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**Cover: A gravid praying mantis just before she laid her ootheca—digital art on procreate. © Aakanksha Komanduri.**

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ARTICLE

# Mitochondrial CO1 gene haplotype diversity of Sumatran Tiger *Panthera tigris sumatrae* (Pocock, 1929) (Mammalia: Carnivora: Felidae)

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**Abstract:** Sumatran Tigers *Panthera tigris sumatrae* inhabit 12 tiger conservation landscapes that stretch across Sumatra Island. Conservation efforts for these species require robust, information-based research, including a genetic approach. This study analyzed the haplotype diversity of *P. t. sumatrae* based on the mitochondrial CO1 (Cytochrome Oxidase Subunit 1) gene. Specifically, a nucleotide guanine at position 121 was found, distinguishing *P. t. sumatrae* from other tiger subspecies. Among the 17 sequences of *P. t. sumatrae*, two haplotypes were detected: 13 individuals were in haplotype 1 (Hap_1), and four individuals were in haplotype 2 (Hap_2). Hap_1 individuals predominantly originated from Riau and North Sumatra, while Hap_2 individuals were primarily from West Sumatra. Haplotype diversity (Hd) (0.382±0.113) and nucleotide diversity (pi) (0.00038±0.00011) confirmed the low genetic diversity. Five seized samples exhibited Hap_2, suggesting they might have originated from Riau and North Sumatra. However, this result cannot be described as current due to the significant changes in *P. t. sumatrae* habitat. Further genetic studies, such as whole-genome analysis, are needed to detect the origin and variation of *P. t. sumatrae* across all landscapes.

**Keywords:** Forest lost, genetic diversity, Illegal trade, mtDNA, PCR, population interactions, species identification, Sumatran forest, wildlife genetic, wildlife forensic.

**Bahasa:** Harimau Sumatera *Panthera tigris sumatrae* menempati 12 area lanskap *Panthera tigris* yang berada di sepanjang Pulau Sumatera. Usaha konservasi spesies ini telah banyak dilakukan dengan melakukan berbagai macam riset, termasuk dengan pendekatan genetika. Penelitian ini dilakukan untuk menganalisis diversitas haplotipe dari *P. t. sumatrae* berdasarkan gen Cytochrome Oxidase Sub Unit 1 (CO1) DNA mitokondria (mtDNA). Dua haplotip ditemukan dari total 17 sekuen sampel *P. t. sumatrae* dengan komposisi 13 individu memiliki haplotip 1 (Hap_1) dan empat individu memiliki haplotip 2 (Hap_2). Haplotip 1 (H_1) cenderung terdapat pada individu-individu dari Provinsi Riau dan Provinsi Sumatera Utara. Haplotip 2 (H_2) cenderung terdapat pada individu-individu dari Provinsi Sumatera Barat. Nilai diversitas haplotipe (0.382) dan diversitas nukleotida (pi) (0.00038) menunjukkan rendahnya variasi genetik dari semua individu yang dianalisis. Lima sampel yang berasal dari sitaan kasus perdagangan ilegal memiliki haplotip 2 yang berarti dapat diasumsikan cenderung berasal dari Riau and Sumatera Utara. Hasil ini tentu belum dapat mendeskripsikan asal sampel *P. t. sumatrae* secara akurat dikarenakan keterbatasan sampel dan habitat *P. t. sumatrae* yang luas. Selain itu perubahan fungsi habitat yang berubah secara signifikan mengharuskan perlunya dilakukan analisis DNA lengkap *P. t. sumatrae* dari individu-individu pada populasi di semua lanskap yang tersisa.

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## INTRODUCTION

The extinction of the Bali Tiger *Panthera tigris balica* and the Javan Tiger *Panthera tigris sondaica* led to the *Panthera tigris sumatrae* being the only tiger subspecies remaining in Indonesia (Seidensticker et al. 1999). The Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) categorizes *P. t. sumatrae* in Appendix I, which means it is prohibited from being traded (Soehartono et al. 2007). *P. t. sumatrae* naturally inhabits the Sumatran forest ecosystem, exhibiting high adaptability supported by prey availability and access to water sources (Nowell & Jackson 1996; Seidensticker et al. 1999). Additionally, factors such as vegetation density and human activity play crucial roles in determining the existence of *P. t. sumatrae* (Sanderson et al. 2006; Linkie et al. 2008; Suyadi et al. 2012).

There are 76 tiger conservation landscapes (TCLs) around the world, 12 of them being home to *P. t. sumatrae* is located on the island of Sumatra, covering approximately 88,000 km$^2$. These TCLs encompass various areas, including Bukit Barisan Selatan, Tesso Nilo, Kerinci Seblat, Bukit Balai Rejang-Selatan, Rimbo Panti-Batang Barat, Leuser, Berbak, Sibolga Kuala Kerumutan, Bukit Rimbang Baling, Rimbo Panti-Batang Timur, and Bukit Tigapuluh (Sanderson et al. 2006). Within these 12 landscapes of *P. t. sumatrae*, there are 18 conservation areas as well as other forested regions, including protected forests and production forests (Soehartono et al. 2007). Human activities have been a significant cause of forest loss, posing a severe threat to the sustainability of *P. t. sumatrae* (Seidensticker et al. 1999; Linkie et al. 2003; Wibisono & Pusparini 2010). Between 2000 and 2010, Sumatra saw a 3% loss in its forests (Wilcove et al. 2013), and deforested areas exhibited a 20% lower occupancy rate for *P. t. sumatrae* compared to areas that remained forested (Wibisono et al. 2011). Empirical evidence has demonstrated that habitat fragmentation, habitat loss, and isolation among populations can lead to changes in genetic composition among living species (Keyghobadi 2007). Consequently, genetic studies of *P. t. sumatrae* have become increasingly important and are a key focus of research in the 21st century.

Genetic diversity within a population plays a critical role in determining a species' ability to survive and avoid extinction. Low or diminished genetic diversity can significantly reduce a population's capacity to adapt to environmental changes and succeed in reproduction (Frankham et al. 2010). Several parameters are used to assess genetic diversity, including genetic distance, haplotype diversity, and nucleotide diversity. A haplotype refers to a group of genes in organisms inherited from the same parent. It is defined as the inheritance of a cluster of single nucleotide polymorphisms (SNPs), which are variations In a single base within DNA sequences among individuals, particularly within the CO1 gene (Frankham et al. 2010; Liang 2013). The CO1 gene is a protein-coding gene located in mitochondrial DNA (mtDNA) and does not undergo recombination because it is maternally inherited (Ladoukakis & Zouros 2017). Consequently, individuals or closely related species will exhibit a high degree of genetic similarity (Folmer et al. 1994).

Recent genetic studies within the felid family have utilized microsatellite loci, as reported by Williamson et al. (2002). They identified an ideal set of 16 microsatellite loci for population genetic analysis. Another study successfully unveiled the phylogenetic and evolutionary relationships among the six tiger subspecies worldwide. This investigation employed three genetic markers, including mitochondrial DNA spanning approximately 6.000 bp, the class II gene DRB, and microsatellites. While these markers showed low variation between subspecies, they exhibited significant distinctions in partitioning among subspecies (Luo et al. 2004). The *P. t. sumatrae*, mitochondrial DNA study was developed with discovered 7891 bp or 46.4% (Kitpipit, Linacre, and Tobe 2009). Previously, Faizah (2008) conducted a study on the mitochondrial DNA genetic markers (Cytochrome b and D-loop) of *P. t. sumatrae*. The study involved designing primers based on the mitochondrial DNA of *Felis catus*. Additionally, Kitpipit et al. (2012) reported the identification of five single nucleotide polymorphisms (SNPs) specific to *Panthera tigris*, three specific SNPs in *P. t. sumatrae*, and three specific SNPs in *P. t. tigris*, based on an approximately 15.000 bp mitochondrial DNA sequence.

The utilization of genetic markers for *P. t. sumatrae* has been extended to various applications, including the reconstruction of *P. t. sumatrae* pedigrees by targeting the D-loop region, species identification, and phylogenetic analyses through the CO1 gene (Setianingsih 2013; Ashrifurrahman et al. 2022). Additionally, Smith et al. (2018) analyzed the impact of habitat loss and fragmentation on the genetic variation of *P. t. sumatrae* using microsatellite markers. Their findings indicated that Sumatran forest deforestation did not have a significant effect on the genetic variation of *P. t. sumatrae*, mainly due to the maintenance of heterozygosity. However, it is crucial to address the deforestation rate promptly to mitigate future risks. In this study, we investigated haplotype diversity and predicted the origin of tiger body part samples traded from three provinces on Sumatra Island. We utilized blood and hair samples from *P. t. sumatrae* with known origins. This study provides valuable information about the geographical origin of the CO1 haplotype, previously reported by Luo et al. (2004) and Xue et al. (2015), which lacked data on the sample origin.

## MATERIALS AND METHODS

Five seized samples (PTS 1, PTS 3, PTS 5, PTS 6, and PTS 8) of *P. t. sumatrae* from illegal trading were collected from the West Sumatra Natural Resources Conservation Agency (BKSDA). These samples consisted of various body parts, including hairs and bones, from *P. t. sumatrae* that had been confiscated from illegal traffickers arrested by authorities on Sumatra Island. In addition, we obtained nine blood samples (PTS 9, PTS 10, PTS 11, PTS 12, PTS 14, PTS 15, PTS 16, PTS 19, and PTS 20) and one hair sample (PTS 4) from the Dharmasraya Sumatran Tiger Rehabilitation Center (PR-HSD), as shown in Figure 1. The blood samples were collected from *P. t. sumatrae* individuals that had been evacuated from conflicts with humans in recent years in three provinces (West Sumatra, East Sumatra/Riau, North Sumatra), except for PTS 11, whose origin was unknown. Then,

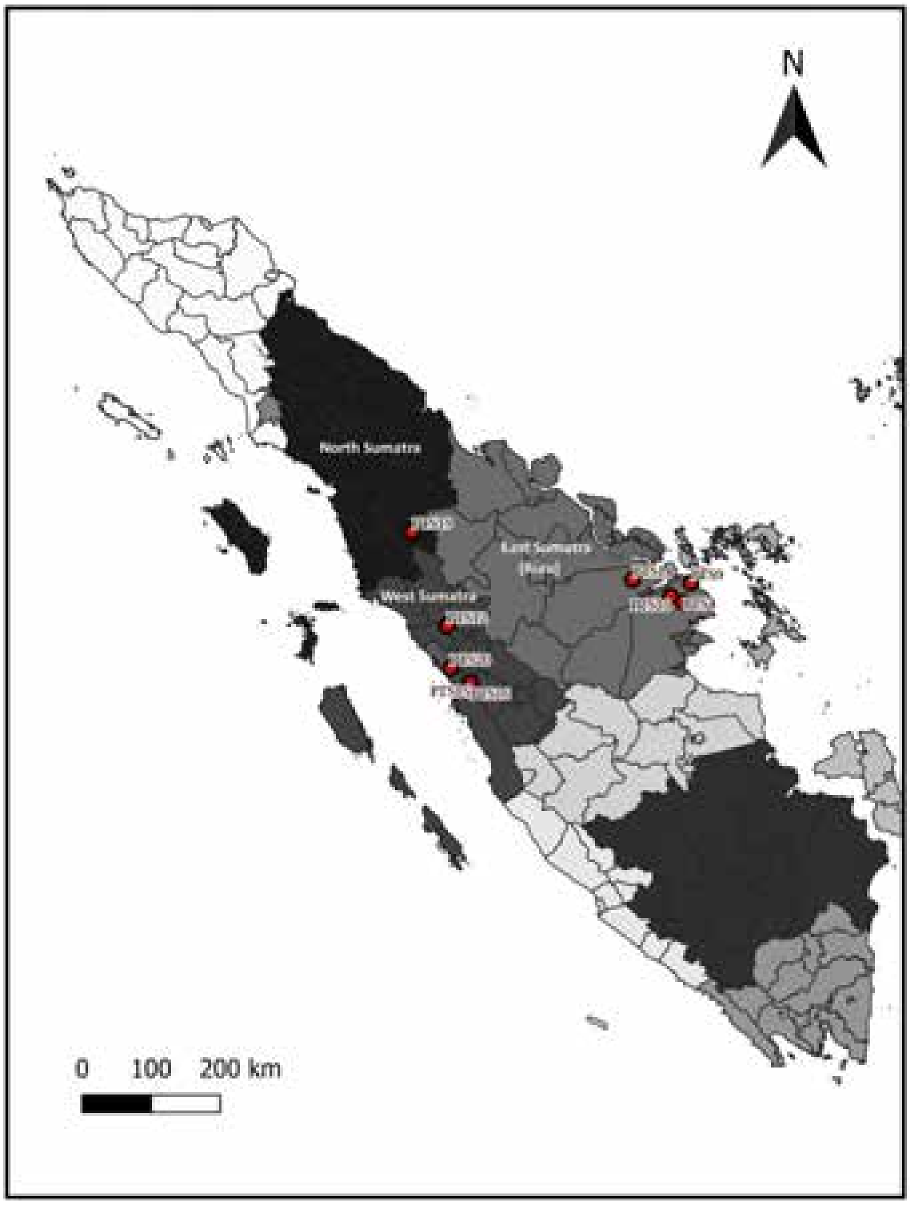

**Figure 1. Geographic locations of *Panthera tigris sumatrae* samples used for molecular genetic analyses.**

the tigers will be rehabilitated to be released back into their habitat. All collected samples were placed into 1.5 ml microtubes, appropriately labeled, photographed, and stored at room temperature. For validation and comparison with previous studies, we utilized sequence data (mtDNA) of *P. t. sumatrae* as assessed by (Kitpipit et al. 2012).

Laboratory methods start with DNA isolation for each sample using GeneAll Exgene Genomic DNA micro. Each type of sample used different protocols according to the kit guide. Then, pairs of primers used to amplify CO1 gene segments were performed using forward primers PTSF 5 'AGTTACTGCCCATGCCTTTG 3' and reverse primers PTSR 5 'CAGGCCTAGGAAATGCTGAG 3' (Ashrifurrahman et al. 2022). The primers would amplify 1129 bp of the Sumatran tiger CO1 gene sequences. Finally, PCR reactions were performed using 25µl reaction volume containing 11 µl supermix of bioline, 9 µl nuclease freewater, 1 µl forward primer, 1 µl reverse primer, 3 µl DNA isolate. PCR machine was set to start from predenaturation at 96°C for 1 minute to ensure complete denaturation, then denaturation was carried out at 96°C for 30 seconds, annealing at 50°C for 30 seconds, and elongation at 72°C for 1.5 minutes. The last cycle at 72°C for 3 minutes, this PCR process runs for 40 cycles.

The amplification product was sent to Firstbase Company in Malaysia to be purified and sequencing reaction. The sequencing process used Applied Biosystems highest capacity-based genetic analyzer platforms and used the BigDye® Terminator v3.1 cycle sequencing kit chemistry. The forward and reverse DNA sequences were then combined using the DNA STAR (Burland 2000). The *P. t. sumatrae* sequences were then aligned using the Clustal X version 1.8. Polymorphism sequence analysis was carried out using DNA sequence polymorphism 5.10. To analyze the changes in the nucleotide base (haplotype), calculating the haplotype diversity and nucleotide diversity (Rozas 2009). MEGA (Molecular Evolutionary Genetics Analysis) version 7 was used for nucleotide base differences analysis (Kumar et al. 2016). The AMOVA (Analysis of Molecular Variance) and FST (Population-based gene flow measures) analysis was calculated with Arlequin 3.5.2.2 (Excoffier et al. 2010).

Furthermore, the various haplotypes identified in the genetic variation analysis were visually represented on a map using QGIS 3.6. The known coordinates of *P. t. sumatrae* were inputted into the QGIS software. Each *P. t. sumatrae* specimen was labeled based on the specific haplotype type that had been determined in the analysis of haplotype diversity. This mapping approach provided a clear visual representation of the distribution of haplotypes among *P. t. sumatrae* populations.

## RESULTS

In total, 17 samples of *P. t. sumatrae* were sequenced for a 999 bp segment of the mtDNA CO1 gene. The analysis revealed the presence of two distinct mtDNA haplotypes: haplotype 1 (Hap_1), consisting of 13 individuals, and haplotype 2 (Hap_2), which was found in four individuals. Hap_1 included PTS 4, PTS 9, PTS 10, PTS 11, PTS 12, PTS 19, PTS 1, PTS 3, PTS 5, PTS 6, and PTS 8, while Hap_2 was identified in PTS 14, PTS 15, PTS 16, and PTS 20. The accuracy of these haplotypes was verified through a thorough examination of the electropherograms obtained during the sequencing process. The analysis was conducted using the MEGA 7.0 program, and DNA-to-protein translation was applied for amino acid translation. It's worth noting that the sequence variability observed in other research studies corroborates the mutations found at these specific sites. Additionally, to ensure the accuracy, several amino acid sites were carefully examined and corrected, with the best frame selected from multiple frames generated by the DNA-to-Protein Translation program. Confirmation was also obtained from NCBI data with accession number AEJ88608.1. Lastly, as part of the DNA-to-amino acid translation process, the initial two nucleotide bases (TT) and the final nucleotide base (A) were removed for consistency and accuracy.

The amino acids were obtained from the translation of the 996 nucleotide bases of *P. tigris* sequences (332 amino acids) with eight various sites (Table 2). Notably, all variations observed in the amino acids were synonymous mutations and transitional mutations. The substitutions detected in the nucleotide base sequences analyzed served to differentiate between tiger subspecies. Specifically, based on subspecies-specific nucleotides, *P. t. amoyesis* is characterized by guanine at position 17, adenine at 121 and 302, and thymine at 422. On the other hand, *P. t. altaica* and *P. t. corbetti* share the same specific nucleotides: adenine at 121, cytosine at 825, and thymine at 920. *P. t. corbetti* does not have a specific site for this study. Of particular significance is the discovery of a specific nucleotide, guanine at position 121, which serves as a distinctive marker distinguishing *P. t. sumatrae* from other tiger subspecies (Table 3).

The genetic variation in the *P. t. sumatrae* population

**Table 1. Haplotype variation, haplotype diversity value (Hd) and nucleotide diversity (Pi) in the *Panthera tigris sumatrae* sequences.**

| | Haplotypes | Samples | Origin | Haplotype diversity (Hd) | Nucleotide diversity (Pi) |
|---|---|---|---|---|---|
| 1 | **Hap_1** | PTS_1 | *Unknown* | **0.382±0,113** | **0.00038±0,00011** |
| 2 | | PTS_3 | *Unknown* | | |
| 3 | | PTS_4 | Riau | | |
| 4 | | PTS_5 | *Unknown* | | |
| 5 | | PTS_6 | *Unknown* | | |
| 6 | | PTS_8 | *Unknown* | | |
| 7 | | PTS_9 | Riau | | |
| 8 | | PTS_10 | Riau | | |
| 9 | | PTS_11 | Unknown | | |
| 10 | | PTS_12 | West Sumatra | | |
| 11 | | PTS_19 | North Sumatra | | |
| 12 | | JF357969_1_*P.t.sumatrae* | *Unknown* | | |
| 13 | | JF357970_1_*P.t.sumatrae* | *Unknown* | | |
| 14 | **Hap_2** | PTS_14 | Riau | | |
| 15 | | PTS_15 | West Sumatra | | |
| 16 | | PTS_16 | West Sumatra | | |
| 17 | | PTS_20 | West Sumatra | | |

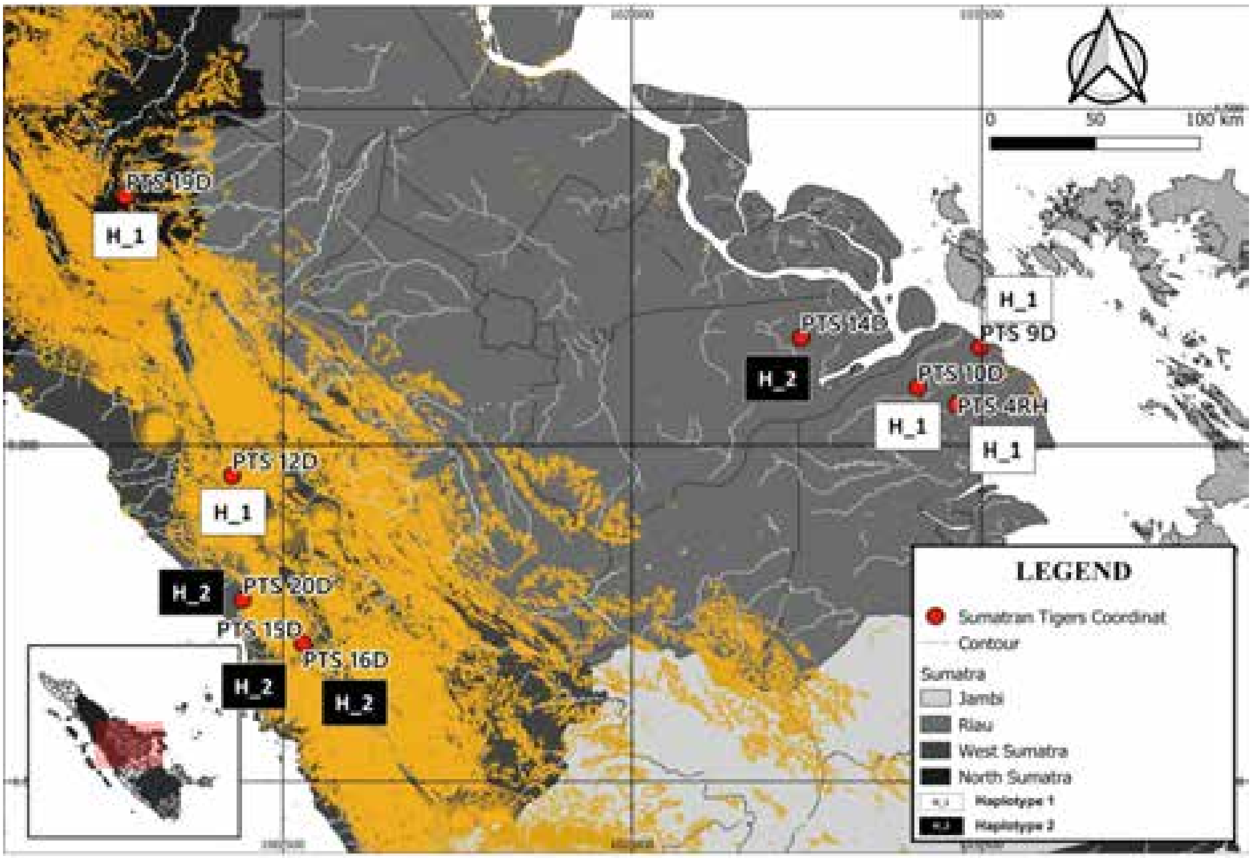

**Figure 2. Haplotypes distribution of *Panthera tigris sumatrae*.**

**Table 2. Variations in the amino acid of *Panthera tigris* cytochrome oxidase subunit 1 gene.**

| | Species | G (Glycine) | G (Glycine) | L (Leucine) | A (Alanine) | L (Leucine) | L (Leucine | I (Isoleucine) |
|---|---|---|---|---|---|---|---|---|
| | | GGA | GGG | CTG | GCC | TTA | TTG | ATC |
| | | 15/5 | 120/40 | 300/100 | 420/140 | 823/275 | 840/280 | 918/306 |
| 1 | PTS 1 | - | - | - | - | - | - | - |
| 2 | PTS 3 | - | - | - | - | - | - | - |
| 3 | PTS 4 | - | - | - | - | - | - | - |
| 4 | PTS 5 | - | - | - | - | - | - | - |
| 5 | PTS 6 | - | - | - | - | - | - | - |
| 6 | PTS 8 | - | - | - | ` | - | - | - |
| 7 | PTS 9 | - | - | - | - | - | - | - |
| 8 | PTS 10 | - | - | - | - | - | - | - |
| 9 | PTS 11 | - | - | - | - | - | - | - |
| 10 | PTS 12 | - | - | - | - | - | - | - |
| 11 | PTS 14 | - | - | - | - | - | TTA | - |
| 12 | PTS 15 | - | - | - | - | - | TTA | - |
| 13 | PTS 16 | - | - | - | - | - | TTA | - |
| 14 | PTS 19 | - | - | - | - | - | - | - |
| 15 | PTS 20 | - | - | - | - | - | TTA | - |
| 16 | *P. t. amoyensis*_HM589215 1 | GGG | GGA | CTA | GCT | - | - | - |
| 17 | *P. t. sumatrae*_JF357969 1 | - | - | - | - | - | - | - |
| 18 | *P. t. sumatrae*_JF357970 1 | - | - | - | - | - | - | - |
| 19 | *P. t. corbetti*_JF357972 1 | - | GGA | - | - | CTA | - | ATT |
| 20 | *P. t. altaica*_KF297576 1 | - | GGA | - | - | CTA | - | ATT |
| 21 | *P. t. altaica*_MH124080 1 | - | GGA | - | - | CTA | - | - |
| 22 | *P. t. altaica*_MH124110 | - | GGA | - | - | CTA | - | ATT |
| 23 | *P. t. altaica*_MN624080 1 | - | GGA | | | CTA | | ATT |

was characterized by two distinct haplotypes: Haplotype 1 (H_1) and Haplotype 2 (H_2), each distinguished by a single nucleotide site. Haplotype 1 (H_1) is characterized by the guanine nucleotide base at position 842, while Haplotype 2 (H_2) features an adenine base at the same position (Table 3). This result aligns with findings by Luo et al. (2004), who reported one nucleotide base variation in eight *P. t. sumatrae* samples based on a 409 bp segment of the CO1 gene sequence. Five of these individuals had guanine at position 7382 bp, while the remaining three had adenine bases. Furthermore, Xue et al. (2015) examined five *P. t. sumatrae* museum samples using the same primers as Luo et al. (2004), with four samples having guanine bases and one sample featuring adenine bases in the same order as previously reported. Despite the reporting of haplotypes, the geographical origin of these haplotype samples within *P. t. sumatrae* has not been previously documented. A map illustrating the distribution of these haplotypes is presented in Figure 2.

Haplotype diversity throughout the population of *P. t. sumatrae* in this study was low at 0.382 ± 0,113 (Table 1). Haplotype diversity values range from 0 to 1, with values exceeding 0.5 indicating high haplotype diversity, while values below 0.5 suggest low diversity (Curry et al. 2015). The nucleotide diversity (pi) value of the 17 sequences was 0.00038±0,00011 From several previous reports, other tiger subspecies also have low mtDNA nucleotide diversity, including *P. t. tigris* 0.000355±0.000256, *P. t. jacsoni* 0.00118±0.000670, respectively.

## DISCUSSION

The low levels of haplotype (0.382±0,113) and nucleotide diversity (0.00038±0,00011) were found in *P.t. sumatrae* from this research. Recent reports on Felidae mtDNA diversity show comparable values. For example,

**Table 3. Specific nucleotide bases in *Panthera tigris* sequences.**

| No | Sample | | *Site* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Luo et al. 2004** | **6479** | **6583** | **6764** | **7130** | **7287** | **7304** | **7382** |
| | | **mtDNA (NC_010642.1)** | **6543** | **6647** | **6828** | **7194** | **7351** | **7368** | **7446** |
| | | ***CO1* (NC_010642.1)** | **263** | **367** | **548** | **668** | **1071** | **1088** | **1166** |
| | | **CO1 in this study** | **17** | **121** | **302** | **422** | **825** | **842** | **920** |
| 1 | PTS 1 | | A | G | G | C | T | G | C |
| 2 | PTS 3 | | . | . | . | . | . | . | . |
| 3 | PTS 4 | | . | . | . | . | . | . | . |
| 4 | PTS 5 | | . | . | . | . | . | . | . |
| 5 | PTS 6 | | . | . | . | . | . | . | . |
| 6 | PTS 8 | | . | . | . | . | . | . | . |
| 7 | PTS 9 | | . | . | . | . | . | . | . |
| 8 | PTS 10 | | . | . | . | . | . | . | . |
| 9 | PTS 11 | | . | . | . | . | . | . | . |
| 10 | PTS 12 | | . | . | . | . | . | . | . |
| 11 | PTS 14 | | . | . | . | . | . | A | . |
| 12 | PTS 15 | | . | . | . | . | . | A | . |
| 13 | PTS 16 | | . | . | . | . | . | A | . |
| 14 | PTS 19 | | . | . | . | . | . | . | . |
| 15 | PTS 20 | | . | . | . | . | . | A | . |
| 16 | JF357969 1 *P t sumatrae* | | . | . | . | . | . | . | . |
| 17 | JF357970 1 *P t sumatrae* | | . | . | . | . | . | . | . |
| 18 | JF357972 1 *P t corbetti* | | . | A | . | . | C | . | T |
| 19 | MH124110 1 *P t altaica* | | . | A | . | . | C | . | T |
| 20 | MN624080 1 *P t altaica* | | . | A | . | . | C | . | T |
| 21 | KF297576 1 *P t altaica* | | . | A | . | . | C | . | T |
| 22 | MH124080 1 *P t altaica* | | . | A | . | . | C | . | . |
| 23 | HM589215 1 *P t amoyensis* | | G | A | A | T | . | . | . |

*Panthera pardus saxicolor* exhibited comparably low diversity levels in haplotypes (0.247) and nucleotides (0.00078) (Farhadinia et al. 2020), *Puma concolor* mtDNA diversity (0,006) (Caragiulo et al. 2013), In the case of *Panthera tigris* in the Sundarbans, haplotype diversity was 0.50, and nucleotide diversity was 0.00266 (Aziz et al. 2022). Previous research by Luo et al. (2004), involving the analysis of several gene sequences in mtDNA (4078 bp), consistently reported low nucleotide diversity values (0.00717±0.00444). The levels of genetic variation, whether high or low, as determined by the CO1 gene play a significant role in determining the genetic relatedness between populations and taxa. Lower genetic variation indicates a closer relationship among individuals or populations of living organisms, especially in the case of tigers. This condition has implications for the geographic isolation of tigers, suggesting that they were separated approximately 100,000 years ago (Luo et al. 2004; Xue et al. 2015).

A total 17 individuals were analyzed there were nine individuals have known wild origin. The nine individuals are spread across three provinces on the island of Sumatra. There are four individuals from West Sumatra Province (PTS 12, PTS 15, PTS 16, PTS 20), four individuals from Riau Province (PTS 14, PTS 10, PTS 4, PTS 9), and one individual from North Sumatra Province (PTS 19). These individuals can serve as representatives to determine the distribution of haplotypes forensic samples that unknown origin (PTS 1, PTS 3, PTS 5, PTS 6, PTS 8) and GeneBank data sequences (*P. t. sumaterae*_JF357969.1 and *P. t. sumaterae*_JF357970). All *P. t. sumatrae* forensic samples showed that conceivable from Riau and North Sumatra.

Haplotype 2 (Hap_2) was found in all seized samples of poaching and illegal trade in *P. t. sumatrae,* suspected from Riau and North Sumatra provinces. However, this assumption is not entirely accurate because Hap_2 also exists in individuals from West Sumatra. This suggests the possibility that confiscated tiger samples could originate from other populations on the island of Sumatra.

For the current number of samples, there was a propensity for all districts to have the same haplotype variation, particularly Riau and West Sumatra. The haplotype distribution indicates the sharing of haplotypes by individuals from West Sumatra and Riau Provinces. Furthermore, the sharing of haplotype 1 (H_1) from Riau and North Sumatra Provinces come to pass. The distribution of haplotypes shown in Figure 2 is not significant indicating the specific haplotypes from each province.

Haplotype distribution in this study indicates that no specific grouping formed between these three provinces. The AMOVA and FST (Population-based gene flow measures) analysis was calculated with Arlequin 3.5.2.2 (Excoffier et al. 2010). FST was not calculated between areas within the North Sumatra population due to contributing only one sample that would have skewed the result. AMOVA analysis, run with each of the main areas within Riau group and West Sumatera group, resulted in an FST of 0.2. In line with Smith et al. (2015) reported on microsatellite analysis of 37 samples of *P. t. sumatrae* originating from the North, West, East and South of Sumatra. The data showed inconsistent group separations between regions using three different software. First, structure analysis shows two subgroups, Northern Riau and the island of Sumatra in general. Second, Tess's analysis shows two subgroups, namely the Southern Way Kambas group and the Sumatra Island group in general. Finally, Geneland's analysis indicates four subgroups, namely Northern Sumatra, Eastern Sumatra, mixed East-West Sumatra, and Southern Sumatra. Gene flow values from west to east are 0.20. This value indicates the existence of a migration history of *P. t. sumatrae* is quite high from the west to the east. At the same time the value of 0.06 gene flow from the main area of Sumatra to the southern region indicates the low gene flow of *P. t. sumatrae* to South Sumatra.

The mitochondrial CO1 haplotypes presented here show historic connectivity between Riau and West Sumatra. The *P. t. sumatrae* sharing haplotype in this study is due to the maternal lineage between populations in each province. Any population of *P. t. sumatrae* seems to be bordered by a mountain range from South to North sumatra. The mountain range might not be a barrier for each population to interact. The adaptability and roaming abilities of the *P. t. sumatrae* are among the factors that support the possibility of interaction between populations in each province. Franklin et al. (1999) reported that the territory of adult male *P. t. sumatrae* is 110 km$^2$ and for adult females around 50–70 km$^2$. Griffiths (1994) also reported home ranges of adult male tigers of about 180 km$^2$ at altitudes ranging 100–600 m, 274 km$^2$ at altitudes of 600–1,700 m. Mitochondrial DNA CO1 genes are inherited maternally. The sharing of the haplotypes of each population from this study shows the distribution of individual females carrying specific haplotypes from the original population. The distance from the origin of the discovery of *P. t. sumatrae* in West Sumatra Province to *P. t. sumatrae* in Riau and North Sumatra Provinces is in the range of 200–400 km. Geographical facts support the possibility of interactions or encounters between populations. Priatna's (2012) research reinforces that female *P. t. sumatrae* can have a home range of 376.8 km$^2$.

The interaction among *P. t. sumatrae* populations on the Sumatra island was estimated to have occurred tens to hundreds years ago. The anthropogenic influence was not great enough to fragment the *P. t. sumatrae* habitat. Currently, it is very unlikely that interactions and breeding between populations naturally because of the fragmentation and reduction of forest areas on the island of Sumatra. From 1985–1997, it was recorded that 61% of the total forest on the island of Sumatra had disappeared (Holmes 2002). Genetic studies of the *P. t. sumatrae* population with microsatellite markers show that the genetic structure of the *P. t. sumatrae* population is still good with preserved heterozygosity values to minimize the risk of genetic drift. However, the high rate of forest fragmentation and loss will accelerate the risk of decreasing genetic variation (Kenney et al. 2014; Smith et al. 2018).

Despite the limitations of our sample size, this study generated the first report of CO1 genetic datasets for *P. t. sumatrae* population in several origin locations. The CO1 mtDNA haplotypes exhibited here show historic connectivity, and maintain genetic connectivity within both East and West Sumatra. The Initial overview of *P. t. sumatrae* gives a basic picture of how the genetic structure (Smith et al. 2018). Furthermore, to detect the confiscated sample origin and accomplish the case of illegal trade with genetic forensic tools, ideally reveal the complete mtDNA and nuclear markers of *P. t. sumatrae* with most recent technology.

## CONCLUSION

Haplotype diversity and sharing haplotypes showed the possibility of interaction by each population in the past. Evidenced by the haplotypes distribution in several regions (West, North, East Sumatra). One variation in *P. t. sumatrae* is the important data and supports the previous studies. The results of this study can also determine the origin of unknown samples, although not too significant. Other genetic studies on the entire population of *P. t. sumatrae* with geological time observation are needed.

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**Author contributions:** Ashrifurrahman contributed to sample collection, methodology, formal data analysis and writing original draft. Dewi Imelda Roesma contributed to designing ideas and methodology. Simamora Saruedi and Rusdiyan Ritonga contributed to sample collection. Djong Hon Tjong, Wilson Novarino, Syaifullah, Rizaldi contributed in designing ideas, improving data analysis and manuscripts.

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ARTICLE

# The population trend of the largest breeding colony of the Indian Swiftlet *Aerodramus unicolor*: is it on the verge of extinction?

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**Abstract:** Fluctuations in animal populations are indicators of environmental change. Populations of the Indian Swiftlet *Aerodramus unicolor* on the Burnt and Old Lighthouse islands of Vengurla rocks, Sindhudurg district, Maharashtra were assessed using the logistic growth model. The study used secondary literature and primary surveys to estimate breeding population sizes on both islands. To understand population dynamics, we calculated the carrying capacity (K) using the Verhulst population growth model, and the percent rate of change in populations. Swiftlet populations on both islands are considered to be the maximum size their habitat can sustain, not exceeding 5,000 and 246 birds on Burnt and Old Lighthouse islands, respectively. These populations were observed to fluctuate between 2020 and 2023, with change rates of 5.5% on Burnt Island, and -53% on Old Lighthouse Island. The logistic growth model indicates that these Indian Swiftlet populations are fluctuating near the carrying capacities of their habitats, which could gradually lead to extinction. This highlights the urgent need for conservation and regular monitoring of these populations in Vengurla rocks.

**Keywords:** Apodid, carrying capacity, cave habitat, conservation, extinction, logistic growth, sindhudurg, swiftlet populations, tropical cyclone, vengurla rocks.

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## INTRODUCTION

The population and distribution of animals are attributed to various aspects of their environment (Morrison 1986). Birds are frequently regarded as markers of environmental change (Temple & Wiens 1989; Gregory et al. 2009). When there is a rapid decrease in a population (>25%), conservation action is triggered, and influences policy decisions (Dunn 2002; Gregory et al. 2002, 2009; Luther et al. 2016). There are several reasons for bird declines, which include habitat loss (Rolstad 1991; Dolman & Sutherland 1995), predation (Cresswell 2011), unsustainable farmland practices (Rigal et al. 2023), overall range decline (Rodríguez 2002), environmental and climate change (Morrison 1986; Wilson & Fuller 2001; Pearce-Higgins et al. 2015). Birds' response to environmental changes at behavioral and physiological levels affects the population trend; which, in turn, affects the geographic range, population density, age structure, sex ratios, and habitat occupancy (Temple & Wiens 1989). Moreover, it may further lead to population extinction (Sæther et al. 2005).

According to Sæther et al. (2005), the extinction of animal populations has been studied using five approaches: (i) estimates of the loss of species in a specific area over time (Pimm et al. 1988; Ferraz et al. 2003; Schoener et al. 2003), (2) species–area relationships (Simberloff 1992), (3) assigning several species to different risk categories (Sæther et al. 2005), (4) patterns in time series of population fluctuations (Inchausti & Halley 2003), and (5) population viability analysis using given a set of preconditions (Morris & Doak 2002). A few empirical studies have been conducted on the theories, highlighting that more variability in population abundance would mean a higher probability of extinction (Inchausti & Halley 2003). Additionally, there are still few studies conducted on the populations and other aspects such as habitat occupancy and carrying capacity for the terrestrial bird species (Chamberlain & Fuller 1999; Sæther et al. 2005; Ramírez-Cruz et al. 2020; Campos-Cerqueira et al. 2021). Even though few such attempts have been made for the wild populations of cave-dwelling birds in their natural habitats (Sankaran 2001; Nguyên et al. 2002; Manchi & Sankaran 2011; Roark et al. 2022) and in the ex situ conditions (Thorburn 2014; Mursidah et al. 2020), none have attempted studying swiftlets' inter-relatedness of the populations, its variability (trends), long-term survival or probability of extinction.

