absents in *Eunaticina*.

Various factors influence the dispersal of naticid species in Indian marine waters. Oceanic currents and ballast water from ships play a major role (Ruiz et al. 2000; Cowen & Sponaugle 2009; Treml et al. 2015). The Bay of Bengal, located in the northern Indian Ocean, shares environmental and habitat characteristics with the central and western Indo-Pacific marine regions. These include high sea surface temperatures, a variety of coastal and open-water habitats, and rich biodiversity, all characteristic of tropical Indo-West Pacific ecosystems (Spalding et al. 2007). Climate change and rising sea temperatures are likely to shift the ecological niches of these species. This could extend their distribution beyond previously established ranges (Wallingford & Sorte 2022; Lin et al. 2024). Changes in species distribution may be hard to establish due to a lack of comprehensive surveys and challenges in species identification, especially given the cryptic nature of *Gennaeosinum perobliquum* (D'Souza & Shenoy 2023). Research on the Naticidae family in India has been limited, particularly along the eastern coast. This highlights the need for further exploration, including the collection of live specimens and the establishment of a DNA database to elucidate intraspecific and interspecific relationships among Indian naticid species.

CONCLUSION

The discovery of *G. perobliquum* underscores significant connections among molluscan fauna in the Indo-West Pacific and highlights the urgent need for a comprehensive taxonomic review of naticid species in India. This study also confirms the limited exploration of malacofaunal diversity along the eastern coast. Additionally, this record expands the regional species list and deepens the understanding of biogeographical patterns in the Indo-West Pacific. These findings are crucial for developing effective conservation strategies and for protecting biodiversity in the region.

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REVIEW

Taxonomic reassessment of *Ompok hypophthalmus* (Bleeker, 1846) (Actinopterygii: Siluriformes: Siluridae) in Indonesia with global implications

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Abstract: Indonesia harbors exceptional freshwater fish diversity, yet taxonomic uncertainty persists for several economically important species, including *Ompok hypophthalmus*. This study provides a critical assessment of the morphological and molecular identification of *O. hypophthalmus* in Indonesia by analyzing published literature and publicly available mitochondrial DNA sequence data retrieved from resources such as NCBI. Morphological revisions by Ng (2003) recognized three distinct taxa within the *O. hypophthalmus* complex (*O. hypophthalmus*, *O. rhadinurus*, and *O. urbaini*), yet subsequent studies have frequently applied species names inconsistently, particularly in Sumatra. Analysis of available cytochrome c oxidase subunit I (COI) and cytochrome b (Cyt b) sequences reveals substantial genetic structuring among river populations, suggesting historical misidentification and possible cryptic diversity. Limited sequence availability and incomplete geographic coverage preclude definitive conclusions regarding species boundaries in some river systems. This study highlights the urgent need for integrative taxonomy, i.e., combining morphology, standardized DNA barcoding, and expanded sampling to resolve species identities and to support effective fisheries management and conservation planning in Indonesian freshwater ecosystems.

Keywords: C oxidase subunit (coi), cryptic diversity, DNA barcoding, fish diversity conservation, mitochondrial dna, morphological identification, molecular taxonomy, river system, species.

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Author contribution: All authors contributed substantially to the conception, design, data collection, analysis, and interpretation of results. All authors participated in drafting and critically revising the manuscript, approved the final version for submission, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Indonesia is home to 4,970 documented fish species, accounting for approximately 15% of the world's fish diversity (Reid et al. 2013; Reis et al. 2016). According to FAO assessments between the late 1990s and 2003, the number of recorded fish species in Indonesia increased by approximately 300. Despite this growth, the global total of fish species rose by over 11,000, reducing Indonesia's proportional share from approximately 25% in 2003 to about 15% by 2016. Of these, approximately 1,258 species represent about 10% of the world's freshwater fish diversity (Gustiano et al. 2021). Dudgeon (2000) estimated that around 1,700 freshwater species are found in Indonesia (Dudgeon 2000). Regarding endemism, 19.5% of the fish species are unique to the region (Gustiano et al. 2021). Among the main Indonesian Islands, Sulawesi boasts the highest number of endemic species, with about 76% of the species on the island being native (Partasasmita et al. 2015). The limited increase in newly documented species and the decline in Indonesia's percentage of global species raise important conservation questions.

Catfishes belonging to the genus *Ompok* (La Cepede) are species within the Siluridae family, commonly found in lentic and lotic systems across southeastern and southern Asia. According to Bornbusch (1995), the genus is paraphyletic, comprised of four distinct clades: group of *O. hypophthalmus* (Bleeker, 1846), group *of O. bimaculatus* (Bloch, 1794), group of *O. eugeneiatus* (Vaillant, 1893), and group of *O. leiacanthus* (Bornbusch, 1995). The group of *O. hypophthalmus* is characterized by cartilaginous plates supporting the mandibular barbels. These plates possess two posterior extensions, in contrast to other conditions where the plates are underdeveloped, exhibit a single dorsolateral extension, or appear elongated and hourglass-shaped without extensions. The term "selais fish" is a local vernacular name used in Sumatra and Kalimantan to refer to several morphologically similar silurid catfishes, primarily within the genus *Ompok*. The non-specific use of this term has contributed to taxonomic ambiguity in both ecological and molecular studies.

The rapid advancement of molecular technology worldwide has significantly enhanced the identification of new species, as highlighted by Kalyankar et al. (2012), Bachry et al. (2019), Patil et al. (2023), and Sontakke et al. (2023). Studies focusing on systematics and evolutionary genetics, including taxonomy and phylogeny, have played a crucial role in documenting genetic resources. The use of DNA barcoding for quick and precise species identification is essential for enhancing fish diversity initiatives (Hubert et al. 2015; Tiknaik et al. 2019). In Indonesia, this method has not been widely adopted, with conventional, taxonomy-based species identification still being the predominant approach.

MATERIALS AND METHODS

Molecular analysis

Mitochondrial DNA sequences of *Ompok* species were retrieved from BOLD Systems and GenBank, including 18 COI and five cytochrome b (Cyt b) sequences with verified locality information. Sequence alignment was performed using MUSCLE implemented in MEGA X v10.x, with manual trimming to equal lengths. Genetic distances were calculated using the Kimura 2-parameter (K2P) model. Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) method with 1,000 bootstrap replicates. Species delimitation was preliminarily explored using GMYC and PTP models based on the COI dataset. Only sequences with unambiguous taxonomic annotation and river-level metadata were included in downstream analyses.

RESULTS

The analysis revealed that the distribution of *O. hypophthalmus* in Indonesia is restricted to specific river systems, suggesting a narrower range than previously reported. The findings highlight the importance of accurate species identification and continuous monitoring, as populations may be vulnerable to environmental changes, habitat fragmentation, and anthropogenic disturbances.

Current Status of *O. hypophthalmus* in Indonesia

The distribution of *O. hypophthalmus* in Indonesia appears to be confined to specific river systems, indicating a more restricted range than previously understood. This emphasizes the need for accurate species identification and continuous monitoring to support effective conservation measures. The habitats of these fish may be vulnerable to environmental changes, habitat fragmentation, and anthropogenic activities.

Morphological identification of *Ompok* species in Indonesia

1. In 2003, Ng reviewed the taxonomy and distribution of the *O. hypophthalmus* complex in

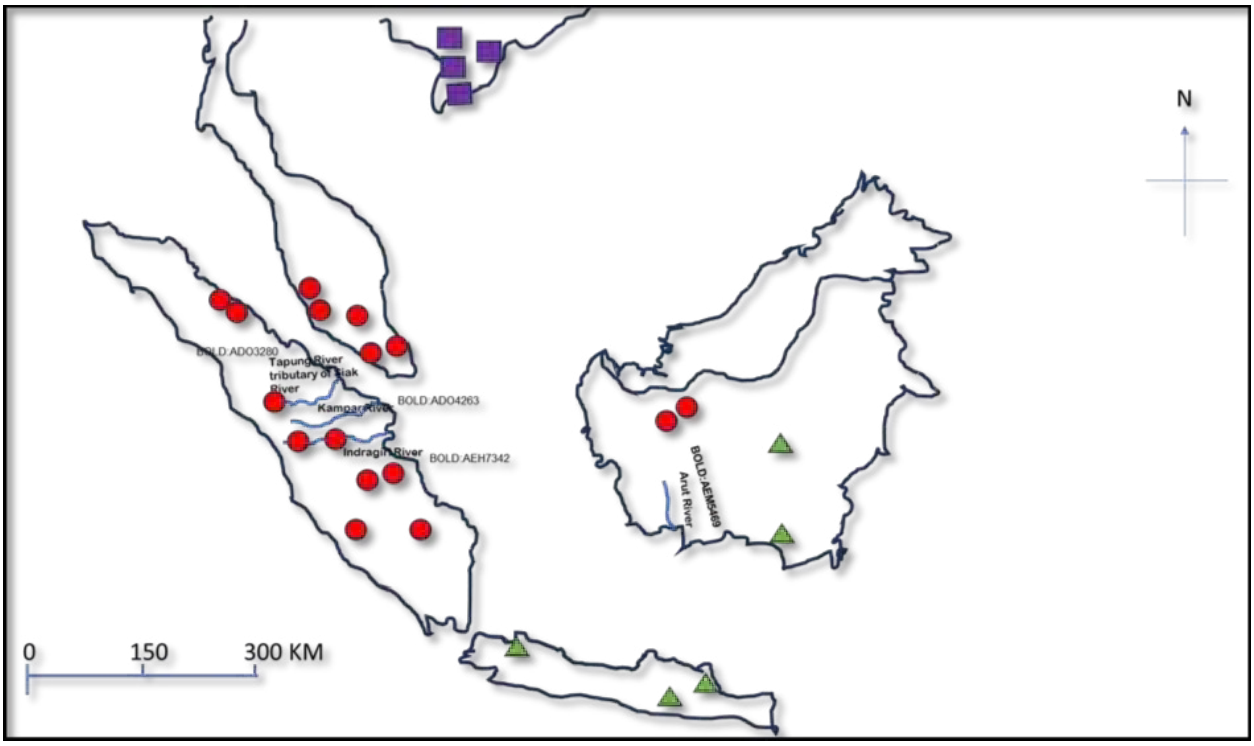

Figure 1. Map of south-east Asia showing distributions according to Ng (2003) of: *Ompok hypophthalmus* ▲, *O. rhadinurus* ●, and *O. urbaini* ■, / indicate rivers.

southeastern Asia, clarifying the identities of *O. hypophthalmus*, *O. urbaini*, and describing a new species, *O. rhadinurus*. The study synonymized *O. macronema* with *O. hypophthalmus* and validated *O. urbaini* as a distinct species, previously misclassified. It also delineated the geographical distributions of these species: *O. hypophthalmus* is found primarily in Java and southern Borneo, *O. urbaini* in Indo-China river systems, and *O. rhadinurus* in Sumatra, Borneo, and the Malay Peninsula. Key morphological differences, such as head width, caudal peduncle depth, and the number of anal-fin rays, were instrumental in distinguishing these species. Ng's findings are illustrated in Figure 1. Despite these clarifications, several subsequent studies appear to have misidentified *O. urbaini* and *O. rhadinurus* as *O. hypophthalmus*.

2. In a biodiversity study conducted in Buaya Lake and the Indragiri River basin in Sumatra, 22 fish species were identified across both habitats. The lake served as a spawning and nursery ground, supporting younger specimens likely originating from the Indragiri River. Among the identified species, *O. hypophthalmus* was reported from the river channel (Nofrizal et al. 2023).