Swiftlets, the members of Genus *Aerodramus*, *Collocalia*, and *Hydrochous*, are among the least-studied bird groups. These paleo-tropical cave-dwelling birds are colonial (Chantler & Driessens 1999). They breed and roost in colonies varying in size from millions, as in the Gomantong Cave, North Borneo (Stimpson 2013), to a few dozen, as in some caves in the Andaman Islands (Sankaran 1998; Gurjarpadhye et al. 2021). Global demand for the edible nest of one swiftlet resulted in uncontrolled nest harvesting, leading to population declines and local extinctions (Sankaran 2001; Manchi & Sankaran 2010; Mursidah et al. 2020).

India is home to four species of swiftlets, including the Indian Swiftlet *Aerodramus unicolor,* with populations from the Western Ghats (Mahabal et al. 2007; Chantler & Kirwan 2020) and Sri Lanka (Chantler & Kirwan 2020). This species is under illegal nest harvesting pressure in different regions in India and Sri Lanka (Sankaran 2001). The largest known colony is located at Vengurla Rocks in Maharashtra, and it was documented to be under illegal nest harvesting pressure until 2002 (Mahabal et al. 2007). A small breeding colony was discovered recently on Old Lighthouse Island of Vengurla Rocks. After 2002, there is no record of these colonies being raided for the swiftlet's nest.

According to the available literature, the Indian Swiftlet population on Burnt Island has been fluctuating within a specific range since 2001 (Mahabal et al. 2007), possibly due to pressure from illegal nest harvesting. Therefore, it is crucial to understand the dynamics of the world's largest population of Indian Swiftlet in the absence of nest collection pressure. In this study, we attempted to understand the status and trends of populations on the Burnt and Old Lighthouse Islands of Vengurla Rocks and predict population trends at both locations.

## METHODS

### Study area: Vengurla Rocks Archipelago, Maharashtra, India

According to the Integrated Coastal and Marine Area Management (ICMAM-PD 2001) through IRS LISS-III satellite imagery, Malwan Bay is a submerged and exposed rocky island chain extending straight towards the south. The Malwan coast forms part of the Western Ghats, where the Sahyadri ranges gradually meet the Arabian Sea. Several islands exist in this chain, including 20 islets of the Vengurla Rocks Archipelago at the southern tip, and Sindhudurg Fort at the northern end. The archipelago extends approximately 5 km north-south and 1.6 km east-west, and consists of rocks rising 20–50 m above sea level (Bhanti 2000). Three islands are of significant size: Burnt, New Lighthouse, and Old Lighthouse Islands. Among the remaining, nine are small islands, and eight are submerged rocks (Mahabal et al. 2007; Image 1a). The base rock of these islands is submerged towards the deeper waters and lies below the exposed sediments (Raju et al. 1991), containing ferruginous quartzite of the Dharwar group (Raju et al. 1991).

A recent study by Manchi et al. (2022) documented the presence of a Swiftlet Cave and a void on Burnt Island, and one void on New Lighthouse Island (Manchi et al. 2022). The Vengurla rocks archipelago bears the Dharwar period of rock formation, fixed mainly between 2,500 and 1,800 million years ago. It is one of the oldest known rock formations in the Indian peninsula (Raju et al. 1991). The Swiftlet Cave is the largest and most accessible cave in the Vengurla Rocks Archipelago (Manchi et al. 2022; Image 1b). The cave is 61 m long with an average height of 18 m (Manchi et al. 2022). It is home to the largest known population of Indian Swiftlet globally.

The Old Lighthouse (Image 1b) is an abandoned structure built in 1876 using laterite and cobblestones (Bhanti 2000). A chamber with a dome inside the structure roughly measures about 5 x 5 m in size and 5 m in height (Mahabal et al. 2007), with five windows and an entrance to the lighthouse. The Indian Swiftlet colony, of 30 breeding pairs nesting on the chamber ceiling, was first documented in 2001 by Mahabal et al. (2007). With continuous and significant deterioration, the structure is not in good condition.

Reviewing the available literature, we compiled Indian Swiftlet population data from Burnt (1940–2006) and Old Lighthouse (2001) Islands. We estimated breeding populations of the Indian Swiftlet by conducting population surveys on both islands using the nest count method between December 2020 and April 2023 (Sankaran 2001; Manchi & Sankaran 2014). After entering the habitat, we meticulously search the cave walls and ceiling. Once located, the nests were counted. These counts are conducted at the end of every month during the breeding season (from December to June) to monitor the breeding populations of the Indian Swiftlet). The highest count obtained usually during the incubation and nestling period (April or May) was taken as the breeding population of that particular cave. The number of nests increases during incubation and nestling periods, as certain nests during the nesting period are camouflaged with the cave wall and are fairly simple to locate once the parents start sitting on eggs for incubation and nestlings hang to or sit in the nests. Since swiftlets are monogamous, each nest is considered to represent a breeding pair (Sankaran & Manchi 2008; Manchi & Sankaran 2014; Gurjarpadhye et al. 2021). Monthly nest counts were done on both islands, and the maximum count in each colony during a season was considered to be the breeding population of each colony for a year.

### Data analysis

Following Mujib et al. (2019), we calculated the carrying capacity (K) for both colonies using the Verhulst (Logistic) Population Model. This logistic model assumes that "at some point, the population will be close to the equilibrium point, i.e., carrying capacity" (Timeneno & Utomo 2008). We used the following formula of the Verhulst (Logistic) Population Model to calculate the carrying capacity:

$K = P_1 (P_1P_0 – 2P_0P + P_1P_2)/ P_1^2 - P_0P_2$ ........... (Equation 1)

Where, K = Carrying capacity, $P_0$ = swiftlet population in 2020, $P_1$ = population in 2021, $P_2$ = population in 2022

The population growth rate was calculated using the following:

$K = P_0 (P_2 - P_1) / P_1 - P_0$ ............... (Equation 2)

We also independently calculated the change in the percent rate of the populations by using the following equation:

Percent change in population = 100 x $((P_f - P_i))/ (P_i)$.... (Equation 3)

Where, $P_i$ = Initial Population, $P_f$ = Final Population

## RESULTS

### Population status of Indian Swiftlet on Burnt Island

Jerdon (1862) first documented the presence of thousands of birds in the cave on Burnt Island. Considering this as the first record, it is comprehended that the Indian Swiftlet's breeding colony on Burnt Island has been known for the last 161 years. The subsequent documentation of this population was by Abdulali (1940, 1962), who recorded ~5,000 birds, or around 2,500 nests. Later, in 2001, when illegal nest collection was brought to arrest, the population estimate was 3,600 birds (Pande et al. 2001), which increased to 5,000 in 2006 (Mahabal et al. 2007). In 2020, during the beginning of the present study, the Indian Swiftlet population was recorded as ~4,000 birds (2,000 nests), and in subsequent years the counts were 4,674 in 2021, 3,920 in 2022, and 4,220 in 2023 (Figure 1a). The overall population change rates were 39% from 2001 to 2006, and 5.5% between 2020 and 2023.

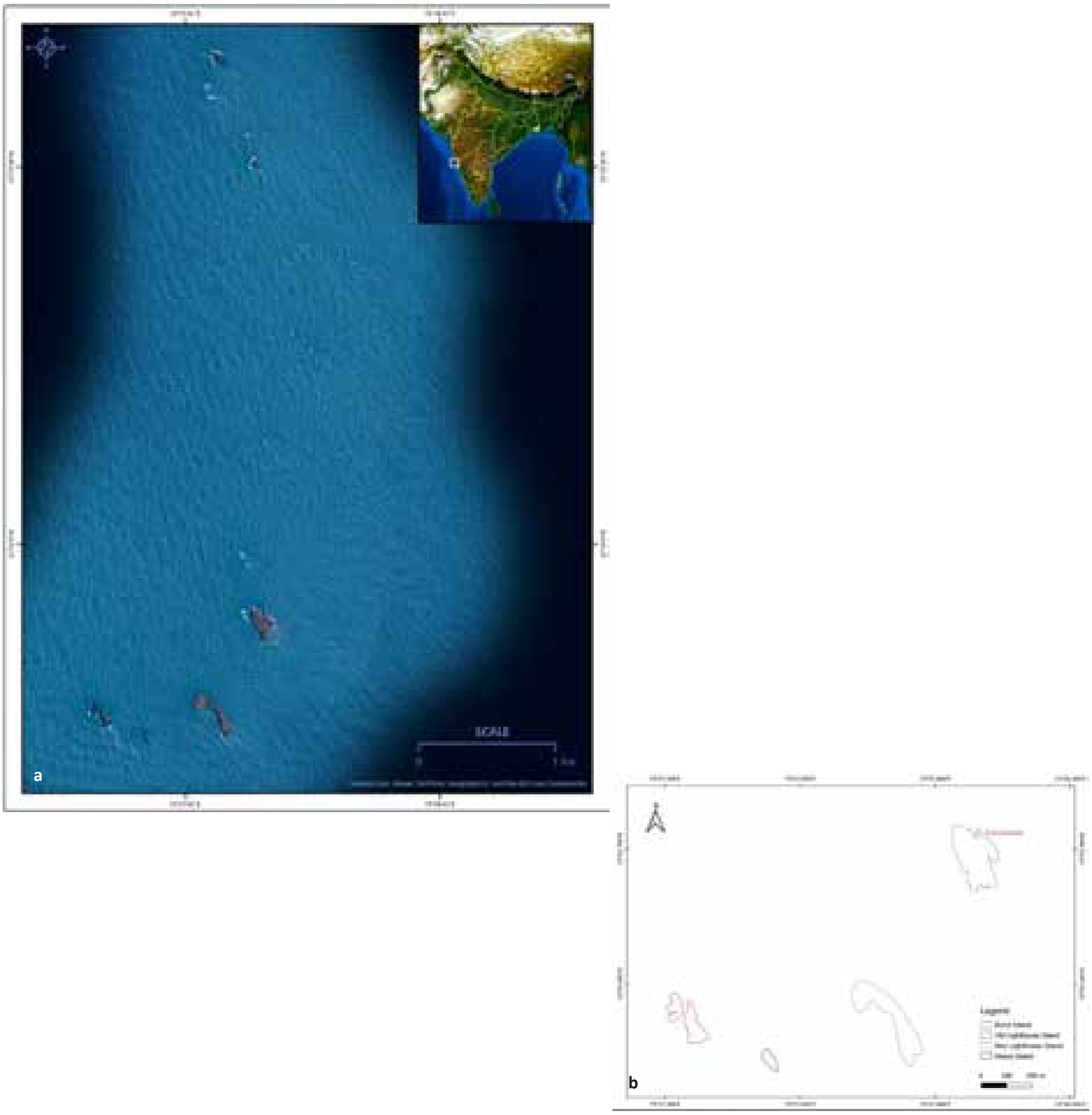

**Image 1. a—The geographical location of the Vengurla Rocks in Sindhudurg, Maharashtra | b—The location of the Old Lighthouse Island and Swiftlet cave in the Vengurla rocks.**

### Population status of Indian Swiftlet in Old Lighthouse Island

The Indian Swiftlet breeding colony on the Old Lighthouse Island is relatively new. The initial population of this colony in 2001 was estimated to be 60 birds, i.e., 30 nests (Mahabal et al. 2007). In 2020 the population was evaluated at 246 birds, and in subsequent years the numbers were 196 in 2021, 92 in 2022, and 116 in 2023 (Figure 1b). The overall population change rate from 2020 to 2023 was -53%.

### Logistic population growth model

We considered the three-year population data (2020–2022) to calculate the carrying capacity of both Islands. Considering that the estimated average population size at Burnt Island has never exceeded 5,000 birds and fluctuates within a specific range (between 5,000 and 3,600 individuals), the population is assumed to be '*k*' type. Also, the logistic growth model (Equation 1) suggested that the carrying capacity of the cave at Burnt Island is 4,041 individuals. Based on the estimated

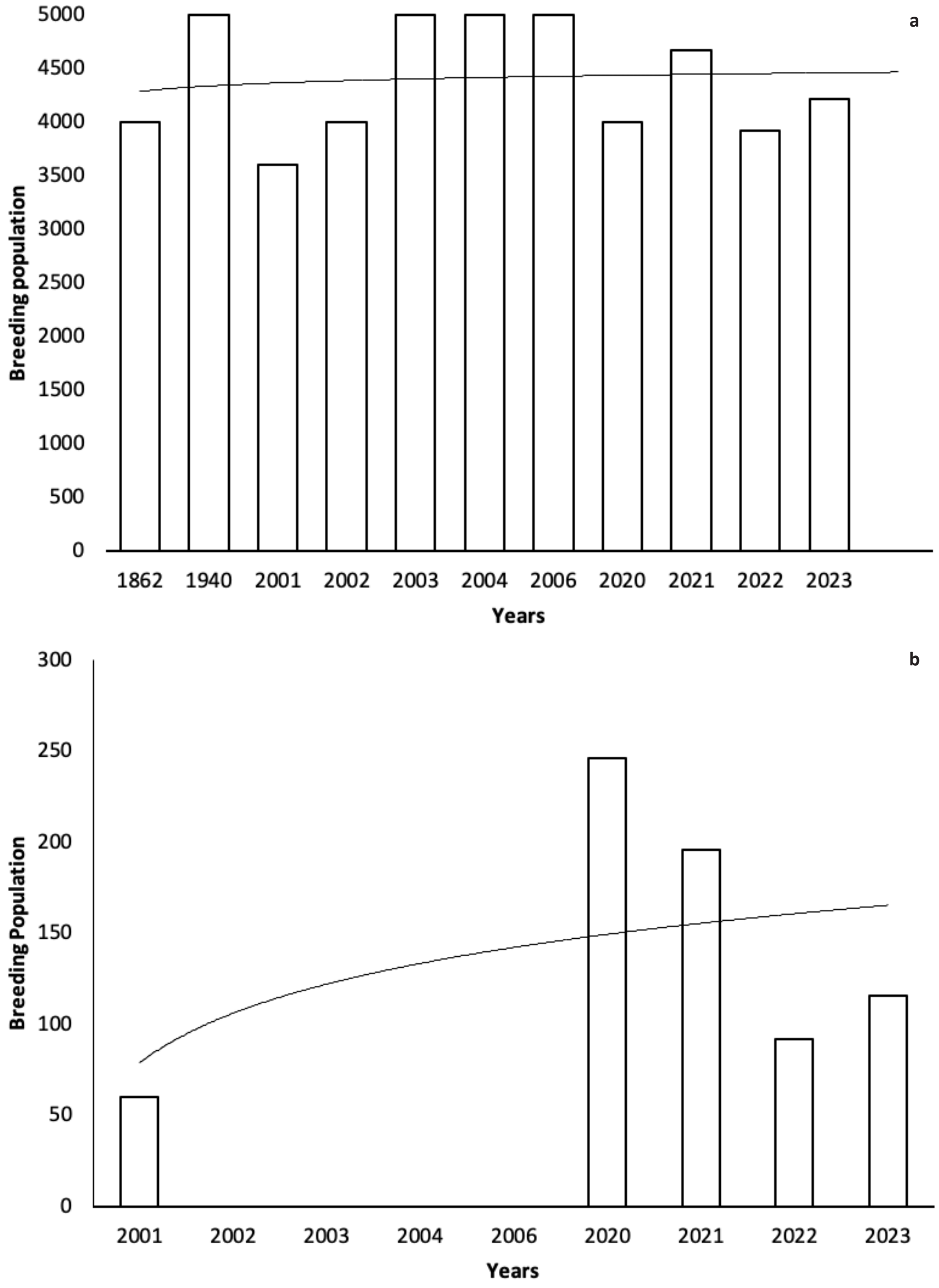

**Figure 1. The population of the Indian Swiftlet from: a—1862 to 2023 in Burnt Island | b—2001 to 2023 in Old Lighthouse Island, Sindhudurg district. Note – the trend line is logarithmic.**

carrying capacity, the following formulae were made using Mujib et al. (2019), to estimate the population of the Indian Swiftlet for the next 50 years.

$P(t) = 4041.79 / 0.010e^{-1.12\,t} + 1$ (Equation 4)

$P(50) = 4041.79 / 0.010e^{-1.12\,*50} + 1$

$P(50) = 3473$ birds

Using this formula, we could predict the swiftlet populations for the next 50 years (Figure 2a), which depicts that this population will have a declining trend over the next 50 years. Similarly, the Indian Swiftlet population on Old Lighthouse Island fluctuated between 246 in 2020 and 116 in 2023. However, as per the logistic growth model, this population is predicted to remain more or less steady for the next 50 years (Figure 2b).

$P(t) = 260.57 / 0.059e^{5.56t} +1$ (Equation 5)

$P(50) = 260.57 / 0.059e^{5.56*50}+1$

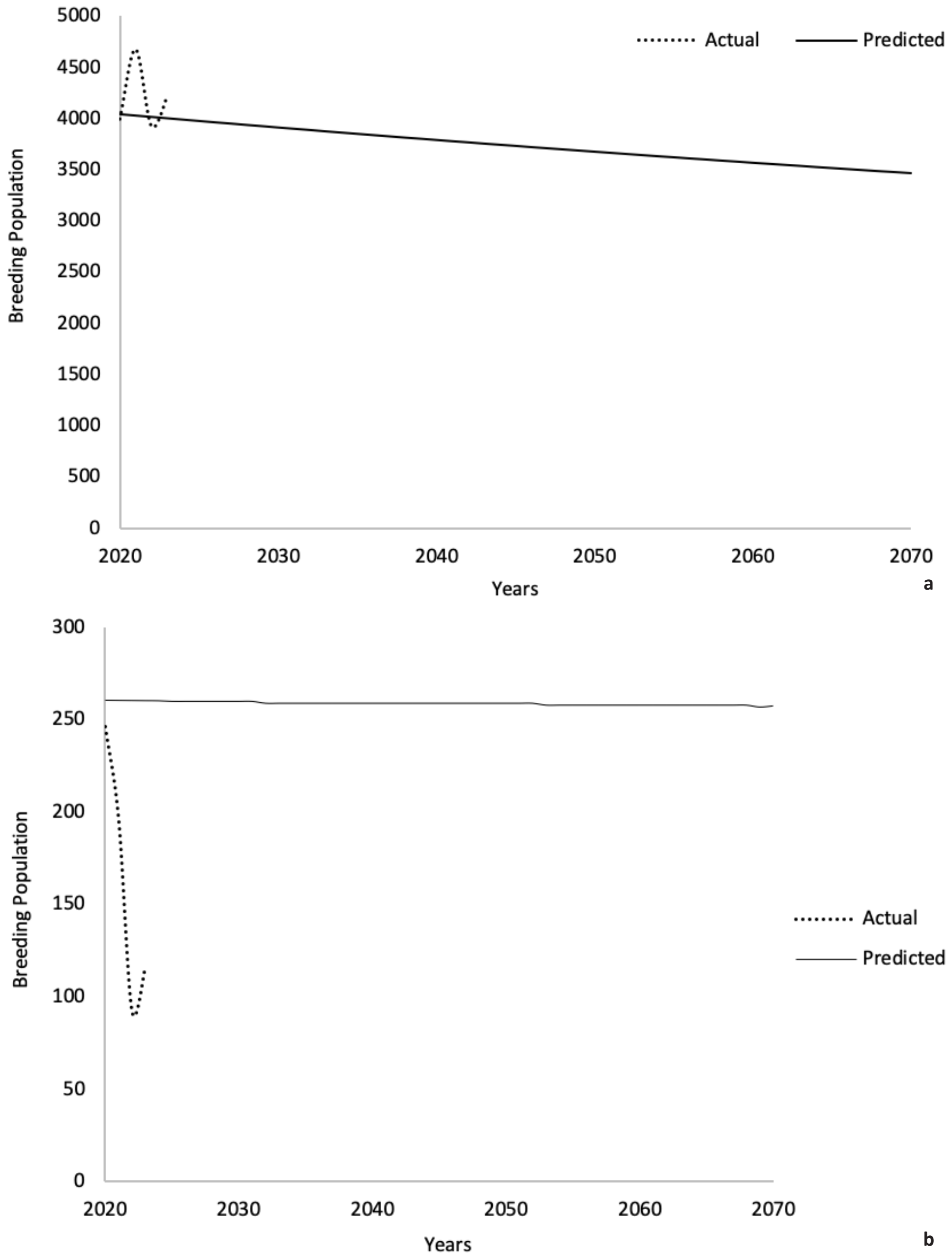

**Figure 2. The actual and predicted populations 2020 to 2070: a—Burnt Island | b—Old Lighthouse Island in the Vengurla rocks, Sindhudurg district.**

P(50) = 257.59 birds

## DISCUSSION

Our estimates for populations of Indian Swiftlet on Burnt and Old Lighthouse Islands of Vengurla rocks indicate fluctuations, as were also observed in the counts between 2000 and 2006 (Mahabal et al. 2007). Similarly, the Indian Swiftlet breeding colony of 60 birds, discovered in 2001 on Old Lighthouse Island (Pande et al. 2001), depicted significant growth. During the present study in 2020, we recorded more than four times increase in the initial population to 246 birds, which later depicted a continuous decline between years 196 (2021), 92 (2022), and 116 (2023). Based on the documented knowledge from other parts of the world, we assume the recent decline of the Indian Swiftlet populations in the Vengurla Rocks Archipelago resulted from the tropical cyclone Tauktae in May 2021. Tarburton & Tarburton (2013)

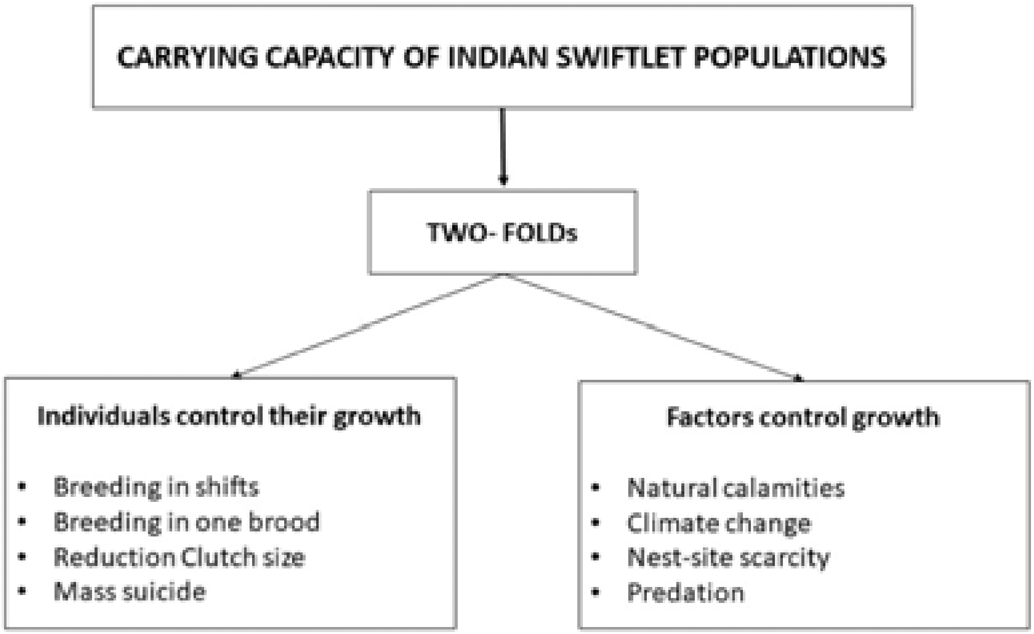

**Figure 3. The hypothetical understanding of the factors based on the two-fold carrying capacity described by Del Monte-Luna et al. (2004) of the Indian Swiftlet populations in Vengurla Rocks.**

have documented that the cyclones caused a significant decline in the populations of the White-rumped Swiftlet *Aerodramus spodiopygius* by either washing down the rock face on which the nests are anchored or partially dissolving the nests, or by the cave or cracks bed filling up until floodwater drowns the nestlings or causes eggs to fail. According to Tarburton & Tarburton (2013), the offshore and coastal colonies, may also be reduced or destroyed by high waves or heavy rainfall during the cyclonic weather. The cyclonic effect was also observed in the other cave-dwelling animals, such as the Pacific Sheath-tailed Bat *Emballonura semicaudata* in Upolu (South Pacific Ocean). Before cyclones Ofa in 1990, and Val in 1991, the species was known to occur in good numbers in several caves. Some American Samoa caves have reported steep declines over the past 10–20 years, perhaps related to cyclone damage (Hutson et al. 2001). Other natural calamities such as earthquakes also affected swiftlet populations. According to Manchi & Sankaran (2009), the changes (rock fall, closure of cave openings, cracks on the rock surfaces and shifting of rocks) caused in the cave structures and microhabitat because of the mega earthquake of December 2004 in the Andaman & Nicobar Islands, the bats and Edible-nest Swiftlet *Aerodramus fuciphagus* lost their roosting caves or shifting their roosting and nesting sites within the caves. Understanding how natural disasters affect various aspects of the swiftlet populations would be a fascinating scientific exploration. Moreover, detailed studies are also required regarding the cave morphology and behavioural responses of the swiftlets towards strong winds and cyclones.

The Tauktae cyclone hit the Arabian Sea between 14 and 19 May 2021 and passed through the study site (Image 1b; Burnt Island and Old Lighthouse Island). The northeastern direction of the cave opening makes this particular cave on Burnt Island a haven for the swiftlet nesting as the south-west monsoon winds do not directly affect the microhabitat inside the cave. Also, as the nests are on the cave walls at a height of 10–16 m and the sea waves cannot reach that height, the nesting place (walls and ceiling) remains dry. At the same time, the storm and strong cyclonic winds can affect the foraging activity and lead to the mortality of the birds foraging around or returning to the cave. For instance, the population decline seen after Tauktae (220 km/h; northward direction parallel to the western coast of India) might be because of a severe effect on the flying birds.

Furthermore, there may be mortality because the swiftlets, the members of Apodidae, cannot resume flight if pushed onto the ground or any other surface. In another case, during the unusual rains in December 2021, several individuals of the Little Swift *Apus affinis* in urban areas of Mumbai, Maharashtra, were observed in the balconies of the high floored buildings and could not survive post-rescue (Aditya Patil, President, Wildlife

Welfare Association, Mumbai, pers. comm.13 December 2021). The reason for the mortality was suspected to be dehydration and starvation. Studies such as by Porter & Aspinall (2013) recorded the populations of the Himalayan Swiftlet *Aerodramus brevirostris* and the Little Swift following a cyclone in the Indian Ocean in November 2007 in Socotra Island (Middle-east) which is far away from their known distribution range. It indicates that the birds may get disoriented during the cyclones and reach a destination out of their distribution range (Elkins & Johnson 2005). The cyclone Tauktae (2021) took place during May (Swiftlet's peak breeding season and nestling period) and when they made multiple visits to the nest to feed the chicks (Nguyên et al. 2002). Sicurella et al. (2015) documented similar observations in Common Swift *Apus apus*, mentioning that the frequent rains and adverse weather conditions affect their foraging activity and result in the mortality of both adults and chicks.

According to Langham (1980), the cessation of breeding in Edible-nest Swiftlet *Aerodramus fuciphagus* was influenced by the onset of monsoon, where the wet weather affects the prey. The heavy rains and strong winds can cause a low abundance of aerial insects and reduce the foraging activity of Germain's Swiftlet *Aerodramus germani* (Petkliang et al. 2017). Öberg et al. (2015) observed that the fledging success of insectivorous birds is negatively related to rainfall (days >10 mm) during nestling periods. Further, according to Blomqvist & Peterz (1984), birds are known to be sensitive to wind conditions during migration or when foraging at sea, and seabirds are particularly vulnerable to windstorms since they cannot find shelter when facing extreme wind conditions in the open sea. Overall, it is seen that the rainfall affects the breeding success and survival of the swiftlets and indirectly affects the recruitment, ultimately affecting the overall populations. Further studies in this regard would help us understand the related dynamics.

According to several studies (Cigna 1968; Badino 2010; Borsato et al. 2015; James et al. 2015), the subsurface air flows are controlled by the cave geometry, its connection with the surface, and variations in external weather and climate. Further, as there are two entrances on the cave ceiling, the continuous rains and winds can enter the cave, adversely affecting the bird populations. Also, a study by Jessel et al. (2019) found that the Edible-nest Swiftlet uses a mechanical overdesign strategy for building the edible nest (safety factor 5–10), however, it has been observed that an extremely violent storm could destroy mud nests which has a safety factor (10) similar to the edible nest (Turner 2006). This directly means that the strong winds might affect the nest of the swiftlet and the nestlings' survival.

The population growth rate of the Indian Swiftlet in Burnt Island from 2001–2006 is 39%, and from 2020–2023 is 5.5%. Until the poaching of the swiftlet nests was brought to a halt in 2001, the Indian Swiftlet population was observed to be dwindling (Mahabal et al. 2007). In the Andaman Islands, continuous poaching reduced swiftlet populations by >80% within a decade (Sankaran 2001). After the conservation actions after the year 2000, the population in protected caves increased by 39%, whereas it declined by 74% in unprotected caves from 2000 to 2008 (Manchi & Sankaran 2014). Similar to our observations on Burnt Island, the study by Manchi & Sankaran (2014) on the Andaman Islands, also assessed the growth rate of ~38–39 % after ceasing the nest collection through participatory conservation efforts.

To explore further population dynamics, in the present study, the logistic population growth model is used to calculate the carrying capacity (K) of the Indian Swiftlet populations in both Burnt (4,041 birds) and Old Lighthouse (260 birds) Islands. The exploration also highlights that both these populations have already reached their thresholds and keep fluctuating around their respective *K* values. As per the model's predictions, the population in both colonies will continue to experience slight fluctuations between 2020 and 2070. However, some of the factors responsible are yet unidentified. Based on the understanding of the two-fold carrying capacity described by Del Monte-Luna et al. (2004), we could identify a few factors that can lead to population declines or fluctuations in the swiftlet populations (Figure 3).

Understanding the findings by Sæther & Engen (2003), many populations fluctuate around their carrying capacity for an extended period before they eventually go extinct. However, it is also evident from Lande et al. (1993) that the average time of extinction of a population and the *K* follows different laws in response to demographic stochasticity, environmental stochasticity, or random catastrophes. Mursidah et al. (2020) observed similar fluctuations in the ex-situ populations in a 1,600 $m^2$ swiftlet house with a productive population of 725 birds in its third year, increasing to 5,500 birds in 23rd year and declining to 400 in the 45th year due to the increased compactness in the breeding colony. According to Stimpson (2013), in the Niah region, Sarawak, Malaysia, the fluctuations in the swiftlet populations since the late Pleistocene result from changes in the environment's carrying capacity and prey resources. Additional efforts are required to understand the factors affecting the decline in swiftlet populations (Caughley 1994).

A few studies have demonstrated the logging and conversion of land to plantations affected on insect diversity and abundance (Koh 2008; Brühl & Eltz 2010), which is a crucial part of the swiftlet diet (Tarburton 1986; Lourie & Tompkins 2000; Nituda & Nuneza 2016). A similar effect of rapid land use change is observed in the northern Western Ghats, India (Munje & Kumar 2022). Hence, comparative studies based on the swiftlet diet and population trend should be conducted to understand the overall effect of these factors to assess the species' extinction risk.

Further, it is of utmost importance that the responses of the population dynamics towards all the factors must be assessed. The Indian Swiftlet populations in the Vengurla Rocks have been known for more than 100 years, and continuous monitoring is required of these populations to understand the population behaviour and variable time before extinction. Also, as the abandoned structure at Old Lighthouse Island has very limited nesting space available for Indian Swiftlet, it is important to create suitable breeding spaces for the species.

Understanding the population status, monitoring trends and predicting their carrying capacity provides a significant opportunity to assess the aspects of the population dynamics (the population growth rates, future population trends, and carrying capacities; Fagan & Holmes 2006) to globally manage the existing and upcoming in situ and ex situ populations of the commercially and ecologically important swiftlets (Manchi et al. 2022). No specific studies have been conducted on the carrying capacity of swiftlets, but many studies have indirectly pointed out the related aspects, such as species habitat requirements and the occupancy of the populations inside caves. Overall, this study provides an interesting perspective on the logistic growth of the Indian Swiftlet populations on Burnt and Old Lighthouse islands and highlights the utmost need to continuously monitor the swiftlet populations worldwide for better conservation action and practice.

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ARTICLE

# DNA barcoding reveals a new population of the threatened Atlantic Forest frog *Sphaenorhynchus canga*

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**Abstract:** Species identification plays a significant role in biodiversity conservation. As many species remain unrecognized, particularly in neotropical hotspots like the Brazilian Atlantic Forest (AF), novel molecular techniques are being widely employed to bridge this gap. In this study, we used DNA barcoding and phylogenetic tools to identify a new population of *Sphaenorynchus canga* in the central region of the Brazilian AF. Our results extend the species' known distribution by approximately 200 km to the south, encompassing a different mountain range than its type locality (Serra do Espinhaço). This disjunct distribution, while not uncommon among amphibians, suggests a historical connection between these two mountain complexes as a biogeographic explanation. Despite the discovery of a new *S. canga* population, the species continues to face numerous anthropogenic threats such as mining, land use, and cattle ranching. Urgent conservation and research efforts are warranted to ensure the survival of *S. canga* populations across these habitats.

**Keywords:** 16S mtDNA, Hylidae, Mantiqueira mountain range, Minas Gerais, species identification.

**Abbreviations:** ASAP – Assemble Species by Automatic Partitioning | CAUFJF – Coleção de Anfíbios da Universidade Federal de Juiz de Fora | ICMBIO – Instituto Chico Mendes de Conservação da Biodiversidade | IUCN – International Union for Conservation of Nature | MCMC – Markov chain Monte Carlo | ML – Maximum Likelihood | mtDNA – mitochondrial DNA | PCR – Polymerase chain reaction | SISBio – Sistema de Autorização e Informação em Biodiversidade | ZUFMS-AMP – Coleção Zoológica da Universidade Federal de Mato Grosso do Sul.

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Species identification is a crucial component of biodiversity research and conservation (Delić et al. 2017; Lyra et al. 2017; Sheth & Thaker 2017). To this end, DNA barcoding has become a widely used molecular technique for identifying species. This approach relies on sequencing a standardized fragment of DNA that can be compared to reference databases to accurately identify species (Gehara et al. 2013; Koroiva & Santana 2022). DNA barcoding has also proven to be effective in delimiting species, and it has been applied across a wide range of taxa, including amphibians (Jansen et al. 2011; Koroiva et al. 2020; Koroiva & Santana 2022).

Delimitation and identification of amphibians using robust methods is paramount, given that they are the most threatened group of terrestrial vertebrates worldwide (Howard & Bickford 2014; Cox et al. 2022; Toledo et al. 2023). Many species of amphibians are being classified under the IUCN Red List categories at the same time they are being formally named (Brasileiro et al. 2007; Caramaschi & Cruz 2011; Assis et al. 2013). Atlantic Forest, a biodiversity hotspot (Myers et al. 2000; Ribeiro et al. 2011; Zachos & Habel 2011), harbors more than 625 amphibian species, 77% of them endemic, and many with very narrow distributions (Rossa-Feres et al. 2017). Since the arrival of the first European colonizers in the early 16th century, the Atlantic Forest has lost most of its original cover, and the remaining is heavily fragmented (Ribeiro et al. 2009, 2011). In Brazil, habitat loss is the main threat to amphibians living in this rainforest (ICMBio 2018); 41 species are in peril, and two are already declared extinct (Ministério do Meio Ambiente 2022). The Atlantic Forest is also the region with the highest amphibian population declines reported worldwide (Toledo et al. 2023). Therefore, identifying and describing the amphibian diversity of the Atlantic Forest is crucial for its conservation and for developing targeted conservation strategies.

The landscape of the southeastern portion of the Atlantic Forest includes many mountain ranges that are considered cradles of amphibian diversity (Leite et al. 2008; Neves et al. 2018; Silva et al. 2018). These mountain chains harbor most of the endemic amphibian species from the Atlantic Forest (Guedes et al. 2020), and many are threatened (Pontes & Guidorizzi 2023). One such species is the Hatchet-faced Canga Lime Treefrog, *Sphaenorhynchus canga*, first described in 2015 (Araujo-Vieira et al. 2015) and known only from a small area in the southern portion of the Espinhaço Mountain range in Minas Gerais (Silveira et al. 2020). The species is classified by the Brazilian Ministry of Environment as Critically Endangered (Ministério do Meio Ambiente 2022; Pontes & Guidorizzi 2023).

During field expeditions in the northern portion of the Mantiqueira Mountain range in southern Minas Gerais in December 2015, January 2020, and November 2021, a series of specimens of *Sphaenorhynchus* were collected. We collected five adult male specimens during visual and acoustic searches in one pond in the countryside of Bom Jardim de Minas, Minas Gerais (-22.004, -44.180; 1,210 m; datum = SAD69). Specimens were euthanized in a 2% lidocaine chlorhydrate solution (MCTIC 2018), fixed in 10% formalin, and preserved in 70% alcohol. Prior to fixation, we collected tissue samples (muscle and liver) and stored them in cryotubes filled with 100% ethanol. Voucher specimens and tissues were deposited in the Coleção de Anfíbios da Universidade Federal de Juiz de Fora (CAUFJF), Juiz de Fora municipality, Minas Gerais, and in the Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP), Campo Grande municipality, Mato Grosso do Sul, Brazil. Collection permits for this study were issued by ICMBIO (SISBio 73975-1 and 72874-1).

DNA was extracted using the QIAGEN DNeasy Blood and Tissue Kit (Valencia, California, USA) following the manufacturer's protocol. Next, a fragment of the mitochondrial 16S gene was amplified using primers 16Sar and 16Sbr (Palumbi et al. 2002). The PCR protocol was configured with one initial phase of 94°C for 3 min, followed by 35 cycles of 94°C for 20s, 50°C for 20s, 72°C for 60s, and a final extension phase of 72°C for 5 min. Purification of PCR products and sequencing were performed by Eurofins Genomics Inc. (Louisville, Kentucky, USA). Comparable 16S sequences of *Sphaenorhynchus* from GenBank and one sequence of *Scinax fuscovarius* to use as an outgroup were downloaded (Supplementary Table 1). All 16S mtDNA gene fragments were aligned using the MAFFT algorithm (Katoh & Toh 2008) in Geneious v9.0.5 with default settings. The final dataset comprised 53 sequences of a 515 base-pair (bp) fragment of the 16S gene. A maximum likelihood tree was inferred in RAxML (Stamatakis 2014) via raxmlGUI 2.0 (Edler et al. 2021). The analysis was conducted using a ML + rapid bootstrap setting with a GTR+I+G substitution model and 1,000 bootstrap replicates. The appropriate substitution model was confirmed with Modeltest (Darriba et al. 2020) in raxmlGUI 2.0. Additionally, PTP and bPTP species delimitation analyses were conducted (Zhang et al. 2013) using the ML Tree. Calculations were performed on PTP webserver (http: //species.h-its.org/ptp/) with 500,000 MCMC generations, thinning set at 100, and burn-in at 10%. In addition, we performed the delimitation method Assemble Species by Automatic Partitioning (ASAP) on the online server (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html) using a simple distance model to compute distances between samples and default parameters (Puillandre et al. 2021). To explore relationships among mtDNA haplotypes, we estimated a 16S haplotype network among species closely related to *S. canga*—*S. botocudo*, *S. cammaeus*, *S. caramaschii*, *S. platycephalus*, and *S. surdus* (Pereira et al. 2022)—in POPART (Leigh & Bryant 2015) using the median-joining network method. We depict each species using different colors in the haplotype network. Lastly, we calculated sequence divergence (uncorrected *p*-distance) among species/individuals using MEGA v10.1.1 (Kumar et al. 2018).

We identified the *Sphaenorhynchus* from Bom Jardim de Minas as *Sphaenorhynchus canga* (Image 1). Our maximum likelihood tree (Figure 1) of the mitochondrial 16S gene confidently (bootstrap = 0.98) placed the sequenced specimens with *Sphaenorhynchus canga*, sister to a clade formed by *S. botocudo* and *S. surdus*. The three species delimitation methods we used yielded the same results, recovering one evolutionary entity for each known species (Figure 1). All three analyses confidently recovered all populations of *Sphaenorhynchus canga* as a single evolutionary lineage. Our haplotype network (Figure 2) shows a clear separation between all species of *Sphaenorhynchus*. The genetic distance between *S. canga* from Bom Jardim de Minas and *S. canga* from the type locality was 0.4% (Supplementary Table 2). Overall, the morphology of *S. canga* from Bom Jardim de Minas also have the standard diagnosis of the species presented in its original description, such as the lack of tympanic membrane, the snout protruding in profile, the presence of a canthal white line, a dorsolateral white line from the eye to sacral region, and a dorsolateral black line from the tip of snout extending beyond the eye and disappearing up to the flanks (Araujo-Vieira et al. 2015). The newly discovered population of *S. canga* in Bom Jardim de Minas extends the distribution of the species by about 200 km southward to a different mountain range, Serra da Mantiqueira (Image 2).

The distribution of *S. canga* in both the southern Espinhaço and the northern Mantiqueira mountain ranges is a pattern observed in other anuran species as well, including *Bokermannohyla feioi*, *Pithecopus ayeaye*, *Physalaemus maximus*, and *Scinax tripui* (Baêta et al. 2007; Magalhães et al. 2017; Silveira et al. 2019; Brunes et al. 2023). This shared distribution pattern has led biogeographers to hypothesize a historical connection between the Espinhaço and Mantiqueira mountain ranges (Magalhães et al. 2017; Neves et al. 2018; Brunes et al. 2023). The discovery of *S. canga* in the Mantiqueira Mountains adds additional support for a historical connection between these mountain ranges and increases the potential area where this species could occur, particularly in protected areas in the northern portion of Serra da Mantiqueira.

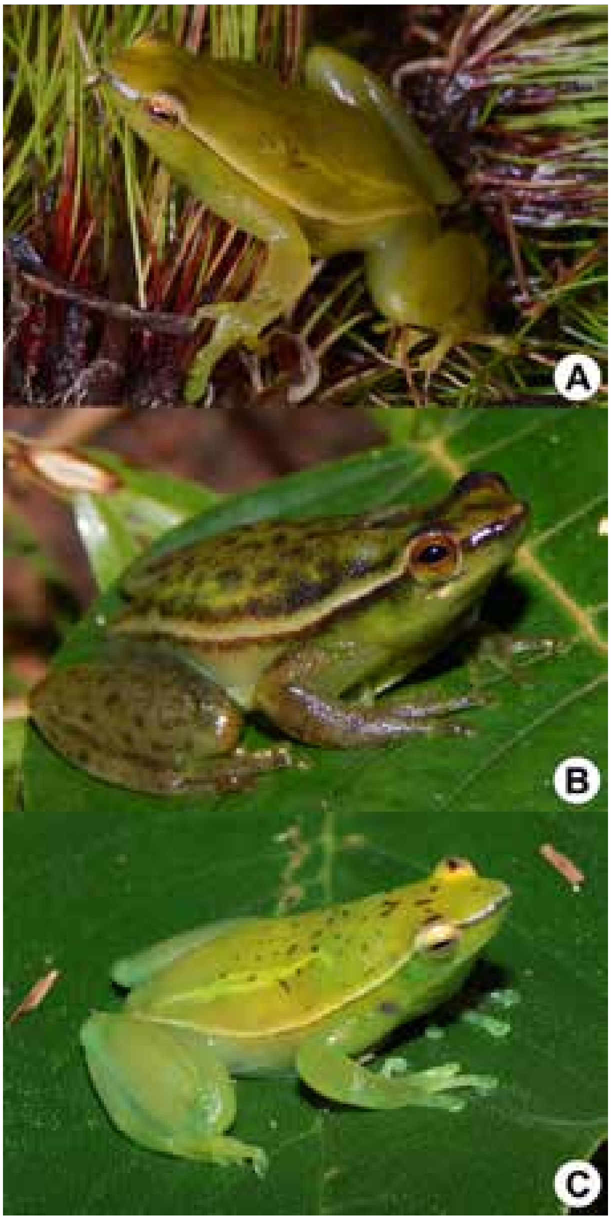

**Image 1. Adult males of *Sphaenorhynchus canga* documented in Bom Jardim de Minas, Minas Gerais, Brazil. A—MAP6807 collected on 1 November 2021 | B–C—unvouchered specimens photographed on 9 December 2015. © A—DJ Santana, B&C—EA Pereira.**

While the International Union for Conservation of Nature (IUCN) Red List of Threatened Species classifies this species as of Least Concern (IUCN & Boitatá 2023),

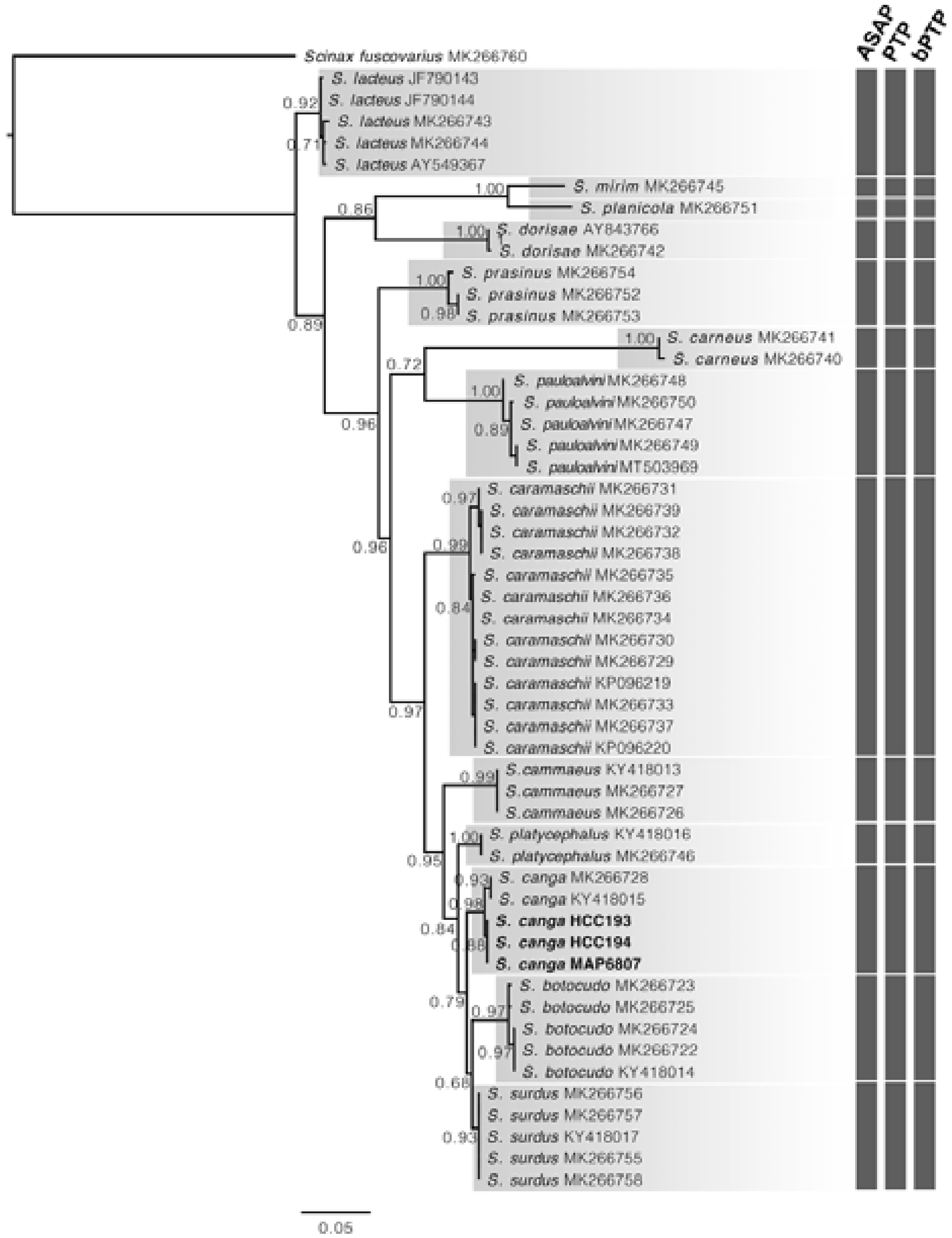

Figure 1. Maximum likelihood tree for *Sphaenorhynchus* based on the mitochondrial 16S gene. Nodes are labeled with Bootstrap values. Vertical grey bars on the right represent each evolutionary entity delimited by ASAP (Assemble Species by Automatic Partitioning), PTP (Poisson Tree Process), and bPTP (Bayesian Poisson Tree Process).

the latest update of the Brazilian Red List, which is based on IUCN criteria, classifies *Sphaenorhynchus canga* as Critically Endangered (Pontes & Guidorizzi 2023) due to its limited geographic distribution and restriction to ponds in ironstone outcrops, a habitat severely impacted by mining (Bastos et al. 2022). The IUCN assessment states that 'there are no ongoing major threats, the species is a habitat generalist occurring even in modified areas, and it is presumed to have a large and stable population' (IUCN & Boitatá 2023). However, mining activity poses a significant threat to *S. canga*. Five out of six ponds where the type series was collected are influenced by mining activities (Pena et al. 2017). Mining activity has resulted in a continuous decline in both the area and quality of *S. canga*'s habitat due to the suppression of ironstone outcrops and vegetation (Bastos et al. 2022). Recent surveys have had some success in finding the species in additional localities and habitats, including perennial small dams and anthropogenic swamps inside or on the edge of semi-deciduous seasonal forests, suggesting that it may have some degree of ecological plasticity; nonetheless, the species' spatial extent has only slightly increased because of these discoveries (Silveira et al. 2020). The discovery of *S. canga* in Bom Jardim de Minas is an important contribution to the conservation of this species, as research on its geographic distribution is among the main priorities (Bastos et al. 2022). Although our discovery has increased the species' distribution by more than 200 km (straight-line distance), it is important to note that is still restricted to high-elevation areas, reproducing in ponds (Araujo-Vieira et al. 2015; Silveira et al. 2020). Furthermore, no known populations of the species occur in protected areas (Bastos et al. 2022; this study).

While this discovery provides a glimmer of hope for the species, it is essential to note that the new population was found adjacent to a dirt road and in areas designated for cattle ranching, where vegetation around marshes is typically burned annually by local farmers. Moreover, the region is experiencing an increase in real estate speculation for allotments, and a proposal is currently under consideration for the installation

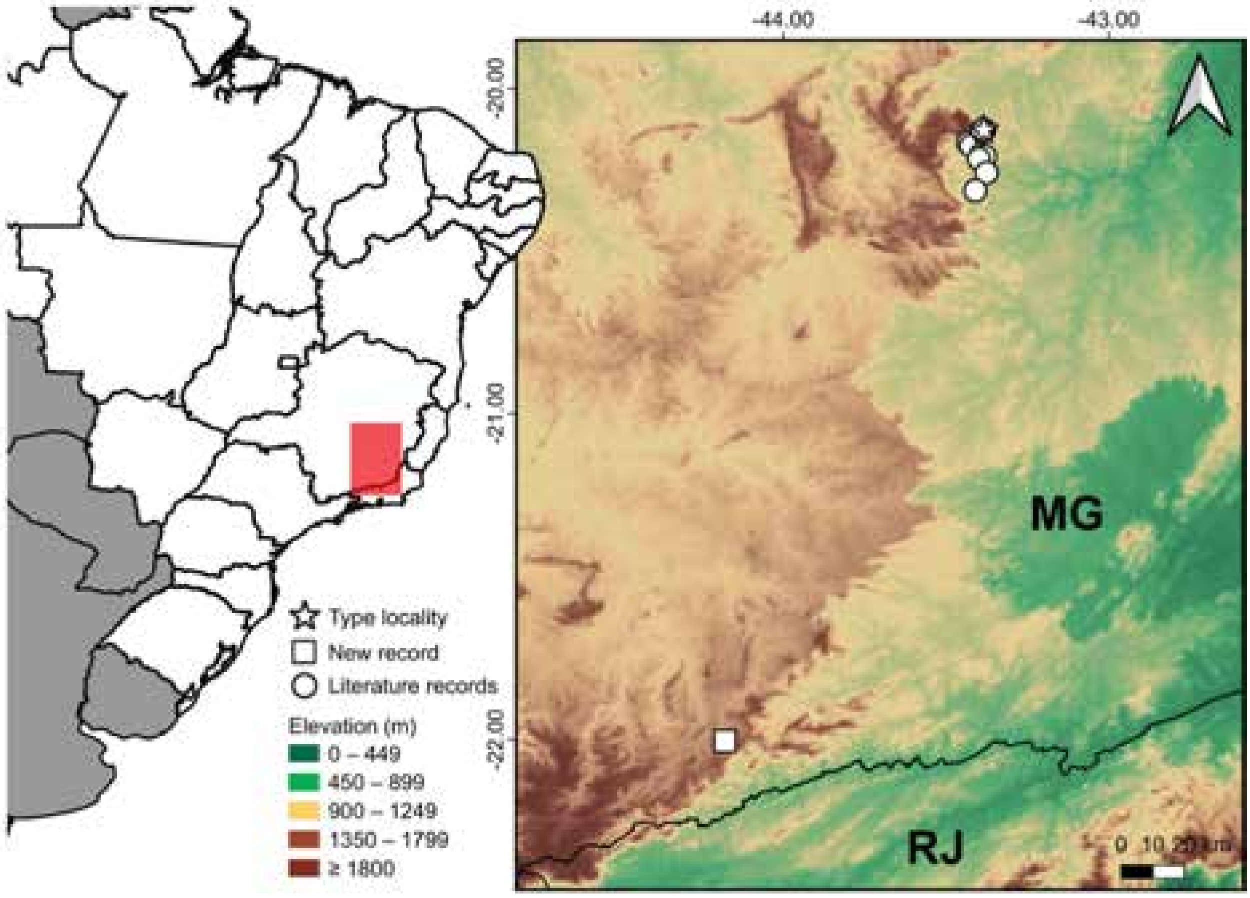

**Image 2. Brazil state and elevation map showing the known geographic distribution of *Sphaenorhynchus canga* in the Espinhaço and Mantiqueira mountain ranges. State abbreviations: MG—Minas Gerais | RJ—Rio de Janeiro.**

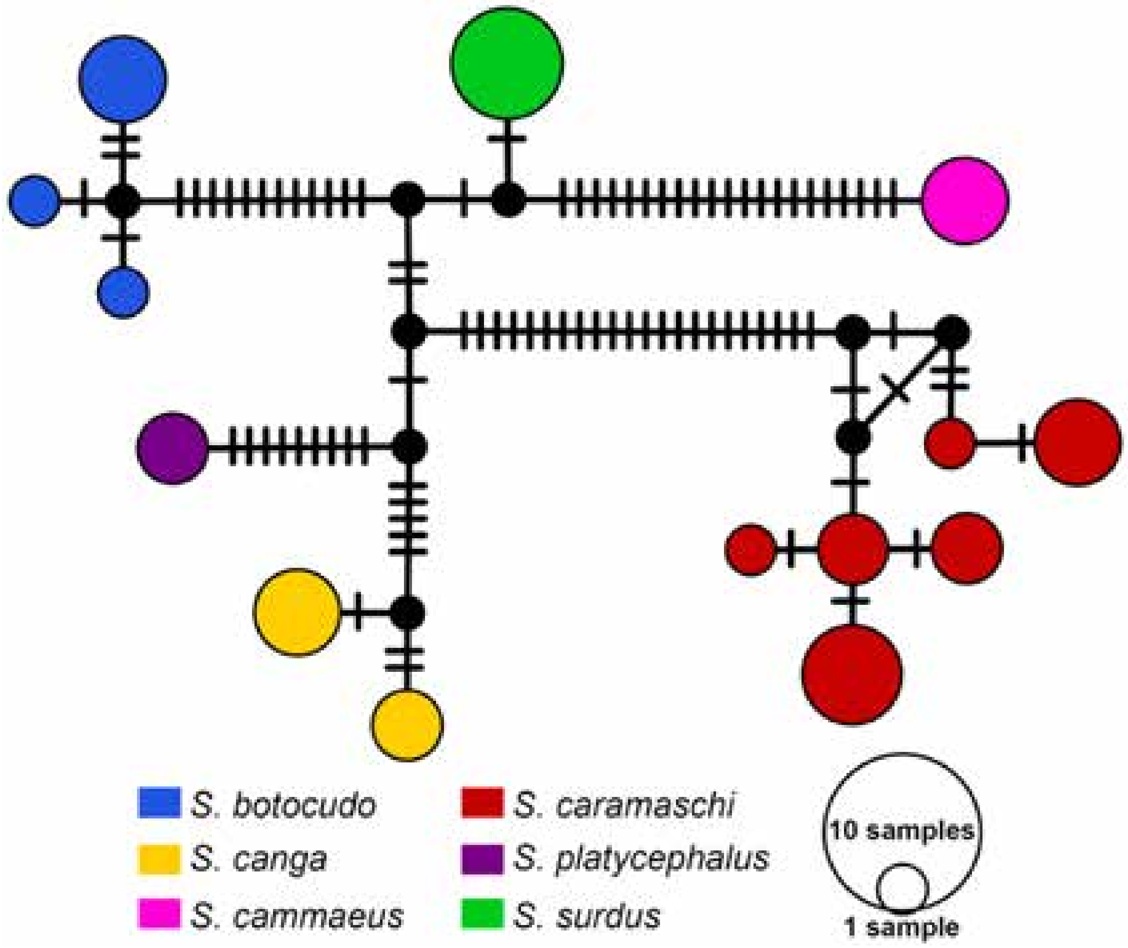

**Figure 2. Median-joining haplotype network of *Sphaenorhynchus canga* and closely related species based on 16S mtDNA. Each haplotype is represented by a circle whose area is proportional to its frequency. Traits indicate additional mutational steps for branches with more than one mutation. Different colors indicate species-level units. The black dots are median vectors (hypothesized sequences).**

of a hydroelectric power plant at the Pacau waterfall (Cachoeira do Pacau), just 5 km from the discovered population. Therefore, future visits to the locality are of utmost importance to monitor this population and to search for additional areas where the species may be present. A reevaluation of the conservation status of *S. canga* based on this discovery is beyond the scope of this work. Nonetheless, it is evident that this finding underscores the urgent need for further research, conservation measures, and advocacy efforts to ensure the survival of this critically endangered species.

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**Supplementary Table 1. GenBank accession numbers for mitochondrial 16S gene sequences of specimens of Sphaenorhynchini (*Sphaenorhynchus* and *Gabohyla*) and the outgroup *Scinax fuscovarius* included in the molecular analyses.**

| Species | Genbank accession number | Reference |
|---|---|---|
| *S. botocudo* | KY418014 | Roberto et al. (2017) |
| *S. botocudo* | MK266722 | Araujo-Vieira et al. (2019) |
| *S. botocudo* | MK266723 | Araujo-Vieira et al. (2019) |
| *S. botocudo* | MK266724 | Araujo-Vieira et al. (2019) |
| *S. botocudo* | MK266725 | Araujo-Vieira et al. (2019) |
| *S. cammaeus* | KY418013 | Roberto et al. (2017) |
| *S. cammaeus* | MK266726 | Araujo-Vieira et al. (2019) |
| *S. cammaeus* | MK266727 | Araujo-Vieira et al. (2019) |
| *S. canga* | KY418015 | Roberto et al. (2017) |
| ***S. canga*** | **HCC193** | **Present Work** |
| ***S. canga*** | **HCC194** | **Present Work** |
| ***S. canga*** | **MAP6807** | **Present Work** |
| *S. canga* | MK266728 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | KP096219 | Araujo-Vieira et al. (2015) |
| *S. caramaschii* | KP096220 | Araujo-Vieira et al. (2015) |
| *S. caramaschii* | MK266729 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266730 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266731 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266732 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266733 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266734 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266735 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266736 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266737 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266738 | Araujo-Vieira et al. (2019) |
| *S. caramaschii* | MK266739 | Araujo-Vieira et al. (2019) |
| *S. carneus* | MK266740 | Araujo-Vieira et al. (2019) |
| *S. carneus* | MK266741 | Araujo-Vieira et al. (2019) |
| *S. dorisae* | AY843766 | Faivovich et al. (2005) |
| *S. dorisae* | MK266742 | Araujo-Vieira et al. (2019) |
| *S. lacteus* | AY549367 | Faivovich et al. (2004) |
| *S. lacteus* | JF790143 | Jansen et al. (2011) |
| *S. lacteus* | JF790144 | Jansen et al. (2011) |
| *S. lacteus* | MK266743 | Araujo-Vieira et al. (2019) |
| *S. lacteus* | MK266744 | Araujo-Vieira et al. (2019) |
| *S. mirim* | MK266745 | Araujo-Vieira et al. (2019) |
| *G. pauloalvini* | MK266747 | Araujo-Vieira et al. (2019) |
| *G. pauloalvini* | MK266748 | Araujo-Vieira et al. (2019) |
| *G. pauloalvini* | MK266749 | Araujo-Vieira et al. (2019) |
| *G. pauloalvini* | MK266750 | Araujo-Vieira et al. (2019) |
| *G. pauloalvini* | MT503969 | Orrico et al. (2021) |
| *S. planicola* | MK266751 | Araujo-Vieira et al. (2019) |
| *S. platycephalus* | KY418016 | Roberto et al. (2017) |
| *S. platycephalus* | MK266746 | Araujo-Vieira et al. (2019) |
| *S. prasinus* | MK266752 | Araujo-Vieira et al. (2019) |
| *S. prasinus* | MK266753 | Araujo-Vieira et al. (2019) |
| *S. prasinus* | MK266754 | Araujo-Vieira et al. (2019) |
| *S. surdus* | KY418017 | Roberto et al. (2017) |
| *S. surdus* | MK266755 | Araujo-Vieira et al. (2019) |
| *S. surdus* | MK266756 | Araujo-Vieira et al. (2019) |
| *S. surdus* | MK266757 | Araujo-Vieira et al. (2019) |
| *S. surdus* | MK266758 | Araujo-Vieira et al. (2019) |
| *Scinax fuscovarius* | MK266760 | Araujo-Vieira et al. (2019) |

**Supplementary Table 2. Average uncorrected (*p*-distance) sequence divergence between different species of *Sphaenorhynchus*. Values in bold along the diagonal are intraspecific divergences. n/c= not calculated.**

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *S. botocudo* | **0.004** | | | | | | | | | | | | |
| 2 | *S. cammaeus* | 0.067 | **0.000** | | | | | | | | | | | |
| 3 | *S. canga* | 0.039 | 0.055 | **0.004** | | | | | | | | | | |
| 4 | *S. caramaschi* | 0.073 | 0.073 | 0.059 | **0.006** | | | | | | | | | |
| 5 | *S. carneus* | 0.151 | 0.140 | 0.143 | 0.137 | **0.004** | | | | | | | | |
| 6 | *S. dorisae* | 0.133 | 0.140 | 0.121 | 0.137 | 0.167 | **0.002** | | | | | | | |
| 7 | *S. lacteus* | 0.107 | 0.102 | 0.106 | 0.099 | 0.137 | 0.090 | **0.005** | | | | | | |
| 8 | *S. mirim* | 0.166 | 0.157 | 0.149 | 0.156 | 0.183 | 0.126 | 0.127 | **n/c** | | | | | |
| 9 | *S. platycephalus* | 0.053 | 0.054 | 0.032 | 0.060 | 0.140 | 0.132 | 0.102 | 0.135 | **0.000** | | | | |
| 10 | *S. pauloalvini* | 0.114 | 0.092 | 0.107 | 0.097 | 0.130 | 0.128 | 0.107 | 0.149 | 0.096 | **0.006** | | | |
| 11 | *S. planicola* | 0.147 | 0.139 | 0.127 | 0.135 | 0.162 | 0.131 | 0.122 | 0.065 | 0.112 | 0.132 | **n/c** | | |
| 12 | *S. prasinus* | 0.110 | 0.089 | 0.087 | 0.090 | 0.137 | 0.125 | 0.091 | 0.142 | 0.090 | 0.096 | 0.144 | **0.007** | |
| 13 | *S. surdus* | 0.032 | 0.045 | 0.023 | 0.060 | 0.140 | 0.135 | 0.107 | 0.157 | 0.028 | 0.105 | 0.139 | 0.092 | **0.000** |

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ARTICLE

# Ecological values of Ourkiss wetland (Oum El Bouaghi province - Algeria), an overview of waterbirds diversity and richness

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**Abstract:** The monitoring of waterbirds' abundance and richness serves as the primary method for scientists to characterize the ecological values and diversity profile of wetlands. This survey was specifically conducted in Ourkiss wetland, situated in the Oum El Bouaghi province of East Algeria, spanning from January to May 2013. The study aimed to elucidate the ecological significance of Ourkiss wetland by analyzing various parameters, including the abundance, richness, diversity profile, and conservation status of its waterbird population. A total of 23 species, representing 11 families, were documented during the survey period, with Anatidae and Rallidae emerging as the most prevalent taxa. Notably, Ourkiss wetland exhibited two distinct populations: the "wintering population" and the "breeding population," with significant waterbird activity observed during migration between the northern and southern regions. The presence of the endangered species *Oxyura leucocephala* further underscores the ecological importance of this wetland. Noteworthy peaks in waterbird diversity were particularly observed in April, as indicated by richness and Shannon indices. To safeguard the ecological integrity of Ourkiss wetland, it is strongly recommended to intensify conservation efforts and implement effective management plans.

**Keywords:** Abundance, conservation, diversity profile, endangered species, management, monitoring, wintering

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**Author contributions:** RA—sampling, the conception of the study, editing the first draft; MB—data analysis, editing first draft, reviewing.

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## INTRODUCTION

Animal population dynamic depends on many intra- and inter-species-specific factors (Mukherjee & Roy 2021). The bio-monitoring of these factors (such as air temperature, rainfall, prey-predator relationship, and trophic availability) is fundamental for understanding ecology, population dynamics, and conservation of animals (Kitahara et al. 2022). Many studies reported that birds are used as bioindicators of ecosystems and their population is significantly influenced by habitat structure and foraging availability (Norris & Marra 2007; Carnicer et al. 2009; Byju et al 2023a). Moreover, adaptive radiations and ecological niches are widely recommended for birds' expansion and diversity (Cooney et al. 2017). Also, VASQUEZ et al. (2007) emphasize that bird expansion and diversity result from the distribution of abundance and richness among individuals.

Green & Elmberg (2014) said that the waterbird species are protected effectively as much as the services and values of ecosystems are identified. This ecological balance of ecosystems (i.e., ecosystems values and services) is indicated throughout avifaunal diversity studies (Byju et al. 2023b; Gyeltshen et al. 2023). It is well known that biodiversity within ecosystems and landscapes is influenced and regulated by the assemblage of birds (Kumar & Sahu 2020).

The distribution of abundance, richness and diversity profile of waterbirds in semi-arid wetlands are little studied in Algeria, and all data published previously were focused on northeastern wetlands (mainly in SKIKDA and ELTARF provinces) (Merzoug et al. 2015; Merzoug et al. 2021; Boubekeur et al. 2020; Loucif et al. 2020; Draidi et al. 2023; Ouarti et al. 2023).

In this study, we aim to describe the waterbird population in Ourkiss wetland (Oum El Bouaghi province). Our approach was focused on a description of the ecological value of Ourkiss wetland by using 1) ecological indices (Shannon, Simpson, Evenness, and Berger-Parker), 2) monthly variation of abundance and richness, and 3) the diversity profile of these waterbirds' population. The survey was initiated based on the geographical location of Ourkiss, situated within the primary migratory flyway "North-South" that connects the northern and southern regions of the country.

## MATERIALS AND METHODS

### Study area

Ourkiss wetland (35.8759$^0$N, 6.9387$^0$E) (Figure 1), is a freshwater dam flooded by Oued Ourkiss. It covers a total surface of 36 ha at an altitude of 930 m above sea level. Under the authority of the Ain Fakroun district (Oum El Bouaghi province - Eastern Algeria), it was built in 2004 by an Algerian hydraulic agency to maintain rainfall water (the irrigation of neighboring lands, arboriculture, and cereal crops). In some parts of this dam, we can observe the emergent aquatic plants "hydrophytes" such as *Typha* sp. and *Phragmites* sp. (Aissaoui, R. pers. obs . 2013). The climate is semiarid, with an annual mean temperature of 15.56°C (minimum 6.53°C recorded in January and maximum 26.13°C recorded in August). The annual rainfall average never exceeds 400 mm (Data provided from ONM). This wetland is not classified as a protected area. It is not cited in the national protected area database.

### Sampling and data analysis

Waterbird counts were conducted three times per month from January to May 2013. The observations were made using an ornithological telescope Konus (60 x 25) during the twilight period of the day from a concealed observation point to minimize disturbance (Lumpkin & Pearson 2013). The punctual abundance indices (PIA) method, recommended by Bara & Segura (2019), was utilized for assessing abundance and richness, as it significantly reduces observer movement and disturbance (Ochando 1988). Observers remained stationary at one point for 15–20 minutes, tallying the abundance of waterbirds (Blondel 1985).

Four ecological indices (Shannon-Weaver, Simpson, evenness, and Berger-Parker) were calculated as per the methods described by Shannon & Weaver (1949), Pielou (1975), and Caruso et al. (2008). The conservation status of species was determined based on the IUCN Red List criteria (https://www.iucnredlist.org/resources/birdlife2021). A statistical analysis, including the Kruskal-Wallis test, was conducted to compare waterbird abundances. A diversity profile was generated to compare the composition of waterbird families, with α values set at α = 0 for richness, α = 1 for the Shannon index, α = 2 for the inverse Simpson index (1/D), and α = a higher value approximating the Berger-Parker index.

The principal component analysis (PCA) was performed to examine the correlation between waterbirds' abundance and their monthly distribution (Pearson, 1901). The selection of two independent

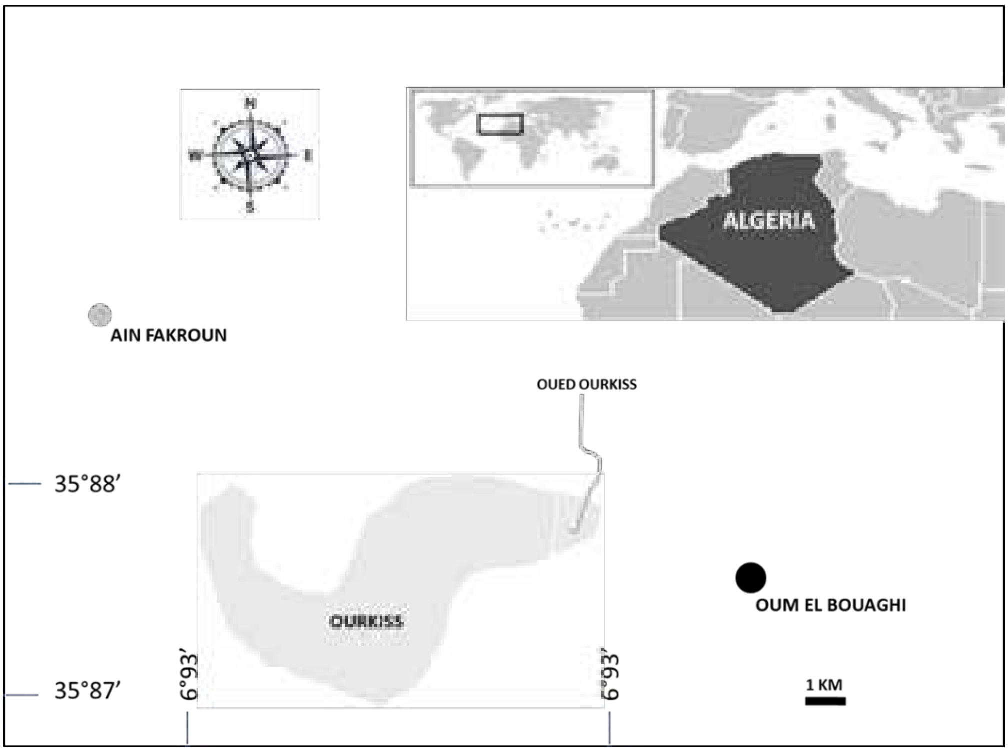

Figure 1. Geographical situation of Ourkiss wetland (Ain Fakroun – Oum El Bouaghi province).

components, labelled "PC1" and "PC2," aids in focusing attention on the primary proportion of information (Litvak & Hansell 1990; Janžekovič & Novak 2012). The high variance explained by these first principal components (PCs) facilitates computational procedures and enhances analysis reporting (Vaughan & Ormerod 2005). Thus, PCA enables the analysis of species abundance and their monthly variation (Blanck et al. 2007).

A statistical matrix of size 6 × 15 was constructed. Waterbird taxa absent ('0') in more than five sampling data points were excluded from the analysis. All statistical analyses and tests, including Kruskal-Wallis, ecological indices, diversity profile, and PCA, were conducted using PAST 4.11 software (Hammer et al. 2012).

## RESULTS

### Waterbird's abundance and richness

During the survey, 23 waterbird species from 11 families were recorded. All recorded waterbirds are classified as Least Concern in the IUCN Red List status, except for the White-headed Duck *Oxyura leucocephala*, which is endangered. The maximum richness (number of species, S) was observed in April (towards the end of the month), while the abundance of the waterbird population peaked in early January (first week, 221 individuals per 36 ha) (Figure 2). Subsequently, the number of individuals decreased to a minimum recorded in mid-May (44 individuals) (Figure 2). A significant monthly difference was observed in the abundance of waterbird families (Kruskal-Wallis: H (chi-2) = 94.09, P (same) = 1.855 E-17). The abundance of Rallidae was higher or significantly higher than other waterbird families, except between "Rallidae/Anatidae", "Rallidae/Podicipididae", and "Rallidae/Ciconidae" (Table 1).

### Ecological indices

In mid-April, the maximum values of Simpson and Shannon indices were recorded (0.79 and 1.9, respectively). The evenness reached a maximum in the end of January 0.76). Berger-Parker index reached the maximum in early January (0.71).

Figure 3 summarizes the monthly trending of four ecological indices. The Berger-Parker index decreased substantially in February 0.32) whereas the abundance corresponded to 160 individuals and the dominance corresponded to 0.23. Except in May where the abundance and dominance of waterbird population noted 44 individuals and 0.32, respectively, the Berger-Parker was 0.52.

Table 1. Dunn's post hoc test comparing the monthly variation of abundance intra waterbirds families. Bonferroni corrected p values.

| | Anatidae | Podicipedidae | Ardeidae | Ciconidae | Gruidae | Ralidae | Recurvirostridae | Sternidae | Accipitridae | Scolopacidae | Corvidae |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Anatidae** | | 1 | 1 | 1 | *** | 1 | 0.06 | *** | 0.69 | *** | *** |
| **Podicipedidae** | 1 | | 1 | 1 | *** | 1 | 0.39 | *** | 1 | *** | ** |
| **Ardeidae** | 1 | 1 | | 1 | 0.49 | ** | 1 | 0.42 | 1 | 0.30 | 1 |
| **Ciconidae** | 1 | 1 | 1 | | * | 0.09 | 1 | * | 1 | * | 0.10 |
| **Gruidae** | *** | *** | 0.49 | 0.04 | | *** | 1 | 1 | 0.94 | 1 | 1 |
| **Ralidae** | 1 | 1 | ** | 0.09 | *** | | *** | *** | ** | *** | *** |
| **Recurvirostridae** | 0.06 | 0.39 | 1 | 1 | 1 | *** | | 1 | 1 | 1 | 1 |
| **Sternidae** | *** | *** | 0.42 | * | 1 | *** | 1 | | 0.81 | 1 | 1 |
| **Accipitridae** | 0.69 | 1 | 1 | 1 | 0.94 | ** | 1 | 0.81 | | 0.59 | 1 |
| **Scolopacidae** | *** | *** | 0.3 | * | 1 | *** | 1 | 1 | 0.59 | | 1 |
| **Corvidae** | *** | ** | 1 | 0.1 | 1 | *** | 1 | 1 | 1 | 1 | |

*p <0.05, ** p <0.01, ***p <0.001

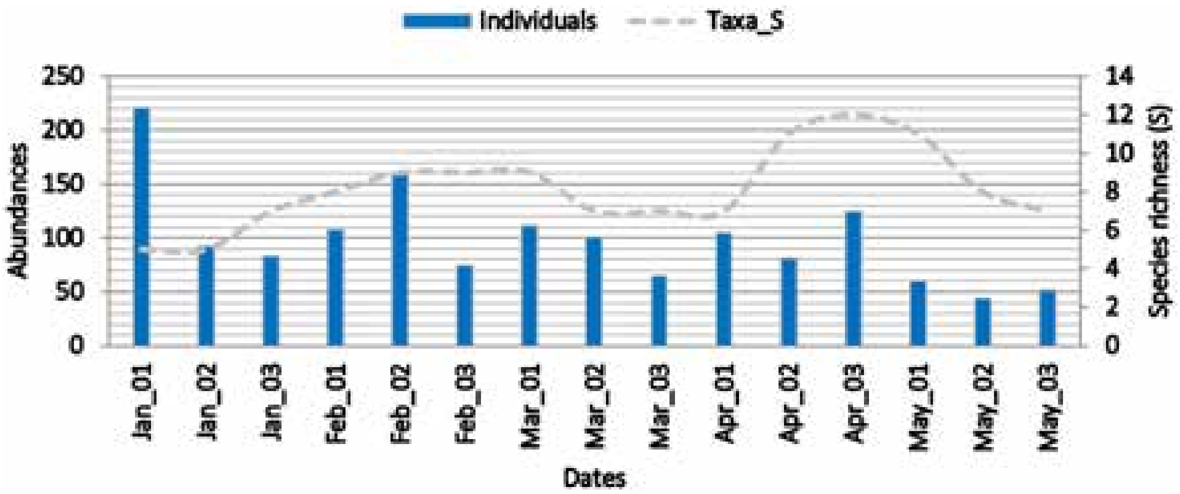

Figure 2. Trend of waterbird abundance and richness in Ourkiss wetland (Oum El Bouaghi province).