3. Further research on the reproductive biology of *O. hypophthalmus* in the Kampar Kiri River floodplain revealed five stages of gonad maturity, confirming the species as a total spawner and iteroparous (Sjafei et al. 2008). Simanjuntak (2008) examined fecundity, maturity, and spawning patterns of *O. hypophthalmus* and *Clarias macrocephalus* in the same region (Simanjuntak et al. 2008).

4. Elvyra et al. (2010) studied the gonadal maturity, fecundity, and conservation needs of *O. hypophthalmus* in the Kampar River, emphasizing the ecological importance of this species, locally known as 'ikan lais' (Elvyra et al. 2010).

5. Eddy & Gema (2019) reported *O. hypophthalmus* from the Arut–Kumai peat waters; notably, *O. rhadinurus*, which Ng (2003) reported from parts of Borneo, was not mentioned in that study, raising the possibility of misidentification.

6. In the Siak River, *O. rhadinurus* was identified by the original authors in fish catches, highlighting the continued taxonomic challenges in distinguishing these species (Budy et al. 2023).

7. A year-long study in the Mahakam River, East Kalimantan, documented the presence of *O. hypophthalmus* and *O. miostoma* among six catfish species sampled across four locations (Jusmaldi et al. 2018).

Table 1. COI gene sequence details of *Ompok hypophthalmus* available in BOLD and NCBI databases.

Listed species	BIN ID	Sample ID / NCBI ID	Location	Name of scientist who generated sequences
O. hypophthalmus	BOLD:AEM5469	MZ634369, MZ634366, MZ634368, MZ634372, MZ634367, MZ634371, MZ634373, MZ634370, MZ634374	Arut River, Central Kalimantan, Indonesia	Kasayev, T. & Arisuryanti, T.
	BOLD:ADO3280	MH732890, MH732889, MH732891, MH732887	Tapung River	Elvyra, R.
	BOLD:AEH7342	MK473379, MK473378, MK473377, MH732888	Indragiri River	Elvyra, R.
	BOLD:ADO4263	MH732886	Kampar River	Elvyra, R.

Molecular identification and DNA barcoding evidence

As of late 2024, only 18 COI gene sequences and five cytochrome b (Cyt b) sequences of *O. hypophthalmus* have been submitted to the BOLD and NCBI databases. These sequences were contributed primarily by two research groups led by Elvyra R. and Kasayev T. (Refer to Table 1).

1. A study by Kasayev & Arisuryanti (2022) analyzed nine COI sequences of selais fish from the Arut River in Central Kalimantan, suggesting their identity as *O. hypophthalmus* (Kasayev & Arisuryanti, 2022). These findings revealed up to 3.6% nucleotide divergence among COI sequences, indicating substantial genetic structuring and raising uncertainty regarding conspecificity.

2. The 18 COI sequences available in the BOLD database are categorized into four Barcode Index Numbers (BINs), each corresponding to a distinct river system: the Arut, the Tapung, the Indragiri, and the Kampar Rivers (Refer to Figure 1 & Table 1).

3. Elvyra et al. (2020) assigned Cyt b sequences to *O. hypophthalmus* based primarily on morphological identification, as comparative Cyt b reference sequences for confirmed *O. hypophthalmus* were not available at the time. Consequently, these assignments should be interpreted cautiously (Elvyra et al., 2020).

4. Arisuryanti et al. (2020) investigated genetic variation in selais fish from the Arut River by analyzing polymorphisms in the partial 16S mitochondrial gene. Their findings revealed intra-population genetic variation and suggested that the selais fish represents a single species, although its specific taxonomic name remains unresolved.

This growing body of molecular evidence underscores notable genetic diversity among populations currently identified as *O. hypophthalmus* and highlights the importance of integrating molecular tools with morphological analyses for accurate species identification.

DISCUSSION

Reassessment of the distribution of *O. hypophthalmus*

This section synthesizes evidence from both morphological taxonomy and molecular barcoding to reassess the reported distribution of *O. hypophthalmus* in Indonesia. Both morphological and molecular approaches complement each other, and each has inherent strengths and limitations. The following synthesis summarizes the reassessment of *O. hypophthalmus* distribution in Indonesia.

Morphological status of *O. hypophthalmus* in Indonesia

1. Ng (2003) provided a comprehensive morphological revision of the *O. hypophthalmus* species complex, recognizing *O. hypophthalmus*, *O. urbaini*, and describing *O. rhadinurus* as a new species. Clear diagnostic characters and geographically structured distributions were established; however, these revisions have not been consistently applied in subsequent Indonesian studies.

2. Following Ng's (2003) revision, several studies from Indonesia did not consistently apply the revised diagnostic criteria and continued to report *O. hypophthalmus* in regions where *O. rhadinurus* was expected.

3. Several studies conducted in the Kampar River have investigated reproductive biology and gonadal development of specimens identified as *O. hypophthalmus* (Simanjuntak et al. 2008; Sjafei et al. 2008; Elvyra et al. 2010). Ng (2003) did not report *O. hypophthalmus* from the Kampar River based on morphological evidence.

4. Most subsequent studies focused on ecological or fisheries-related aspects rather than taxonomic validation, and only a limited number explicitly referenced Ng's (2003) revision (Akbar et al. 2020; Nofrizal et al. 2023).

5. In 2023, Budy and Isnaniah mentioned that the *O. rhadinurus* species was observed in Benayah Village,

Table 2. Distance matrix within and between the BIN ID. (Values outside parentheses represent maximum K2P distance (%), while values in parentheses represent mean distance).

BIN ID	BOLD:AEM5469	BOLD:ADO3280	BOLD:AEH7342	BOLD:ADO4263
BOLD:AEM5469	0 (0)			
BOLD:ADO3280	4.55 (1.9)	0.17 (0.09)		
BOLD:AEH7342	4.03 (1.66)	1.61 (0.89)	0	
BOLD:ADO4263	16.57 (3.27)	15.53 (6.09)	16.16 (6.29)	0

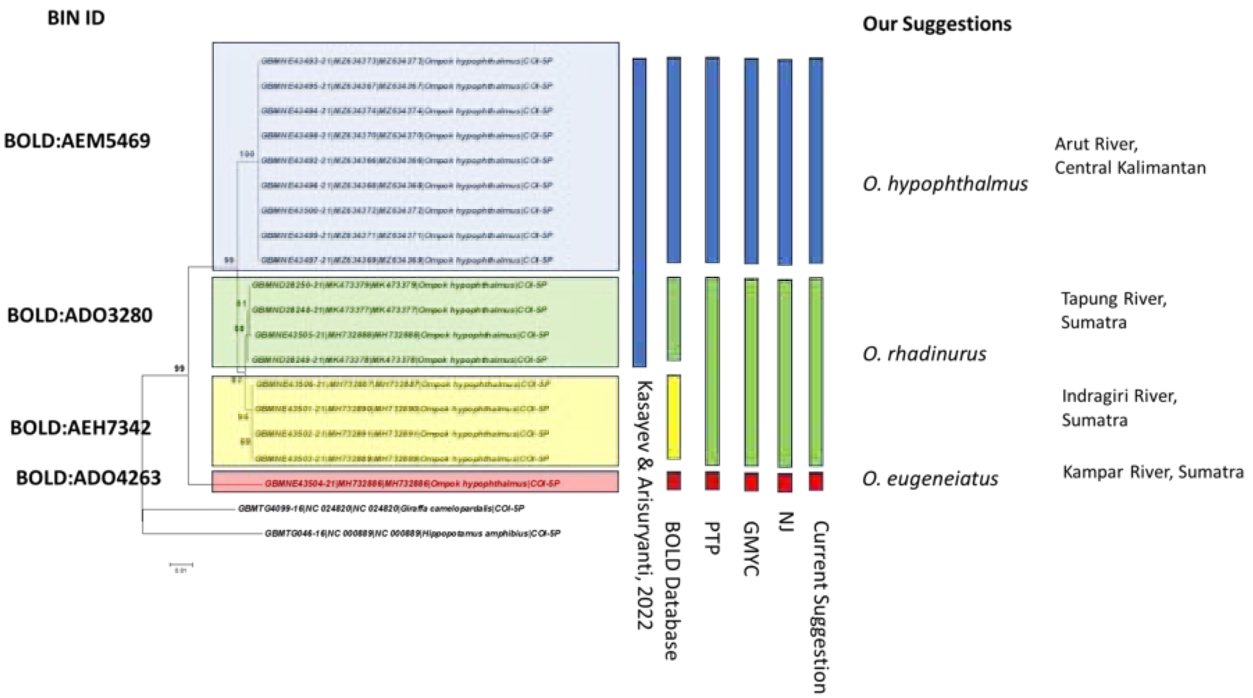

Figure 2. Phylogenetic tree relationships inferred using NJ, GMYC, PTP based on COI sequences; bootstrap values >50% are shown.

Pusako District, Siak Regency (Budy et al. 2023).

Molecular identification status of *O. hypophthalmus*

1. Elvyra et al. (2020) investigated the molecular characteristics and phylogenetic relationships of silurid catfishes from the Kampar River in Indonesia, focusing on the cytochrome b gene. They found four species, including *Kryptopterus limpok*, *O. eugeneiatus*, *O. hypophthalmus*, and *Phalacronotus apogon* (Elvyra et al. 2020).

2. A study by Kasayev & Arisuryanti (2022) mentioned selais fish from the Arut River in central Kalimantan, confirming their identity as *O. hypophthalmus*. In the same study, COI sequences from specimens collected in the Indragiri River were assigned to *O. hypophthalmus*; based on Ng's (2003) morphological framework and observed genetic divergence exceeding 3%, these assignments remain uncertain. This level of divergence suggests the possibility of taxonomic misidentification or cryptic diversity; additional integrative analyses are required before proposing taxonomic speciation (Kasayev & Arisuryanti 2022).

3. Arisuryanti et al. (2020) investigated 16S genetic variation in selais fish from the Arut River; the precise taxonomic identity of these specimens remained unresolved.

Proposed reassessment of *Ompok* species distributions in Indonesian Rivers

Based on the synthesis of available morphological and molecular evidence, the following interpretations are proposed:

Kampar River

1. Available morphological and Cyt b data suggest the presence of at least two silurid taxa in the Kampar River (*O. eugeneiatus* and specimens currently identified

as *O. hypophthalmus*). The absence of comparative COI data prevents definitive confirmation of species identity.

Indragiri & Tapung Rivers

2. Based on Ng's (2003) morphological revision and available COI divergence values, specimens from the Indragiri and Tapung Rivers are more consistent with *O. rhadinurus* than *O. hypophthalmus*. Nevertheless, additional integrative sampling is required to confirm this reassignment.

CONCLUSION

This study highlights persistent taxonomic inconsistencies in the identification of *O. hypophthalmus* in Indonesia. While molecular data reveal clear genetic structuring among river populations, current evidence is insufficient to fully resolve species boundaries across all regions. Integrative taxonomy combining standardized morphological assessment and expanded DNA barcoding is essential before definitive biogeographic conclusions can be drawn. Accurate species identification is critical for fisheries management, conservation planning, and preventing the perpetuation of taxonomic confusion in Indonesian freshwater fishes. Accurate taxonomic recognition of *O. hypophthalmus* is critical for fisheries management and conservation assessments, as historical misidentifications may have influenced stock evaluation, biodiversity estimates, and regional Red List assessments across its reported distribution. The conclusions of this study are limited to a small number of publicly available sequences, uneven geographic sampling, and the lack of integrated morphological examination of sequenced specimens.