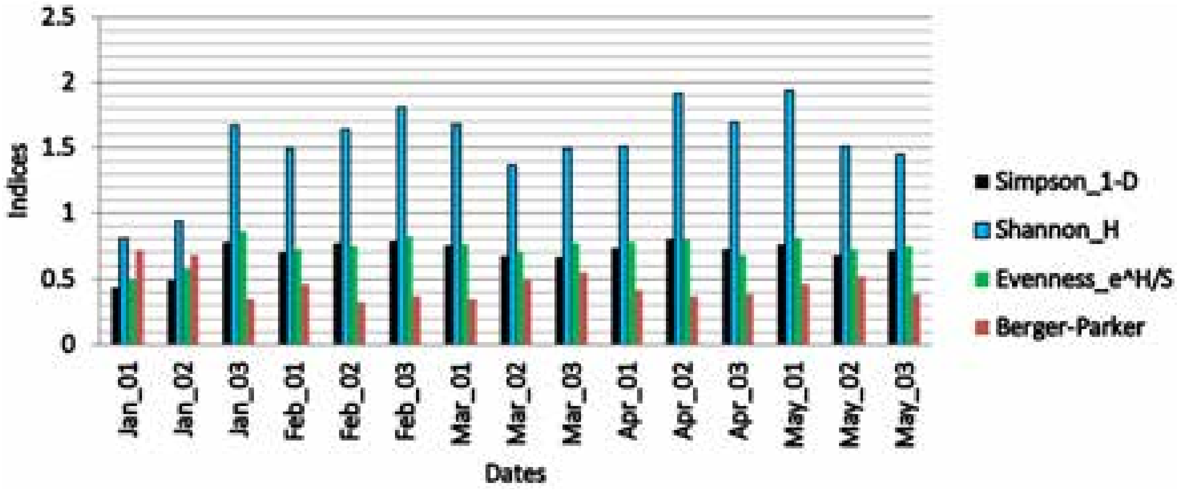

Figure 3. Monthly variability of Shannon, Simpson, evenness, and Berger-Parker indices in Ourkiss wetland (Oum El Bouaghi province).

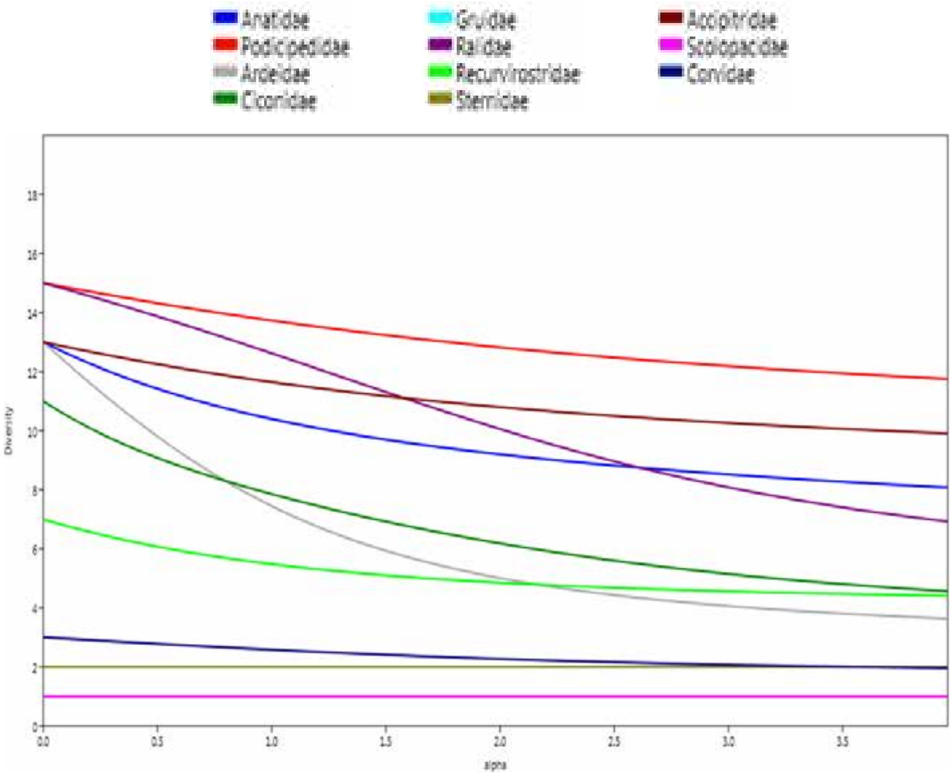

**Figure 4. An overview of the alpha diversity profile in Ourkiss wetlands (Oum El Bouaghi province).**

### Diversity profile

Figure 4 exposed the template of alpha diversity according to waterbird families. Rallidae, Anatidae, and Podicipedidae were the most abundant families according to the diversity profile (higher values when alpha = 0) (Figure 4). Indeed, the total abundance of these three families was 684, 384, and 107, respectively. The podicipididae abundance decreases substantially and shows a fallen curve (Figure 4).

The diversity profile of Gruidae, Corvidae, Scolopacidae, and Sternidae show a steady shape with low values of abundance (40, 10, six, and four respectively) along an alpha axis (Figure 4). The curve representing Ciconidae and Recurvirostridae was smoother along the profile (total abundance reached 152 and 47 individuals, respectively).

### The monthly variation of abundance

**Table 2. Principal component analysis describing the monthly variation of waterbird abundance in Ourkiss (Oum el Bouaghi province).**

| PC | Eigenvalue | % variance |
|---|---|---|
| 1 | 6703.4 | 85.02 |
| 2 | 713.257 | 9.0464 |
| 3 | 394.493 | 5.0034 |
| 4 | 43.9466 | 0.55738 |
| 5 | 29.3746 | 0.37256 |

The monthly variation of waterbird abundance was reported by PCA components with Eigenvalues, % variance and plots provided in Table 2 and Figure 5. The primary information was reported by PC1 and PC2 which collectively accounts for 94% of the variance. PC1 (85%) effectively distinguished between Rallidae/Anatidae and other families (Podicipedidae, Ciconidae, Accipitridae, Ardeidae). PC2 (9%) indicated that Anatidae were predominantly associated with Ourkiss wetland during the winter period (from late January to early April), while the remaining waterbird families were observed in early January (first and second weeks) and from April to May. Additionally, the abundance of Rallidae and Anatidae exhibited a negative correlation with the rest of the waterbird families (refer to Figure 5).

## DISCUSSION

The richness of waterbirds was less than other neighboring wetlands in northeast Algeria (in Lake

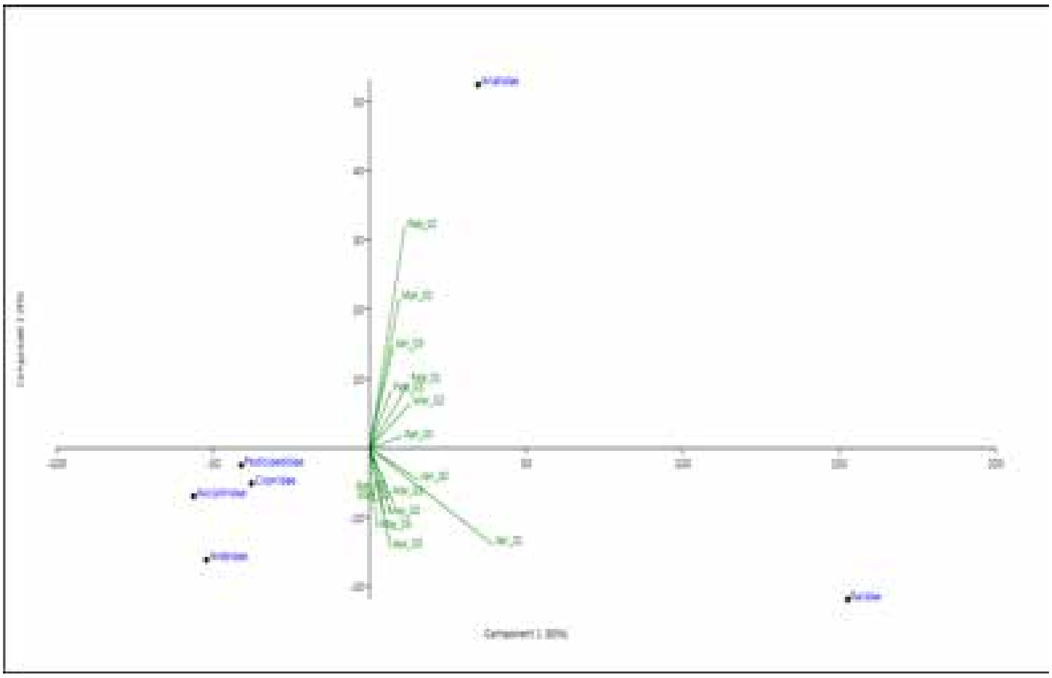

Figure 5. Projection of the waterbirds' families on the PCA map according to monthly trending in Ourkiss wetland (Oum El Bouaghi province).

TONGA (Loucif et al. 2020) and in Garaet HADJ TAHAR (Bara et al. 2020) both reported 35 species. While the number of species in Ourkiss reaches 23 species in spite of the restricted area in Ourkiss (36 ha). The number of species here represented 65% of the total richness reported in the Algerian avifauna database. Also, according to the total area, Ourkiss wetland is smaller than Lake Tonga (2,400 ha) and Garaet Hadj Tahar (100 ha). This data shows that the size of wetlands is not a deterministic factor of waterbird richness.

The Rallidae and Anatidae were the most dominant waterbirds (noted during the study period). The only species of Podicipedidae (The little Grebe *Tachybaptus ruficollis*) showed a preference for open shallow wetlands (Mukherjee & Roy 2021), and recorded during the entire study period

The Accipitridae (mainly the Western Marsh Harrier *Circus aeruginosus*) never exceeded five individuals but were recorded throughout the study period. This species is known as a predator in open wetlands and a wintering species in the Mediterranean region (Agostini & Panuccio 2010). The birds of families Corvidae, Sternidae, Gruidae, Scolopacidae, and Recurvirostridae were recorded as irregular birds (with a low abundance, they are observed as sporadic or occasional birds). The waterbirds' abundance is limited by conditions encountered in migration. Mainly, the food supply can reduce the number of individuals (Newton 2006). Now it is unclear to what extent different waterbird species overlap in their roles as vectors and how robust this pattern is to changes in the waterbird population (Green & Elmberg 2014). However, this abundance is recognized as an asymmetric interaction network. This pattern suggests that bottom-up processes have a greater influence than top-down processes in these networks (Shurin et al. 2002). Kumar & Sahu (2020) reported that the complexity of food resources can organize the trophic guilds of birds. Also, the habitat structure (such as water level) can be a deterministic factor in the distribution pattern of aquatic birds (Malik & Joshi 2013; Kumar et al. 2016). However, recent waterbird abundance and distribution data have shown a notable increase related to these deterministic factors (mostly the draught in Ourkiss induced by low rainfall and intensive agriculture).

The ecological indices reached the maximum in April and January. We observed that during these two months, the waterbirds changed their phenology status (wintering versus breeding). The Anatidae associated with the wintering period (i.e., Northern Shoveler Spatula *clypeata* and Common Shelduck *Tadorna tadorna*) are known as wintering birds in Algeria (Loucif et al. 2020). Except for the White-headed Duck which is a sedentary in Ourkiss it is known as a breeding bird in Lake Tonga (Chettibi et al. 2013).

The Shannon and Simpson indices reached a maximum in April-May, this period corresponded to breeding. But, the Evenness and Berger-Parker indices reached their maximum in January. On the other side, under disturbance waterbird population can share a dominant pattern (Caruso et al. 2008) and this population was dominated by some sedentary species (such as ducks, coots, and grebes), it results a high value of the Berger-Parker index in January. The rest of the species were opportunistic and did not record in the first week of January.

The number of waterbirds decreasing significantly in this dam (many ducks recorded previously, were not observed) was recorded, this observation can be explained by a large scale of agricultural activities (which use a high quantity of water) and water deficiency (caused by a little rainfall level recorded this decade).

Also, many wetlands lose their ecological functions and values (by losing richness and abundance) (Sekercioglu et al. 2004). Many studies reported that waterbirds' dynamics and number of individuals were influenced by the seasonal interactions, "The seasonal interactions will depend on the degree of migratory connectivity between periods of the year" (Norris & Marra 2007).

It is known that the monthly distribution of waterbirds was related to the behavior and the phenology of each species. A large part of Anatidae had a wintering status (observed during the winter). The variables clustering shown in our PCA map gives an easier way to explain this assembling (see PCA map).

An urgent conservation plan for Ourkiss wetland is strongly recommended, along with a comprehensive survey of the site to potentially classify it as an Important Bird Area (IBA), particularly considering the possible breeding of the White-headed Duck as suggested by many scientists. Besides this, various threats such as the intensive agriculture that assigns the ecological integrity of Ourkiss. This survey can allow the classification of Ourkiss wetland as a protected area. In this context, a global bird conservation perspective by regular long-term monitoring can accelerate this classification.

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ARTICLE

# Elliptic Fourier analysis of leaf shape of *Callicarpa pedunculata* and *Callicarpa rubella* (Lamiaceae)

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**Abstract:** Leaves play an important role in species discrimination. An elliptic Fourier analysis (EFA) based morphometric technique was used to assess divergence between the poorly differentiated species, *Callicarpa pedunculata* and *C. rubella*. Using leaf specimen images from herbarium collections, principal components (PCs) were extracted from the Fourier coefficients and used to describe leaf outline and leaf shape descriptors: circularity, aspect ratio, and solidity. The results indicate that symmetric (54%) and asymmetric (35%) components of the leaves of *C. pedunculata* and *C. rubella* are sources of shape variation, as shown in the width and leaf tips among the samples. MANOVA revealed significant interspecific differences ($P = 0.03$) between *C. pedunculata* and *C. rubella.* The jack-knife cross-validation showed 71% of correctly classified species both in *C. pedunculata* and *C. rubella.* Furthermore, the results of this study were able to reveal significant leaf shape descriptors like aspect ratio, circularity, and solidity as important diagnostic characters in discriminating *C. pedunculata* and *C. rubella.* Thus, in conclusion, leaf serrations, leaf size, and leaf lobes are important characteristics in discriminating between *C. pedunculata* and *C. rubella*.

**Keywords:** Aspect ratio, Callicarpa, circularity, correlation, evolution, geometric morphometrics, leaf, principal component analysis, solidity, symmetry.

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

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## INTRODUCTION

*Callicarpa* is a genus of Lamiaceae characterized by branched hair; inflorescences axillary; flowers polysymmetric, 4(−5) merous; anthers porose; stigma peltate or capitate; and fruit a drupe (Linnaeus 1753; Munir 1982; Leeratiwong et al. 2009; Bramley 2013). Several species of *Callicarpa* have been classified and formally recognized from different parts of the world, including the Philippines and Borneo. *C. pedunculata* R. Br. and *C. rubella* Lindl. show extensive distribution in the southeastern Asian region, but both are geographically and taxonomically controversial. *C. pedunculata* is not found in Sumatra, Java and Borneo, while *C. rubella* is rather more extensive, occupying a wider range in the Asian continent. In contrast, *C. pedunculata* is widely distributed in the Philippines, while *C. rubella* is not present (BGCI 2024; Arvidsson 2020). Taxonomically, the relationship between the two taxa was not clear due to ambiguous morphological characters.

C. *pedunculata* and *C. rubella* were usually differentiated by their leaf size and presence of glandular hairs (Bramley 2013): *C. pedunculata* has wider leaves and lacks glandular hairs, while *C. rubella* has narrower leaves and hairs are present. Although its morphology has been previously described by Bramley (2013, 2019), *C. pedunculata* is easily confused with *C. rubella* due to misleading morphological characters. Likewise, several taxonomists have linked other species with *C. pedunculata* and *C. rubella*, e.g., the long-established *C. caudata* Maxim and doubtful *C. cuspidata* Roxb. were linked to *C. rubella* based on indumentum and leaf serrations (Roxburgh 1820; Lam & Bakhuizen 1921) and leaf bases (Bramley 2013), while *C. cuspidata* has been reported as a synonym of *C. pedunculata* (Munir 1982) which adds to the confusion between the two taxa. Likewise, no direct studies have identified the relationship between *C. pedunculata* and *C. rubella* to further separate or combine the two species. Thus, the taxonomic status of *C. pedunculata* and *C. rubella* was becoming uncertain due to the overlapping of morphological characters.

The taxonomic transcription among *C. pedunculata* and *C. rubella* and its closely related species were originally described by Roxburgh (1820) and revised by Munir (1982), but, according to Bramley (2013), they did not indicate any specimen or type to describe the species. Consequently, Bramley (2013), considered the description of Roxburgh (1820) and Munir (1982) unsuitable for correct identification due to lack of data and poor vouchering. In a previous study of *Callicarpa* in Thailand and the Philippines (Leeratiwong et al. 2009; Bramley 2013), *C. rubella* was recognized as distinct from other *Callicarpa* species through its cordate or obliquely cordate leaf base, while *C. pedunculata* was defined by its attenuation to cuneate leaf bases. Currently, our knowledge of these two species is known only from collections made early in the twentieth century, and recent studies were mostly based on herbarium specimens. The lack of updated distribution listings and exhaustive data contributes to species taxonomic challenges. This also raises several questions on the current conservation status and taxonomic relationship of *C. pedunculata* and *C. rubella.* While *C. rubella* is thought to be absent in the Philippines, its current natural distribution is also difficult to determine with precision because of the potential impact of human use in different countries. In the southeastern Asian region, *C. pedunculata* and *C. rubella* were reported to have medicinal properties (Brown 1920; Tu et al. 2013) collected from twigs, roots, and leaves, while their fruits are used for human consumption. Thus, the natural distribution of most species may have been changed by its dispersal based on human actions affecting local or even global distributions (Di Marco & Santini 2015; Newbold et al. 2015). The change in the environment and distribution of species were highly influential in plant structures, especially on leaves which serve as indicators of environmental change (Gupta et al. 2019; Zhang & Li 2019).

In this paper, the authors discuss leaf morphometrics using a more comprehensive quantification of leaf shape, where measurements of individual parameters were obtained as a basis of species discrimination. This technique, elliptical Fourier descriptors (EFD) utilizes the sum of ellipses over contours to quantify outlines and silhouettes in an image (McLellan & Endler 1998; Hearn 2009; Godefroy et al. 2012), based on the instructions taken from Klein and Svoboda (2017) on geometric morphometric analysis. Aside from the typical leaf extraction, leaf shape descriptors: Circularity, measured as 4π (area/perimeter$^2$) related to serrations and lobing; Aspect ratio (AR), the ratio of the major to the minor axis and influenced by length and width; and, Solidity, measured as area or convex hull and sensitive to leaf deep lobes (Cope et al. 2012) were incorporated into the downstream analysis. As leaf shapes vary among or within species, it is also important to quantify leaf shapes to understand broader aspects of plant adaptation to the environment (Chitwood, et al. 2014). Leaf morphological traits such as length, width, and veins are controlled by the environment, whether to stabilize or to adjust to certain environmental conditions (Alonso-Forn et al. 2020). This study describes for the first-time accessions of *C. pedunculata* and *C. rubella* through leaf morphometrics, contributing to a better understanding of the species variation through leaf shapes. Furthermore, this study aimed to discriminate *C. pedunculata* and *C. rubella* leaf shape descriptors: circularity, aspect ratio (AR), and solidity between the two taxa, and predict the correlation among the three leaf descriptors.

## MATERIALS AND METHODS

### Study Site

A total of 46 individual herbarium samples of *C. pedunculata* and *C. rubella* were used in the study (Image 1). Twenty samples of *C. pedunculata* were collected in the secondary forests and forest edges of Palanan, Isabela in the Philippines while 26 samples of *C. rubella* were carried out from selected digital herbarium of AMD, FLMNH, K, MSU, NY, US, and USTH (Image 2) through online accessions in the Global Biodiversity Information Facility (GBIF) database via the web interface (Table 1). The online images and details were downloaded using the 'Darwin Core Archive' format which contains the URLs and information of the samples in GBIF (Table 9). On the other hand, samples

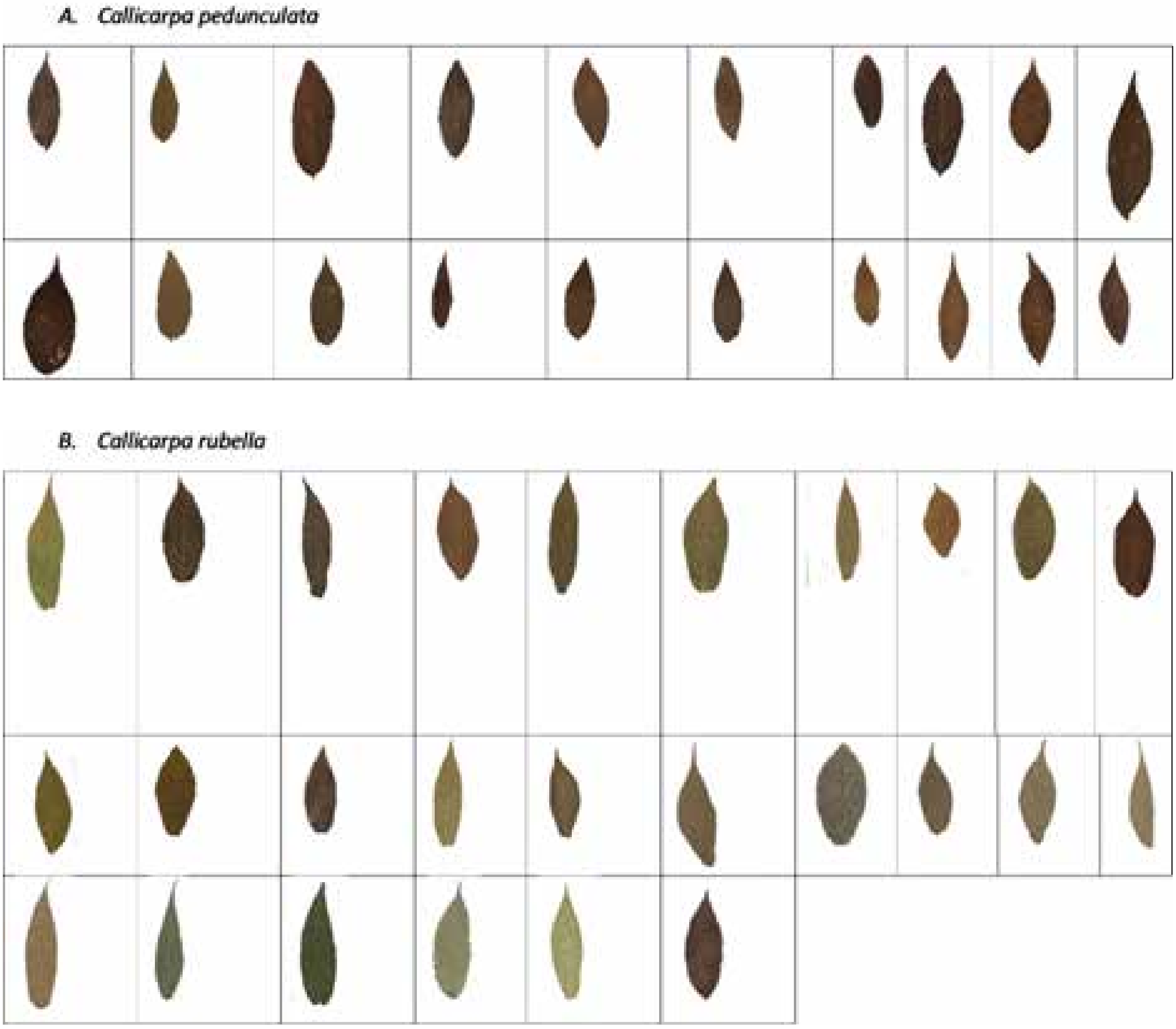

**Image 1. The sample of *C. pedunculata* and *C. rubella* leaves used in the study from selected digital herbaria.**

**Image 2. Map of southeastern Asian region indicating the localities of selected *C. pedunculata* and *C. rubella* species used in the study: 1—China | 2—Indonesia | 3—Myanmar | 4—Taiwan | 5—Thailand | 6—Vietnam | 7—Philippines. (Map: www.scribblemaps.com).**

from the fieldwork have undergone herbarium protocol from the securing of the permit for the collection of specimens, preparation of materials, pressing of the specimen, mounting in herbarium sheet, identification, and labeling to the deliberation of voucher specimen to the University of Santo Tomas Herbarium (USTH) in the Philippines.

### Procedures

In this study, herbarium samples were the main source of datasets to build shape descriptors from the leaf outline. The collected digital images were subjected to leaf isolation using Adobe Photoshop version 22.0.0 (Adobe System San Jose, USA). After all leaves have been isolated from the scans, the software SHAPE (Iwata & Ukai 2002) which uses binary leaf outline image files in BMP format converts images to black and white. SHAPE converts the image outlines to chain code and then normalized EFDs. A maximum number of harmonics were set to 20 to recapitulate leaf shape and the normalization method was set to the longest radius for the initial orientation of the images. From the obtained EFD coefficients, the analysis focused on coefficients a and d, as well as coefficients b and c. These correspond to the symmetric and asymmetric components of leaf shapes, respectively, following the approach outlined by Lexer et al. (2009). Subsequently, principal component analysis (PCA) was conducted on the EFD coefficients to identify variations in leaf shape across the entire set of leaf samples. Prinprint program was used to view the Eigen leaves or leaf contours of each principal component. Then an analysis of leaf shape descriptors was obtained using ImageJ version 1.52a, Java 1.8.0_112 (64-bit) (Ambramoff et al. 2004) software. After all images of *C. pedunculata* and *C. rubella* were measured based on AR, circularity, and solidity, the resulting data were imported to PAST version 4.06b software (Hammer et al. 2001) for further analysis.

**Table 1. Populations and samples of *C. rubella* and *C. pedunculata were* used in this study.**

| Species | Localities | Accession number | Herbarium |
|---|---|---|---|
| *C. rubella* Lindl. | Myanmar | 2648823 | The New York Botanical Garden (NY) |
| | China | 2787428 | United States National Herbarium, Smithsonian Institution (US) |
| | China | FLAS 269814 | Florida Museum of Natural History (FLMNH) |
| | China | FLAS 269815 | Florida Museum of Natural History (FLMNH) |
| | Thailand | L 0534717 | Naturalis Biodiversity Center (AMD) |
| | Thailand | L 0534080 | Naturalis Biodiversity Center (AMD) |
| | Malaysia | L 2754590 | Naturalis Biodiversity Center (AMD) |
| | Malaysia | L 2754591 | Naturalis Biodiversity Center (AMD) |
| | China | L4212486 | Naturalis Biodiversity Center (AMD) |
| | Malaysia | L0534846 | Naturalis Biodiversity Center (AMD) |
| | Vietnam | P00991455 | The New York Botanical Garden (NY) |
| | Taiwan | K000674727 | Royal Botanic Gardens Kew (K) |
| | Indonesia | K000194757 | Royal Botanic Gardens Kew (K) |
| | Indonesia | K000194756 | Royal Botanic Gardens Kew (K) |
| | Vietnam | MW0756909 | Moscow State University (MSU) |
| | Vietnam | MW0757612 | Moscow State University (MSU) |
| | China | 103972 | The New York Botanical Garden (NY) |
| | China | 193971 | The New York Botanical Garden (NY) |
| | China | 103960 | The New York Botanical Garden (NY) |
| | China | 103959 | The New York Botanical Garden (NY) |
| | China | 103961 | The New York Botanical Garden (NY) |
| | China | 525329 | The New York Botanical Garden (NY) |
| | Vietnam | 2808318 | The New York Botanical Garden (NY) |
| | Vietnam | 2808046 | The New York Botanical Garden (NY) |
| | Myanmar | 3231815 | The New York Botanical Garden (NY) |
| *C. pedunculata* R.Br. | Philippines | JDS001 | University of Santo Tomas Herbarium (USTH) |

**Table 2. Eigenvalues and contribution of the first 10 principal components before data partitioning.**

| Component | Eigenvalue | Proportion (%) | Cumulative (%) |
|---|---|---|---|
| 1 | 0.000682534 | 36.65 | 36.65* |
| 2 | 0.000385342 | 20.69 | 57.34* |
| 3 | 0.000166940 | 8.96 | 66.30* |
| 4 | 0.000144030 | 7.73 | 74.04* |
| 5 | 0.000106694 | 5.73 | 79.77 |
| 6 | 0.000085726 | 4.60 | 84.37 |
| 7 | 0.000063535 | 3.41 | 87.78 |
| 8 | 0.000048451 | 2.60 | 90.38 |
| 9 | 0.000029872 | 1.60 | 91.99 |
| 10 | 0.000025266 | 1.36 | 93.34 |

*Only the first four are significant based on the broken stick method.

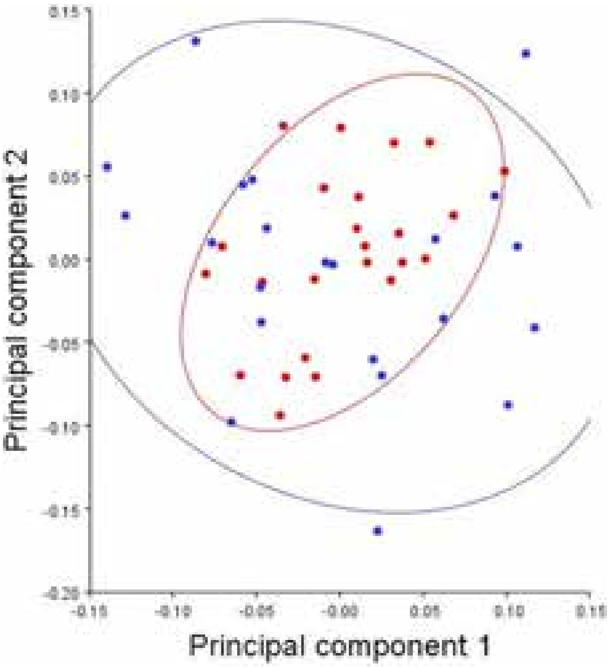

**Figure 1. Principal component analysis based on variance-covariance matrix of the unpartitioned dataset from elliptic Fourier coefficients of C. pedunculata (red dots) and C. rubella (blue dots). The plot shows PC1 and PC2, which explained 39.26% and 22.17% of the total variation, respectively.**

## RESULTS AND DISCUSSION

### Principal Component Analysis (PCA)

Independent shape variables were identified by PCA of EFD. Table 2 shows the relative contributions of the first 10 PCs of the whole dataset are accounted for 93% of the total variance while significant variations in the first four PCs (PC1, PC2, PC3, and PC4) equal to 74% cumulative variance based on broken stick method (MacArthur 1957). Most of the samples of *C. pedunculata* and *C. rubella* were densely overlapping than scattered in the scatter plot. (Figure 1). The

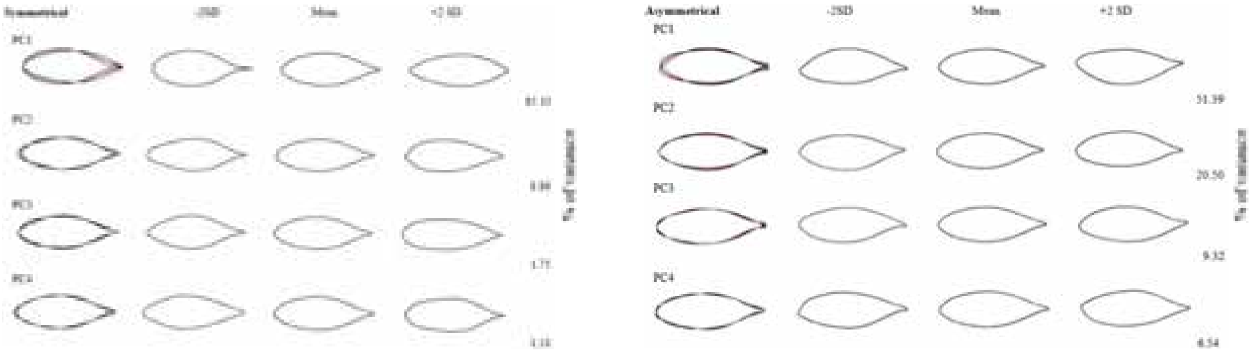

**Figure 2. Leaf shape reconstructions using the elliptical Fourier descriptor (EFDs) along the first four PCs from the symmetric and asymmetric data. The first column shows the overlaid drawings of the next three columns along with each PC.**

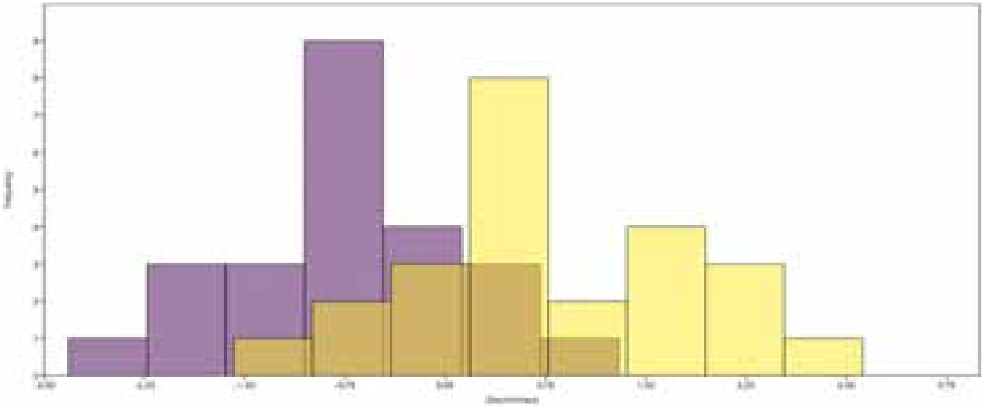

**Figure 3. Discriminant analysis (DA) of the leaf shape of *C. pedunculata* and *C. rubella*. Blue bars *C. pedunculata*; Yellow bars *C. rubella* Hotelling's $T^2$ = 36.83, F = 2.2419, P = 0.08146. Computed in PAST vers. 4.06b (Hammer et al. 2001).**

**Table 3. The relative contribution of symmetric and asymmetric components to leaf shape in two *Callicarpa* species.**

| **Eigenvalues** | | | | | |
|---|---|---|---|---|---|
| | **PC1** | **PC2** | **PC3** | **PC4** | **Percentage contribution to overall shape *** |
| Symmetric | $6.76 \times 10^{-4}$ | $1.36 \times 10^{-4}$ | $1.13 \times 10^{-4}$ | $8.11 \times 10^{-5}$ | 54.01% |
| Asymmetric | $3.80 \times 10^{-4}$ | $1.51 \times 10^{-4}$ | $6.88 \times 10^{-5}$ | $4.83 \times 10^{-5}$ | 34.80% |

* Total percentage contribution from PC1 to PC4 only.

ordination plot of the two taxa in a two-dimensional space was highly defined by PC1 and PC2. It suggests that the plots of *C. pedunculata* and *C. rubella* are similar along PC1 and PC2, with positive values but few data points were positioned in the negative values in both PCs which results in overlap in the interspecific comparison suggesting similarities between the two taxa. Likewise, the similarity in leaf shape has been reflected in the discriminant analysis (DA), where there is no significant difference between the means (Figure 3, Hotelling's $T^2$ = 36.83, F = 2.2419, $P$ = 0.08146) of *C. pedunculata* and *C. rubella*. Additionally, the jack-knife cross-validation showed 71% of correctly classified species both in *C. pedunculata* and *C. rubella* (Table 4). Despite similarities in the ordination of plots between the two taxa, the comparisons showed relevant variations in their leaf mean shapes in multivariate analysis of variance (MANOVA) as the significant difference between the *C. pedunculata* and *C. rubella* exists based on leaf shapes (Wilk's λ = 0.6196, F = 2.272, d.f. = 10 and 37, $P$ = 0.03431) (Figure 3).

The effects of shape variables in the Eigen leaves or leaf contours were determined based on the scores of the first four PCs to identify symmetric (54%) and asymmetric variations (35%). In Figure 4, symmetric variation highlights PC1 (85%) which explains leaf shape changes in width and leaf tips among samples of *C. pedunculata* and *C. rubella*. These variations were represented by discernible width expansion and transformation of leaf tips from acuminate to acute. Since PC1 accounts for the

**Table 4. Cross-validation matrices from canonical variates analysis (CVA) of leaf shape in *C. pedunculata* and *C. rubella*.**

| | *C. rubella* | *C. pedunculata* | Total | % correct |
|---|---|---|---|---|
| **A. confusion matrix without the jackknife** | | | | |
| *C. rubella* | 20 | 4 | 24 | 83 |
| *C. pedunculata* | 5 | 19 | 24 | 79 |
| Total | 25 | 23 | 48 | |
| **B. confusion matrix with the jackknife** | | | | |
| *C. rubella* | 17 | 7 | 24 | 71 |
| *C. pedunculata* | 7 | 17 | 24 | 71 |
| Total | 24 | 24 | 48 | |

Classification using PC scores computed from the original matrix. B. Jackknife classification.
Computed in PAST ver. 4.06b. (Hammer et al. 2001).

highest variations, it revealed that leaf tips and width expansion contribute to the overlapping of the two taxa. PC2 score (8.9%) describes cuneate, oblique to cordate leaf bases among samples, whereas PC3 (1.75%) and PC4 (1.53%) describe fine leaf changes along its margin that exhibit variations in the basal portion of the leaf. On the other hand, asymmetrical outline reconstruction shows basal and apical leaf variations on PC1 (51.4%) while remaining PCs (PC2 20.5%; PC3 9.32%; PC4 6.54%) revealed imperceptible variations across species. Thus, multivariate analyses were more restricted to the symmetric dataset due to the inadequate contribution of the asymmetric component.

In the recent study of two closely related genera,

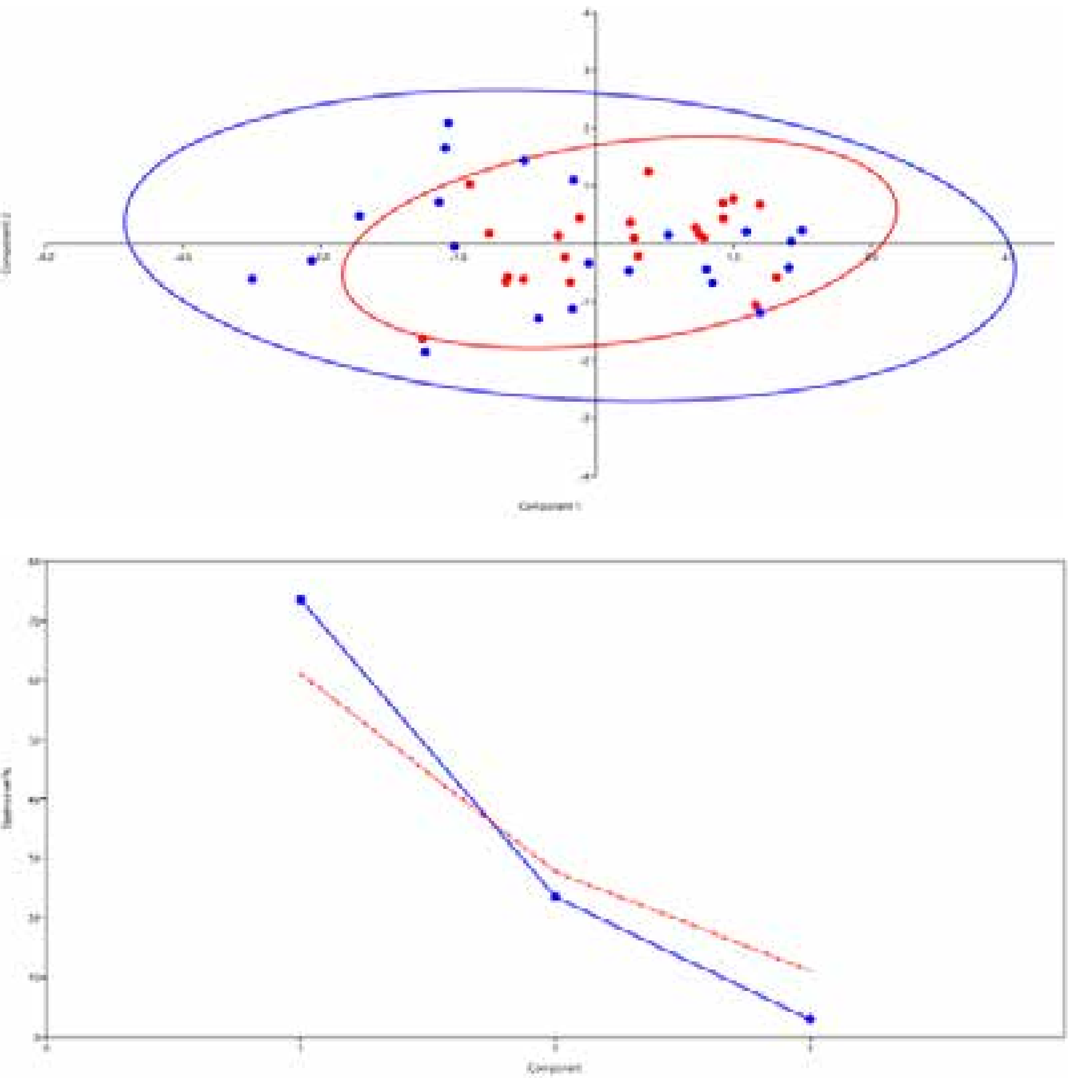

**Figure 4. Principal component analysis (PCA) of the leaf of 46 individuals of *C. pedunculata* and *C. rubella*. (A) The plot of principal components 1 and 2 (PC1 and PC2) shows 95% confidence ellipses of the samples based on leaf shape descriptors. Red dots *C. pedunculata*, Blue dots *C. rubella*. (B) The broken stick method shows the retained number of principal components used in this study.
(Red—Broken stick rule; Blue—Proportion of variance). Computed in PAST 4.06b software (Hammer et al., 2001).**

**Table 5. Leaf shape trait values across 46 selected species of (A) *C. pedunculata*, (B) *C. rubella*, and (C) overall accessions.**

| Trait | Range | Mean | SD | PCV (%) |
|---|---|---|---|---|
| Circularity | | | | |
| (A) | 0.36–0.57 | 0.47 | 0.06 | 13.51 |
| (B) | 0.29–0.57 | 0.44 | 0.09 | 19.68 |
| (C) | 0.29–0.57 | 0.45 | 0.08 | 16.6 |
| Aspect ratio | | | | |
| (A) | 2.05–3.73 | 2.76 | 0.35 | 12.54 |
| (B) | 1.90–4.12 | 2.92 | 0.71 | 24.12 |
| (C) | 1.90–4.02 | 2.84 | 0.55 | 19.33 |
| Solidity | | | | |
| (A) | 0.87–0.97 | 0.94 | 0.02 | 2.46 |
| (B) | 0.87–0.97 | 0.93 | 0.03 | 3.28 |
| (C) | 0.87–0.97 | 0.94 | 0.03 | 2.89 |

PCV, phenotypic coefficient of variation

**Table 6. The eigenvalue of principal component analysis (PCA) of the leaf descriptors in 46 individuals of *C. pedunculata* and *C. rubella*.**

| PC | Eigenvalue | % variance | Eig 2.5% | Eig 97.5% |
|---|---|---|---|---|
| 1 | 2.21911 | 73.970* | 65.116 | 83.207 |
| 2 | 0.69498 | 23.166 | 14.095 | 32.331 |
| 3 | 0.0859082 | 2.8636 | 1.3203 | 4.5508 |

* Only the first PC is significant based on the broken stick method.

**Table 7. Coefficients of correlation among PC1 to PC3 and the leaf shape descriptors**

| | PC 1 | PC 2 | PC 3 |
|---|---|---|---|
| Circ | 0.65380 | -0.06060 | 0.75423 |
| AR | -0.56176 | 0.62891 | 0.53749 |
| Solidity | 0.50692 | 0.77511 | -0.37714 |

**Table 8. Pearson correlation coefficients between three leaf-shape descriptors.**

| | Circularity | Aspect ratio | Solidity |
|---|---|---|---|
| Circularity | | $3.91 \times 10^{-11}$ | $6.64 \times 10^{-7}$ |
| Aspect ratio | -0.8067 | | 0.10703 |
| Solidity | 0.67839 | -0.31056 | |

*Callicarpa* and *Geunsia*, the effect of environment and genetic factors were mentioned as the probable cause of the taxonomic overlap between the two taxa (Danila & Alejandro 2021). In geometric morphometrics, this overlap indicates morphological similarities among species and may occur due to the presence of hybrid among samples (Adebowale et al. 2012). In recent years, there has been an increase in the number of hybrids in the genus *Callicarpa*, e.g., *C. japonica* Thunb. with *C. kochiana* Makino or *C. mollis* Siebold & Zucc., and *C. dichotoma* (Lour.) K.Koch with *C. kwangtungensis* Chun. (Yamanaka 1988, Tsukaya et al. 2003). The emergence of hybrids has brought several consequences in the population including introgression of plant traits or even the formation of new species which affect the interaction between plants and the environment (Orians 2000).

Despite the overlap, one clear finding in this study showed that symmetric variations on the leaf bases play a key role in determining leaf shape variations between *C. pedunculata* and *C. rubella*. In contrast to the symmetric variations, asymmetric PC1 also showed an interspecific variation focusing on the appearance of lobes in the basal portion of the leaf (Figure 2). In the leaf shape morphometric study conducted by Danila & Alejandro (2021) of the genus *Geunsia* and *Callicarpa,* the two taxa showed the possible occurrence of fluctuating asymmetry (FA). This results when the same species were unable to go through an identical development of the body organ on both sides resulting in uneven growth (van Valen 1962). Likewise, the occurrence of FA in leaves is a poor sign of environmental and genetic stress which happens when two closely related species mate and produce offspring (Sander & Matthies 2017). Hence, evidence of overlap in leaf shape variations and FA suggests that environmental and genetic factors affect variations in the leaf shape of *C. pedunculata* and *C. rubella.*

## Analysis of Leaf Shape Descriptors

In this study, the first two principal components (PC1 and PC2) showed the most variation among the three leaf shape descriptors having 74% and 23%, respectively (Table 6). However, it shows that shape trends in most samples were mostly observed in PC1 (74%). The bar plot (Figure 5) and coefficient of correlation (Table 7) among PCs showed a significant relationship among the three leaf-shape descriptors. PC1 is more related to circularity (0.65380) and solidity (0.50692) but inversely related to AR (-0.56176) while PC2 is more associated with AR (0.62891) and solidity (0.77511) but inversely related to circularity (-0.06060). On the other hand, PC2 marked a high coefficient of correlation in solidity and AR, but the proportion of variability in PC2 is relatively low (23.17%). Therefore, the first principal component (PC1) was considered a statistically significant PC based on the broken stick method (MacArthur 1957) (Figure 4–6).

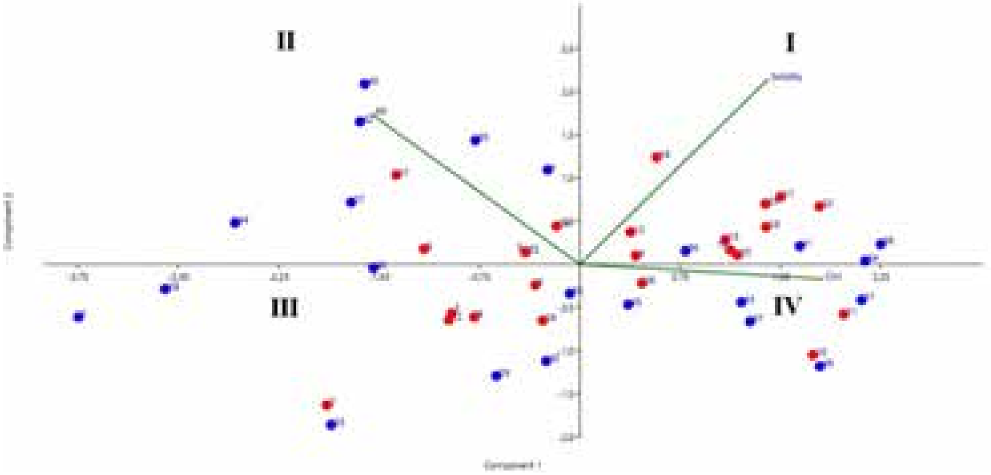

**Figure 5. Principal component analysis (PCA) biplot showing the multivariate variation among the leaf descriptors of *C. pedunculata* (red dots) and *C. rubella* (blue dots) based on two principal components. Vectors (green line) indicate the direction and strength of each leaf descriptor to the overall distribution.**

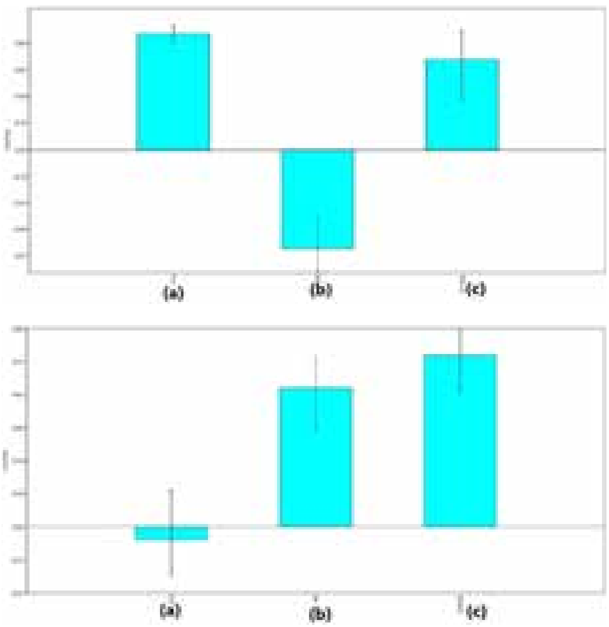

**Figure 6. The barplot which represents factor loadings of the first two principal components shows the magnitude of each variable: (a) circularity, (b) aspect ratio, and (c) solidity.**

The overall results showed that AR is the most variable leaf shape descriptor with a Phenotypic Coefficient of Variation (PCV; ((standard deviation/mean) × 100), estimates indicated the existence of a significant amount of variability among species, with 19.33% followed by circularity with 16.60% (Table 5). Additionally, both AR and circularity have a high distribution range of 1.90–4.02 and 0.29–0.57, respectively, meaning a high degree of variation was observed among samples. On the other hand, solidity is the least variable with the narrowest distribution (0.87–0.97) and the lowest PCV of 2.89%. Almost all samples of *C. pedunculata* and *C. rubella* exhibited a high AR (>1.90) which manifested an increase in leaf width relative to the length, or vice versa. However, it shows that *C. rubella* has higher PCV values (24.12%) compared to *C. pedunculata* (12.54%) which indicates that the former has higher diversity in length-width ratio. While an increase in AR manifests an increase in the size of the leaf width relative to length, or vice-versa (Gupta et al. 2019). Some leaves of *C. rubella* were narrower but with high AR, that is, a larger major axis either on its length or width, affects the overall AR of the taxa. On the other hand, variations in circularity were observed in all accessions, where 30 samples indicated a low circularity (<0.50) while 16 samples had moderate circularity (0.50–0.57), meaning the lower the circularity values, the more prominent serrations are. Based on the observations, more specimens in *C. pedunculata* (45%) have more prominent serrations than in *C. rubella* (30%). Thus, these observations revealed that serrations and leaf size were useful in discriminating the two taxa. Moreover, the results showed a significant relationship between leaf serrations to leaf size, that is, as the leaf size increases, serrations decrease, or vice versa. Lastly, solidity showed a narrow distribution (0.87–0.97) and low PCV values (2.89%) indicating that most samples of *C. pedunculata* and *C. rubella* do not have lobed leaves. However, few accessions of *C. rubella* have been observed to show slightly rounded projections from the base of the leaf blade. Likewise, these samples of *C. rubella* were observed to have a lower solidity value representing cordate to oblique-cordate leaf bases. In the study (Bramley 2013) of *Callicarpa* species in the Philippines, it has been noted that most *Callicarpa* species have either acute, acuminate, rounded, cuneate, oblique, or obtuse leaf bases which are all features of species with a high solidity (>0.87). Thus, we can conclude that solidity is also globally important as a diagnostic character to distinguish species between *C. pedunculata* and *C. rubella*.

### Correlation among leaf shape descriptors

Figure 5 presents a biplot that simultaneously draws information from 46 individual samples of *Callicarpa* based on three leaf shape descriptors: AR, Circularity, and Solidity. The three leaf shape descriptors were positioned on the first, second, and fourth quadrants while data points of samples were distributed in all four quadrants based on their PCA scores. However, the distribution among individuals of *C. pedunculata* and *C. rubella* has found a minimal group differentiation due to a large degree of overlap. Although overlap has been observed among samples, the three leaf-shape descriptors produced a comparable level of relationship. In Table 8, the vectors of the variables circularity and solidity were closer to each other which suggests a positive correlation (+0.6784) between them. On the other hand, the greater distance close to 180 degrees found between circularity and AR suggests a negative correlation (-0.8067) while vectors of solidity and AR show almost an angle of 90 degrees which indicates that the variables were weakly correlated (-0.3106).

As mentioned above, AR and circularity were found to be the two most important variables in the discrimination of *C. pedunculata* and *C. rubella*. These leaf shape descriptors were highly influenced by length, width, and leaf margin. Since AR and circularity were found to be negatively correlated, variables like the length and width of the leaf were inversely proportional to the presence of serrations, that is, when the magnitude of the leaf decreases, the degree of serrations increases or vice versa. These observations exist among samples of *C. pedunculata* and *C. rubella*, where each taxon exhibits a corresponding trait relative to leaf serration and size. On the other hand, circularity and solidity indicate a moderate positive correlation that shows an impact of serrations in the projections of the leaf blade. Although a positive correlation was found between circularity and solidity the interval between the PCV values (circularity 16.6%; solidity 2.89%) is high, the two variables are related but exhibit different percentages in terms of their effects on the leaf shapes. This observation was evident among samples of *C. rubella* in the occurrence of fine leaf lobes and discernible leaf serration. While the weak correlation was observed between solidity and AR where the former, unlike circularity, is little or not affected by serrations and leaf lobes (Figure 5).

Several studies (Thomas & Bazzaz 1996; Piazza 2005; Royer & Wilf 2006; Chitwood et al. 2013) have identified several factors in the evolution of leaf shapes and sizes, including the adaptation of plants to various types of environments. Likewise, different environmental factors showed a significant effect on morphological characters of closely related species (Jones 1995; Wolfe & Liston 1998; Royer et al. 2008). However, the adaptation mechanism in response to environmental variation in most species is still incomprehensible (Jump & Panuelas 2005). Since *C. pedunculata* and *C. rubella* have been identified to grow in a different environment, the two taxa showed distinct characteristics to discriminate the two species of *Callicarpa*. However, it also revealed that *C. pedunculata* and *C. rubella* showed similar leaf traits which can be considered as a plesiomorphic character of the two taxa. *C. pedunculata* has been described to show more serrations than *C. rubella*, while *C. rubella* exhibits a larger leaf size than *C. pedunculata* based on AR values. According to Peppe et al. (2011), leaf characters including sizes and shapes strongly correlate to environmental factors and prove that there is a biological basis for this relationship. The variations in serrations and leaf size between *C. pedunculata* and *C. rubella* are likely adaptations suited to specific environments. These distinctive features contribute to the species' ability to thrive in different ecological niches. To gain a more comprehensive understanding of the distribution and evolutionary relationships within the *Callicarpa* genus, it is strongly recommended to undertake a thorough phylogenetic study. This broader investigation will offer valuable insights into the geographic distribution of *Callicarpa* species and enhance our understanding of their adaptive evolution.

## CONCLUSION

A statistically significant difference in leaf shape between *C. pedunculata* and *C. rubella* was observed, although there is considerable interspecific assessment, possibly due to environmental and genetic factors. Nevertheless, this study identifies aspect ratio and circularity as the two most informative variables in discrimination between the two species, emphasizing the importance of length, width, and leaf serrations as key diagnostic characteristics. The finding suggests

**Table 9. Specimen examined. Authors and URLs of the digital images obtained from the online herbaria used in this study.**

| Author | HTTP url |
|---|---|
| Bijmoer. R., M. Scherrenberg & J. Creuwels (2021). Naturalis Biodiversity Center (NL) - Botany. Naturalis Biodiversity Center. Occurrence dataset https://doi.org/10.15468/ib5ypt accessed via GBIF.org on 2021-08-29. | https://www.gbif.org/occurrence/2516551448 |
| | https://www.gbif.org/occurrence/2516532469 |
| | https://www.gbif.org/occurrence/2516548469 |
| | https://www.gbif.org/occurrence/2517253874 |
| | https://www.gbif.org/occurrence/2516548469 |
| | https://www.gbif.org/occurrence/2516381494 |
| MNHN & S. Chagnoux (2021). The vascular plants collection (P) at the Herbarium of the Muséum national d'Histoire Naturelle (MNHN - Paris). Version 69.223. MNHN - Museum national d'Histoire naturelle. Occurrence dataset https://doi.org/10.15468/nc6rxy accessed via GBIF.org on 2021-08-29 | https://www.gbif.org/occurrence/2270292394 |
| Orrell, T & Informatics Office (2021). NMNH Extant Specimen Records. Version 1.45. National Museum of Natural History, Smithsonian Institution. Occurrence dataset https://doi.org/10.15468/hnhrg3 accessed via GBIF.org on 2021-08-29. | https://www.gbif.org/occurrence/1852124824 |
| Perkins, K.D. (2021). University of Florida Herbarium (FLAS). Version 11.1454. Florida Museum of Natural History. Occurrence dataset https://doi.org/10.15468/v5wjn7 accessed via GBIF.org on 2021-08-29 | https://www.gbif.org/occurrence/2433456102 |
| | https://www.gbif.org/occurrence/2433458107 |
| Ramirez, J., K. Watson, B. Thiers & L. McMillin (2021). The New York Botanical Garden Herbarium (NY). Version 1.38. The New York Botanical Garden. Occurrence dataset https://doi.org/10.15468/6e8nje accessed via GBIF.org on 2021-08-29. | https://www.gbif.org/occurrence/1929638283 |
| | https://www.gbif.org/occurrence/1930601756 |
| | https://www.gbif.org/occurrence/1930296336 |
| | https://www.gbif.org/occurrence/1930106241 |
| | https://www.gbif.org/occurrence/1929663090 |
| | https://www.gbif.org/occurrence/1929049006 |
| | https://www.gbif.org/occurrence/1928131180 |
| | https://www.gbif.org/occurrence/1929940867 |
| | https://www.gbif.org/occurrence/1931232274 |
| Royal Botanic Gardens, Kew (2021). Royal Botanic Gardens, Kew - Herbarium Specimens. Occurrence dataset https://doi.org/10.15468/ly60bx accessed via GBIF.org on 2021-08-29. | https://www.gbif.org/occurrence/912528324 |
| | https://www.gbif.org/occurrence/912176780 |
| | https://www.gbif.org/occurrence/912176785 |
| Seregin, A. (2021). Moscow University Herbarium (MW). Version 1.195. Lomonosov Moscow State University. Occurrence dataset https://doi.org/10.15468/cpnhcc accessed via GBIF.org on 2021-08-29. | https://www.gbif.org/occurrence/3004116377 |
| | https://www.gbif.org/occurrence/3004100339 |

leaf serrations and leaf size were important *to C. pedunculata* and *C. rubella*, respectively, and considered as an adaptive feature of the two taxa. Likewise, fine-scale variations in the basal region, e.g., presence of leaf lobes, also show significance in the discrimination of the two taxa. Thus, this research provides new experimental support for future taxonomic, genetics, or even ecological studies of *Callicarpa* species in the relevance of leaf size, leaf serrations, and leaf lobes.

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COMMUNICATION

# Checklist and comparison of the bird diversity from the Himachal Pradesh Agricultural University, India

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**Abstract:** Agricultural ornithology plays a crucial role in managing and sustaining agroecosystems. In agriculture, birds such as insectivores and raptors serve as natural controllers of insect and rodent pests, contributing to integrated pest management strategies. In this study, a checklist of birds was compiled using data collected over three years (2019–2022) from the agricultural landscape surrounding Himachal Pradesh Agricultural University, Palampur (HPAU), India. The study area comprises varied habitats including agricultural fields, forest patches, water bodies, and tea orchards. A total of 116 avian species, spanning 17 orders and 44 families were documented. Muscicapidae emerged as the most dominant family, comprising 16 species and exhibiting the highest relative diversity index value (13.79). A comparison with previous records revealed that 40 avian species were absent, while 34 bird species were reported for the first time in the study area. These finding revealed the significant shift in avian diversity at HPAU compared to previous assessments (HPAU 2019). The observed decline in avian diversity may be attributed to rapid habitat degradation driven by large-scale shrub trimming and other development activities, particularly construction projects.

**Keywords:** Avifauna, conservation, Kangra, Palampur, species richness, tea orchards.

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**Author contributions:** Praveen Kumar—data collection, data analysis, methodology, writing the original draft. Bharti Parmar—manuscript review & comments. Pardeep Kumar—writing, review & editing.

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## INTRODUCTION

Agricultural ornithology deals with regular monitoring and collection of scientific information on bird diversity in agroecosystems (Dhindsa & Saini 1994), which is a prerequisite for their management sustainably. The birds form a wide range of feeding guilds, viz., frugivore, granivore, insectivore, and nectarivore, and in an ecosystem, they act as primary consumers (herbivorous) to top carnivorous (fish-eating birds) (Kumar 2021a). Birds are an integral part of the food chain and contribute to a healthy ecosystem due to various ecological services rendered by them, viz., seed dispersal and pollination (Burin et al. 2016). In an agricultural landscape, avian diversity plays an essential role in controlling the insect-pest population (Railsback & Johnson 2014), and is thus useful for integrated pest management.

The montane landscapes of the Indian Himalayan Region (IHR) are a biodiversity hotspot (Myer 2000) and contribute about 80% of the avian diversity of the Indian subcontinent (Price et al. 2003; Chandra et al. 2018). In many countries like India, scientific information on bird diversity is limited, particularly for the agriculture landscape for providing input in agricultural sustainability. The large-sized university premises are also the subject of interest to understand the human-induced urban environment and vegetation association (Ali et al. 2013; Aggarwal et al. 2016; Chakdar et al. 2016; Rajashekara & Venkatesha 2017). Many campuses are unexplored and need to be evaluated for preparing a systematic management plan. Agriculture universities are known to possess monoculture or mixed crop with limited wildlife (Şekercioğlu et al. 2019). However, in hilly terrain such universities comprise a variety of habitats and a large area occupied with mixed vegetation that may enhance the bird diversity. The checklist of bird diversity prepared for such areas may be quite helpful for biodiversity conservation and long-term integrated pest management (IPM).

Considering the importance of bird diversity, the present study was carried out at the Himachal Pradesh Agriculture University (HPAU), India. An annotated checklist was prepared that provides baseline information for the conservation and management of bird diversity in a sustainable way.

### STUDY AREA

The present study was conducted in the university premise of HPAU Palampur, Himachal Pradesh, India (76.5489°N & 32.1029°E). The study area comes under the agro-climatic zone II, which covers sediments derived from a geologically complex environment with a long-term erosion history that leads to varied geo-botanical landscapes. The study area is located in the foothill region of the Dhauladhar ranges characterized by snow-clad peaks in steep slopes (16–30 % gradient), while the university premise has an area of moderate topography with a 10–15 % gradient. The university premise is situated along the national highway (NH-154) criss-crossed by many linked roads and seasonal drainage.

The study area comprises varied habitat diversity such as agriculture (A), forest (F), grassland (G), tea orchards (T), wasteland (W), and water bodies (WB) (Figure 1). The agricultural fields and organic farms are extensively cultivated with seasonal crops, namely, maize *Zea mays*, wheat *Triticum aestivum*, okra *Abelmoschus esculentus*, cole crops *Brassica* spp. and their genotypes; while the rest of the area is cover with scattered patches of tea garden, wasteland and mixed vegetation forest. The main tree species include *Bauhinia variegata, Callistemon viminalis*, *Cedrus deodara*, *Jacaranda mimosifolia, Populus* sp., and *Salix babylonica*. There are many edible fruit plants such as *Morus alba*, *Psidium guajava*, *Pyrus pashia*, and *Rubus* spp., and many others, as reported by Kumar (2021b) for the foothill region of Dhauladhar ranges. The annual rainfall varies 1,500–1,800 mm. The climate of the study area is a monsoonal-influenced humid subtropical climate (Cwa) as per Köppen & Geiger's classification (Peel et al. 2007).

## MATERIALS AND METHODS

An annotated checklist of bird diversity was meticulously compiled following an extensive field survey conducted across 315 sites (refer to Figure 1) from 2019 to 2022. The survey encompassed both planned observations and numerous opportunistic sightings. The main survey sessions were carried out mainly from 0700 h to 0900 h and in the evening from 1700 h to 1830 h. Opportunistic sightings, on the other hand, were made near experimental farms, playground areas, and water bodies, adding valuable data to checklist. Several surveys were conducted along a specific track from gate 1 to gate 5 under streetlight condition between 1930 h to 2130 h throughout 2019–2020, with the exception of the COVID-19 lockdown period. While a subset of these surveys was meticulously planned and executed over 2–3 days per month, the majority were opportunistic, occurring 3–4 days each week. This survey encompasses

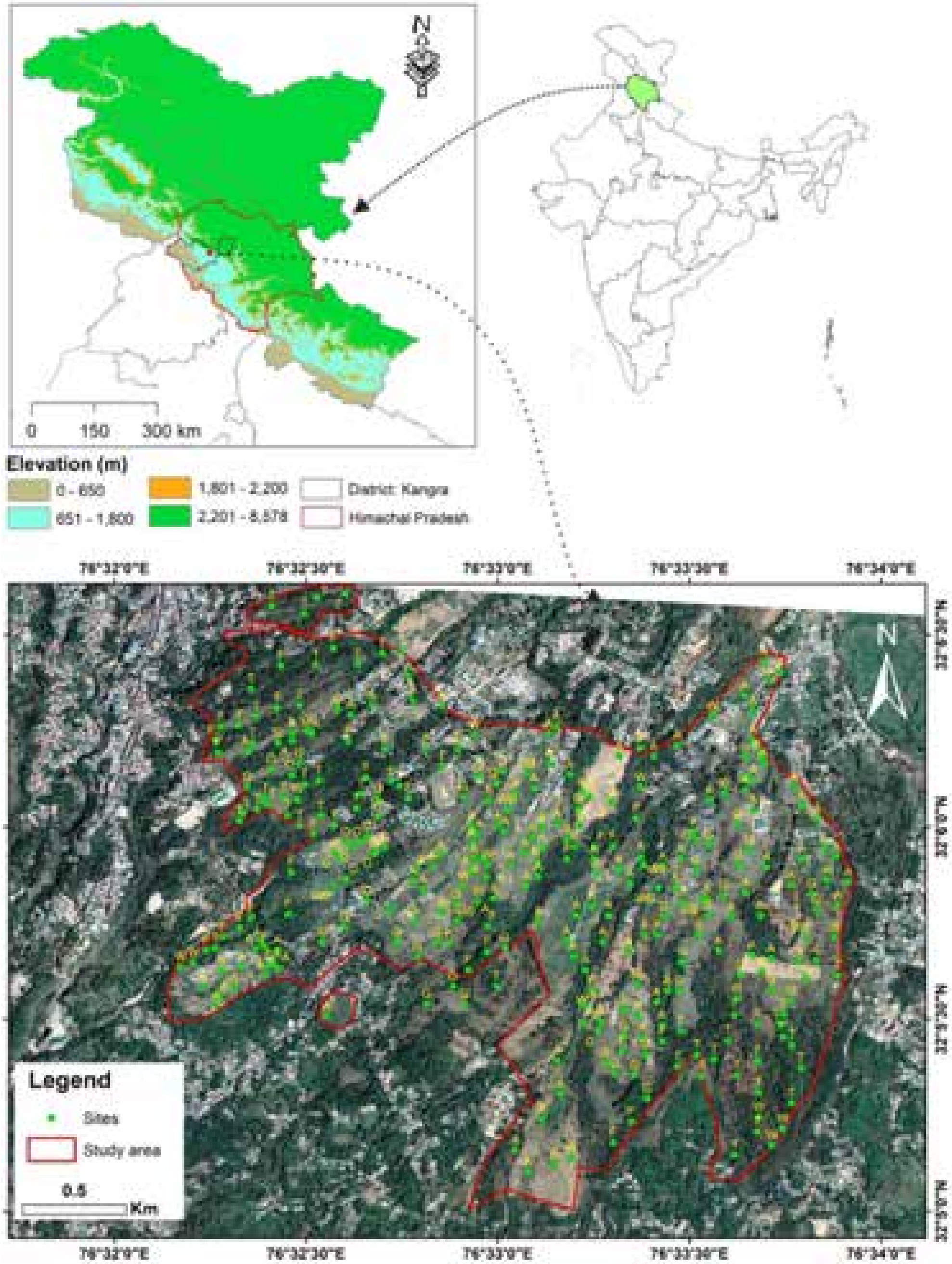

**Image 1. Survey sites in the study area and the location with respect to agroclimatic zone II (651–1,800 m), Himachal Pradesh, India. (Abbreviation: A—agriculture | F—forest | G—grassland | O—orchard | T—tea orchard | W—wasteland | WB—water bodies).**

transects and points established within the university premises across different habitats (A = 130; F = 32; G = 52; O = 17; T = 55; W = 21; WB = 8). Additionally, numerous opportunistic surveys were conducted during university activities, yielding rare sightings recorded once or twice in the study period. The opportunistic surveys primarily occurred while moving to experimental fields, near playground areas, and near water bodies. Observations were recorded using a Hanumex 30 x 60 binocular and Nikon 3300 camera with 70–300 mm zoom lens, while a large proportion of these records were geotagged using a Nikon p900 camera. Bird acoustic signals were also employed for species location. The identification of bird species was facilitated by various field guides (Singh 2015; Grimmett et al. 2016; Grewal & Bhatia 2017; Dhadwal 2018; Kalsi et al. 2020). The study area, characterized by a variety of habitats, necessitated a combination of belt transects (50 m wide), point surveys and call surveys. The transect length varied 300–500 m depending on habitat accessibility, while point surveys lasted approximately 20–30 minutes at specific locations.

The data collected from the well-planned survey (replicated twice) and opportunistic survey in the study period were complied and categorized based on encounter rate and sightings frequency into very common (VC), common (C), and rare (R) categories (MacKinnon & Phillipps 1993). VC denoted species sighted over 10 times across all seasons, C represented sightings occurring 7–9 times in specific habitats, and R indicated species sighted once or twice during the study period. The relative diversity (RDi) of families was calculated using a specified formula (Torre-Cuadros et al. 2007).

$$\text{RDi} = \frac{\text{Number of bird species in a family}}{\text{Total number of species}} \text{ x } 100$$

## RESULTS

The annotated checklist of the HPAU- 2019–2022 revealed that a total of 116 bird species belonging to 17 orders and 44 families occur on the university premises. Muscicapidae family dominates over other families. The checklist reveals the Muscicapidae family contributes to 16 species followed by Picidae (7), Cisticolidae (6), Columbidae (5), Accipitridae, Ardeidae, Corvidae, Paridae, Psittaculidae, Strigidae, Sturnidae (4 each), Cuculidae, Dicruridae, Hirundinidae, Motacillidae, Phasianidae, Pycnonotidae, Timaliidae (3 each), and Fringillidae, Megalaimidae, Nectariniidae, Passeridae, Phylloscopidae, Rallidae, & Stenostiridae (2 each). While the remaining 19 families are poorly reported (Table 1). The RDi value was also calculated highest for the family Muscicapidae followed by other Picidae and Cisticolidae (Table 2). The family Muscicapidae was also reported as dominant in various studies (Sankar et al. 2006; Yaseen et al. 2011; Koli 2014). It is the largest family of birds in the Indian context (Manakadan & Pittie 2001).

The present checklist was also compared with the annotated checklist prepared by Kottawa-Arachchi (2022) and the checklist prepared for the Central University of Himachal Pradesh (CUHP) 2015–2018 located in the foothill region of Dhauladhar ranges, Himachal Himalaya, India (Kumar 2021a). The CUHP is a university operating on a temporary campus with no agricultural activities, while the HPAU is characterized by a wide agricultural landscape and a large area under unmanaged tea orchards. These universities (viz., HPAU and CUHP) are separated by an aerial distance of approximately ~40 km. Table 1 represents the checklists and their comparison for the occurrence of bird species in two university premises and previously published records. The opportunistic sightings particularly near playground areas, agricultural fields, and wetland habitats provided a significant contribution to the observation of rare birds species. A comparison with the previous records, specifically HPAU-2019, revealed notable differences in the presence and abundance of common species. Many previously common species were not found during the study period, while others that were once abundant were either missing or now considered rare. Therefore the primary focus of this study lies in comparing the diversity and distribution across similar and varied landscape (refer to Table 1). Bird species newly recorded in the study area, compared to HPAU-2019, are shown in Images 2 & 3. Additionally, Kumar (2021a) has already provided photographic records of common species observed in CUHP 2015–2018 and HPAU 2019.

The present checklist HPAU 2019–2022 showed 98 common bird species and 18 species are new records while comparing the checklist CUHP 2015–2018. The difference in species composition can be correlated to the variation in habitat diversity, human intervention, and size of the study area. The checklist HPAU 2019–2022 was also compared with the previous records (i.e., HPAU 2019) that showed 82 species are common while a huge difference with new records, which are reported 34 in number, while, 40 bird species were found absent even considering the wide timeframe. Many common bird species (viz., Scaly-breasted Munia *Lonchura punctulata,* Fire-breasted Flowerpecker *Dicaeum ignipectus*, Brahminy Starling *Sturnia pagodarum*, Indian Robin *Saxicoloides fulicatus*, Black-throated Thrush *Turdus atrogularis*, Green Bee-eater *Merops orientalis*) are not reported, which can easily be seen in their specific habitats as per their time of seasonal migration in the study area (Table 1).