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SHORT COMMUNICATION

Recent sighting of Black Baza *Aviceda leuphotes* Dumont, 1820 (Aves: Accipitriformes: Accipitridae) in Nandhaur Wildlife Sanctuary, Uttarakhand, India

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Abstract: The Black Baza *Aviceda leuphotes* is a small migratory raptor of the family Accipitridae, primarily distributed across northeastern India, the eastern Himalaya, and southeastern Asia, with scattered seasonal records from peninsular and western India. Validated records from northern India remain limited. A single individual was observed on 20 December 2025 in a semi-evergreen forest-edge habitat near Chorgaliya, Uttarakhand. The identification was confirmed using diagnostic plumage characters and photographic evidence. Previous records from Maharashtra, Odisha, Tamil Nadu, and Puducherry suggest that the species exhibits broader seasonal occurrence patterns across India than previously understood. The present observation represents the first scientifically documented record of the species from the Nandhaur landscape and constitutes a notable northwestern range extension within the Indian subcontinent. The occurrence during winter further supports the possibility of seasonal dispersal or migratory movement from eastern populations. This record highlights the ecological importance of the Terai–Bhabar landscape as a potential stopover or seasonal foraging habitat for migratory raptors and emphasizes the need for long-term monitoring of avifaunal movements in the Himalayan foothills.

Keywords: Avifauna, Himalayan foothills, migratory raptor, northern India, passage migrant, seasonal dispersal, species distribution, Terai-Bhabar landscape.

The Black Baza *Aviceda leuphotes* is a small, stocky, pigeon-sized raptor belonging to the family Accipitridae. It is primarily distributed across the eastern Himalayas, northeastern India, southern and southeastern Asia, and parts of peninsular India, with migratory tendencies observed in several regions of its range (Ali & Ripley 1978; Ferguson-Lees & Christie 2001; Purohit et al. 2017). In India, the species is typically associated with broad-leaved evergreen forests of the Western Ghats, northeastern India, and the Andaman Islands, though sporadic and seasonal records suggest a wider distribution (Rasmussen & Anderton 2012).

Morphologically, the Black Baza is characterized by a distinctive long, spiky crest, black head and upperparts, and contrasting white and chestnut barring on the underparts. In flight, it exhibits a striking black-and-silver pattern with broad, paddle-shaped wings. Sexual dimorphism is evident, with females generally larger

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NANDHAUR WILDLIFE SANCTUARY

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and exhibiting more prominent barring than males (Ferguson-Lees & Christie 2001; Sivakumar & Prakash 2004). Ecologically, the species is usually solitary or found in small family groups. It feeds primarily on lizards, frogs, and insects, often employing aerial sallies from canopy perches to capture prey (Ali & Ripley 1978). Due to its reliance on intact forest canopies and diverse prey availability, the Black Baza is considered an indicator of healthy forest ecosystems.

Across the Indian peninsula, the species has been recorded as a passage migrant or rare winter visitor, including regions such as the Eastern and Western Ghats, Maharashtra, and parts of eastern India (Rane & Borges 1987; Bapat & Wadatkar 2015). Verified occurrences from Bhimashankar (Rane & Borges 1987), Vidarbha (Bapat & Wadatkar 2015), and the Gupteswar forests of Odisha (Purohit et al. 2017) suggest a broader and more dynamic distribution pattern than previously understood (Rane & Borges 1987; Bapat & Wadatkar 2015). Historical and recent sightings from southern India, including Chennai, Puducherry, and Kancheepuram, further support its status as a seasonal migrant in certain regions (Santharam 1981, 2009; Boobalan 2017; Nagarajan 2017).

The December 2025 bird census conducted in Nandhaur Wildlife Sanctuary represents the first scientifically documented record of the Black Baza in this landscape, thereby extending its known distribution range in northern India. The species is currently classified as Least Concern on the IUCN Red List and is protected under Schedule I of the Wildlife (Protection) Act, 1972. Although considered locally uncommon and sensitive to habitat degradation, its presence signifies good forest canopy integrity, adequate prey availability, and minimal anthropogenic disturbance, reinforcing its importance as an ecological indicator species.

Methods

The study was conducted in the Nandhaur Wildlife Sanctuary, established in 2012, located in the Terai–Bhabar landscape of Uttarakhand, India (Mehra 2015). The sanctuary extends between 79.675°–80.009° E & 29.138°–29.184° N and is bounded by the Gola River in the west and the Sharda River on the eastern side, providing connectivity with Shuklaphanta National Park. It forms a part of the Terai Arc Landscape (TAL). The vegetation is primarily tropical moist deciduous forest dominated by *Shorea robusta*, along with associated species such as *Terminalia tomentosa* and *Adina cordifolia* (Champion & Seth, 1968). The sanctuary covers approximately 270 km² and comprises four ranges: Nandhaur, Jaulasal, Danda, and Sharda, with perennial rivers such as Nandhaur and Kalaunia and several seasonal streams.

Field observations were carried out through systematic visual surveys during early morning and mid-afternoon across forest clearings, canopy zones, and edges. The Black Baza was identified based on distinctive plumage, crest, and flight characteristics (Purohit et al., 2017). Line transect and point count methods were employed, with notable sightings supported by photographic documentation and GPS coordinates, following standard ornithological protocols (Bapat & Wadatkar 2015).

Birds were primarily observed with binoculars (Olympus 8 × 40, Nikon 12 × 48, Nikon 8 × 25), while distances and perch heights were estimated using a Nikon Forestry Pro II Laser Rangefinder. Geographical coordinates of all sightings were recorded using a Garmin eTrex 30x GPS device. Species verification and record-keeping were supported by the Merlin Bird ID and eBird mobile applications. Photographs were captured using Nikon and Canon cameras, and plumage identification was cross-referenced with a standard field guide Grimmett et al. (2011).

Results

On 20 December 2025 at 1407 h, during the bird census in Nandhaur Wildlife Sanctuary, a single Black Baza was observed foraging above the forest canopy in a mixed deciduous forest-edge patch near Chorgaliya in the Haldwani Forest Division (29°07'52"N, 79°42'19"E ; elevation ~350 m) (Figure 1; Table.1). The bird was seen soaring briefly above the canopy before making short aerial movements, suggestive of active foraging behaviour. The individual was identified based on distinct morphological features, including black upperparts, prominent crest, and contrasting white markings on the flight feathers (Image 1). The underwing pattern and overall flight silhouette were consistent with descriptions provided by Purohit et al. (2017) and Bapat & Wadatkar (2015), confirming the species as Black Baza. The habitat comprised semi-evergreen forest with a mosaic of dense canopy and open woodland patches. The bird remained in the area for a short duration before flying deeper into the forest and was not relocated during subsequent observations. No evidence of nesting or breeding behaviour was recorded during the survey period.

Discussion

The confirmed sighting of a Black Baza *Aviceda leuphotes* in Nandhaur Wildlife Sanctuary on 20 December 2025 represents the first scientifically

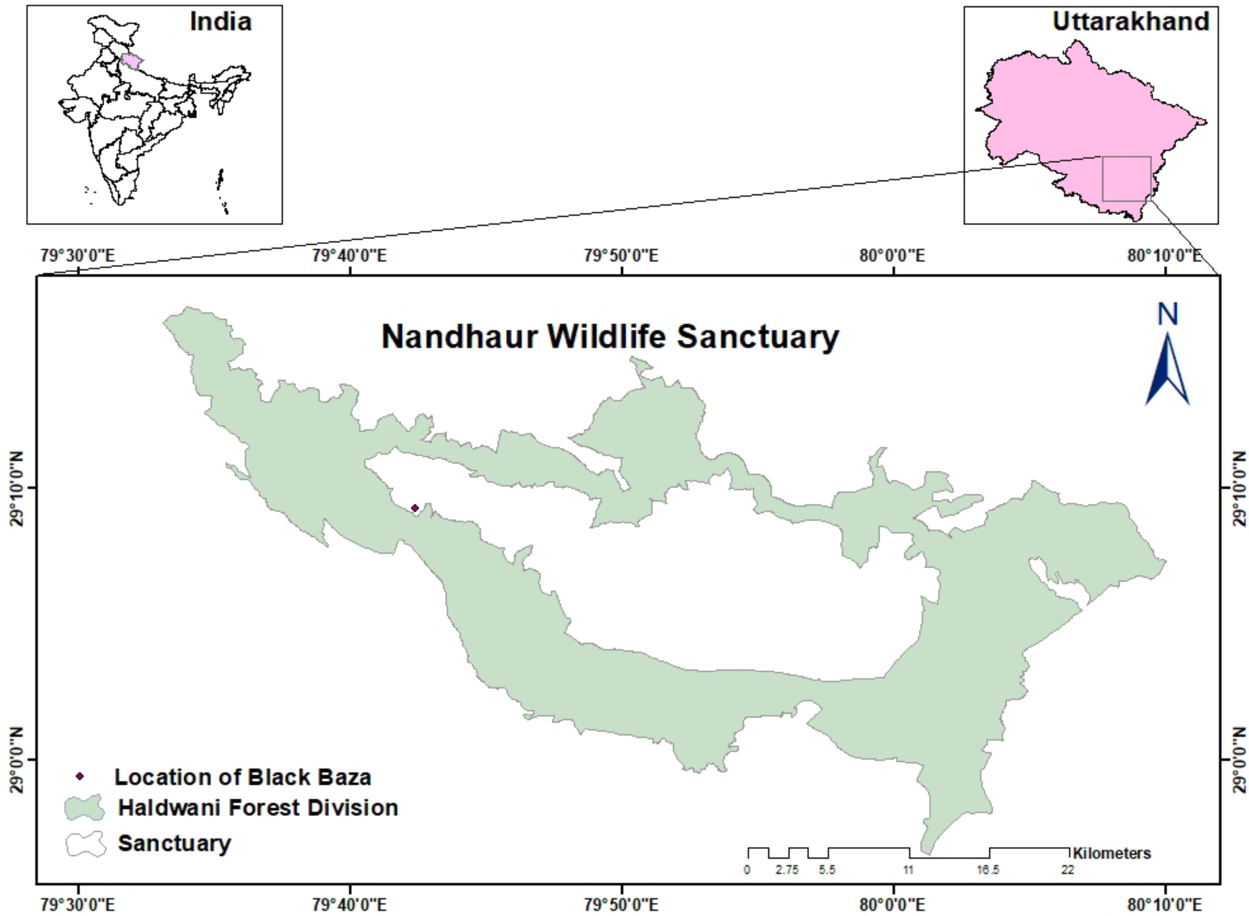

Figure 1. The map outlining the Haldwani Forest Division and Nandhaur Wildlife Sanctuary (NWS), Uttarakhand, India. The purple dot shows the location of the Black Baza observation.

Table 1. Selected published records of Black Baza from western, peninsular, and eastern India.

Location	State	Season / Month	No. of Individuals	Remarks	Source
Bhimashankar	Maharashtra	October	1	Passage / seasonal sighting	Rane & Borges (1987)
Vidarbha	Maharashtra	Winter	1	First regional record	Bapat & Wadatkar (2015)
Gupteswar Forest	Odisha	November	2	Forest-associated occurrence	Purohit et al. (2017)
Chennai	Tamil Nadu	October–December	Multiple sightings	Seasonal migrant	Santharam (2009)
Puducherry	Puducherry	Winter	1	Migratory observation	Boobalan (2017)
Kancheepuram	Tamil Nadu	Winter	1	Seasonal occurrence	Nagarajan (2017)
Nandhaur Wildlife Sanctuary	Uttarakhand	December	1	First documented record from the Terai–Bhabar landscape	Present study

documented record of this species in the northern Terai–Bhabar landscape of Uttarakhand. This observation expands the known distribution of the species in India, and adds to the growing number of scattered records from western and peninsular regions outside its core distribution range in northeastern India and southeastern Asia.