Considering the similar timeframe, many species such as Little Cormorant *Microcarbo niger*, Brahminy Starling *Sturnia pagodarum*, and Black-throated Thrush *Turdus atrogularis*, were reported each year, found absent. Some raptor species, viz., Indian Scops Owl *Otus bakkamoena*, Barn Owl *Tyto alba*, and Brown Boobook *Ninox scutulata*, were also found unnoticed. Many common species recorded in the previous checklist (HPAU 2019) were either absent (40) or rarely seen. It has been reported that food resources, safe roosting sites,

**Table 1. Checklist of the bird diversity from the Himachal Pradesh Agriculture University (HPAU 2019-2022) along with the previous records.**

| | English name | Scientific name | Abundance | CUHP (2015–2018) | HPAU (2019) | HPAU (2019–2022) present study |
|---|---|---|---|---|---|---|
| GALLIFORMES | | | | | | |
| Phasianidae (partridges, pheasants, grouse) | | | | | | |
| 1 | Common Quail | *Coturnix coturnix* (Linnaeus, 1758) | R | + | - | + |
| 2 | Black Francolin | *Francolinus francolinus* (Linnaeus, 1766) | R | + | + | + |
| 3 | Red Junglefowl | *Gallus gallus* (Linnaeus, 1758) | R | + | + | + |
| COLUMBIFORMES | | | | | | |
| Columbidae (pigeons) | | | | | | |
| 4 | Rock Pigeon | *Columba livia* (Gmelin, JF, 1789) | VC | + | + | + |
| 5 | Oriental Turtle Dove | *Streptopelia orientalis* (Latham, 1790) | C | + | + | + |
| 6 | Eurasian Collared Dove | *Streptopelia decaocto* (Frivaldszky, 1838) | R | + | - | + |
| 7 | Spotted Dove | *Streptopelia chinensis* (Scopoli, 1786) | VC | + | + | + |
| 8 | Asian Emerald Dove | *Chalcophaps indica* (Linnaeus, 1758) | R | - | + | + |
| CUCULIFORMES | | | | | | |
| Cuculidae (cuckoos) | | | | | | |
| 9 | Greater Coucal | *Centropus sinensis* (Stephens, 1815) | C | + | + | + |
| 10 | Indian Cuckoo | *Cuculus micropterus* (Gould, 1838) | - | - | + | - |
| 11 | Asian Koel | *Eudynamys scolopaceus* (Linnaeus, 1758) | R | + | + | + |
| 12 | Banded Bay Cuckoo | *Cacomantis sonneratii* (Latham, 1790) | - | - | + | - |
| 13 | Common Cuckoo | *Cuculus canorus* (Linnaeus, 1758) | R | + | - | + |
| 14 | Common Hawk-cuckoo | *Hierococcyx* varius (Vahl, 1797) | - | - | + | - |
| GRUIFORMES | | | | | | |
| Rallidae | | | | | | |
| 15 | Brown Crake | *Zapornia akool* (Sykes, 1832) | C | + | - | + |
| 16 | White-breasted Waterhen | *Amaurornis phoenicurus* (Pennant, 1769) | R | + | + | + |
| PELECANIFORMES | | | | | | |
| Ardeidae (herons) | | | | | | |
| 17 | Indian Pond Heron | *Ardeola grayii* (Sykes, 1832) | R | + | + | + |
| 18 | Cattle Egret | *Bubulcus ibis* (Linnaeus, 1758) | C | + | + | + |
| 19 | Great Egret | *Ardea alba* (Linnaeus, 1758) | - | - | + | - |
| 20 | Grey Heron | *Ardea cinerea* (Linnaeus, 1758) | R | - | - | + |
| 21 | Black-crowned Night Heron | *Nycticorax nycticorax* (Linnaeus, 1758) | R | - | - | + |
| SULIFORMES | | | | | | |
| Phalacrocoracidae (cormorants) | | | | | | |
| 22 | Little Cormorant | *Microcarbo niger* (Vieillot, 1817) | R | + | - | + |
| 23 | Indian Cormorant | *Phalacrocorax fuscicollis* (Stephens, 1826) | - | - | + | - |
| CHARADRIIFORMES | | | | | | |
| Charadriidae (plovers & lapwings) | | | | | | |
| 24 | Red-wattled Lapwing | *Vanellus indicus* (Boddaert, 1783) | C | + | + | + |
| Scolopacidae (sandpipers) | | | | | | |
| 25 | Green Sandpiper | *Tringa ochropus* (Linnaeus, 1758) | R | + | - | + |
| 26 | Common Sandpiper | *Actitis hypoleucos* (Linnaeus, 1758) | - | - | + | - |
| ACCIPITRIFORMES | | | | | | |
| Accipitridae (kites, hawks and eagles) | | | | | | |
| 27 | Egyptian Vulture | *Neophron percnopterus* (Linnaeus, 1758) | R | + | + | + |
| 28 | White-rumped Vulture | *Gyps bengalensis* (Gmelin, J.F. 1788) | - | + | - | - |

| | **English name** | **Scientific name** | **Abundance** | **CUHP (2015–2018)** | **HPAU (2019)** | **HPAU (2019–2022) present study** |
|---|---|---|---|---|---|---|
| 29 | Shikra | *Accipiter badius* (Gmelin, J.F. 1788) | R | + | + | + |
| 30 | Eurasian Sparrowhawk | *Accipiter nisus* (Linnaeus, 1758) | - | + | - | - |
| 31 | Black Kite | *Milvus migrans* (Boddaert, 1783) | C | + | + | + |
| 32 | Besra | *Accipiter virgatus* (Temminck, 1822) | - | - | + | - |
| 33 | Mountain Hawk-eagle | *Nisaetus nipalensis* (Hodgson, 1836) | - | - | + | - |
| 34 | Lesser Fish-eagle | *Haliaeetus humilis* (S. Müller & Schlegel, 1841) | - | - | + | - |
| 35 | Oriental Honey Buzzard | *Pernis ptilorhynchus* (Temminck, 1821) | - | - | + | - |
| 36 | Himalayan Buzzard | *Buteo refectus* (Portenko, 1935) | R | - | - | + |
| CAPRIMULGIFORMES | | | | | | |
| Apodidae | | | | | | |
| 37 | House Swift | *Apus nipalensis* (Hodgson, 1837) | - | - | + | - |
| STRIGIFORMES | | | | | | |
| Strigidae (owls) | | | | | | |
| 38 | Asian Barred Owlet | *Glaucidium cuculoides* (Vigors, 1831) | C | + | + | + |
| 39 | Collared Owlet | *Taenioptynx brodiei* (Burton, E. 1836) | - | - | + | - |
| 40 | Barn Owl | *Tyto alba* (Scopoli, 1769) | R | - | - | + |
| 41 | Indian Scops Owl | *Otus bakkamoena* (Pennant, 1769) | C | - | - | + |
| 42 | Brown Boobook | *Ninox scutulata* (Raffles, 1822) | R | - | - | + |
| BUCEROTIFORMES | | | | | | |
| Bucerotidae (hornbills) | | | | | | |
| 43 | Indian Grey Hornbill | *Ocyceros birostris* (Scopoli, 1786) | C | + | + | + |
| Upupidae (hoopoes) | | | | | | |
| 44 | Common Hoopoe | *Upupa epops* (Linnaeus, 1758 | R | + | + | + |
| PICIFORMES | | | | | | |
| Picidae (woodpeckers) | | | | | | |
| 45 | Speckled Piculet | *Picumnus innominatus* (Burton, E. 1836) | R | + | + | + |
| 46 | Back-rumped Flameback | *Dinopium benghalense* (Linnaeus, 1758) | R | + | - | + |
| 47 | Lesser Yellow-naped Woodpecker | *Picus chlorolophus* (Vieillot, 1818) | R | + | - | + |
| 48 | Grey-headed Woodpecker | *Picus canus* (Gmelin, J.F. 1788) | R | + | + | + |
| 49 | Grey-capped Pygmy Woodpecker | *Dendrocopos canicapillus* (Blyth, 1845) | C | + | + | + |
| 50 | Fulvous-breasted Pied Woodpecker | *Dendrocopos macei* (Vieillot, 1818) | R | + | + | + |
| 51 | Brown-fronted Pied Woodpecker | *Dendrocopos auriceps* (Vigors, 1831) | - | + | - | + |
| 52 | Scaly-bellied Woodpecker | *Picus squamatus* (Vigors, 1831) | - | - | + | - |
| Megalaimidae | | | | | | |
| 53 | Great Barbet | *Psilopogon virens* (Boddaert, 1783) | C | + | + | + |
| 54 | Brown-headed Barbet | *Psilopogon zeylanicus* (Gmelin, J.F. 1788) | - | + | - | - |
| 55 | Blue-throated Barbet | *Psilopogon asiaticus* (Latham, 1790) | C | + | + | + |
| 56 | Coppersmith Barbet | *Psilopogon haemacephalus* (Müller, PLS, 1776) | - | - | + | - |
| CORACIIFORMES | | | | | | |
| Coraciidae (rollers) | | | | | | |
| 57 | Indian Roller | *Coracias benghalensis* (Linnaeus, 1758) | - | + | - | - |
| Alcedinidae (kingfishers) | | | | | | |
| 58 | White-throated Kingfisher | *Halcyon smyrnensis* (Linnaeus, 1758) | R | + | + | + |

| | **English name** | **Scientific name** | **Abundance** | **CUHP (2015–2018)** | **HPAU (2019)** | **HPAU (2019–2022) present study** |
|---|---|---|---|---|---|---|
| FALCONIFORMES | | | | | | |
| Falconidae (falcons and caracaras) | | | | | | |
| 59 | Common Kestrel | *Falco tinnunculus* (Linnaeus, 1758) | R | + | + | + |
| PSITTACIFORMES | | | | | | |
| Psittaculidae (Old World parrots) | | | | | | |
| 60 | Slaty-headed Parakeet | *Psittacula himalayana* (Lesson, 1832) | R | + | - | + |
| 61 | Plum-headed Parakeet | *Psittacula cyanocephala* (Linnaeus, 1766) | R | + | + | + |
| 62 | Alexandrine Parakeet | *Psittacula eupatria* (Linnaeus, 1766) | C | + | + | + |
| 63 | Rose-ringed Parakeet | *Psittacula krameri* (Scopoli, 1769) | R | + | - | + |
| PASSERIFORMES | | | | | | |
| Campephagidae (minivets and cuckooshrikes) | | | | | | |
| 64 | Orange Minivet | *Pericrocotus flammeus* (Forster, J.R. 1781) | R | + | - | + |
| 65 | Long-tailed Minivet | *Pericrocotus ethologus* (Bangs & Phillips, 1914) | - | - | + | - |
| 66 | Small Minivet | *Pericrocotus cinnamomeus* (Linnaeus, 1766) | - | - | + | - |
| Dicruridae (drongos) | | | | | | |
| 67 | Black Drongo | *Dicrurus macrocercus* (Vieillot, 1817) | C | + | + | + |
| 68 | Ashy Drongo | *Dicrurus leucophaeus* (Vieillot, 1817) | C | + | + | + |
| 69 | Hair-crested Drongo | *Dicrurus hottentottus* (Linnaeus, 1766) | R | + | + | + |
| 70 | Crow-billed Drongo | Dicrurus annectens (Hodgson, 1836) | | - | + | - |
| Cinclidae | | | | | | |
| 71 | Brown Dipper | *Cinclus pallasii* (Temminck, 1820) | - | - | + | - |
| Pittidae | | | | | | |
| 72 | Indian Pitta | *Pitta brachyura* (Linnaeus, 1766) | - | - | + | - |
| Cettiidae | | | | | | |
| 73 | Brownish-flanked Bush Warbler | *Horornis fortipes* (Hodgson, 1845) | - | - | + | - |
| 74 | Grey-sided Bush Warbler | *Cettia brunnifrons* (Hodgson, 1845) | - | - | + | - |
| Phylloscopidae | | | | | | |
| 75 | Ashy-throated Warbler | *Phylloscopus maculipennis* (Blyth, 1867) | - | - | + | - |
| 76 | Blyth's Leaf Warbler | *Phylloscopus reguloides* (Blyth, 1842) | - | - | + | - |
| 77 | Greenish Leaf Warbler | *Phylloscopus trochiloides* (Sundevall, 1837) | - | - | + | - |
| 78 | Whistler’s Warbler | *Phylloscopus whistleri* (Ticehurst, 1925) | - | - | + | - |
| Alaudidae | | | | | | |
| 79 | Indian Bushlark | *Mirafra erythroptera* (Blyth, 1845) | - | - | + | - |
| 80 | Oriental Skylark | *Alauda gulgula* (Franklin, 1831) | - | - | + | - |
| Dicaeidae | | | | | | |
| 81 | Fire-breasted Flowerpecker | *Dicaeum ignipectus* (Blyth, 1843) | R | - | - | + |
| Sylviidae | | | | | | |
| 82 | Yellow-eyed Babbler | *Chrysomma sinense* (Gmelin, J.F. 1789) | - | - | + | + |
| Acrocephalidae | | | | | | |
| 83 | Blyth's Reed Warbler | *Acrocephalus dumetorum* (Blyth, 1849) | - | - | + | - |
| Estrildidae | | | | | | |
| 84 | Indian Silverbill | *Euodice malabarica* (Linnaeus, 1758) | - | - | + | - |
| Laniidae | | | | | | |
| 85 | Brown Shrike | *Lanius cristatus* (Linnaeus, 1758) | - | - | + | - |
| 86 | Long-tailed Shrike | *Lanius schach* (Linnaeus, 1758) | - | + | + | - |

| | **English name** | **Scientific name** | **Abundance** | **CUHP (2015–2018)** | **HPAU (2019)** | **HPAU (2019–2022) present study** |
|---|---|---|---|---|---|---|
| Rhipiduridae (fantails) | | | | | | |
| 87 | White-throated Fantail | *Rhipidura albicollis* (Vieillot, 1818) | R | + | + | + |
| Corvidae (crows and jays) | | | | | | |
| 88 | Rufous Treepie | *Dendrocitta vagabunda* (Latham, 1790) | C | + | - | + |
| 89 | Grey Treepie | *Dendrocitta formosae* (Swinhoe, 1863) | R | + | - | - |
| 90 | Yellow-billed Blue Magpie | *Urocissa flavirostris* (Blyth, 1846) | C | + | + | + |
| 91 | Red-billed Blue Magpie | *Urocissa erythroryncha* (Boddaert, 1783) | R | + | + | + |
| 92 | Large-billed Crow | *Corvus macrorhynchos* (Wagler, 1827) | C | + | + | + |
| 93 | Black-headed Jay | *Garrulus lanceolatus* (Vigors, 1830) | - | - | + | - |
| Monarchidae (monarchs & paradise flycatchers) | | | | | | |
| 94 | Indian Paradise-flycatcher | *Terpsiphone paradisi* (Linnaeus, 1758) | R | + | + | + |
| Nectariniidae (sunbirds) | | | | | | |
| 95 | Purple Sunbird | *Cinnyris asiaticus* (Latham, 1790) | R | + | - | + |
| 96 | Crimson Sunbird | *Aethopyga siparaja* (Raffles, 1822) | R | + | + | + |
| Estrildidae (waxbills) | | | | | | |
| 97 | Scaly-breasted Munia | *Lonchura punctulata* (Linnaeus, 1758) | C | + | - | + |
| Passeridae (sparrows, snowfinches, and allies) | | | | | | |
| 98 | House Sparrow | *Passer domesticus* (Linnaeus, 1758) | VC | + | + | + |
| 99 | Russet Sparrow | *Passer cinnamomeus* (Gould, 1836) | VC | + | + | + |
| Motacillidae (wagtails and pipits) | | | | | | |
| 100 | Paddyfield Pipit | *Anthus rufulus* (Vieillot, 1818) | C | + | + | + |
| 101 | Long-billed Pipit | *Anthus similis* (Jerdon, 1840) | - | - | + | - |
| 102 | Grey Wagtail | *Motacilla cinerea* (Tunstall, 1771) | R | + | + | + |
| 103 | White-browed Wagtail | *Motacilla maderaspatensis* (Gmelin, J.F. 1789) | C | + | - | - |
| 104 | White Wagtail | *Motacilla alba* (Linnaeus, 1758) | C | + | + | + |
| Fringillidae (finches, euphonias, and Hawaiian honeycreepers) | | | | | | |
| 105 | Common Rosefinch | *Carpodacus erythrinus* (Pallas, 1770) | R | + | + | + |
| 106 | Yellow-breasted Greenfinch | *Chloris spinoides* (Vigors, 1831) | R | + | + | + |
| Emberizidae (Old World buntings) | | | | | | |
| 107 | White-capped Bunting | *Emberiza stewarti* (Blyth, 1854) | R | + | - | - |
| Stenostiridae (fairy-flycatcher and crested-flycatchers) | | | | | | |
| 108 | Yellow-bellied Fairy-fantail | *Chelidorhynx hypoxanthus* (Blyth, 1843) | C | + | + | + |
| 109 | Grey-headed Canary-flycatcher | *Culicicapa ceylonensis* (Swainson, 1820) | R | + | + | + |
| Paridae (tits, chickadees) | | | | | | |
| 110 | Coal Tit | *Periparus ater* (Linnaeus, 1758) | R | + | - | + |
| 111 | Cinereous Tit | *Parus cinereus* (Vieillot, 1818) | VC | + | - | + |
| 112 | Himalayan Black-lored Tit | *Machlolophus xanthogenys* (Vigors, 1831) | R | + | + | + |
| 113 | Green-backed Tit | *Parus monticolus* (Vigors, 1831) | - | - | + | - |
| 114 | Black-throated Tit | *Aegithalos concinnus* (Gould, 1855) | - | - | + | + |
| Sittidae | | | | | | |
| 115 | White-tailed Nuthatch | *Sitta himalayensis* (Jardine & Selby, 1835) | - | - | + | - |
| Cisticolidae (cisticolas) | | | | | | |
| 116 | Zitting Cisticola | *Cisticola juncidis* (Rafinesque, 1810) | R | + | - | - |
| 117 | Grey-breasted Prinia | *Prinia hodgsonii* (Blyth, 1844) | C | + | + | + |
| 118 | Jungle Prinia | *Prinia sylvatica* (Jerdon, 1840) | R | + | + | + |

| | English name | Scientific name | Abundance | CUHP (2015–2018) | HPAU (2019) | HPAU (2019–2022) present study |
|---|---|---|---|---|---|---|
| 119 | Ashy Prinia | *Prinia socialis* (Sykes, 1832) | R | + | + | + |
| 120 | Common Tailorbird | *Orthotomus sutorius* (Pennant, 1769) | C | + | + | + |
| 121 | Plain Prinia | *Prinia inornata (Sykes, 1832)* | R | - | + | + |
| 122 | Himalayan Prinia | *Prinia crinigera* (Hodgson, 1836) | R | - | + | + |
| Hirundinidae (swallows) | | | | | | |
| 123 | Red-rumped Swallow | *Cecropis daurica* (Laxmann, 1769) | VC | + | + | + |
| 124 | Barn Swallow | *Hirundo rustica* (Linnaeus, 1758) | R | + | + | + |
| 125 | Wire-tailed Swallow | *Hirundo smithii* (Leach, 1818) | R | - | - | + |
| Pycnonotidae (bulbuls) | | | | | | |
| 126 | Black Bulbul | *Hypsipetes leucocephalus* (Gmelin, J.F. 1789) | C | + | + | + |
| 127 | Himalayan Bulbul | *Pycnonotus leucogenis* (Gray, J.E. 1835) | VC | + | + | + |
| 128 | Red-vented Bulbul | *Pycnonotus cafer* (Linnaeus, 1766) | VC | + | + | + |
| Phylloscopidae (Old World leaf warblers ) | | | | | | |
| 129 | Lemon-rumped Warbler | *Phylloscopus chloronotus* (J.E. & G.R. Gray, 1847) | C | + | + | + |
| 130 | Grey-hooded Leaf Warbler | *Phylloscopus xanthoschistos* (Gray, JE and Gray, GR, 1847) | C | + | + | + |
| Aegithalidae (long-tailed tits) | | | | | | |
| 131 | Black-throated Tit | *Aegithalos concinnus* (Gould, 1855) | R | + | + | + |
| Zosteropidae (white-eyes and yuhinas) | | | | | | |
| 132 | Oriental White-eye | *Zosterops palpebrosus* (Temminck, 1824) | C | + | + | + |
| Timaliidae (scimitar babblers and allies) | | | | | | |
| 133 | White-browed Scimitar Babbler | *Pomatorhinus schisticeps* (Hodgson, 1836) | R | + | - | - |
| 134 | Rusty-cheeked Scimitar Babbler | *Erythrogenys erythrogenys* (Vigors, 1831) | C | + | + | + |
| 135 | Black-chinned Babbler | *Cyanoderma pyrrhops* (Blyth, 1844) | R | + | + | + |
| 136 | Puff-throated Babbler | *Pellorneum ruficeps* (Swainson, 1832) | R | + | + | + |
| Leiothrichidae (babblers, laughing thrushes, and allies) | | | | | | |
| 137 | Jungle Babbler | *Argya striata* (Dumont, 1823) | VC | + | - | - |
| 138 | Streaked LaughingThrush | *Trochalopteron lineatum* (Vigors, 1831) | R | + | - | - |
| 139 | Rufous Sibia | *Heterophasia capistrata* (Vigors, 1831) | R | + | + | + |
| Certhiidae (treecreepers) | | | | | | |
| 140 | Bar-tailed Treecreeper | *Certhia himalayana* (Vigors, 1832) | C | + | + | + |
| Sturnidae (starlings) | | | | | | |
| 141 | Common Starling | *Sturnus vulgaris* (Linnaeus, 1758) | - | + | - | - |
| 142 | Brahminy Starling | *Sturnia pagodarum* (Gmelin, J.F. 1789) | R | + | - | + |
| 143 | Chestnut-tailed Starling | *Sturnia malabarica* (Gmelin, J.F. 1789) | R | + | + | + |
| 144 | Common Myna | *Acridotheres tristis* (Linnaeus, 1766) | VC | + | + | + |
| 145 | Jungle Myna | *Acridotheres fuscus* (Wagler, 1827) | R | + | + | + |
| Muscicapidae (chats and flycatchers) | | | | | | |
| 146 | Indian Robin | *Copsychus fulicatus* (Linnaeus, 1766) | C | + | - | + |
| 147 | Oriental Magpie Robin | *Copsychus saularis* (Linnaeus, 1758) | VC | + | + | + |
| 148 | Rufous-bellied Niltava | *Niltava sundara* (Hodgson, 1837) | R | + | - | + |
| 149 | Verditer Flycatcher | *Eumyias thalassinus* (Swainson, 1838) | VC | + | + | + |
| 150 | Spotted Forktail | *Enicurus maculatus* (Vigors, 1831) | R | + | - | + |
| 151 | Blue Whistling Thrush | *Myophonus caeruleus* (Scopoli, 1786) | VC | + | + | + |

| | English name | Scientific name | Abundance | CUHP (2015–2018) | HPAU (2019) | HPAU (2019–2022) present study |
|---|---|---|---|---|---|---|
| 152 | White-tailed Rubythroat | *Calliope pectoralis* (Gould, 1837) | C | + | - | - |
| 153 | Slaty-blue Flycatcher | *Ficedula tricolor* (Hodgson, 1845) | R | + | + | - |
| 154 | Blue-fronted Redstart | *Phoenicurus frontalis* (Vigors, 1831) | C | + | - | + |
| 155 | Plumbeous Water Redstart | *Phoenicurus fuliginosus* (Vigors, 1831) | VC | + | + | + |
| 156 | White-capped Water Redstart | *Phoenicurus leucocephalus* (Vigors, 1831) | VC | + | + | + |
| 157 | Chestnut-bellied Rock Thrush | *Monticola rufiventris* (Jardine & Selby, 1833) | R | + | + | + |
| 158 | Common Stonechat | *Saxicola torquatus* (Pallas, 1773) | R | + | + | + |
| 159 | Pied Bushchat | *Saxicola caprata* (Linnaeus, 1766) | R | + | + | + |
| 160 | Asian Brown Flycatcher | *Muscicapa dauurica* (Pallas, 1811) | - | - | + | + |
| 161 | Rusty-tailed Flycatcher | *Ficedula ruficauda* (Swainson, 1838) | - | - | + | - |
| 162 | Rufous-gorgeted Flycatcher | *Ficedula strophiata* (Hodgson, 1837) | - | - | + | - |
| 163 | Blue-capped Redstart | *Phoenicurus coeruleocephala* (Vigors, 1831) | R | - | - | + |
| 164 | Grey Bushchat | *Saxicola ferreus* (Gray, JE and Gray, GR, 1847) | VC | + | + | + |
| 165 | Himalayan Bush Robin | *Tarsiger rufilatus* (Hodgson, 1845) | R | - | - | + |
| Turdidae (thrushes) | | | | | | |
| 166 | Grey-winged Blackbird | *Turdus boulboul* (Latham, 1790) | R | + | + | - |
| 167 | Black-throated Thrush | *Turdus atrogularis* (Jarocki, 1819) | C | - | - | + |
| CORACIIFORMES | | | | | | |
| Meropidae | | | | | | |
| 168 | Blue-tailed Bee-eater | *Merops philippinus* (Linnaeus, 1767) | R | - | + | - |
| 169 | Green Bee-eater | *Merops orientalis* (Latham, 1801) | R | - | - | + |

C—common | VC—very common | R—rare.

human disturbances, and environmental factors such as air, light, and noise pollution, and global warming affect the functional diversity of birds (Dutta 2017; Rajashekara & Venkatesha 2019; Matuoka et al. 2020). These factors also contribute to the varied distribution of bird diversity in the study area.

## DISCUSSION

The study represents the avifaunal diversity in the university premise located in the hilly terrain of the agro-climatic zone-II, Himachal Pradesh, India. The university lies in the foothill region of Dhauladhar ranges which are also known as flyover of many raptor species and is the major passage for local migration of many bird species to low land areas of the valley sub-region. The agricultural landscape within the study area exhibits diverse habitats, encompassing the expanse under agricultural fields. Conversely, the built-up locations predominately reflect the impact of developmental activities and habitat degradation. Areas designated as grasslands and forests are characterized by mixed vegetation, which serves as a significant contributor to bird diversity. Notably, the study area functions as a transition zone between human habitation and agricultural landscapes, encompassing unmanaged tea gardens, patches of forests, and various water bodies. These diverse features effectively draw in both migratory and resident bird species, distinctly augmenting the overall biodiversity of the area.

The data collected over three years for bird diversity was compared with the previous records. We observed that the present checklist (HPAU 2019–2022) showed many discrepancies/variations with the previous record (HPAU) 2019 compiled by Kottawa-Arachchi (2022). Several avian species, viz., Banded Bay Cuckoo *Cacomantis sonneratii*, Indian Cormorant *Phalacrocorax fuscicollis*, Lesser Fish-eagle *Icthyophaga humilis*, Oriental Honey-buzzard *Pernis ptilorhynchus*, Collared Owlet *Glaucidium brodiei*, Scaly-bellied Woodpecker *Picus squamatus*, Coppersmith Barbet *Psilopogon haemacephalus*, and many warbler, shrike, and minivet species were found absent. Most of these species even not recorded outside the university premises and surrounding areas of Palampur city. Some avian species, viz., White-tailed Nuthatch *Sitta himalayensis* and Black-

**Table 2. Relative diversity (RDi) of avian families in Agricultural University Himachal Pradesh, India.**

| | Family | RDi |
|---|---|---|
| 1 | Phasianidae | 2.59 |
| 2 | Columbidae | 4.31 |
| 3 | Cuculidae | 2.59 |
| 4 | Rallidae | 1.72 |
| 5 | Ardeidae | 3.45 |
| 6 | Phalacrocoracidae | 0.86 |
| 7 | Charadriidae | 0.86 |
| 8 | Scolopacidae | 0.86 |
| 9 | Accipitridae | 3.45 |
| 10 | Strigidae | 3.45 |
| 11 | Bucerotidae | 0.86 |
| 12 | Upupidae | 0.86 |
| 13 | Picidae | 6.03 |
| 14 | Megalaimidae | 1.72 |
| 15 | Alcedinidae | 0.86 |
| 16 | Falconidae | 0.86 |
| 17 | Psittaculidae | 3.45 |
| 18 | Campephagidae | 0.86 |
| 19 | Dicruridae | 2.59 |
| 20 | Dicaeidae | 0.86 |
| 21 | Sylviidae | 0.86 |
| 22 | Rhipiduridae | 0.86 |
| 23 | Corvidae | 3.45 |
| 24 | Monarchidae | 0.86 |
| 25 | Nectariniidae | 1.72 |
| 26 | Estrildidae | 0.86 |
| 27 | Passeridae | 1.72 |
| 28 | Motacillidae | 2.59 |
| 29 | Fringillidae | 1.72 |
| 30 | Stenostiridae | 1.72 |
| 31 | Paridae | 3.45 |
| 32 | Cisticolidae | 5.17 |
| 33 | Hirundinidae | 2.59 |
| 34 | Pycnonotidae | 2.59 |
| 35 | Phylloscopidae | 1.72 |
| 36 | Aegithalidae | 0.86 |
| 37 | Zosteropidae | 0.86 |
| 38 | Timaliidae | 2.59 |
| 39 | Leiothrichidae | 0.86 |
| 40 | Certhiidae | 0.86 |
| 41 | Sturnidae | 3.45 |
| 42 | Muscicapidae | 13.79 |
| 43 | Turdidae | 0.86 |
| 44 | Meropidae | 0.86 |

headed Jay *Garrulus lanceolatus* were recorded outside the university premises (Table 1). The previous checklist by Kottawa-Arachchi (2022) also reported many doubtful records such as Crow-billed Drongo *Dicrurus annectens* and Indian Pitta *Pitta brachyura*. Some of these species are not even reported in many birding sites surrounding the study area; moreover, many bird species have few records in the hilly state of Himalaya. Some of these species are mainly widespread residents in the Shivaliks and the foothills region. As most of the species are geotagged with an inbuilt Nikon p900 camera, the huge gap in previous records (HPAU 2019) seems to arise due to misidentification and sampling errors that may cause such reporting.

The more records in HPAU comparison to CUHP was due to the varied habitat diversity and larger study area. The absence of common species indicates the influence of unscientific anthropogenic activities and habitat loss. The results are in line with the findings that suggest the loss of habitat and development activities influences bird diversity (Rajashekara & Venkatesha 2019; Mbiba et al. 2021). The agricultural landscape in the Indian Himalayan region is predominately characterized by human modifications, with the agriculture university premise also exhibiting sign of habitat degradation and fragmentation. Throughout the survey, activities such as clearing new areas for experimental trials, developmental endeavours, large-scale shrub trimming, and unauthorized livestock grazing from the nearby villages have led to significant habitat destruction. However, despite these challenges, the hilly terrain of the agriculture university premise hold vast potential for habitat diversity, featuring wastelands, scattered patches of forest, grasslands, and water bodies. Nonetheless, the agricultural landscape and built-up areas are significantly impacted by habitat degradation, affecting the visitation and migration pattern of many shy bird species. Protection measures are essential for areas far from human habitation to prevent habitat fragmentation. Furthermore, the sites adorned with patches of forest, unmanaged tea orchards, and water bodies hold promise as potential areas for developing conservation strategies aimed at safeguarding avian species.

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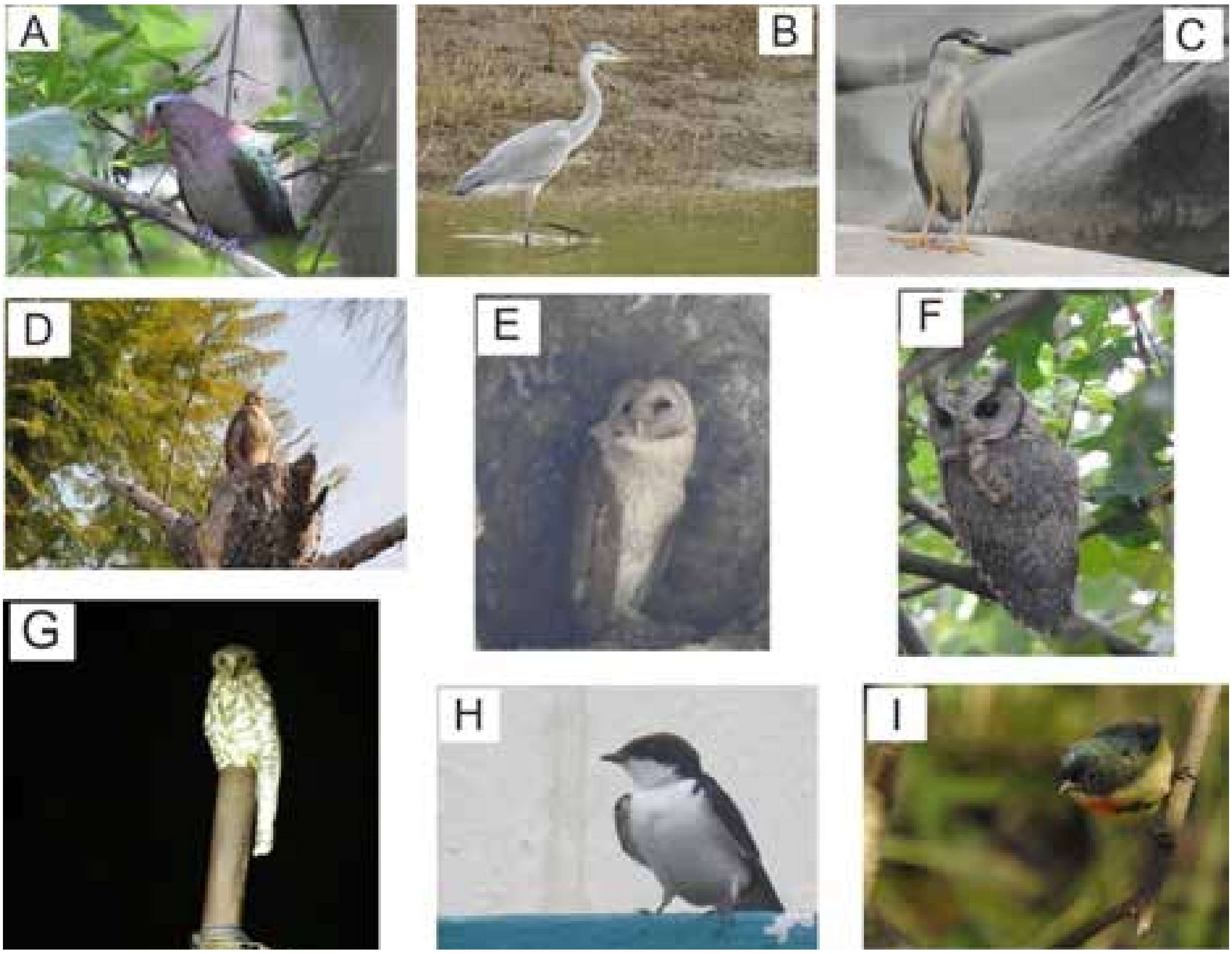

**Image 2. Photographic records of some additional bird species based on present survey [HPAU (2019–2022)] in comparison to previous checklists [CUHP (2015–2018) & HPAU (2019)]. A—Asian Emerald Dove *Chalcophaps indica* | B—Grey Heron *Ardea cinerea* | C—Black-crowned Night Heron *Nycticorax nycticorax* | D—Himalayan Buzzard *Buteo refectus* | E—Barn Owl *Tyto alba* | F—Indian Scops Owl *Otus bakkamoena* | G—Brown Boobook *Ninox scutulata* | H—Wire-tailed Swallow *Hirundo smithii* | I—Fire-breasted Flowerpecker *Dicaeum ignipectus*. © Praveen Kumar.**

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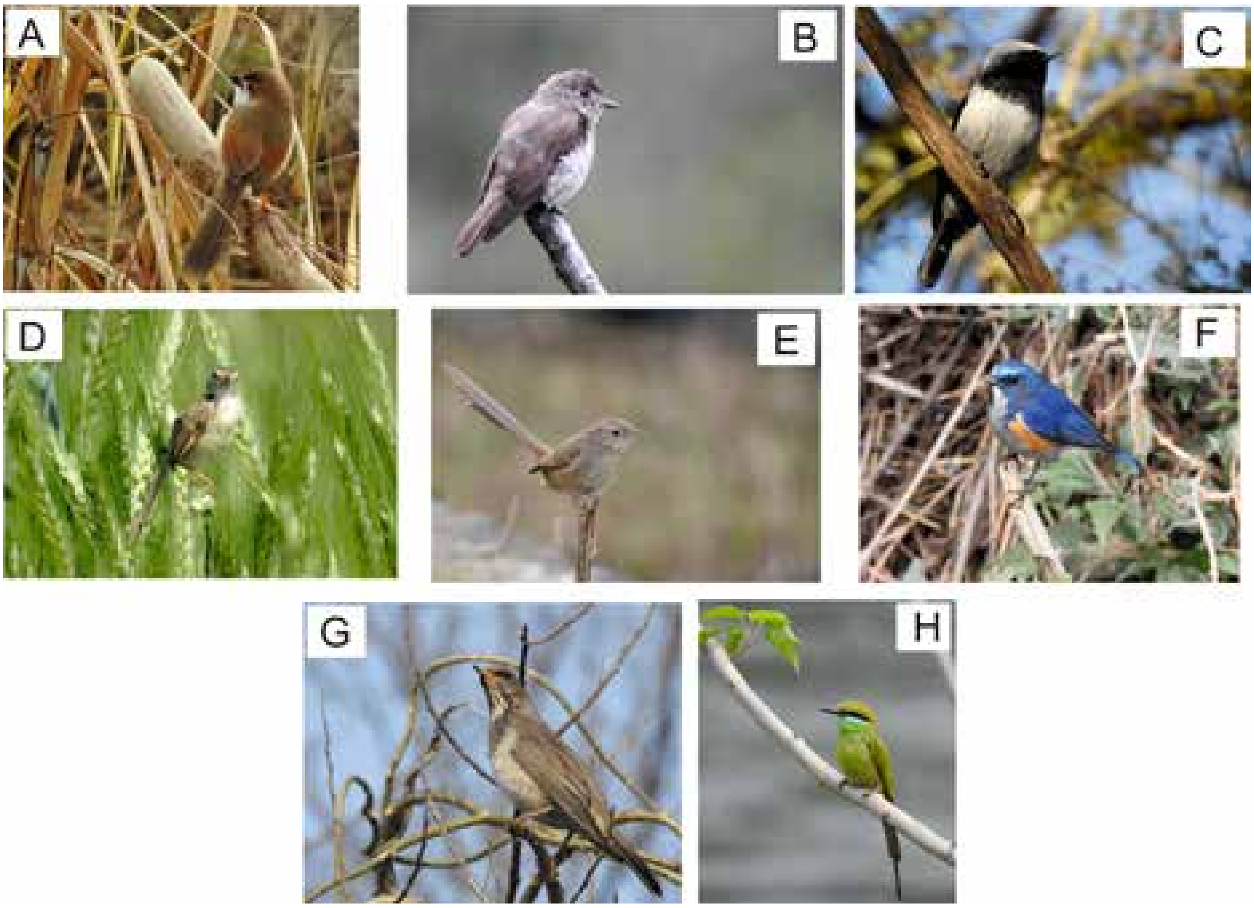

Image 3. Photographic records of some additional bird species based on present survey [HPAU (2019–2022)] in comparison to previous checklists [CUHP (2015–2018) & HPAU (2019)]. A—Yellow-eyed Babbler *Chrysomma sinense* | B—Asian Brown Flycatcher *Muscicapa dauurica* | C—Blue-capped Redstart *Phoenicurus coeruleocephala* | D—Plain Prinia *Prinia inornata* | E—Striated Prinia *Prinia crinigera* | F—Himalayan Bluetail *Tarsiger rufilatus* | G—Black-throated Thrush *Turdus atrogularis* | H—Green Bee-eater *Merops orientalis*. © Praveen Kumar.

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COMMUNICATION

# Aquatic insects as bioindicators of stream water quality - a seasonal analysis on Western Ghats river, Muthirapuzha, in central Kerala, India

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**Abstract:** This study was conducted to assess the water quality of Muthirapuzha River, Idukki using aquatic insects as bioindicators. Insects were collected on a seasonal basis from February 2014 to January 2015 from 12 sampling stations. Insects were sampled using standard collection methods and were identified up to family level. A total of 3,278 individuals belonging to seven orders and 37 families were collected during the study period. The greatest number of taxa was represented by order Ephemetroptera during monsoon (27%) and post-monsoon (25%), while Diptera (22.7%) dominated the pre-monsoon season. Shannon-Weiner diversity index, Simpson dominance index, and Margalef's richness index was highest at post-monsoon. The EPT score in Muthirapuzha was for normal waters, however, pre-monsoon values were lowest, indicating pollution load during this period. Hilsenhoff's family biotic index (HFBI) was used to estimate the status of organic pollution along the river based on representative families of aquatic entomofauna; values were highest at pre-monsoon season. The overall organic water quality level in the Muthirapuzha was good to fair based on this study.

**Keywords:** Biomonitoring, diversity indices, EPT scores, Hilsenhoff's family biotic index, macro-invertebrates, Margalef's richness index, Munnar, Muthirapuzha River, Periyar River, Shannon Weiner diversity index, Simpson dominance index.

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**Author contributions:** MH—data collection and manuscripts preparation. LJ—correction of manuscripts, language check. VSJ—conceptual support, Identification of macroinvertebrates, correction of final draft.

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## INTRODUCTION

Rivers provide fresh water for agricultural, industrial and domestic needs (Ridoutt et al. 2010; Sunil et al. 2010) that can create enormous environmental pressures, including pollution leading to deteriorated water quality adversely affecting aquatic life (Kamboj & Kamboj 2019; Sinha et al. 2020). Biological communities provide a faithful reflection of environmental conditions, since they are continually exposed to them (Rosenberg & Resh 1993). Water quality changes are directly reflected by aquatic fauna, which can be assessed to measure the health of their ecosystems (Mulani et al. 2009; Saxena & Singh 2020). This approach is widely exploited as a reliable technique for assessing point and non-point sources of pollution of water bodies via biomonitoring protocols. Benthic macroinvertebrates representing different visible aquatic phyla exhibit a relatively wide range of response to chemical and physical water quality stressors like pH, temperature, dissolved oxygen, organic pollutants, heavy metals and sediments that can serve as a biological indicator of water pollution (Marzelai et al. 2008). Latha & Thanga (2010) identified macroinvertebrates as useful bioindicators in estuaries. Stream insect communities were suggested for aquatic biomonitoring protocol by Morse et al. (1994) and Subramanian & Sivaramkrishnan (2005). Diversity of aquatic insects is relatively easy to measure for assessing the health status of streams, and many biomonitoring studies are reported from southern Indian rivers (Sheeba & Ramanujan 2009; Priyanka & Prasad 2014). Stream entomofauna were targeted in Killi Ar, an urban river of Trivandrum corporation area, to assess the pollution status of the stream (Dinesh et al. 2017).