Published observations from Bhimashankar in Maharashtra (Rane & Borges 1987), Vidarbha (Bapat & Wadatkar 2015), Gupteswar forests of Odisha (Purohit et al. 2017), and southern India including Chennai, Puducherry, and Kancheepuram (Santharam 1981,

Image 1. a & B—Black Baza captured in Nandhaur Wildlife Sanctuary, Uttarakhand, India.

2009; Boobalan 2017; Nagarajan 2017) indicate that Black Baza occurs sporadically across different parts of the Indian peninsula. Notably, many of these records are concentrated during post-monsoon and winter months, suggesting that at least some populations undertake seasonal movements or dispersal. The December record from Nandhaur Wildlife Sanctuary is consistent with this broader temporal pattern and may represent part of a wider migratory or passage movement from eastern populations.

Across its primary range in northeastern India and southeastern Asia, the Black Baza is known to exhibit migratory behaviour, with seasonal local movements have been reported in response to prey availability and climatic conditions (Ferguson-Lees & Christie 2001; Rasmussen & Anderton 2012). In this context, the present observation may represent an occasional dispersing or migratory individual moving westward from eastern populations. Alternatively, the increasing number of scattered observations from different parts of India may indicate that the species is more widespread than currently understood but remains overlooked because of its elusive behaviour, short seasonal occurrence, and resemblance in flight to other small raptors. Although the available evidence is insufficient to confirm residency in Uttarakhand, repeated surveys and long-term monitoring may help determine whether the species occurs regularly as a passage migrant or seasonal visitor in the Himalayan foothills.

The habitat in which the bird was observed, comprising semi-evergreen and mixed deciduous forest with relatively intact canopy structure, corresponds well with the species' known habitat preferences. The observed aerial sallies above the canopy are also consistent with its documented feeding ecology, which includes the capture of insects and small vertebrates during short foraging flights (Ali & Ripley 1978; Purohit et al. 2017). The absence of breeding or nesting evidence during the survey suggests that the individual was likely transient rather than resident.

The present record also has conservation relevance. If Black Baza occurs in the region primarily as a migrant or passage visitor, then Nandhaur Wildlife Sanctuary may function as an important stopover or seasonal foraging habitat within the Terai–Bhabar landscape. Conversely, if future observations indicate repeated or prolonged occurrence, the area could hold greater significance for sustaining a previously overlooked local population. In either case, the sighting highlights the ecological value of intact forest habitats in the Himalayan foothills and underscores the importance of continued avifaunal monitoring in the Terai Arc Landscape.

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SHORT COMMUNICATION

Rapid increase in artificial-substrate nesting by White-bellied Sea Eagle *Haliaeetus leucogaster* (Gmelin, 1788) (Aves: Accipitriformes: Accipitridae) in Tamil Nadu, India

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Abstract: White-bellied Sea Eagle *Haliaeetus leucogaster* is a coastal apex predator whose nesting ecology is associated with the availability of tall trees along water bodies. An increase in anthropogenic pressure along the southeastern coast of India has led to nesting adaptations, including the use of artificial substrates. Here, unusual observations on four nests constructed on artificial structures within a single breeding season (2025–2026) in Ramanathapuram District, Tamil Nadu, of which three were successful are reported. Only one artificial substrate nest was recorded in the previous decade. This represents a notable increase compared to previous records from the region and suggests a potential behavioural shift. Nesting activity patterns, breeding success, and conservation implications in the context of habitat modification and ecological traps are discussed.

Keywords: Apex predator, breeding, coastal wetland, electric pylon, nest-site selection, raptor, Ramanathapuram.

The White-bellied Sea Eagle (WBSE) *Haliaeetus leucogaster* is a large, diurnal raptor distributed across the coastal regions of the Indian subcontinent, southeastern Asia, and Australia (del Hoyo et al. 1994). As a sentinel species of ecosystem health, its presence and breeding success are key indicators of thriving coastal and wetland habitats (Amal & Roshnath 2025). Traditionally, WBSEs exhibit high nest-site fidelity, constructing large, stick-platform nests (eyries) in the upper canopies of tall, mature trees, which provide security from terrestrial predators and afford optimal vantage points for foraging (Azman et al. 2013). WBSE accounts of nesting on communication towers or windmills are reported from Australia and Thailand, but documentation from the Indian subcontinent remains scarce (Palei et al. 2014). Although with increasing frequency of nesting from Odisha (Pattnayak et al. 2025a) and Tamil Nadu (Byju et al. 2023a), they prefer to nest on electric pylons and telecommunication towers. Habitat loss resulting from the decline of mature coastal trees due to urbanization, cyclones, and shoreline development has forced the species to increasingly adopt artificial nesting substrates (Byju et al. 2023a; Pattnayak et al. 2025a). Artificial nesting may represent behavioural plasticity, emerging evidence suggests that these structures may function as ecological traps, often associated with lower breeding success and higher mortality risks (Pattnayak et al. 2025a). This study documents an unusual clustering of nests on artificial substrates in Ramanathapuram, contributing to the growing body of evidence on this adaptive shift and its conservation implications.

Study area

The observations were made along the coastal belt of Ramanathapuram District, southeastern Tamil Nadu (Figure 1). The region comprises a mosaic of tidal creeks, mudflats, aquaculture ponds, and fragmented coastal vegetation. The area has undergone substantial

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land-use change, including the loss of mature nesting trees, expansion of aquaculture and increased human settlement and infrastructure. Such changes are likely influencing nesting site selection by WBSEs.

Methods

Field observations were carried out during the breeding season (November–March) of 2025–2026, following roadside surveys are described in the previous study (Byju et al 2023a). Nests built on artificial substrates (n = 4) were monitored at least once every 10 days to assess occupancy, breeding activity (pair presence, incubation, and nestling rearing), outcomes (success/failure), and fledging rates. Behavioural observations included nest-building, prey delivery, and defensive responses. A nest was considered successful if at least one chick survived the fledging stage (~80 days for WBSE), which represents a key threshold for independent survival (Debus 2008; Dennis et al. 2012), irrespective of parental care. Nests were classified as unsuccessful if they were abandoned during any stage or if no nestlings reached fledging. Observations were made from 50 m to 100 m using binoculars and spotting scopes to mitigate disturbance. Abandonment was confirmed when repeated visits showed no adult presence, absence of nest maintenance, and no evidence of chicks (Liu et al. 2009).

Results and Discussion

Nest distribution and success

A total of four nests (N1–N4) were recorded on artificial substrates within a relatively small spatial extent. Three nests were active and successful, producing chicks (Table 1). One nest (N4) functioned as a secondary or alternate nest. This represents an unusually high density of artificial-substrate nesting for the region within a single breeding season. The height of the nest in the pylon and towers was approximately 18 m from ground level (Byju et al. 2023a).

Behavioural observations

Adults were observed reinforcing nests with sticks, indicating prolonged prior use of certain nests (N1) before egg-laying, whereas the other nests appeared to

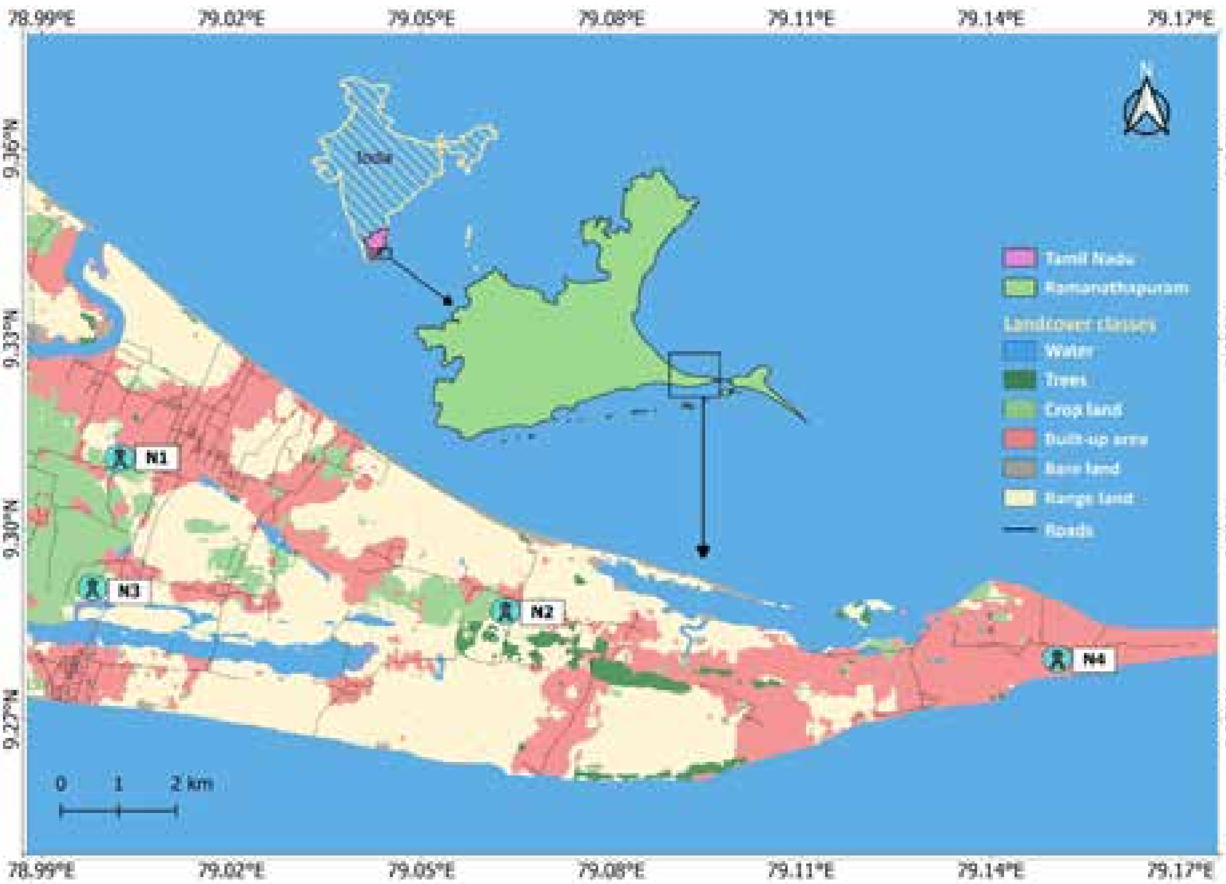

Figure 1. Map showing the four nesting locations of White-bellied Sea Eagle in the Ramanathapuram District.

Table 1. Four nests of White-bellied Sea Eagle with their breeding biology and nest locations.

Nest ID	Substrate type	Status	Breeding cycle	Outcome	Notable behaviour	GPS location
N1	Electric	Active (same nest as in 2022–2023)	Chick reared -2	Successful	Strong territorial defense	9.289° N, 78.998° E
N2	Telecommunication tower	Active	Chick reared -2	Successful	Regular prey delivery (fish)	9.285° N, 79.064° E
N3	Telecommunication tower	Active	Chick reared -1	Successful	Nest repair observed	9.310° N, 79.003° E
N4	Telecommunication tower	Secondary	Nest built; no eggs	Unsuccessful	Secondary/inactive	9.278° N, 79.152° E

Note: Active nests have egg-laying confirmed.

have been constructed in the months leading up to egg-laying. Use of multiple nests (including a non-breeding 'false nest') was recorded in the first observation (Byju et al. 2023a), likely as a predator avoidance strategy, and was not recorded in the current year's observations. Feeding consisted predominantly of fish, consistent with the species' foraging behaviour.