Many tools are employed in biological monitoring to assess the quality of water resources (Buss et al. 2003). The effective use of these tools leads to a better understanding of aquatic organisms that influence on biotic index results, and occurrence of bioindicators (Czerniawska-Kusza 2005). Distribution of bioindicator taxa is influenced by hydrological characteristics, nutrient supply, substrate type, predation pressure and natural or anthropogenic disturbances, in addition to variation in water quality, that makes these biotic indices important tools for evaluate the health of water ecosystems (Silveira et al. 2004). Comparative analyses of biotic indices are now available to determine which index best reflects ecosystem health (Gonçalves & Menezes 2011). William Hilsenhoff formulated family-level (Hilsenhoff 1988) versions of a biotic index, and tabulated interpretive criteria based on known sensitivities of arthropod taxa to organic enrichment (i.e., sewage pollution). This has been widely used in to characterize the health of freshwater streams (Reynoldson & Metcalfe-Smith 1992; Hu et al. 2007).

The river Periyar, the longest river in Kerala State (PWD 1974; CESS 1984) is considered to be the life line of central Kerala. Muthirapuzha River, the major tributary of the Periyar, forms the main drainage system south of Anamudi. This river is the major water resource of five panchayath in Devikulam Taluk of Idukki District. The Muthirapuzha watershed includes Kannan Devan Tea plantations along with Eravikulam National Park, and forms the highest watershed of the Western Ghats. Munnar Township, one of major tourist destinations in Kerala, extends along the banks of this stream. Thus this river is experiencing active anthropogenic pressure chiefly due to tourism and agricultural activities. In this study we undertook a rapid assessment of the status of this river utilizing a biomonitoring protocol targeting aquatic insects as bioindicators for stream water quality.

## MATERIALS AND METHODS

### Study area

The Muthirapuzha is located at 10.172–9.951 $^0$N & 77.077–76.983 $^0$E (Figure 1). It originates from Umaya Mala near Anamudi Peak and flows through Deikulam, Munnar, Pallivasal, Vellathooval and Konnathadi panchayths of Devikulam and Udumbanchola of Idukki District, and joins the Periyar River at Panamkutti, covering a distance of 34 km.

### Macroinvertebrate analysis

Macroinvertebrates were sampled once every four months from February 2014 to January 2015 at twelve selected stations on the Muthirapuzha to capture seasonal variations. A D-frame aquatic net (0.5 mm mesh) was used to collect benthic organisms present in a 10 $m^2$ area (Hellawell 1986). After each jab and sweep, the net was rinsed in a sieve bucket (250 µm mesh) to collect all the macroinvertebrates. Samples were washed, separated through three sieves (2 mm, 1 mm, and 0.3 mm), transferred to glass bottles after labeling and preserved in 5% formalin in the field immediately after each collection. Each animal was then brush picked, preserved in 4% formalin, sorted and identified in the laboratory according to Edmondson (1992) and Pennak (1978). Aquatic insects were counted and identified using a stereo microscope (Headz-HD600D) with the help of standard keys (McCafferty 1983; Morse et al. 1994

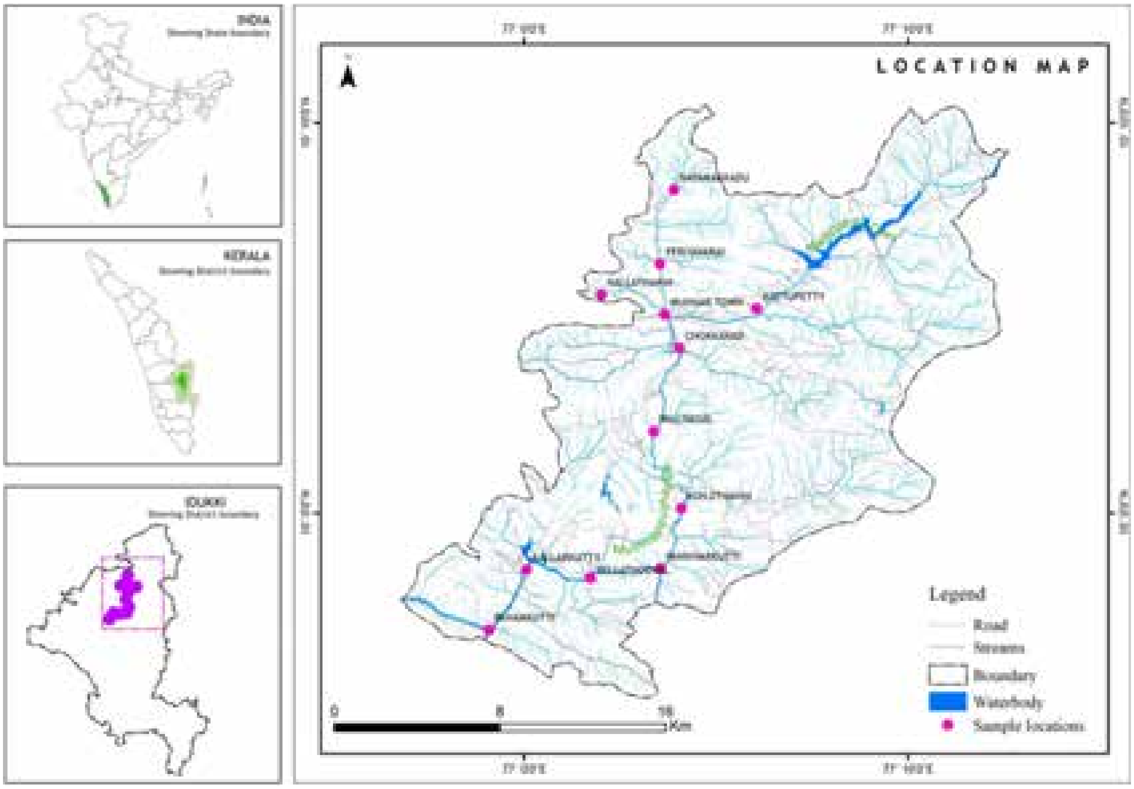

Figure 1. Sampling stations of river Muthirapuzha.

& Yong & Yule 2004) up to the family level. Taxonomic indices used for analyses of aquatic insects include Shannon-Weiner diversity index, Simpson dominance index, Margalef richness index (Shannon & Weiner 1963; Simpson 1949; Margalef 1958; Pielou 1966) and Hilsenhoff's Family Biotic Index (HFBI) (Hilsenhoff 1988) to estimate the level of organic pollution. Biodiversity indices were calculated using PAST ver. 1.34 software (Hammer et al. 2005).

## RESULTS AND DISCUSSION

The present study identified 55 taxa represented by 37 families belonging to eight orders among the 3,278 total aquatic insects collected during the study period in pre-monsoon, monsoon, and post-monsoon seasons. Table 1 shows the overall numbers of insects collected during the sampling period. The number of individuals found in the pre-monsoon season was 1,313, 270 in monsoon, and 1,695 post monsoon. The greatest numbers of taxa were represented by order Ephemeroptera in monsoon (27%) and post-monsoon (25%), while Diptera (22.7%) dominated in pre-monsoon. The overall analysis of aquatic insects indicated that the most abundant taxa were Ephemeroptera (22%), followed by, Odonata (18.5%), Diptera (18%), Trichoptera (11%), Hemiptera (10%), Coleoptera (9.7%), and Plecoptera (7.9%) (Figure 2).

The biological indices of aquatic insects computed for 12 sampling sites are represented in Table 2, 3, & 4. Shannon-Weiner diversity index for pre monsoon season ranged between 3.807–3.211 and were found to be maximum at station 2 and minimum at station 10. During monsoon it was highest at station 1 (3.266) and lower index value was reported in station 10 (2.306). Shannon-Weiner diversity index was varying between 3.752 and 3.428; these values are represented in stations 1 and 10, respectively. Simpson dominance index also showed similar relation and varied from 0.974 to 0.943 in pre-monsoon. Maximum dominance index was found in station 2 and minimum in station 10. Index values were between 0.956 to 0.879 in monsoon and 0.972 to 0.948 in post- monsoon seasons. Margalef's richness index showed comparatively low value in monsoon season and the lowest value (2.954) was identified from station 6, Chokkanadu which is an urbanized site and higher (7.452) in station 1, Nayamakkadu near the origin of stream. Richness index was higher in pre-monsoon and post-monsoon seasons compared to monsoon. In

Table 1. Aquatic insects collected from river Muthirapuzha over different seasons (2014–15).

| ORDER | FAMILY | PRM* | MON** | POM*** |
|---|---|---|---|---|
| **Diptera** | Simuliidae | 33 | 2 | 39 |
| | Chironomidae | 155 | 10 | 129 |
| | Culicidae | 68 | 20 | 52 |
| | Tipulidae | 42 | 5 | 38 |
| **Hemiptera** | Nepidae | 20 | 3 | 16 |
| | Velliidae | 19 | 3 | 19 |
| | Hydrometridae | 9 | 7 | 26 |
| | Belostomatidae | 12 | 2 | 27 |
| | Gerridae | 85 | 13 | 66 |
| **Ephemeroptera** | Ephemeridae | 34 | 9 | 53 |
| | Heptageniidae | 24 | 11 | 68 |
| | Leptohyphidae | 64 | 14 | 108 |
| | Caenidae | 94 | 16 | 110 |
| | Ephemerellidae | 18 | 3 | 25 |
| | Baetidae | 27 | 20 | 63 |
| **Plecoptera** | Perlidae | 94 | 14 | 151 |
| **Odonata** | Coenagrionidae | 114 | 3 | 125 |
| | Chlorocyphidae | 24 | 3 | 31 |
| **Odonata** | Eupaeidae | 25 | 2 | 25 |
| | Calopterygidae | 17 | 0 | 17 |
| | Lestidae | 7 | 2 | 13 |
| | Platystictidae | 13 | 0 | 15 |
| | Cordullidae | 7 | 0 | 7 |
| | Gomphidae | 43 | 6 | 39 |
| | Aeshnidae | 22 | 5 | 43 |
| **Megaloptera** | Corydalidae | 17 | 4 | 30 |
| **Trichoptera** | Helicopsychidae | 27 | 8 | 47 |
| | Hydropsychidae | 11 | 15 | 46 |
| | Glossosomatidae | 18 | 1 | 26 |
| | Polycentropodidae | 6 | 4 | 21 |
| | Leptoceridae | 21 | 31 | 80 |
| **Coleoptera** | Haliplidae | 10 | 7 | 17 |
| | Hydrophildae | 68 | 11 | 55 |
| | Gyrinidae | 14 | 12 | 33 |
| | Dytiscidae | 51 | 4 | 35 |

PRM*—Pre-monsoon | MON**—Monsoon | POM***—Post-monsoon

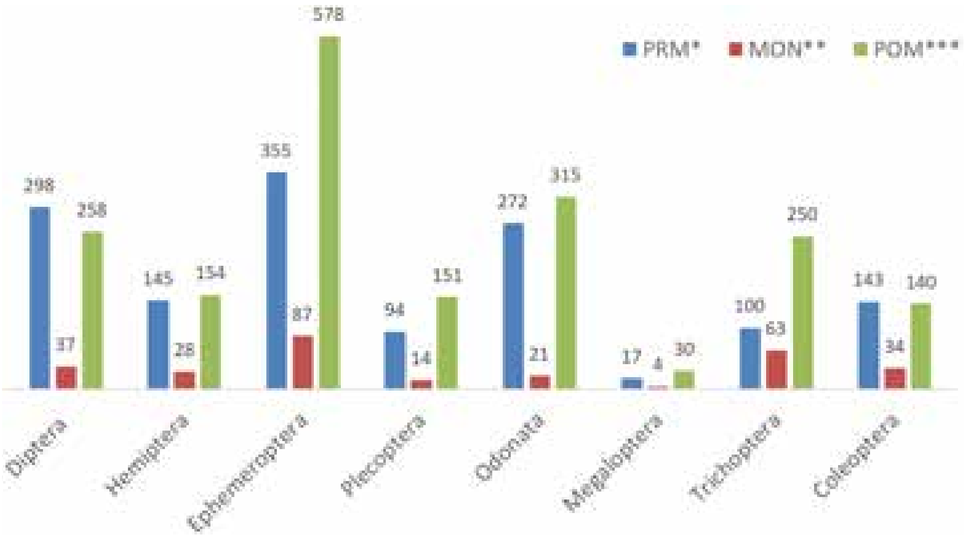

Figure 2. Aquatic insects collected from river Muthirapuzha during 2014–15.

pre-monsoon the maximum Margalef richness index was found in station 2 (10.98) and minimum in station 11 (7.015). In post-monsoon season the richness index varied from 10.08 to 7.856, respectively from station 2 and station 9. Highest taxonomic indices were observed in post-monsoon season.

Among aquatic insects, Ephemeroptera, Plecoptera, and Trichoptera (EPT) have a great role in low and medium order stony cobble streams. The percentage of EPT in river Muthirapuzha during the study period was represented in Table 5. These organisms are sensitive to environmental perturbations and occur in clean and well oxygenated waters. Therefore, EPT assemblages are frequently considered to be good indicators of water quality (Rosenberg & Resh 1992; Priyanka & Prasad 2014), EPT is widely used for the measure of health of

**Table 2. Biodiversity indices of aquatic insects in pre-monsoon season (2014–15).**

| Stations | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxa_S | 46 | 53 | 50 | 48 | 43 | 39 | 39 | 37 | 32 | 33 | 31 | 30 |
| Individuals | 132 | 114 | 128 | 151 | 149 | 135 | 100 | 114 | 81 | 77 | 72 | 60 |
| Simpson_1-D | 0.969 | 0.974 | 0.968 | 0.956 | 0.954 | 0.947 | 0.962 | 0.964 | 0.956 | 0.949 | 0.955 | 0.953 |
| Shannon_H | 3.647 | 3.807 | 3.652 | 3.49 | 3.369 | 3.261 | 3.451 | 3.452 | 3.298 | 3.211 | 3.242 | 3.223 |
| Margalef | 9.216 | 10.98 | 10.1 | 9.368 | 8.393 | 7.747 | 8.252 | 7.601 | 7.054 | 7.367 | 7.015 | 7.083 |

**Table 3. Biodiversity indices of aquatic insects in monsoon season (2014–15).**

| Stations | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxa_S | 30 | 23 | 23 | 15 | 12 | 9 | 14 | 14 | 12 | 12 | 12 | 12 |
| Individuals | 49 | 33 | 30 | 22 | 19 | 15 | 18 | 22 | 17 | 21 | 20 | 18 |
| Simpson_1-D | 0.956 | 0.949 | 0.951 | 0.922 | 0.903 | 0.871 | 0.92 | 0.905 | 0.899 | 0.879 | 0.89 | 0.901 |
| Shannon_H | 3.266 | 3.061 | 3.078 | 2.626 | 2.406 | 2.119 | 2.582 | 2.5 | 2.395 | 2.306 | 2.346 | 2.399 |
| Margalef | 7.452 | 6.292 | 6.468 | 4.529 | 3.736 | 2.954 | 4.498 | 4.206 | 3.883 | 3.613 | 3.672 | 3.806 |

**Table 4. Biodiversity indices of aquatic insects in post-monsoon season (2014–15).**

| Stations | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Taxa_S | 55 | 55 | 52 | 48 | 45 | 41 | 44 | 42 | 38 | 41 | 40 | 41 |
| Individuals | 228 | 212 | 182 | 170 | 141 | 147 | 118 | 105 | 111 | 102 | 90 | 89 |
| Simpson_1-D | 0.972 | 0.966 | 0.965 | 0.971 | 0.953 | 0.959 | 0.958 | 0.956 | 0.948 | 0.96 | 0.955 | 0.957 |
| Shannon_H | 3.752 | 3.668 | 3.649 | 3.683 | 3.402 | 3.416 | 3.486 | 3.434 | 3.248 | 3.481 | 3.409 | 3.42 |
| Margalef | 9.946 | 10.08 | 9.8 | 9.151 | 8.891 | 8.015 | 9.013 | 8.81 | 7.856 | 8.649 | 8.667 | 8.911 |

fresh water ecosystem (Wallace & Jackson 1996).

In this study the percentage of EPT was very high in sampling stations 1, 2, & 3 in three sampling seasons. But it was gradually decreased in the middle and lower streams of river Muthirapuzha. Especially the middle sampling sites representing Munnar Township and nearby inhabited area exhibit a very low percentage of EPT level. This clearly indicates that the water quality was badly affected by pollution related activities at this stretch of river. The percentage of EPT in lower stream varied from station to station which means that each sampling stations were under different types of pollution stress mainly due to anthropogenic and tourism related activities along the river, Muthirapuzha. The overall mean percentage of EPT score indicated that the pre-monsoon season was polluted in nature compared to the other two seasons (Figure 3)

Hilsenhoff family biotic index (HFBI) is one of the most effective bio monitoring tool in stream ecology and is used to assess the level of organic pollution in water bodies (Hilsenhoff 1988). HFBI of river Muthirapuzha (Table 6) categorizes the water quality based on the families identified from 12 stations along this river. Water quality grade according to HFBI index is shown in table 7. HFBI indicated that the water quality varies in each sampling station ranging from excellent to fairly poor and the degree of organic pollution was comparatively low in Muthirapuzha. Based on this study the water of Muthirapuzha could be classified into four categories using the HFBI, 'excellent', 'very good', 'good', and 'fair'. The HFBI values were higher in pre-monsoon and lower during monsoon seasons indicating the organic loading during pre-monsoon.

When classifying water quality during monsoon, the HFBI index gave scores of 'excellent' to 'good', however, station 11 was under some organic pollution (Table 6) otherwise the overall water quality was very good during this period. During post-monsoon season the HFBI ranged 3.78–5.34 which indicated the water quality in between very well to fair (Table 6). Station 5, 6, 8, 11, & 12 came under 'fairly substantial pollution likely' (Table 7) during this season. Finally in pre-monsoon HBFI

Table 5. Percentage of EPT in river Muthirapuzha (2014–15).

| Stations | PRM* | MON** | POM*** |
|---|---|---|---|
| 1 - Nayamakkadu | 42.73 | 33.84 | 46.18 |
| 2 - Periyavarai | 45.74 | 47.61 | 48.56 |
| 3 - Mattupetty | 46.05 | 30.61 | 45.56 |
| 4 - Nallathanni | 22.7 | 15.21 | 28.78 |
| 5 - Munnar Town | 20.68 | 15.55 | 23.78 |
| 6 - Chokkanadu | 15.95 | 21.42 | 27.21 |
| 7 - Pallivasal | 27.78 | 54.57 | 36.87 |
| 8 - Kunjithanni | 31.13 | 44.82 | 39.59 |
| 9 - Panniyarkutti | 27.47 | 32.14 | 42.95 |
| 10 - Vellathooval | 24.09 | 55.17 | 36.71 |
| 11- Kallarkutti | 29.67 | 24.32 | 41.07 |
| 12 - Panamkutti | 25 | 56.1 | 46.28 |
| **Mean** | **29.91** | **35.93** | **38.62** |

PRM*—Pre-monsoon | MON**—Monsoon | POM***—Post-monsoon

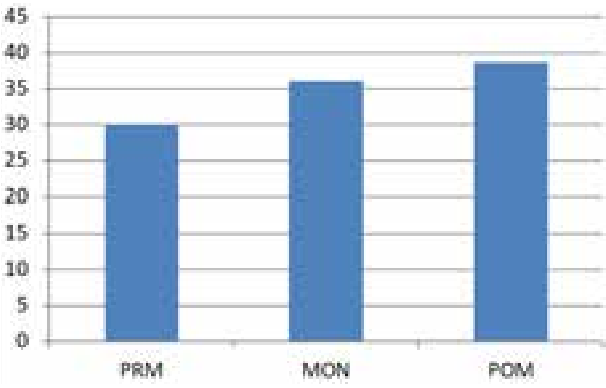

Figure 3. Mean percentage of EPT in river Muthirapuzha (2014–15).

Table 6. Hilsenhoff family biotic index of river Muthirapuzha (2014–15).

| Stations | PRM | MON | POM |
|---|---|---|---|
| S1 (Nayamakkadu) | 3.78 | 3.22 | 3.99 |
| S2 (periyavarai) | 4.33 | 3.45 | 3.78 |
| S3 (Mattupetty) | 4.51 | 3.37 | 4.12 |
| S4 (Nallathanni) | 5.29 | 3.57 | 4.34 |
| S5 (Munnar Town) | 5.75 | 3.8 | 5.19 |
| S6 (Chokkanadu) | 5.8 | 3.78 | 5.34 |
| S7 (Pallivasal) | 5.21 | 3.6 | 4.92 |
| S8 (Kunjithanni) | 5.33 | 4.18 | 5.13 |
| S9 (Panniyarkutti)) | 5.3 | 4.23 | 4.83 |
| S10 (Vellathooval) | 4.82 | 4.4 | 5.04 |
| S11 (Kallarkutti) | 5.12 | 4.54 | 4.61 |
| S12 (Panamkutti) | 5.41 | 3.38 | 4.94 |

PRM*—Pre-monsoon | MON**—Monsoon | POM***—Post-monsoon

Table 7. Hilsenhoff family biotic index for water quality grades.

| HFBI | Water quality | Degree of organic pollution |
|---|---|---|
| 0.00–3.75 | Excellent | Organic Pollution Unlikely |
| 3.76–4.25 | Very Good | Possible Slight Organic Pollution |
| 4.26–5.00 | Good | Some Organic Pollution Probable |
| 5.01–5.75 | Fair | Fairly Substantial Pollution Likely |
| 5.76–6.50 | Fairly Poor | Substantial Pollution Likely |
| 6.51–7.25 | Poor | Very Substantial Pollution Likely |
| 7.26–10.00 | Very Poor | Severe Organic Pollution Likely |

was comparatively higher with the other two seasons; the water quality values come under the categories of ‘very good’ to ‘fairly poor’. Sampling stations 5 and 6 reported ‘substantial pollution likely’ (Table 6, 7) during this period. It may be noted that these sampling stations are representing the Munnar township segment of the stream. ‘Poor’ and ‘very poor’ water qualities were not reported at any sampling stations during the course of sampling period.

According to the HFBI, overall water quality was very good in monsoon, good in post monsoon and fair in pre-monsoon seasons (Figure 3). Though the sampling points were located within populated area except the first three, the HFBI did not reflect obvious anthropogenic pressure on this river. Munnar Township and some small towns are located in the middle and lower reaches of river Muthirapuzha, which reported ‘fairly poor’ status of water at these stretches but the overall water quality falls between very good to fair scale of HFBI. Present study shows a temporal variation in bioassessment of Muthirapuzha River that influence the judgment of the sites. Studies shows temporal variations in bioassessment based on benthic macroinvertebrates (Linke et al. 2001; Nukeri et al. 2021). Substrate heterogeneity as well as land use changes are generally the determinants of the macroinvertebrate distribution along streams (Semwal & Mishra 2019). Spatio-seasonal flux of benthic macroinvertebrate assemblages as indicators of water quality in a coastal basin of southern Chile was assessed by applying HFBI (Fierro et al. 2012). River Muthirapuzha seems sensitive to anthropogenic activities due to tourism as indicated by the macroinvertebrate community based biotic index.

## CONCLUSION

River Muthirapuzha one of the major tributary of river Periyar, a mountain stream originated and flow through the higher elevations of Western Ghats. There are 33 small and large streams contribute water to river Muthirapuzha at various stretches. The taxonomic indices of aquatic insects collected from this river established a clear view of level of stream health. The season-wise analysis of taxonomic Indies indicated that the water quality was good on monsoon season and comparatively higher pollution in other two seasons. The EPT scores indicated average water quality in the river, except at the middle stream sampling sites, the anthropogenic pressure due to tourism activates affects the water quality in this area. The study identified the water quality of the river Muthirapuzha varied seasonally at every sampling station, and the overall water quality was good based on HFBI category, although pollution load was evident in pre-monsoon season.

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SHORT COMMUNICATION

# New distribution record of *Alstonia sebusii* (Van Heurck & Müll. Arg.) Monach. from Manipur, India

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**Abstract:** The genus *Alstonia* belonging to the Apocyanaceae family is represented by 44 species distributed worldwide. In India, the taxon is represented by eight species, reported from Andaman & Nicobar Islands, Western Ghats, Eastern Ghats, western Himalaya, and northeastern regions. The present study reported the occurrence of *Alstonia sebusii* from Pudunamei village, Mao, a new addition to the flora of Manipur. Detailed morphological descriptions and measurements were recorded based on living plant specimens. The plant is locally called 'Topfiira Koso Pro' having ethno-medicinal properties and widely been used by local people of the state to treat various ailments. The plant is rare and found to occur at specific location at Pudunamei village.

**Keywords:** New addition, rare, Topfiira Koso Pro, traditional medicine.

The genus *Alstonia* R.Br. is an important timber producing taxon (Soerianegara & Lemmaens 1993; Sidiyasa 1998) described by Robert Brown (1810) and named in honor of Charles Alston, a Scottish physician and professor of botany at the University of Edinburgh. *Alstonia* is the largest genus in the subtribe Alstoniinae of tribe Plumerieae of the family Apocyanaceae represented by 44 species distributed worldwide (POWO 2023), out of which eight species are reported from India (Datta & Nayar 2021; BSI 2023). The genus is distributed in central America, tropical Africa, and from the Himalaya and China to New South Wales in Australia, and has its centre of diversity in the Malaysian region (Sidiyasa 1998). Some of the species of *Alstonia* provide important timber for commerce, and many species were used in local traditional medicines (Sidiyasa 1998).

During the field exploration in Pudunamei-Mao, Senapati District, Manipur, the author came across an interesting plant species of *Alstonia*. On further investigation and critical analysis of the plant specimen with available literature (Hooker 1880–1882; Kanjilal et al. 1939; Monachino 1949; Sidiyasa 1998; Singh et al. 2000; Eshuo & Chaturvedi 2011; Mao & Gogoi 2016; Datta & Nayar 2021; Eshuo 2023; Eshuo & Lokho 2023) and herbaria photograph images from https://powo.science.kew.org, the species is identified as *Alstonia sebusii* (Van Heurck & Müll. Arg.) Monach., hitherto unknown from Manipur. The occurrence of *A. sebusii* is an addition to the flora of Manipur as well as an extended distribution range from Sikkim through Assam to Manipur in the Indo-Burma region. This plant has ethno-medicinal properties and has been used in treating various ailments like urinary tract infection, agalactorrhea, hypertension, stomach upset by local people of Mao Naga tribe of Manipur State.

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**Competing interests:** The author declares no competing interests.

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## MATERIALS AND METHODS

The collection, pressing and preparation of herbarium specimens were done as per the conventional herbarium techniques (Jain & Rao 1976) and the herbarium specimen was deposited at Herbarium, Botany Department of Dhanamanjuri College of Science (Accession No.: 1.2020), Imphal and at Herbarium, Botanical Survey of India, Eastern Regional Centre (Accession No.: 101280), Shillong for future reference. The live plant photos were taken with the help of Sony SLT-A58 and Canon SX120 digital camera. All the morphological descriptions, measurements were based on observation of the live plant specimens in the field.

## TAXONOMIC TREATMENT

*Alstonia sebusii* (Van Heurck & Müll. Arg.) Monach., Pacific Sci. 3: 157. 1949; Datta, A. & Nayar, M.P., Fasc. Fl. India (P.V. Prasanna ed.) 30: 31. 2021. *Blaberopus sebusii* Van Heurck & Müll. Arg. in Van Heurck, Observ. Bot. 2: 188. 1871. (Image 1 & 2).

**Image 1. *Alstonia sebusii* (Van Heurck & Müll. Arg.) Monach. a—habit | b—flower twig showing the front view of a flower | c—flower twig showing corolla tube | d—fruits | e—stem with colleters and lenticels. © Kazhuhrii Eshuo.**

Trees 2–4 m tall, bark glabrous, young stem lenticellate, grey to yellowish-brown, branches terete. Leaves in 2–4 whorls, leaves elliptic or ovate-elliptic 12–18 x 3–5 cm, glabrous or puberulous, coriaceous, lateral veins 65–80 pairs, stipules dry and scaly, petioles 1–2.5 cm long. Inflorescence cymose, terminal, peduncles 1–2.5 cm long; flower creamy white, 6–7 mm in diameter. Calyx imbricates, connate at base, glabrous, persistent; corolla pink or pinkish-red, tube 8–10 mm long, widened above the middle, indumentum at the mouth of the tube, corolla tube, corolla lobes, lobes triangular, 3–4 x 3–4 mm, epipetalous, basifixed. Ovary ovoid, glabrous, carpels two, style 4 mm long, stigma pagoda like. Follicles in pair, up to 9 cm long, split

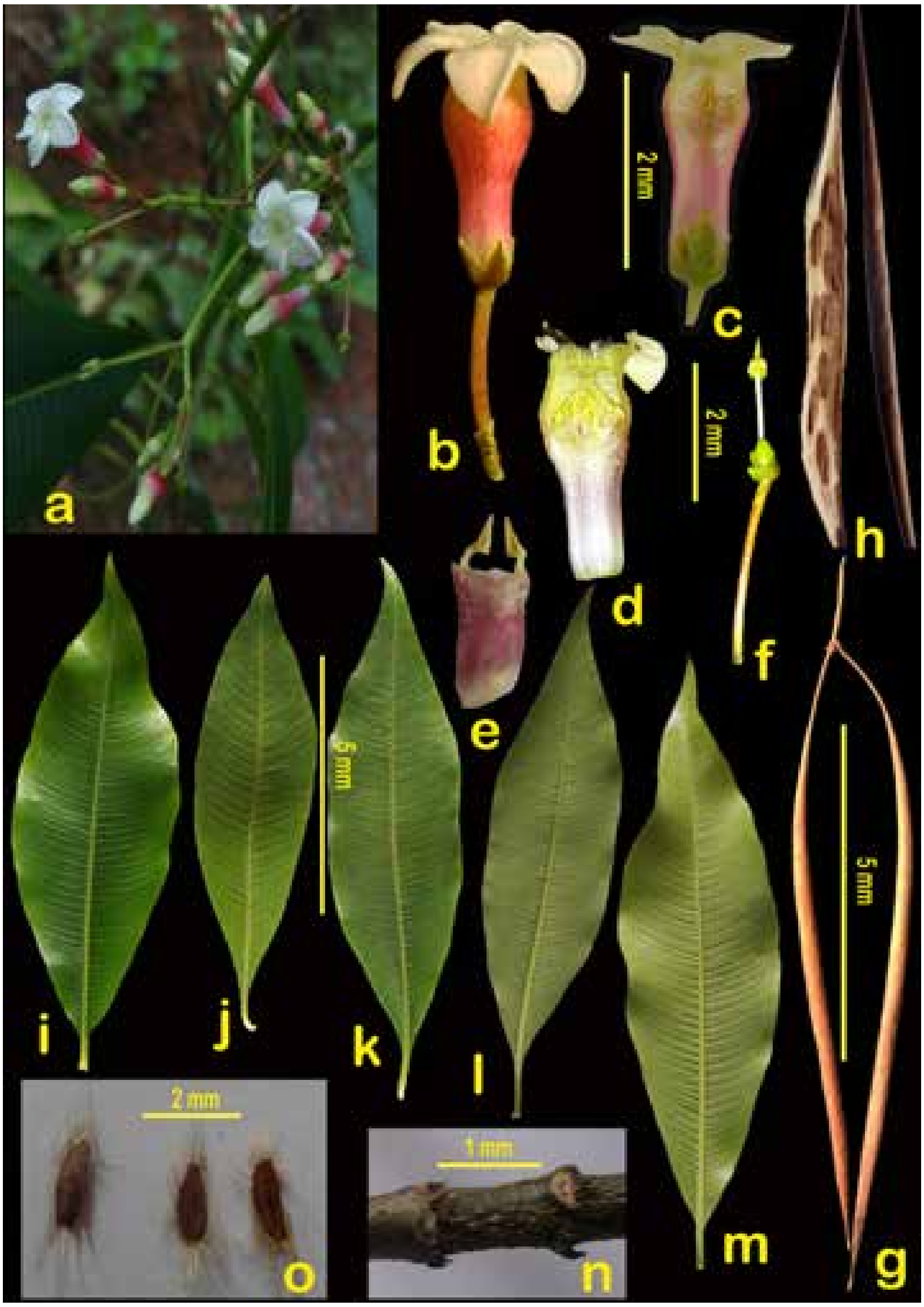

**Image 2. *Alstonia sebusii* (Van Heurck & Müll. Arg.) Monach: a—flowering twig | b—single flower side view | c–d—L.S. of flower | e—stamens | f—gynoecium showing ovary, pagoda —stigma and style | g—mature fruit follicles | h—open follicle showing seeds | i–k—leaves dorsal view | l–m—leaves ventral view | n—enlarged view of colleters | o—seeds. © Kazhuhrii Eshuo.**

**Image 3. *Alstonia sebusii* (Van Heurck & Müll. Arg.) Monach. herbarium specimen submitted to the herbarium, Botany Deartment, Dhanamanjuri College of Science, Imphal, Manipur.**

longitudinal. Seed dry, flattened, both ends rounded, hairy, 8 x 3 mm in size.

Flowering: Almost round the year.

Fruiting: June–January.

Specimen examined: India: Manipur: Pudunamei: KE 100015: 1,650–1,800 m: 25.314⁰N & 94.092⁰E (Image 3).

Ecology: Plants grow along with other herbs, shrubs or trees in the wild and home garden ornamental plants for medicinal usage.

Distribution: India (Assam, Sikkim, Manipur [present report]), Bhutan, China, Myanmar.

## Medicinal Uses

The people of Mao especially Pudunamei villagers have been using *Alstonia sebusii* (Locally called 'Topfiira koso pro') for treating urinary problems, hypertension, stomach upset, agalactorrhea—a condition where a mother fails to produce breast milk after giving birth.

A woman who suffers agalactorrhea or insufficient milk syndrome was given this plant decoction believing that latex produced by this plant can help in milk production for the mother. The plant is rare and found to occur at specific locations believing by locals that it is a 'gift from gods' to heal and cure various ailments. In recent days, a few people have started planting this plant in their home garden for their ethno-medicinal usage and also as an ornamental plant because of its foliage beauty and flowers that bloom almost throughout the year. Out of the various ailments mentioned, village people mostly used this plant to treat urinary tract infection problems. There is no previous record on the traditional uses of *A. sebusii* by any other researchers till date (Mao 1993, 1999; Lokho & Narasimhan 2013). This is the first report on the use of *A. sebusii* plant in the ethnomedicine by the Mao Naga tribe of Manipur.

Preparation and part used: About 4–6 fresh leaves are taken, washed, cut into two to three pieces and boiled in 100 ml of water. The decoction is taken orally to relieve irritation and difficulty in urination problems, hypertension, stomach upset and agalactorrhea or insufficient milk syndrome lactating mother whose breast milk fails to produce or the volume of breast milk production is less after child birth.

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SHORT COMMUNICATION

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# New distribution record of fungi *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc. (Mycenaceae) from the Konkan region of Maharashtra, India

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**Abstract:** Bioluminescence is the generation and emission of light by living things. In the present investigation, *Mycena chorophos* is reported for the first time from the Konkan region of Maharashtra, India. We observed tiny, luminous clumps of *Mycena chlorophos* on a rotten bamboo substratum. The fungi grow in clusters of one or more individuals.

**Keywords:** Biodiversity, Bioluminescence, chemiluminescence, emission, fungi, Konkan, Mycena chlorophos, luciferin.

Mycologists have always been amazed by the fascinating diversity and evolution of bioluminescent fungi. Bioluminescence is the generation and emission of light by living things. It is a type of chemiluminescence. Many marine animals and invertebrates (Amaral et al. 2016), as well as certain fungi (Aravindakshan et al. 2012), microorganisms (Balachandar et al. 2010) and terrestrial arthropods (like fireflies) (Barua et al. 2007) exhibit bioluminescence (Chatragadda 2020). A complex compound such as luciferin is converted to light energy through oxidation under the action of luciferase which acts as a catalytic enzyme (Pandey & Sharon 2017). A full description of a biochemical process that produces bioluminescence in fungi has been published (Kotlobay et al. 2018).

Bioluminescence is recorded across 17 phyla and more than 700 genera, both in marine and terrestrial environments (Lee 2015). A new distribution record of Roridomyces, a bioluminescent fungus has been recorded from Namdapha National Park, Arunachal Pradesh, India (Duta et al. 2023). Desjardin et al. (2008) in their review noted 64 luminescent species. Scientists have recognized a total of 109 luminescent fungi, which can be classified into four molecular lineages (Chew et al. 2015; Mihail 2015; Cortes-Perez et al. 2019; Chang et al. 2020; Karunarathna et al. 2020): 12 in the *Omphalotus* lineage, 10 in the *Armillaria* lineage, 85 in the Mycenoid lineage (mostly Mycenaceae), and two in the Lucentipes lineage.

Konkan, a biodiversity region in the Western Ghats, is home to a wide range of fungal species, yet there is still a dearth of information about bioluminescent fungi. In India, over the past few years, there have been a few reports on bioluminescence from fungi such as *Nothopanus eugrammus* and *Omphalotus olearius* (Vrinda et al. 1999), followed by a unique taxon from Kerala, *Mycena deeptha* (Aravindakshan &

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Manimohan 2014) *Mycena chlorophos* (Arya et al. 2021) & *Armillaria mellea* (Patil & Yadav 2022). In India there are 54 species of *Mycena* species reported in Table 1 and some bioluminescent fungi from mushroom families reported in Table 2. In this article, we report the new distribution of *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc. (Agaricales, Mycenaceae) from Hodavade 15.866° N, 73.725° E (Image 1; Habitat) which is located in Tehsil Vengurla from Sindhudurg District a Konkan region of Maharashtra.

As we conducted a nocturnal survey on 22 July 2023, at 2010 h, for a study on nocturnal animals, we opportunistically discovered a small glowing clump of *Mycena chlorophos* on a rotten bamboo substratum (Image 2). We noted down the observable morphological characters and photographs were taken under both light and dark conditions (Image 3; Habit).

Field photographs of specimens were taken by Canon 760D with a 100 mm macro lens and Nikon 7500D with Tamron 90 mm lens. The fungus was identified based on the articles available (Moser 1977; Kushwaha & Hajirnis 2016; Arya et al. 2021) and also record cited on www.indexfungorum.org. *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc., Syll. Fung. (Abellini) 5: 301 (1887) Figs 1–2 Index Fungorum number: IF147895; Faces of fungi number: FoF10625. The fungi grow in clusters of one or more individuals. Early on, the developing body, or pileus, is conical; as it matures, it becomes more rounded. Gills cover the hymenium.