Breeding success

Interestingly, 75% nesting success (3/4 nests) in this observation contrasts with larger-scale studies indicating a lower fledging success on artificial structures and up to 39% failure rates on pylons (0.87 fledglings per attempt) compared to near 100% (higher productivity, 1.74 fledglings per attempt) in natural trees (Pattnayak et al. 2025b). This discrepancy may be due to proximity to productive foraging habitats, lower disturbance levels at specific sites and early-stage adaptation before long-term negative effects manifest. Higher fledgling rates may be due to the proximity of a water body adjoining the nests (N1 and N2 had two chicks compared to N3 with one chick). In principle, a closer foraging site can reduce foraging time and energy expenditure, enhance the delivery of fish or other aquatic prey to the nest, and thus increase nestlings' survival (Pattnayak et al. 2025b).

Artificial structures as potential ecological traps

The shift in nesting patterns is likely driven by the decline of mature coastal trees due to developmental activities and cyclone impacts. At the same time, expanding infrastructure has introduced elevated artificial structures that offer alternative nesting sites. Similar trends have been reported from eastern India, where habitat alteration influences raptor breeding ecology (Palei et al. 2022). Breeding outcomes differ markedly between natural and artificial sites, suggesting that many artificial structures function as ecological traps (Pattnayak et al. 2025a). These sites are often located in disturbed environments, closer to roads and human settlements, where increased disturbance can elevate stress, disrupt foraging and lower long-term reproductive success (Bilney & Emison 1983). Therefore, although artificial structures may compensate for the loss of nesting sites in the short term, they are unlikely to sustain healthy population growth over the long term.

Conservation implications

The observations highlight both opportunity and concern. The opportunities include **alternative nesting substrates** in landscapes where natural sites are irrevocably decreased or lost; these structures can provide essential nesting platforms, potentially preventing local breeding collapse. This can lead to **conservation partnerships** with infrastructure companies (power utilities, telecom). This can be leveraged for collaborative conservation, such as 'raptor-friendly' infrastructure design.

Meanwhile, severe threats include electrocution as a paramount risk. WBSEs have large wingspans (~1.8–2.2 m). Nesting on or taking off from live power pylons poses a high probability of wing-to-conductor or conductor-to-ground electrocution, a leading cause of mortality for raptors globally (Jenkins et al. 2010; Manigandan et al. 2021). Collisions with wires supporting communication towers are often invisible to fast-flying birds, leading to fatal strikes**.** Chicks are at risk of falling or exposure to extreme weather from **n**est platforms on towers, which are often smaller, more exposed, and less stable than those of natural tree nests.

Recommendations

Proactive, evidence-based management is required, including an urgent need for a district-wide systemic survey to map all WBSE nests (natural and artificial) to assess the scale of artificial nests. To mitigate risk at nest sites, collaboration with electricity distribution companies (e.g., TANGEDCO) can enable retrofitting of active pylon nests with insulated covers or perch deterrents on live components. For communication towers, bird-flight diverters on guy wires can be

Image 1. White-bellied Sea Eagle *Haliaeetus leucogaster*: Nest 1 (N1)—adult in the nest | Nest 2 (N2)—parents with two chicks | Nest 3 (N3)—adult in nest | Nest 4 (N4)—abandoned nest. © N. Raveendran.

installed. A promising strategy is the erection of purpose-built, safe artificial nest platforms (ANPs) on tall poles in secure locations near foraging areas. These ANPs can be designed to be more attractive (stable, spacious) and safer than power towers. Long-term conservation must focus on protecting and restoring mangrove corridors with large, old trees to support natural nesting, as this region is already having the first Asian record of Light-mantled Albatross *Phoebetria palpebrata* (Byju & Raveendran 2022), breeding of Noddy (Byju et al. 2025a), Terns (Byju et al. 2025b) and regional endemic Hanuman Plover *Anarhynchus seebohmi* (Byju et al. 2023b). Continuous engagement with local communities, fisherfolk, and infrastructure managers is crucial to building stewardship and reporting nesting activity.

Conclusion

The documentation of four nests of WBSEs on artificial structures, with three successful breeding attempts, represents a significant and possibly emerging trend in Ramanathapuram. While this behaviour reflects adaptability to changing landscapes, it also signals underlying habitat degradation. It is a clear ecological signal of habitat transformation and species adaptability. Understanding whether such nesting strategies represent a sustainable adaptation or a potential ecological trap is critical. Continued monitoring and targeted conservation interventions are essential to ensure the long-term survival of this iconic coastal raptor.

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NOTE

A photographic record of the Chinese Pangolin *Manis pentadactyla* (Linnaeus, 1758) (Mammalia: Pholidota: Manidae) from Pakyong District, Sikkim, India

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Pangolins, commonly known as "scaly anteaters", derive their name from the Malayan phrase *pengguling*, meaning "rolling ball", as they curl into a tight ball when threatened (Atkins 2004). They are nocturnal, elusive, solitary, non-aggressive, insectivorous mammals adapted for burrowing (Gaubert 2011). Globally, eight pangolin species are recognised, four in Africa and four in Asia. Currently, all species are listed as threatened on the IUCN Red List (IUCN 2022).

The Chinese Pangolin *Manis pentadactyla* Linnaeus, 1758 is distributed across southern and southeastern Asia, including Nepal, Bhutan, northern India, Bangladesh, Myanmar, China, Hainan, and Taiwan (Challender et al. 2019). In India, the species has been reported from several northeastern states such as Arunachal Pradesh, Meghalaya, Nagaland, Manipur, Tripura, Mizoram, and Assam (Zoological Survey of India 2002; Srinivasulu & Srinivasulu 2012), with additional sightings from northern West Bengal and Sikkim (Misra & Hanfee 2000). During faunal assessments conducted in the protected areas of Sikkim by the Forest Department, the Chinese Pangolin was not recorded (Lepcha et al. 2017), despite earlier studies documenting its occurrence in the state (Avasthe & Jha 1999; Sathyakumar et al. 2011b). Later, Naulak & Pradhan (2020) included the species in a checklist of mammals from the Darjeeling-Sikkim Himalaya landscape, indicating its continued presence in both regions. Recently, the occurrence of this species has been confirmed through camera trapping and local informant interviews as part of an environmental impact assessment in Sikkim, India (ADB 2026).

The species is currently listed as 'Critically Endangered' on the IUCN Red List due to rapid population declines caused by illegal trade, habitat loss, and fragmentation (Challender et al. 2019). In India, *M. pentadactyla* is protected under Schedule I of the Wildlife Protection (Amendment) Act, 2022 and is listed under CITES Appendix I, providing the highest level of legal protection, making hunting and trade a punishable offense. Despite all these efforts, pangolins remain the most trafficked animal globally, largely driven by the illegal demand for their meat and scales (Aditya et al.

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2021).

The present observation was recorded from Barapathing Village, Pakyong District, Sikkim, India (27.248° N, 88.697° E), at an elevation ranging from 1,150–1,700 m. The landscape encompasses subtropical to temperate broad-leaved forests combined with agricultural land and human settlements. In recent years, the region has experienced increased anthropogenic pressure due to road construction and upgradation, particularly associated with National Highway 717B.

On the evening of 31 March 2024, a local resident observed an unfamiliar animal wandering within their backyard at Barapathing, apparently lost or in search of food. The resident, unfamiliar with the species, initially attempted to drive the animal away but, the animal did not flee. Despite pangolins being characteristically shy and elusive (Martin et al. 2024), this individual showcased passive behaviour, possibly indicating tolerance to human-dominated landscapes.

The animal was temporarily sheltered inside a room, and the Barapathing Territorial Range, Forest & Environment Department, was contacted for rescue. On the following day, range personnel successfully rescued the individual and rehabilitated it into a downstream subtropical forest. The animal was identified as an adult Chinese Pangolin, *Manis pentadactyla*, based on distinct morphological characteristics, such as overlapping keratinous scales, an elongated snout, and robust body form (Image 1 & 2).

Photographs obtained during the rescue and rehabilitation process (Images 1 & 2) confirm the presence of *M. pentadactyla* from Barapathing, Pakyong District, Sikkim, India. Locally, the species is known as "Saalak" in the Nepali language in the region. According to local residents, the species had never previously been observed in so close proximity to human habitation in the area.

Later, the species identity *Manis pentadactyla* was confirmed by Dr. Vikram Aditya, principal scientist and faculty, Centre for Wildlife Studies, Bengaluru, Karnataka, India (Member of IUCN SSC Pangolin Specialist Group) through photographs and description.

Habitat loss and fragmentation arising from deforestation, forest fires, and road development exert considerable threats to pangolins throughout their range (Suwal et al. 2020). Sikkim has recorded one of the highest decadal growth rates of national highways in India (NEDFi 2024), and the upgradation of roads near Barapathing Village may have contributed to habitat disruption, increasing human-wildlife encounters.

Behavioural adaptation may allow temporary persistence in altered landscapes (Wong & Candolin 2014), such changes often increase susceptibility to illegal hunting, stress, and population decline. Illegal hunting and transboundary trafficking are an escalating threat to Chinese Pangolins in Sikkim and nearby states. A report by The Statesman (2021) documented the seizure of a dead pangolin near the Bengal-Sikkim border, where five individuals from Sikkim were arrested while allegedly attempting to smuggle the animal to Bhutan. The mammal was reportedly killed in a wildlife sanctuary in Sikkim, and the report further indicated

Image 1. Chinese Pangolin *Manis pentadactyla* inside the home until the arrival of the rescue unit of Barapathing Territorial Range. © Sangita Ruchal.

Image 2. Chinese Pangolin after being rehabilitated into the forest by the Barapathing Territorial Range. © Prem Kumar Chettri.

possible links with an international wildlife trafficking network operating across the eastern Himalayan region. Therefore, there is a need for baseline ecological data on *M. pentadactyla* in Sikkim for conservation planning. This short communication provides the photographic and additional record of the Chinese Pangolin *Manis pentadactyla* from Barapathing, Pakyong District, Sikkim, India. It highlights the escalating conservation challenges and threats linked to development-related habitat fragmentation, illegal hunting and transboundary trafficking. This record serves as a catalyst for focused field surveys, habitat connectivity interventions and community-based awareness programmes to reduce anthropogenic pressure and support the long-term survival of this Critically Endangered species in Sikkim, India.

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NOTE

First camera-trap records of three wild carnivores from Corbett Tiger Reserve, India

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Global environmental changes driven by land use and climate change are causing shifts in species distributions, leading to shrinking and fragmented ranges (Schipper et al. 2008). Anthropogenic pressures and competition from invasive species cause habitat loss contributing to species extinctions and biodiversity loss (Ceballos et al. 2015). Certain species are more vulnerable to such local extinctions owing to their inherent biological traits like reproductive rate, habitat specialization, body size, and geographic range (Cardillo et al. 2008). Canids are one such group that are affected by urbanization and climate change, where some species may expand their ranges while others may contract due to differences in their climatic tolerance, behavioural flexibility, and habitat specialization (Filazzola et al. 2024). For instance, Indian Foxes *Vulpes bengalensis* are predicted to experience range contraction, while the range of Red Foxes *Vulpes vulpes* might expand owing to differences in their climatic tolerance (Porto et al. 2024).

The Indian Fox, or Bengal Fox, is a medium-sized canid endemic to the Indian subcontinent, preferring semiarid flats, grasslands, scrub, and dry deciduous forests (Gompper & Vanak 2006), and present in varying densities across its range (Home & Jhala 2010; Kumara & Singh 2012). In contrast, the Red Fox is a widespread generalist, found in both natural and human-dominated landscapes (Hoffmann & Sillero-Zubiri 2021), with a range extending across the Himalaya and deserts in India (Ghoshal et al. 2016). The Striped Hyena *Hyaena hyaena*, a nocturnal scavenger distributed in arid and semi-arid regions south of the Himalaya (Menon 2014), feeds mainly on carcasses but occasionally preys on live animals (Prater 1971; Alam & Khan 2015). Despite their dog-like appearance, hyenas are classified within the Feliformia suborder on the basis of their cranial, dental, and other anatomical features and are placed in the distinct family Hyaenidae (Agnarsson et al. 2010).

Major threats to these species include habitat loss,

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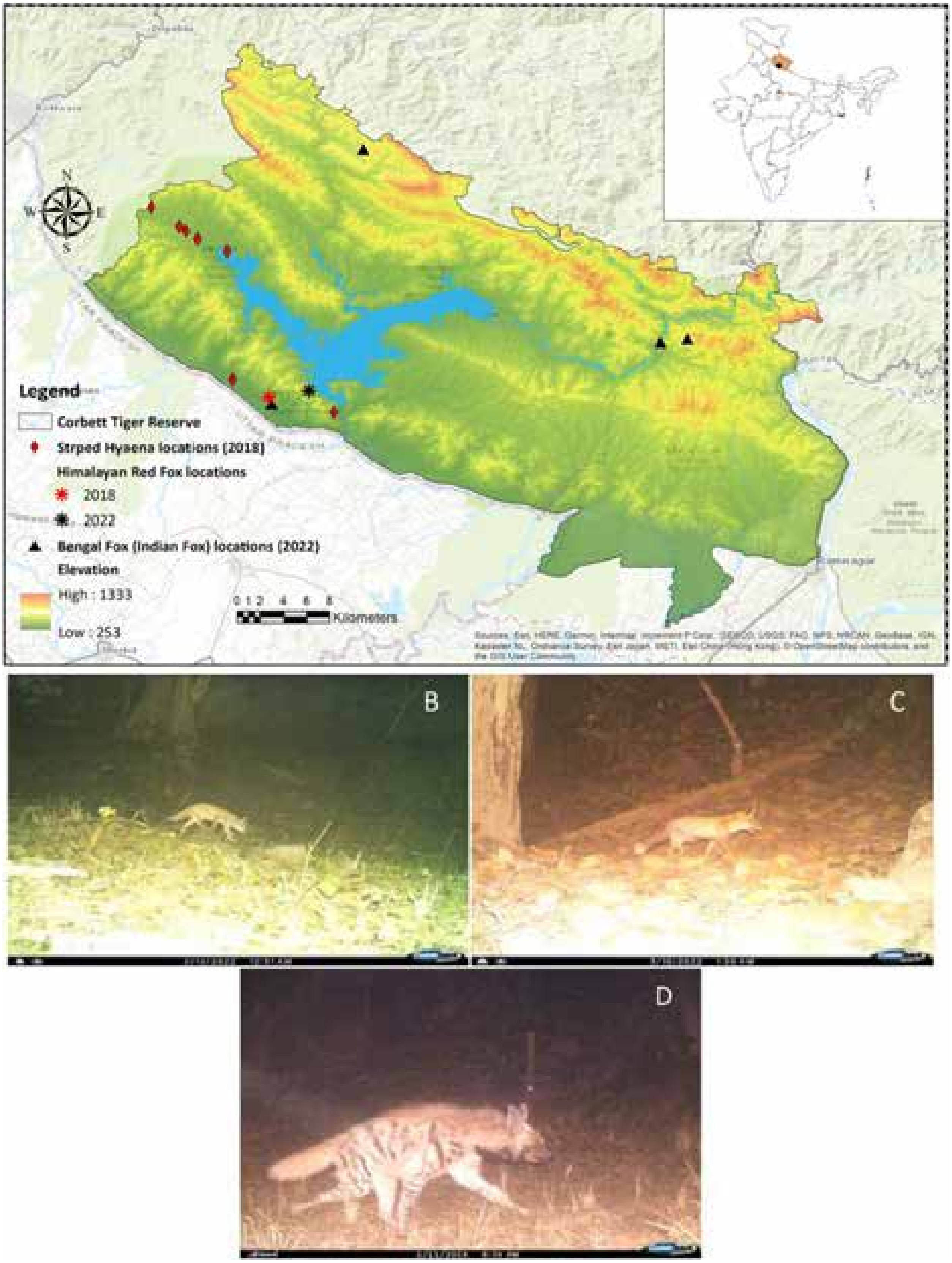

Image 1. A—Map showing the locations of camera trap captures of Indian Fox, Red Fox, and Striped Hyena in 2018 (Red) and 2022 (Black) in Corbett Tiger Reserve | B—Indian Fox | C—Red Fox | D—Striped Hyena.

Table 1. Detailed information on the location of first records of Indian Fox *Vulpes bengalensis*, Red Fox *Vulpes vulpes*, and Striped Hyena *Hyaena hyaena* in Corbett Tiger Reserve, Uttarakhand, India.

	Year	Date	Time	Vegetation type	Elevation (m)	Distance from settlement (m)
Species: Indian Fox *Vulpes bengalensis* (Least Concern)						
Capture 1	2022	13.ii.2022	1231 h	Khair Sissoo Forest	290	1,346
Capture 2	2022	27.ii.2022	0154 h	Moist Siwalik Saal Forest	756	1,774
Capture 3	2022	11.iii.2022	1946 h	Moist Siwalik Saal Forest	839	1,976
Capture 4	2022	11.iii.2022	0831 h	Moist Siwalik Saal Forest	506	3,488
Species: Red Fox *Vulpes vulpes* (Least Concern)						
Capture 1	2019	18.i.2019	2209 h	Dry deciduous scrub	313	1,538
Capture 2	2022	19.iii.2022	0130 h	Moist Siwalik Saal Forest	393	3,596
Species: Striped Hyena *Hyaena hyaena* (Near Threatened)						
Capture 1	2019	11.i.2019	2306 h	Dry Deciduous Scrub	402	8,926
Capture 2	2019	11.i.2019	2039 h	Moist Mixed Deciduous	375	10,058
Capture 3	2019	11.i.2019	2253 h	Dry Deciduous Scrub	395	9,121
Capture 4	2019	11.i.2019	2230 h	Dry Deciduous Scrub	384	9,377
Capture 5	2019	12.i.2019	1225 h	Moist Siwalik Sal	369	8,391
Capture 6	2019	17.i.2019	1758 h	Plantation	294	732
Capture 7	2019	26.ii.2019	1207 h	Moist Siwalik Sal	353	1,940

fragmentation, urbanization, and competition from free-ranging dogs (Macdonald & Reynolds 2004; Vanak & Gompper 2010; Bhandari et al. 2021). The Striped Hyena is also declining due to persecution, habitat destruction, and reduced food availability, partly because of the decline of sympatric carnivores whose kills they scavenge (Alam et al. 2015).

The study area, Corbett Tiger Reserve (hereafter CTR), is situated within the ecologically significant Bhabar-Terai belt, a transitional zone between the Himalayan foothills and the Indo-Gangetic plains, in Uttarakhand. It covers an area of 1,288.32 km², with an altitude ranging from 280–1,138 m. Temperatures vary from 23–46 ^{0}C in summer and 4–21 ^{0}C in winters. Annual rainfall varies from 1,400–2,800 mm, depending on altitude and topography. The climate is tropical, characterized by three distinct seasons: summer, monsoon, and winter. Vegetation of CTR consists of dry and moist deciduous forest, riverine forest, mixed or miscellaneous forest, alluvial grassland, open scrub and plantation. Three major types of forest are classified according to Champion & Seth (1968) namely, northern moist deciduous (3C), northern tropical dry deciduous (5B), and Himalayan subtropical pine forest. The Park is enriched with perennial water streams that support a thriving diversity of fauna.

As part of the All-India Tiger estimation exercise, camera trapping was carried out in CTR from October 2018 to March 2019 and from October 2021 to June 2022. Camera traps were deployed along trails at 520 and 514 locations, respectively, within 2 km² grid cells to maximize detections for tigers and leopards (Jhala et al. 2020; Qureshi et al. 2023). Cameras were placed in pairs facing each other on either side of the trails, and were run for an average of 54 days, resulting in an effort of 28,438 days in 2018–19 and for 70 days on average, constituting an effort of 36,407 days in 2022 (Jhala et al. 2020; Qureshi et al. 2023). On 11 January 2019, a Striped Hyena was photo-captured for the first time in CTR through camera traps. Subsequently, a Red Fox was recorded on 18 January 2019, followed by an Indian Fox on 13 February 2022, their first confirmed records in CTR through camera-trapping efforts. Hyena captures were recorded from seven camera-trap locations, but no subsequent captures were recorded during the 2021–2022 survey (Table 1, Image 1). Red Fox had singular captures in both 2019 and 2022 (Table 1, Image 1). The Indian Fox was photo-captured a total of four times from different locations in 2022 (Table 1, Image 1). Continued monitoring is essential to determine the status and activity of these transient species.

Sighting records such as those made during the present study are important as they expand the known range of these species, providing insights into their adaptability and highlighting the habitat suitability of CTR, which is primarily known for its tiger population.

The presence of tigers and leopards in high densities in CTR (Jhala et al. 2020) may also influence how mesopredators use the landscape, as apex predators are known to shape the spatial distribution, habitat use, and activity patterns of smaller carnivores through interference competition and risk avoidance (Ritchie & Johnson 2009; Ripple et al. 2014). Such observations emphasise the utility of data from targeted camera trap surveys in revealing the presence of lesser-studied species. These findings could significantly influence management and conservation strategies by promoting a more comprehensive approach that considers a multi-species perspective. This approach could lead to enhanced biodiversity monitoring programs and targeted studies on the interactions, population dynamics, and habitat requirements of these species.

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NOTE

Photographic record of the Eastern Bronzeback Tree Snake *Dendrelaphis* cf. *proarchos* (Wall, 1909) from Dudhwa Tiger Reserve, Uttar Pradesh, India

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The Eastern Bronzeback Tree Snake *Dendrelaphis proarchos* (Wall, 1909), is a species of diurnal, arboreal, colubrid snake found in northeastern India and adjacent regions of Indochina including Bangladesh, Bhutan, China, Laos, Myanmar, and Vietnam (Vogel & van Rooijen 2011). In India, the species has been reliably reported from several northeastern states, such as Arunachal Pradesh, Assam, Mizoram, Meghalaya, Nagaland, Sikkim, Tripura, and West Bengal through museum and field records, western and central India (e.g., Gujarat, Maharashtra, Madhya Pradesh) (Vogel & van Rooijen 2011; Biakzuala et al. 2022; Sharma & Verma 2023; Parmar et al. 2024; Mohandas & Dnyaneshwar 2025). While most Indian records from the northeastern India are in conformity with geographic range characterised by the taxonomic revision which revalidated *D. proarchos*, some outlier records from central and western India have been considered of dubious, anthropogenic origin (Sharma & Verma 2023; Parmar et al. 2024; Mohandas & Dnyaneshwar 2025). A few reports of "*D. pictus*", an Indo-Malayan species (fide Vogel & van Rooijen 2011) from Nepal (Kästle et al. 2013; Rai et al. 2022) remain unverified, requiring confirmation of the presence of *D. proarchos* in central Himalaya and Shivalik landscape. In this study, we report a photographic record of *Dendrelaphis* cf. *proarchos* from Dudhwa Tiger Reserve, Uttar Pradesh, India.

The present record is a result of sustained field efforts by the authors in Dudhwa Tiger Reserve, wherein systematic biodiversity documentation across the reserve since 2022, comprising approximately 400 field-days and more than 2,400 person-hours

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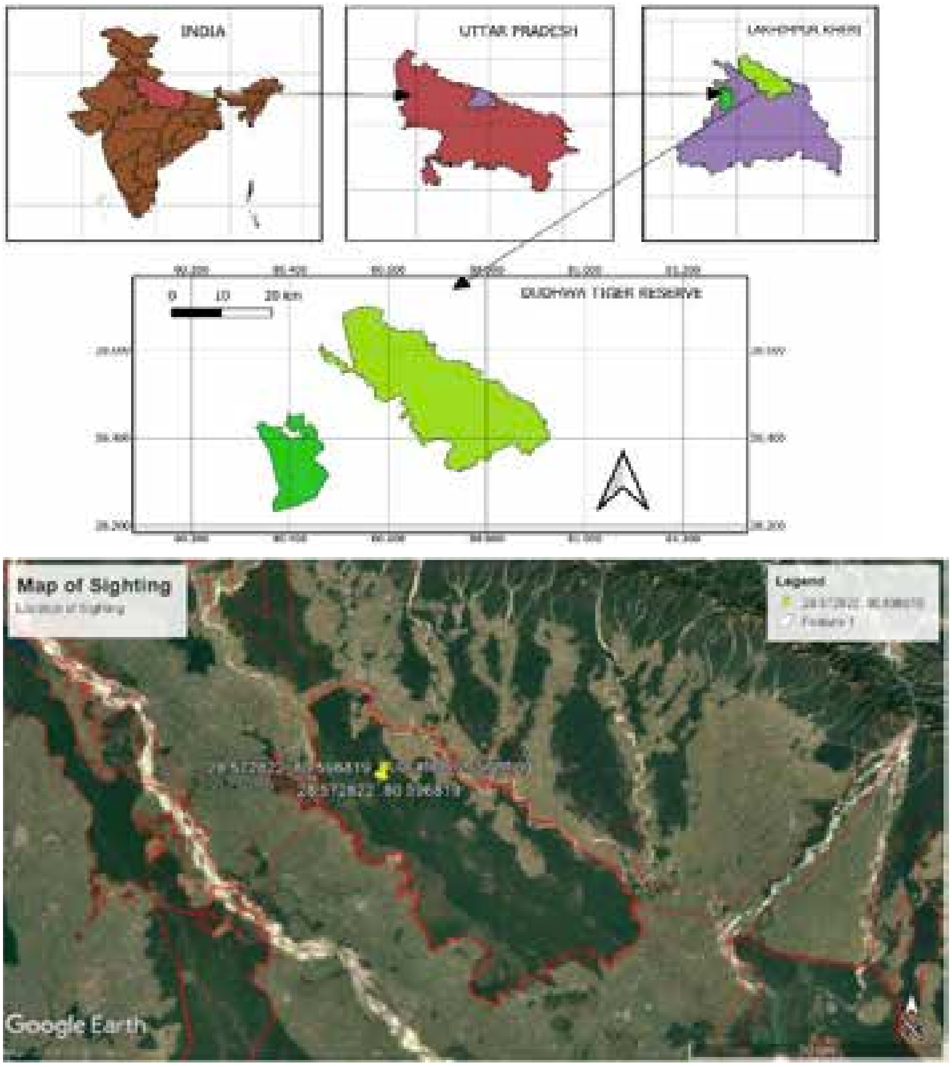

Image 1. Location map of *Dendrelaphis* cf. *proarchos* sighting in Dudhwa Tiger Reserve, Uttar Pradesh, India. (Source: QGIS Desktop 3.44.3; Google Earth).

of structured surveys have been carried out. These surveys were conducted across the core zone ranges of south Sonaripur, Kakrah, Dudhwa, Sathiana, and the Kishanpur Wildlife Sanctuary, using a combination of day and night transects, road-killed monitoring along the Dudhwa–Gauriphanta highway, camera trapping, and herpetological pitfall surveys. These efforts have yielded documented records of over 350 vertebrate species, including 58 reptile species. Despite this sustained effort, no live or dead specimen of any *Dendrelaphis* species had been encountered prior to 10 July 2025, underscoring the genuine rarity of the present record. The present observation was made during a routine patrol along Dudhwa–Gauriphanta forest road, which passes through the interior of the core zone and is primarily used for management and regulated vehicular movement.

On 10 July 2025, at 0930 h, during a field visit, a fresh, mildly trampled road-killed snake about 1 m long was observed on the Dudhwa–Gauriphanta road near Bankati-Piprola junction (28.572822^0 N, 80.596819^0 E; 205 m) in Palia, within the core area of Dudhwa Tiger Reserve (Image 1). The snake was photographed and then identified as an adult *Dendrelaphis* cf. *proarchos* (Wall, 1909) (Image 2). This photographic observation was posted on the iNaturalist citizen-science portal (https://www.inaturalist.org/observations/334567974). The mortality appeared accidental and was likely caused by vehicular impact, as suggested by visible tyre marks on the road surface, a common cause of snake mortality in forested landscapes (Kichloo et al. 2022). Although the mid-body region of the snake was damaged, the head, neck, and posterior portion remained intact,

Image 2. Road-killed *Dendrelaphis* cf. *proarchos* in Dudhwa Tiger Reserve, Uttar Pradesh, India, showing a— whole body | b—forebody | c—head profiles. © Vipin Kapoor Sainy.

allowing reliable identification**.** The specimen was photographed using a Canon EOS 200D Mark II camera with an 18–55 mm lens, and identification was confirmed using standard morphological characters described in authoritative taxonomic literature (Vogel & van Rooijen 2011; Biakzuala et al. 2022; Parmar et al. 2024). Modern herpetological studies increasingly emphasise the importance of photographic or specimen-based confirmation for validating species distribution records (Uetz et al. 2023). However, we were unable to collect or deposit the specimen as a voucher due to prevailing legal stipulations. But the present study documented the photographic evidence of *Dendrelaphis* cf. *proarchos* from Uttar Pradesh.

Identification of the Dudhwa specimen as *Dendrelaphis* cf. *proarchos* was based on (Wall 1909; Vogel & van Rooijen 2011, Biakzuala et al. 2022): slender, elongated body with a head broader than neck, large eyes with round pupils, dorsum bronze-brown with a thick black lateral stripe along the body, bordered below by yellowish-white, and further below by a thin black lateral stripe, smooth dorsal scales arranged obliquely, with enlarged vertebral scales, black post-ocular stripe, venter pale yellowish to cream, sometimes with ventrolateral light stripes bordered by darker lines, loreal 1, preocular 1, postoculars 2, supralabials 8–9 (5^{th}, 6^{th} contacting eye). It is not *D. biloreatus* as it has a single loreal on each side, not *D. cyanochloris* as it has thick black side stripes throughout the trunk and not *D. tristis* as it has lateral black side stripes (Biakzuala et al. 2022). The specimen does not depict any feature not diagnostic of *D. proarchos*. Ventral, subcaudal and dorsal row counts of this specimen are needed for confirming identification and hence we conservatively report it as *D.* cf. *proarchos*.

Distribution records of *Dendrelaphis* cf. *proarchos* in India have been refined through successive faunal surveys and taxonomic revisions (Wall 1909; Wallach et al. 2014). Within India, the species has been reliably reported from several northeastern states, such as Arunachal Pradesh, Assam, Mizoram, Meghalaya, Nagaland, Sikkim, Tripura, and West Bengal through museum, snake rescues and field records, from parts of western and central India (e.g., Gujarat, Maharashtra, Madhya Pradesh) (Vogel & van Rooijen 2011; Biakzuala et al. 2022; Sharma & Verma 2023; Parmar et al. 2024; Mohandas & Dnyaneshwar 2025). While recent morphological and molecular studies have confirmed the validity of *Dendrelaphis proarchos* from northeast India (Mizoram) and reassigned earlier northern Indian records of *D. pictus* to *D. proarchos* (Vogel & van Rooijen 2011), the present record from Dudhwa Tiger Reserve represents the first confirmed occurrence of this species from the Terai landscape of Uttar Pradesh, significantly extending its known distribution westwards by nearly 900 airline km, from the nearest place Darjeeling. Previous studies from Uttar Pradesh, including Dudhwa, have only reported *D. tristis* (Das et al. 2012; Boruah et al. 2020). This shows that similar species are present in the region, but there has been no record of *D. proarchos* before this study.

Patterns of range expansion in snakes are increasingly being recognised across Indian subcontinent. For example, *Ahaetulla longirostris* was described from Bihar and Meghalaya (Mirza et al. 2024), but later records have indicated a range extension, including reports from the Dudhwa Tiger Reserve. Similarly, *Trimeresurus salazar* was originally described from the eastern Himalayan region of Arunachal Pradesh, India (Mirza et al. 2020), but later studies have reported it from more areas, such as central India, showing a westward range extension (Vogel et al. 2022). Similarly, *Trimeresurus erythrurus*, historically known from parts of northeastern India and adjacent Indochina, has also witnessed range updates in recent years, with new distributional records emerging from eastern peninsular India (Deuti et al. 2021). Such examples highlight that cryptic, arboreal, snakes often have wider range extensions than previously documented, particularly in under surveyed regions. Therefore, the present record of *Dendrelaphis* cf. *proarchos* from Dudhwa Tiger Reserve may represent a similar pattern of natural distributional expansion rather than an isolated or human-mediated occurrence (Parmar et al. 2024). As the record originates from the core forest area of a Tiger Reserve, chances of human-mediated introduction is very minimal, if not nil. This record further highlights the underexplored reptile diversity of the Terai Arc Landscape (Das et al. 2012; Boruah et al. 2020; Hakim & Ashar 2025) and underscore the conservation value of Dudhwa Tiger Reserve for both well-known and cryptic species.

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NOTE

Rhododendron pendulum (Ericaceae) from Singalila National Park: an addition to the flora of West Bengal, India

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The European botanist Joseph Dalton Hooker surveyed the eastern Himalaya between 1849 and 1851 and identified 34 new species of *Rhododendron*, including *Rhododendron pendulum* (Hooker, 1849–1851). Furthermore, he opened the *Rhododendron* treasure in the Sikkim Himalaya (Mainra et al. 2010).

The eastern Himalaya have a distinct geographical distribution of *Rhododendron pendulum*, including Bhutan, Nepal, China (South Xinjiang and Tibet), and India (Arunachal Pradesh and Sikkim) (Wangyel et al. 2018; Gogoi et al. 2022). This species inhabits the temperate to subalpine zones of the eastern Himalaya at elevations ranging 2,500–3,800 m. Various sources have reported slightly different elevation ranges, such as 2,000–3,500 m and 1,800–3,500 m (Gogoi et al. 2022).

This species is endemic to the previously classified eastern Himalayan biodiversity hotspot (Tiwari et al. 2006). This species, belonging to the Edgeworthii series, has bud-shaped peltate hairs, as characterised by Cowan (1950).

The Darjeeling Hills comprise 21 taxa of *Rhododendron* from Singalila National Park (Rai et al. 2013), as well as a new variety of *Rhododendron grande* var. *singalense* (Rai et al. 2014). Thapa (2016) found *R. camelliiflorum* in the Darjeeling Hills and revised the total *Rhododendron* taxa to 22.

The study was conducted in the Singalila National Park, which is in the Darjeeling District of West Bengal, India, 27.2208° N & 88.0308° E (Figure 1) . During several field visits to the Park in April, May, and September, we encountered an interesting miniature plant resembling *Rhododendron edgeworthii* (Image 1) at elevations of 3,046.611 m and 3,064.341 m (Images 2). The species was partially distributed across a few sites within the Park. The collected specimens were identified using previous studies (Hooker 1849; Clarke 1882; Hara 1966; Long & Rae 1991; Fang et al. 2005; Sekar et al. 2010; Gogoi et al. 2022). After a comprehensive examination of eHerbaria, the Botanical Survey of India [CAL0000017234], the National Herbarium of Nepal, Kathmandu [KATH098019], the Bhutan National Herbarium, Thimphu [THIM18973], and through the literature cited, we gained a clear understanding of the species, ensuring the accuracy and reliability of our findings.

Herbarium was prepared following the method outlined by Jain & Rao (1976). The voucher specimen was submitted to the Sikkim Botanical Survey of India, and they have provided authentication with the

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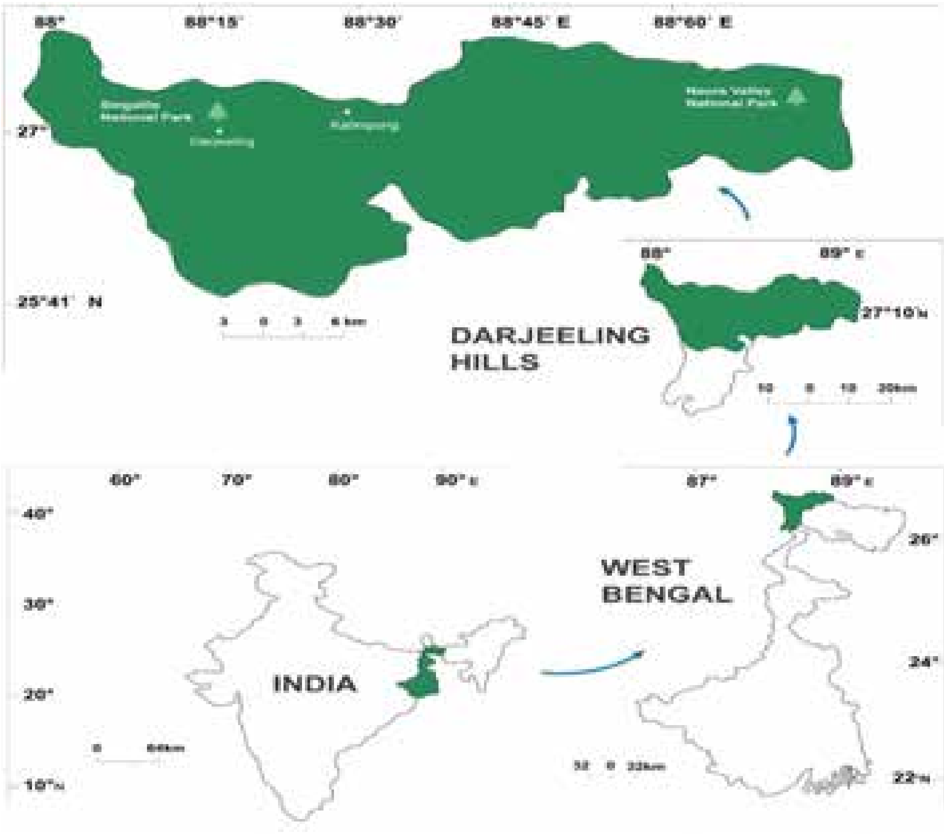

Figure 1. Study area in Darjeeling Hills.

Image 1. a—*Rhododendron pendulum* & b—*Rhododendron edgworthii*. © Sulaxana Baraily.

accession number 0313. A detailed morphological description, along with photographs and sketches, is included (Image 3 & 4).

Specimen Examined: *Rhododendron pendulum* Hook.f., Rhododendron of Sikkim Himalaya t.13 n.14 (1849–1851); Long & Rae, Flora of Bhutan, Vol. 2, Part 1:379 (1991); Pradhan, K.C., The Rhododendron of Sikkim 26–29 (2008).

Morphology: *Rhododendron pendulum* Hook.f. is characterized as an epiphytic as well as a lithophyte. They reach a height of 0.5–1.5 m. Young shoots are densely tomentose. Leaves are oblong-elliptic, 2.8–5 cm long, 1.5–2.8 cm broad, apex obtuse, mucronate, base rounded, margin entire, hairy, slightly revolute, abaxial surface woolly, scaley, adaxial surface dark green, smooth, shiny, pubescent, veins reticulate, anastomosis. Petiole marginal, green to brown, slightly reddish, tomentose, 0.4–1 cm long. Inflorescence terminal, 1 or 2 flowered, without fragrance, pedicel 0.5–0.8cm long, hairy. Corolla tubular, 1.5–1.8 cm long, 2.5–3 cm broad at mouth, broadly funnel-shaped, wavy, five-lobed, white to pale yellow, flushed with pink outside, with brownish spots inside. Calyx well developed, regular, five-lobed, 0.4–0.9cm long, green or tinged pink, ciliated, oblong to ovate. Bracts hairy, ovate, 0.3–0.8 cm long. Stamen 10, almost equal. 0.9–1cm long, filaments hairy at base, anthers light brown, 0.3–0.4 cm. Style slightly hairy, 0.4–6 cm long, stigma capitate, style slightly deflexed, 0.3–0.5 cm, ovary ovoid, hairy, 0.1–0.3 cm long. Capsule ovate, 0.7–1.2 cm long, 0.8–1 cm in diameter, with five valves, tomentose.

Vernacular Name: Jhundinae Chimal (Nepali of Sikkim)

Flowering & Fruiting: April to May & September to October.

Habitat**:** They grow in moist, shady, and mountainous environments within temperate to sub-alpine regions as epiphytes or lithophytes. GPS Location: 27^{0}08'96" N & 88^{0}04'50" E at an elevation of 3,064.341 m in Rimbick fatak, Bikhay Banzang, Singalila National Park, Darjeeling, West Bengal.

Distribution: Bhutan, Nepal, China, Sikkim and West

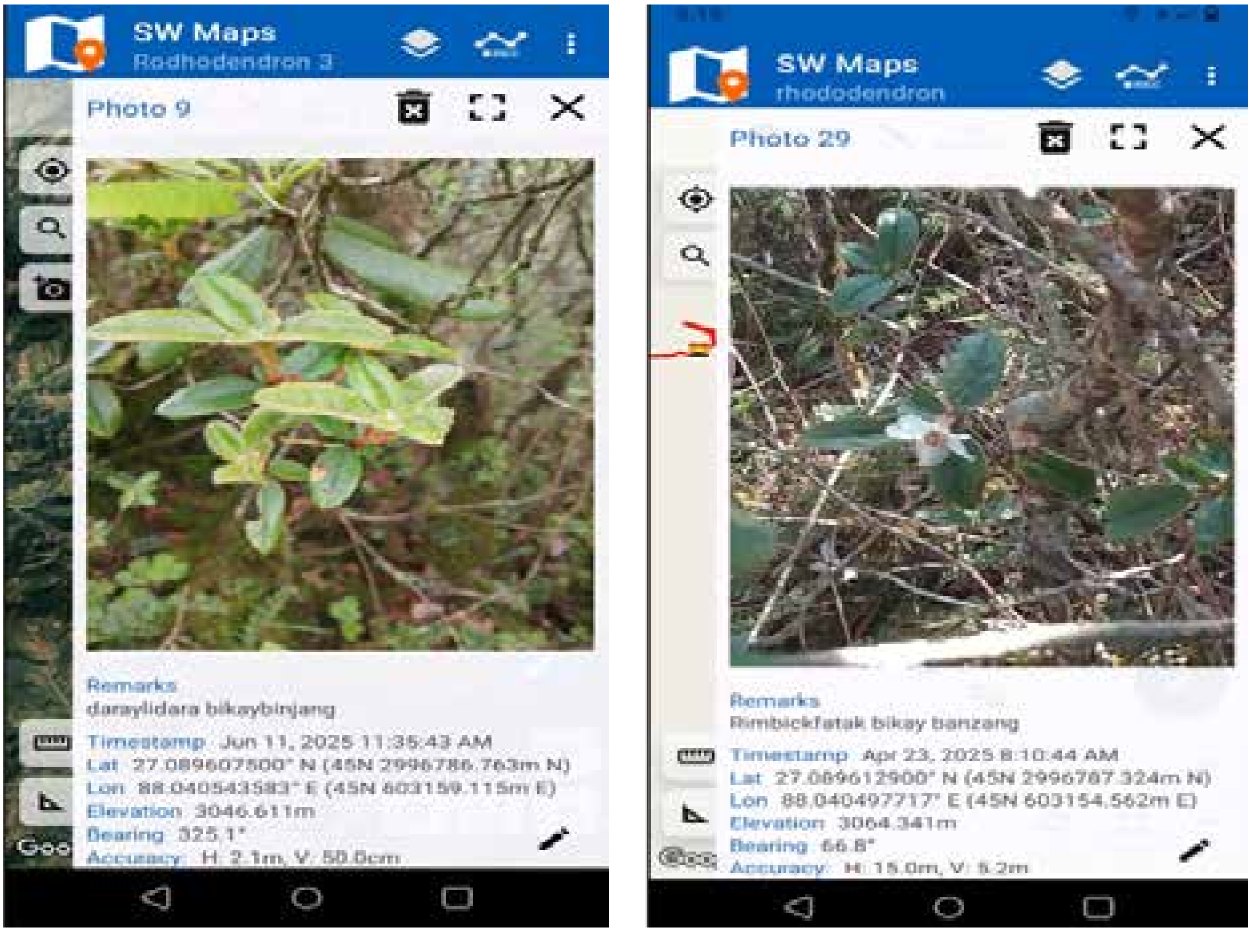

Image 2. GPS location. © Sulaxana Baraily.

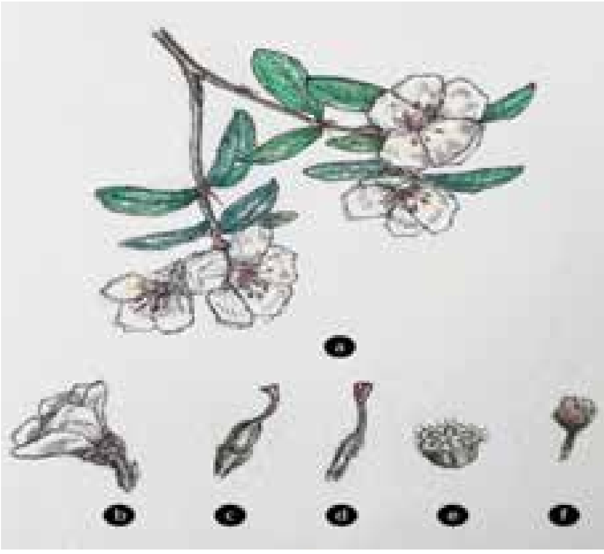

Image 3. Sketch of the specimen: a—Inflorescence | b—Flower with Calyx | c—Gynoecium | d—Stamen | e—Ovary (T.S.) | f—Capsule. © Aswin Diyali.

Bengal (Darjeeling).

Status in the Park: Rare.

Our research added one more *Rhododendron* species to the flora of West Bengal, India. The Darjeeling Himalaya now comprises 23 Rhododendron taxa, including *Rhododendron pendulum* Hook.f. This species is considered rare within the Park. Consequently, from a conservation perspective, we have provided at least two capsules of *Rhododendron pendulum* for seed germination at the Chatakpur nursery in Sonada, Darjeeling, under the supervision of R.O. (HRR-II) Bimal Pradhan. The discovery of *Rhododendron pendulum* in West Bengal opens up possibilities for more ecological and genetic studies on this species. This new finding also highlights the diversity of species in the Himalaya, which is being driven by climate change. Since *Rhododendron pendulum* typically inhabits higher elevations, monitoring its presence at this site over time could help

Image 4. *Rhododendron pendulum*: A—Habitat | B—Inflorescence | C—Flower | D—Dorsal & Ventral sides of leaf | E1—Corola | E2—Carpel | E3—Stamen | E4—Calyx with bract | F—Capsule. © Sulaxana Baraily.

identify changes in its altitude range due to a changing climate.

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