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**Image 1. Habitat of *Mycena chlorophos*.**

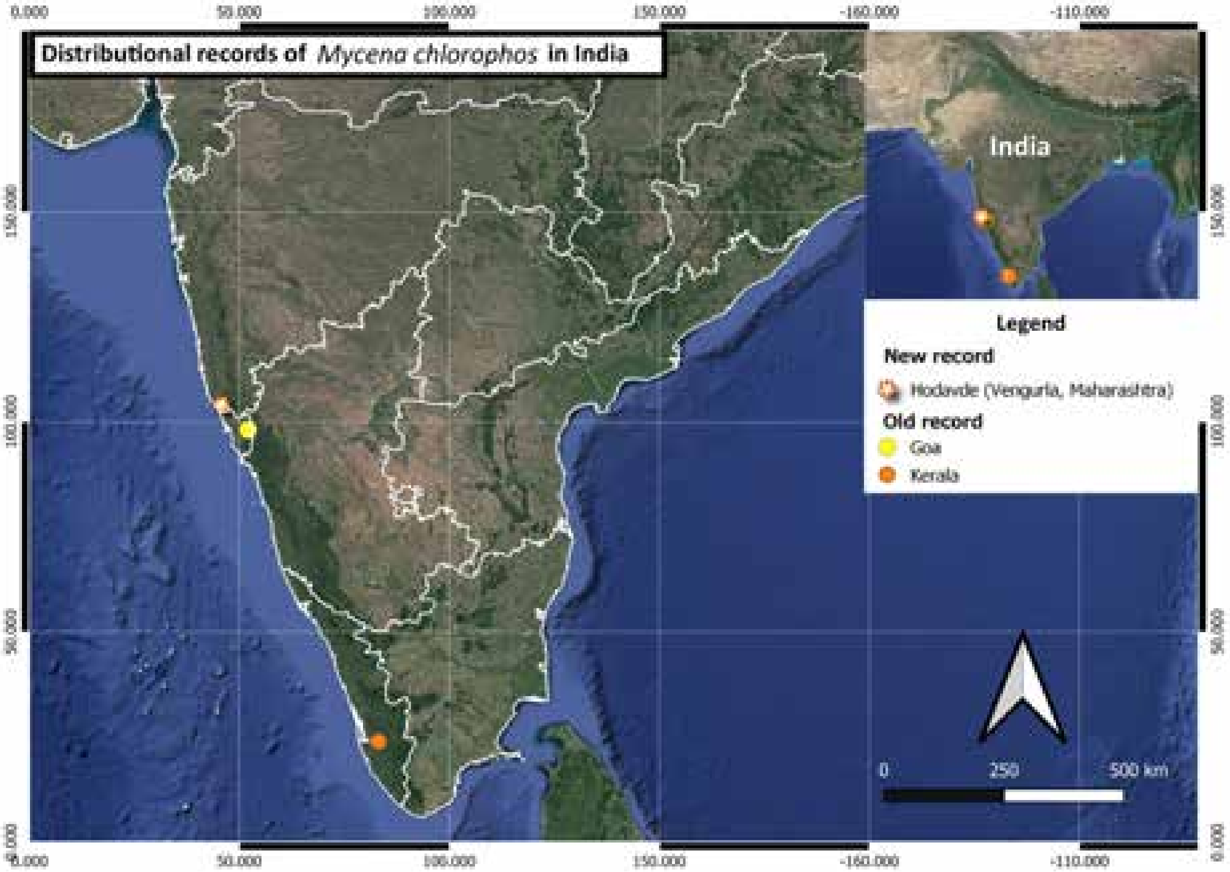

**Image 2. Map of Sindhudurg district with distribution record of *Mycena chlorophos*.**

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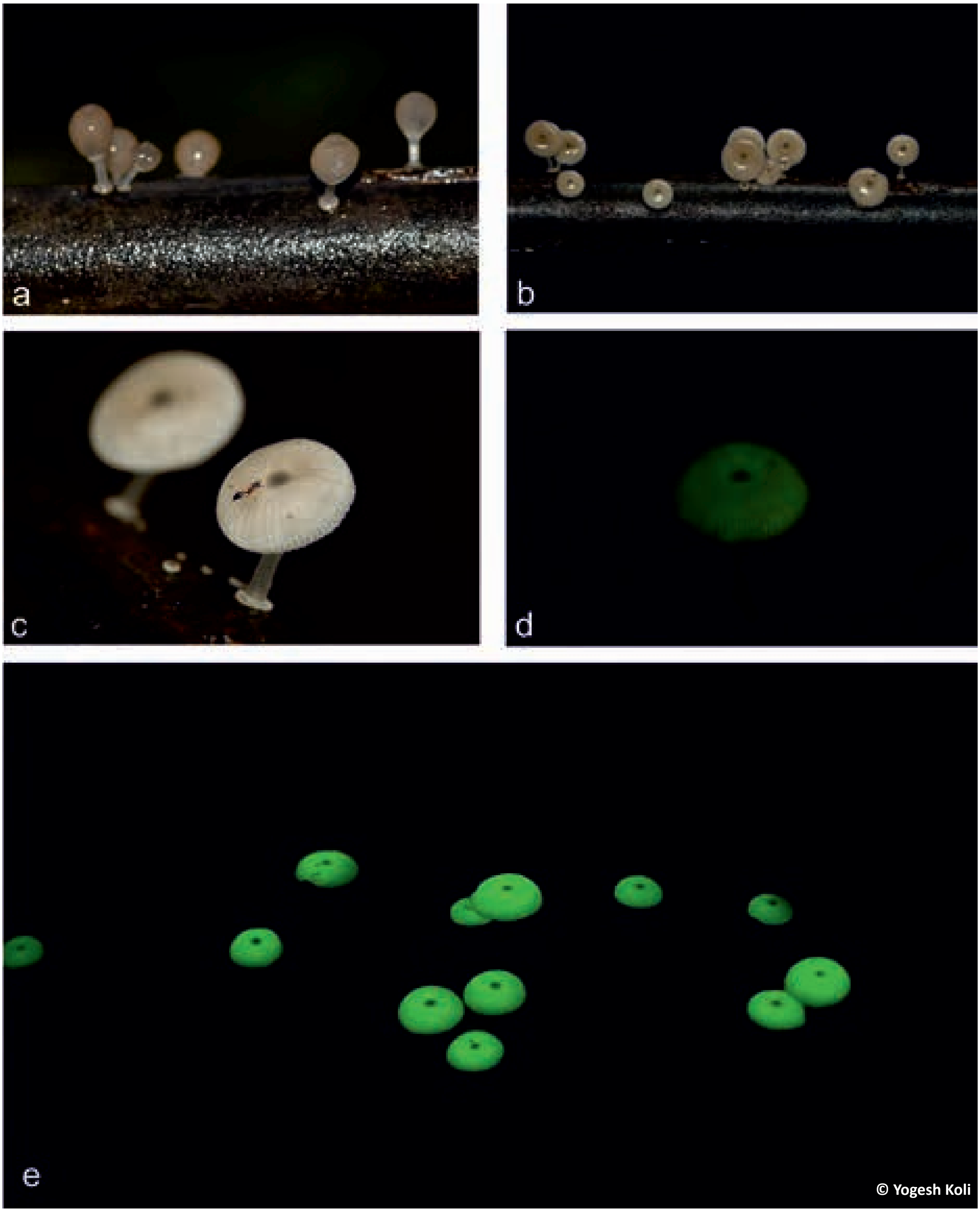

**Image 3 (a–e). Fruiting bodies of *Mycena chlorophos*: a—Early staged fruiting bodies on rotten bamboo substratum | b—Fruiting bodies in cluster on the substratum | c—Mature staged fruiting bodies on rotten bamboo | d & e—Bioluminescent fruiting bodies on the substratum.**

**Table 1. Mycena species recorded from state/regions of India.**

| | Species | State/ Region | Author & Year |
|---|---|---|---|
| 1 | *Mycena abietina* Maas Geest. | Kashmir | Maas (1992d) |
| 2 | *Mycena acrocephala* Maas Geest. & E. Horak. | Sikkim | Maas & Horak (1993) |
| 3 | *Mycena aetites* (Fr.) Quél. | Jammu & Kashmir | Watling & Gregory (1980) |
| 4 | *Mycena alcalina* (Fr.) P. Kumm. | Maharashtra | Sathe & Sasangan (1977) |
| 5 | *Mycena alphitophora* (Berk.) Sacc. | Kerala | Manimohan & Leelavathy (1989) |
| 6 | *Mycena arata* (Berk.) Sacc. | Sikkim | Berkeley (1850) |
| 7 | *Mycena atrocyanea* (Batsch) Gillet. | Jammu & Kashmir | Watling & Gregory (1980) |
| 8 | *Mycena auroricolor* (Berk. & Broome) Petch | Kerala | Manimohan et al. (1988) |
| 9 | *Mycena avenacea* (Fr.) Quél. | Maharashtra | Trivedi (1972) |
| 10 | *Mycena babruka* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2013b) |
| 11 | *Mycena bicrenata* (Berk.) Sacc. | West Bengal | Berkeley (1850) |
| 12 | *Mycena colligata* (Berk.) Sacc. | Sikkim | Berkeley (1852) |
| 13 | *Mycena conocephala* Henn. | Uttar Pradesh | Hennings (1901) |
| 14 | *Mycena coalita* Maas Geest. | Uttar Pradesh | Maas (1992d) |
| 15 | *Mycena deeptha* Aravind. & Manim. | Kerala | Aravindakshan et al. (2012) |
| 16 | *Mycena dentosa* (Berk.) Sacc. | Sikkim | Berkeley (1852) |
| 17 | *Mycena discors* (Berk.) Sacc. | Sikkim | Berkeley (1852) |
| 18 | *Mycena elegantula* Peck. | Tamil Nadu | Natarajan & Ravin |
| 19 | *Mycena epipterygia* (Scop.) Gray | Sikkim | Berkeley (1852) |
| 20 | *Mycena flavominiata* (Berk.) Sacc. | Sikkim | Berkeley (1852) |
| 21 | *Mycena galericulata* (Scop.) Gray. | West Bengal | Berkeley (1852) |
| 22 | *Mycena galopus* (Pers.) P. Kumm. | Maharashtra | Thite & Patil (1983) |
| 23 | *Mycena haematopus* (Pers.) P. Kumm. | Kerala | Bhavanidevi & Nair (1983) |
| 24 | *Mycena himalayana* Rawla | North Western Himalayas | Rawla & Aarya (1991) |
| 25 | *Mycena inclinata* (Fr.) Quél. | Himachal Pradesh | Das (2010) |
| 26 | *Mycena indica* Sarwal & Rawla | Himachal Pradesh | Sarwal & Rawla (1983) |
| 27 | *Mycena jatila* Aravind. & Manim. | Kerala | Aravindakshan & Manimoham (2014) |
| 28 | *Mycena juncicola* (Fr.) Gillet | Mumbai, Maharashtra | Sathe & Deshpande (1982) |
| 29 | *Mycena lohitha* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2013a) |
| 30 | *Mycena lohwagii* Singer | Tamil Nadu | Natarajan & Ravindran |
| 31 | Mycena lomamaya Aravind. & Manim. | Kerala | Aravindakshan & Mania (2014) |
| 32 | *Mycena lomavritha* Manim. | Kerala | Manimohan & Leelavathy (1988) |
| 33 | *Mycena indica* Manim. & Leelav. | Kerala | Aravindakshan & Manimoham (2014) |
| 34 | *Mycena macrothela* (Berk.) Sacc. | West Bengal | Berkeley (1852) |
| 35 | *Mycena manipularis* (Berk.) Sacc. | Himalaya | Berkeley (1850) |
| 36 | *Mycena mridula* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2013) |
| 37 | *Mycena metata* (Secr. ex Fr.) P. Kumm. | Uttar Pradesh | Hennings (1901) |
| 38 | *Mycena mridula* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2013) |
| 39 | *Mycena myriadea* (Berk.) Sacc. | Sikkim | Berkeley (1850) |
| 40 | *Mycena pelava* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2014) |
| 41 | *Mycena prasia* (Berk.) Sacc. | Sikkim | Berkeley (1850) |
| 42 | *Mycena profusa* Manim. & Leelav. | Kerala | Manimohan & Leelavathy (1988a) |
| 43 | *Mycena puberula* (Berk.) Sacc. | Sikkim | Berkeley (1850) |
| 44 | *Mycena pura* (Pers.) P. Kumm. | Sikkim, Uttar Pradesh, Kerala | Berkeley (1852); Maas (1992d); Mohanan (2011) |

| | Species | State/ Region | Author & Year |
|---|---|---|---|
| 45 | *Mycena rasada* Aravind. & Manimohan. | Kerala | Aravindakshan & Manimohan (2013) |
| 46 | *Mycena rufopicta* (Berk.) Sacc. | West Bengal: | Berkeley (1850 |
| 47 | *Mycena russulina* (Berk.) Sacc. | West Bengal: | Berkeley (1850 |
| 48 | *Mycena saloma* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2011) |
| 49 | *Mycena saparna* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2012) |
| 50 | *Mycena snigdha* Aravind. & Manim. | Kerala | Aravindakshan & Manimohan (2013) |
| 51 | *Mycena speirea* (Fr.) Gillet. | Tamil Nadu | Nataraj (1982) |
| 52 | *Mycena stylobates* (Pers.) P. Kumm. | Maharashtra | Sathe & Deshpande (1982) |
| 53 | *Mycena subcaerulea* (Peck) Sacc. | Pune, Maharashtra | Sathe & Deshpande (1982) |
| 54 | *Mycena xanthophylla* (Berk.) Sacc. | West Bengal | Berkeley (1850) |

**Table 2. Bioluminescent fungi from the mushroom family.**

| Name of the species | Family | Reference |
|---|---|---|
| *Roridomyces phyllostachydis* | Mycenaceae | Karunarathna et al. 2020 |
| *Mycena galopus* (Pers.: Fr.) P.Kumm. | Mycenaceae | Desjardin et al. 2016 |
| *Mycena lucentipes* Desjardin, Capelari & Stevani | Mycenaceae | Bechara 2015 |
| *Mycena rosea* Gramberg | Mycenaceae | Chew et al. 2014 |
| *Mycena chlorophos* (Berk. & M.A.Curtis) Sacc. | Mycenaceae | Kenichi et al. 2011 |
| *Mycena luxaeterna* | Mycenaceae | Desjardin et al. 2010 |
| *Armillaria mellea* (Vahl:Fr.) P.Kummer | Physalacriaceae | Mihail 2015 |
| *Flammulina velutipes* (Curtis) Singer | Physalacriaceae | Desjardin et al. 2008 |
| *Omphalotus nidiformis* (Berk.) O.K.Mill. | Marasmiaceae | Weinstein et al. 2016 |
| *Neonothopanus nambi* (Speg.) R.H.Peterson & Krisai, Persoonia | Marasmiaceae | Bondar et al. 2011 |
| *Neonothopanus gardneri* (Berk. Capelari, Desjardin, B.A.Perry, T.Asai & Stevani) | Marasmiaceae | Capelari et al. 2011 |
| *Gerronema viridilucens* (Desjardin, Capelari & Stevani) | Marasmiaceae | Mendes et al. 2008 |
| *Nothopanus eugrammus* (Mont.) Singer | Marasmiaceae | Vrinda et al. 1999 |
| *Omphalotus olearius* (DC ex Fr.) Singer | Marasmiaceae | Vrinda et al. 1999 |

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NOTE

# Potential first record of parrotfish *Scarus zufar* (Randall & Hoover, 1995) (Actinopterygii: Labriformes: Scaridae) from Indian waters, at Netrani Island, Karnataka, India

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Parrotfish are a diverse group of largely coral reef, rocky reef or seagrass dependent fish (Abu-Taweel et al. 2023). Taxonomically, they are placed within the subfamily Scarinae in the family Labridae, which also includes the wrasses (Westneat & Alfaro 2005). Members of the genus *Scarus* comprise 52 living species (Parenti & Randall 2011) while Nair & Kumar (2015) have listed 10 species from the genus from Indian waters. Parrotfish are functionally important species on coral reefs, due to their role as both grazers and bioeroders (Mumby 2009). In this note, we record the new occurrence of *Scarus zufar* (Randall & Hoover, 1995) from Indian waters.

Scuba surveys were conducted in March and April of 2023 at Netrani Island, a small (0.26 km$^2$) rocky island 18 km from the town of Murudeshwar in the Uttara Kannada district of Karnataka, India. The reefs around the island are predominantly rocky with encrustations of coral, but a few areas harbour complex coral growth with large colonies of *Porites.* We surveyed six sites around the island at a depth range between 8 m and 15 m. We observed 15 individuals of the species of interest in our 32, 50 x 5 m transects across the sites.

We were unable to collect a specimen, so we used images to identify the species. We conclude that this parrotfish is likely *S. zufar* owing to its colouration and morphological characteristics, which based on the original description by Randall & Hoover (1995) include: a yellow patch at the base of the tail and a reddish pectoral fin in adult individuals; truncated caudal fin with produced lobes; a green band running from the lower lip and chin extending to and surrounding the eye and the turquoise blue dorsal fin margin (Image 1). These characteristics are not seen in other parrotfish, making it highly likely that the observed specimen was *S. zufar.* To confirm this identity of the species, a specimen would have to be collected.

*Scarus zufar* (Randall & Hoover, 1995) was originally described from the coast of Oman and, for two decades, was thought to be endemic to this area of the Arabian Sea. It belongs to a clade that includes the wide-ranging *Scarus psittacus* and *S. russelli* as well as the more restricted *S. collana* (Choat et al. 2012). The species was later recorded in Pakistan (Psomadakis et al. 2015) and recently in Bangladesh (Hasan & Parvej 2020). Previous surveys of fish diversity around Netrani did not record the species, possibly misidentifying it as *S. hoelferi*, a

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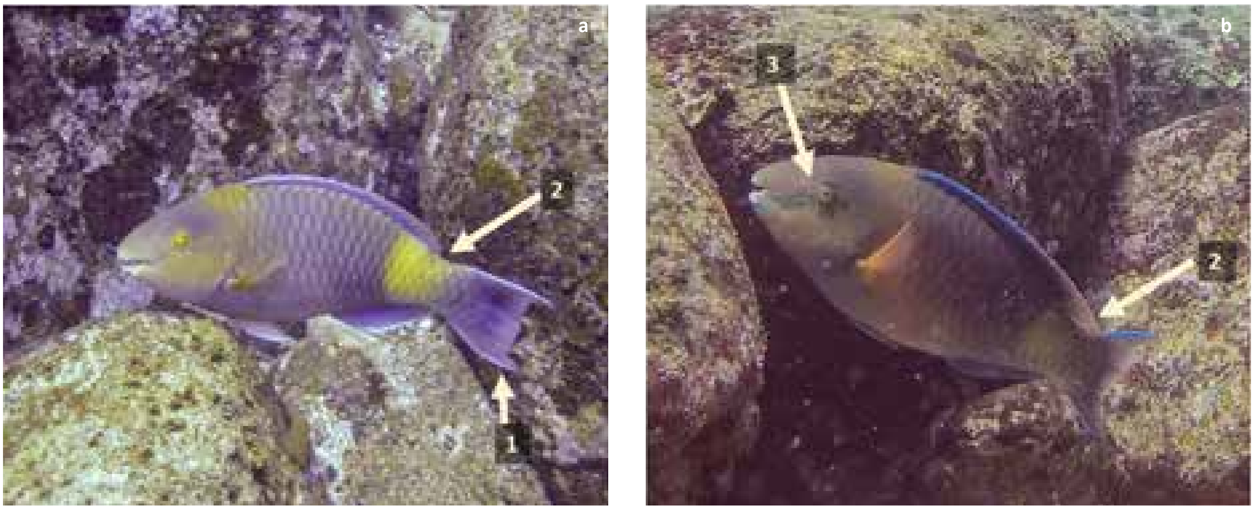

Image 1. Images of *Scarus zufar* adults taken at Netrani Island. 1—Diagnostic features such as the truncated pectoral fin | 2—Yellow patch at the base of the tail | 3—Green band around the eye are clearly visible. © a—Abhishek Jamalabad | b—Jeremy Josh.

species restricted to the Atlantic Ocean (Zacharia et al. 2008; Thomas et al. 2011). *S. zufar* was also not recorded in biodiversity surveys in other reef areas along India's West coast, such as Malvan Marine Sanctuary (De et al. 2021) and Grande Island (Sreekanth et al. 2015) or in the Gulf of Mannar, a major coral area on India's eastern coast (Ramesh et al. 2020).

The species was seen on multiple occasions on our surveys, making its omission in previously published lists surprising. This record shows a need for further in-depth research, and consistent, rigorous observation of the biodiversity and ecology of the reefs of western India.

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NOTE

# First record of the phoretic association between *Pediculaster* sp. (Pygmephoridae) mites and *Musca crassirostris* (Muscidae) flies in India

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The arthropods include many classes; one of them is Arachnida, which is great and assorted as it contains scorpions, spiders, ticks, and mites. It contains 1,14,275 species; among them merely Acari has 55,214 species of mites and ticks (Zhang 2013). In general, many small organisms exploit another larger organism for their movement from one place to another which is usually called phoresy. The word phoresy derives from the Greek phorein, which means 'to carry' (White et al. 2017). According to Farish & Axtell (1971), "Phoresy is a phenomenon in which one animal actively seeks out and attaches to the outer surface of another animal for a limited time during which the attached animal (termed the phoretic) ceases both feeding and ontogenesis, such attachment presumably resulting in dispersal from areas unsuited for further development, either of the individual or its progeny". In simple words, we can say that phoresy is the short-term relation in which transportation takes the place of one animal by other animals. In mites, long-distance dispersal is chiefly determined by phoresy, aerial migration, and anemochory (dispersal by wind) (Szymkowiak et al. 2007).

The phenomenon of phoresy has been pragmatic in many organisms, be it flies & mites, flies & ticks, beetles & bees, nematodes & flies/slugs, and bugs & mantids (White et al. 2017). An examination of a specific phoront on a carrion can authenticate the existence of its carrier even when the carrier is absent. Fast colonised mites have developed unique behaviours in which they have become phoront to flies for mobility and dispersal (Athias-Binche 1994; Siepel 1994). Upon reaching their preferred habitat, such as a corpse or other biological waste, the mites detach from the carrier and commence the process of maturing into their reproductive stage (Halliday 2000). Berlese (1918) was the first to report on phoretic mites found on carcasses. In this article, the description of a phoretic pygmephorid mite on a muscid fly in India marks its initial documentation. Pygmephorid mites fall under the order Prostigmata and the family Pygmephoridae.

Thirty-two mites were collected in July 2019 from Khajjiar town, which is located at 32.5558$^0$N, 76.0656$^0$E with 1,920 m elevation in the Chamba district of Himachal Pradesh, India. Collected mites were present on the muscid flies that were captured by a collection net from the bird corpse (*Acridotheres tristis*). Subsequently, collected flies along with mites were stored in the 70% alcohol. Mites were then detached from the flies with the help of forceps and cleared in lactophenol solution (for 48 hours). Slides of mites were mounted

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with Hoyer's medium. Afterward, photography was done with the help of the micro photographic unit (Leica, DM4000 B LED) in the Sophisticated Instrumentation Centre of Punjabi University, Patiala. Mites were identified with the help of keys given by Krantz & Walter (2009) and fly species by Emden (1965). Fly species were further confirmed with the help of molecular techniques by amplifying the mitochondrial DNA COI (Barcoding) region. The type material was deposited in the collection of the Department of Zoology and Environmental Sciences, Punjabi University, Patiala.

Mites were identified as species of the genus *Pediculaster* belonging to the family Pygmephoridae. Image 1 shows adults of Pediculaster sp. mites attached to the prothorax region of *Musca crassirostris* fly; Image 2 shows adults of *Pediculaster* sp.; Image 3 shows a close view of the gnathosoma part of the mite; and Image 4 shows a close view of the idiosoma part of the mite. The flies were recognized as *Musca crassirostris*, a member of the Muscidae family. Additionally, using LCO/HCO primers provided by Folmer et al. (1994), the mitochondrial COI gene (Folmer area) has been amplified to guarantee the identification of fly species. For sequencing purposes, amplified DNA was delivered to the Agrigenome labs.The species *Musca crassirostris* was validated by BLAST analysis of the sequence acquired after sequencing in the NCBI database.Upon submission of the sequence to the NCBI database, a distinct accession number, MH243421, was obtained (Table 1).It was the first instance of a phoretic connection between *Pediculaster* mites and *Musca crassirostris*.

Various muscid species were recognised as a carriers for the *Pediculaster* mites Kheradmand et al. (2006) recorded that *Pediculaster fletchmanni* mites used *Musca domestica* as a carrier. Camerik & Coetzee (1998) examined that *Pediculaster corpridis* mites attached to *Musca confiscate* for their dispersal. Masan & Kristofik (1992) described that *Pediculaster mesembriane* mites used *Fannia manicata* flies for phoresy. *Cheyletus eruditus* & *Ereynetes* which are members of prostigmatic mites used Muscidae flies along with Lepidopteran as carriers.

Astigmatic mite species are used as well as muscid flies for their dispersal. Flies belonging to family Histiostomatidae like *Copronomoia sphaerocerae*, *Histiostoma muscae*, *Myianoetus diadematus*, *Myianoetus ovatus*, *Myianoetus parvus*, *Myianoetus muscarum*, *Myianoetus longisetosus* (Masan & Kristofik 1992; Chinniah & Mohanasundaram 1995; Greenberg & Carpenter 1960; Greenberg 1961) and family Winterschmidtiidae like *Vidia* sp. (Ho 1990) used muscid flies as a carrier for the dispersion. In company with Asitgmatic types mites, Mesostigmatic mites also used Muscid flies for phoresy as well. Numerous mites species belonging to Macrochelidae family like *Glyptholaspis confusa* used *Musca domestica* (Niogret et al. 2006); *Macrocheles bertrandi* used *Stomoxys calcitrans* (Niogret & Nicot 2008); *Macrocheles glaber* used *Hydrotaea dentipes* (Masan & Kristofik 1992); *Macrocheles muscaedomesticae* used *Musca domestica* (Pereira & Castro 1947) and *Musca sorbens* along with *Ophyra chalcogaster* (Ho 1990); *Macrocheles mykytowyczi* used many species of Muscid flies (Halliday 2000); *Macrocheles ovoidalis* used *Stomoxys calcitrans* (Niogret & Nicot 2008); *Macrocheles perglaber* used *Musca domestica* (Niogret et al. 2006); *Macrocheles robustulus* used *Musca domestica* (Axtell 1964); *Macrocheles subbadius* used *Stomoxys calcitrans*, *Musca domestica*, *Haematobia irritans* (Axtell 1964; Krantz & Whitaker 1988; Niogret et al. 2006) as a carrier. A few


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**Image 1. Adults of *Pediculaster* sp. mites attached on the prothorax region of *Musca crassirostris* fly.**


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**Image 2. Adult of mite *Pediculaster* sp.**

Image 3. Close view of Gnathosoma part of *Pediculaster* mite.

Image 4. Close view Idiosoma part of *Pediculaster* mite.

Table 1. *Musca crassirostris* fly sequence submitted to the NCBI.

```
>MH243421
TTTGGAGCATGATCTGGTATAGTAGGAACTTCATTAAGAATTTTAATTCGAGCCGAATTAGGACACCCTGGTGCTTTAATTGGTGACGATCAAATTTATAAT-
GTTATTGTAACAGCTCATGCTTTTATTATAATTTTCTTTATAGTTATGCCTATTATAATTGGAGGATTTGGAAATTGATTAGTTCCTTTAATGTTAGGAGCTCCT-
GATATAGCATTTCCTCGAATAAATAATATAAGTTTCTGACTTTTACCTCCTGCTCTTACTTTATTATTAGTTAGAAGTATAGTAGAAAAGGGGGCTGGGACAG-
GATGAACAGTTTATCCACCTTTATCTTCAATTATTGCTCATGGAGGGGCTTCTGTTGATTTAGCTATTTTTTCTCTTCATTTAGCCGGAATTTCTTCAATTTTAG-
GAGCAGTAAATTTTATTACTACTGTAATTAATATACGAGCTACTGGAATTACATTTGATCGAATACCTTTATTTGTATGATCAGTTGTAATTACTGCTTTATTACTTT-
TACTTTCTTTACCAGTTTTAGCCGGAGCTATTACTATACTATTAACAGATCGAAATTTAAATACTTCGTTCTTTGACCCAGCAGGAGGAGGTGA
```

mites belonging to the Parasitidae family which includes *Gamasodes spiniger* exploit *Hydotaea* species as carriers. *Uroseius* sp. and *Halolaelaps* sp. species of families Trachytidae and Halolaelapidae employ *Musca domestica*, *Musca stabulans,* and *Hydrotaea dentipes* as dispersal carriers (Masan & Kristofik 1992; Perotti 1998).

The present study is the first of its kind in India but it needs to be done to a great extent directly to compile data about the species exactitude of mites and their habitat penchant. If knowledge about the carriers, life cycles, behaviour, and habitat particulars of mites is acquired then it will help in estimating the postmortem interval (PMI). Forensic acarology should formulate superior exploit of this and hastily expand into a helpful alternate input into forensic analysis.

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NOTE

# *Uniyala multibracteata* (Gamble) H.Rob. & Skvarla (Asteraceae: Vernoniae): notes on its identity and rediscovery

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The genus *Vernonia* Scherb. was first described by Johann Christian Daniel Von Schreber in 1791. It comprises c. 335 accepted taxa in the world (POWO 2023). The genus *Uniyala* was segregated from the broad genus *Vernonia* (Robinson & Skvarla 2009) and the genus *Uniyala* consists of 11 species (Senniappan & Kumar 2022), which are endemic to India and Sri Lanka. Nine species of *Uniyala* are strictly endemic to the southern Western Ghats. The genus *Uniyala* is characterized by long sweeping hairs on style branches, squamiform outer series of the pappus, 3–5-costate achenes, capillary bristles of the inner pappus and suboblate pollen grains (Robinson & Skvarla 2009). On the other hand, some botanists do not follow Robinson's concept and still follow the broad generic concept of *Vernonia* (Bhattacharjee 2020).

During the explorations in the southern Western Ghats, an interesting specimen of *Vernonia* was collected from Odamala, Idukki District, Kerala (15 March 1998), which was identified by Augustine (2022) and deposited at CALI (Coll. No. 17785) as *V. multibracteata* Gamble. Recently, as part of the systematic study of genus *Uniyala*, we collected specimens of five reported species from the Western Ghats. While confirming the identity of these, we found that all the previously labelled specimens as *V. multibracteata* at Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI) were misidentified specimens of *V. ramaswamii* (Hutch.) Rob. & Skvarla, *V. bourdillonii* (Gabmle) H.Rob. & Skvarla and *V. comorinensis* (W.W.Smith) H.Rob. & Skvarla. Kumar et al. (2012) reported the rediscovery of *Uniyala multibracteata* (= *V. multibrateata*) based on the collection from Agasthyamala. But the critical study on the voucher materials confirmed that these were also *U. comorinensis* (= *V. comorinensis*). Therefore, the previous collection by one of the authors from Odamala and the recent recollections of this species from Memala, Vagamon are reported here as the rediscovery of *U. multibracteata* (= *V. multibracteata*) after its type collection.

## Taxonomy

***Uniyala multibracteata*** (Gamble) H.Rob. & Skvarla. *Proc. Biol. Soc. Washington* 122(2): 153.2009. *Vernonia multibracteata* Gamble, *Bull. Misc. Inform. Kew* 1920: 340.1920.

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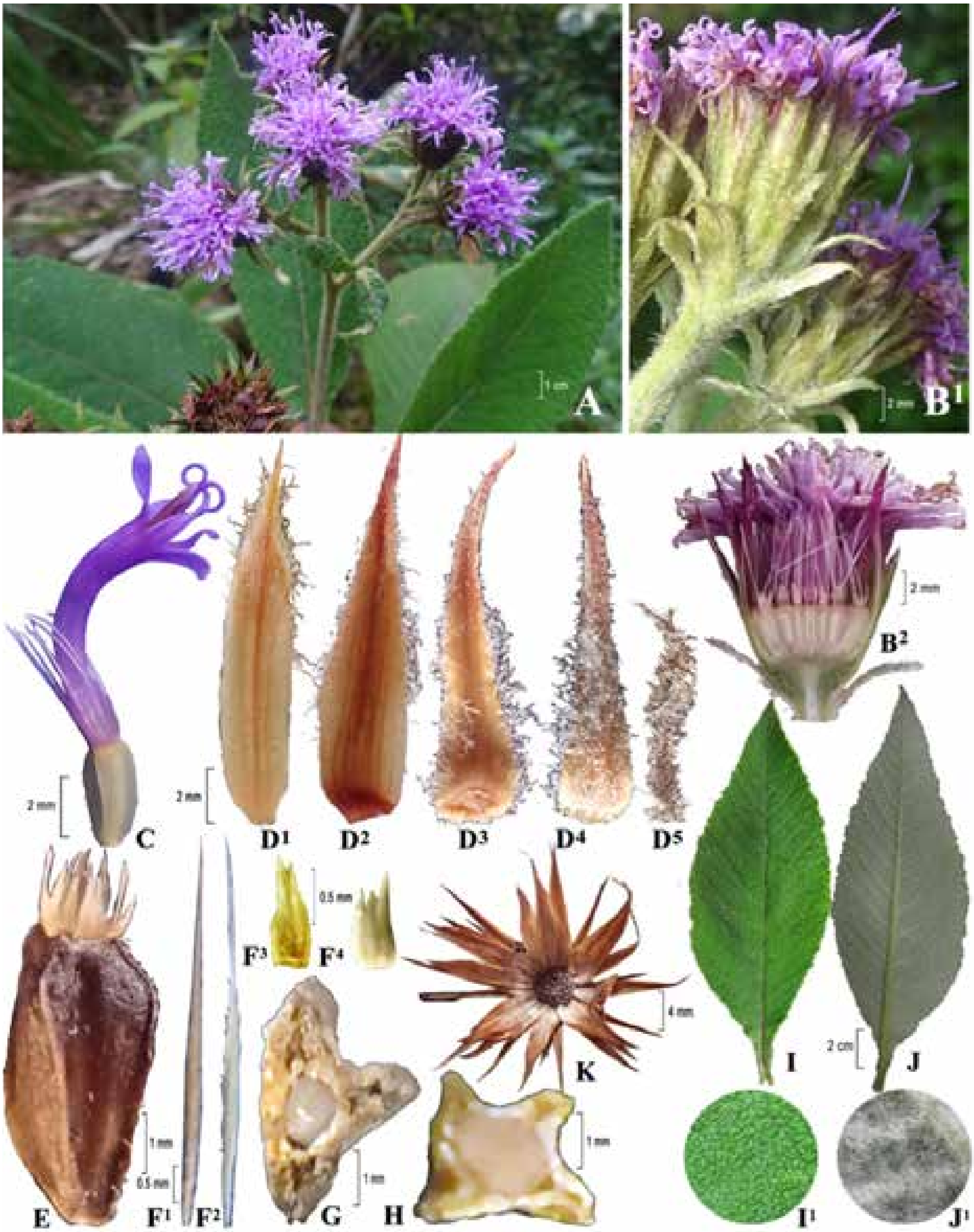

**Image 1.** *Uniyala multibracteata* (Gamble) H.Rob. & Skvarla: A—Habit | B$^1$—Single head | B$^2$—Single head L.S. | C—Single flower | D$^1$ to D$^5$—Involucral bracats | E—Cypsella with paleaceous pappus | F$^1$ & F$^2$—Setaceous pappus | F$^3$ & F$^4$—Paleaceous pappus | G—Cross section of outer achenes | H—Cross section of inner achenes | I & I$^1$—Leaf upper surface | J & J$^1$—Leaf lower surface | K—Head after dispersal of the fruits. © Jomy Augistine.

Lectotype: Southern India, Travancore, Peerumedu, 12.1880, *R.H. Beddome*s n. (K, barcode K000372520! & CAL, barcode CAL0000016750!)

Shrubs, erect, 2–3 m high; stems branched; branchlets thick, 12–20 mm across, white woolly; internode 5–30 mm long; leaf scars present, lunar. Leaves spirally alternate; petiole 1–2.5 cm long, woolly, compressed; lamina 7–20 × 3–7 cm, elliptic to lanceolate, acute to acutely acuminate at apex, attenuate at base, shortly decurrent; margins crenulate, irregular, wavy; adaxially coarsely rugose with hairs of 0.781 × 0.071 mm, multicellular, white; abaxially white woolly, webbed, 0.85–1.13 × 0.014–0.028 mm; tender leaves more woolly; lateral nerves 15–20 pairs, parallel, reticulate, dichotomously branching near margin; nerves depressed above. Inflorescence terminal corymbose cyme, 8–15 cm broad; peduncles elongate,3–14 cm long, white woolly. Heads 2–4 together,2–3.5 cm broad, secondary peduncle 2.5 cm long, stout, tomentose. Flowers similar, tubular, violet to purple, 30–42 per head; receptacle flat, 7–8 mm across; involucral bracts multi–layered, four types, elliptic to lanceolate; outer most 12 × 1.5 mm, lanceolate, sharply acuminate at apex, aristate – mucronate, white woolly, glabrous inside; intermediate one elliptic to lanceolate, 12–15 × 2 mm, sparsely pubescent, glabrous inside, acuminate at apex, aristate-mucronate; inner one elliptic–subulate 11 mm long, narrower towards apex, white woolly; inner most bract 8–10 × 3 mm, elliptic to lanceolate, pubescent on apex, glabrous inside, apex acuminate, mucronate; corolla violet, glabrous, tube cylindrical, 6–8 × 0.56–0.85 mm; corolla lobes 5, linear-oblong, recurved, apex acute, 3–4 mm long, violet to purple, valvate; stamens 5, anther tube 2.5–3 mm long, 0.639–0.78 mm broad, cylindrical, yellowish-brown; anthers sagittate at base; pollen spherical, 0.0284–0.042 mm, spinate, spines 0.004–0.006 mm long; staminal filaments 1.7–2 mm long, attached near half of the corolla tube; style 7–9 mm long, with sweeping hairs on upper part; stigma bifid, 2–2.8 mm long, puberulent, hairs 0.04–0.09 × 0.0142–0.028 mm; ovary 2–3 mm long, 3-angled, glabrous, white with scattered glands on angles. Achene 3–4.5 mm long, inner tetragonous, and outer trigonous (Figure 1 G&H); peripheral achenes broader, 2.5–4 mm broad; tending to trigonous; inner ones relatively narrow, 1–1.5 mm broad, tetragonous; straw coloured, smooth, angles winged; wings 0.4–1 mm broad, glabrous; pappus white–creamy, biseriate; outer ones paleaceous, linear–lanceolate to oblong with fimbriate to serrate, 0.5–1 mm long, 5–8 in number, subequal; inner pappus deciduous, setaceous, 8–12 in number, 4–6 mm long; hairs on pappus 0.071–0.127 × 0.007–0.0142 mm (Image 1).

Flowering & Fruiting: October–March.

Distribution: Endemic to Idukki district of Kerala State; less than 500 individuals.

Ecology: The habitat of this endemic species is open rocky grasslands and margins of small patches of evergreen forests in rocky cuttings. The canopy height is of 10–20 m. Associated species of trees are *Litsea wightiana* (Nees) Wall. ex Hook.f.*, Cinnamomum sulphuratum* Nees*, Vernonia arborea* Buch.-Ham.*, Bhesa indica* (Bedd.) DingHou*, Hydnocarpus alpina* Wight*, Actinodaphne bourdillonii* Gamble*, Nothopaegia colebrookiana* (Wight) Blume*, Dimocarpus longan* Lour.*, Gomphandra coriacea* Wight*, Croton caudatus* Geis., and associated herbs are *Cymbopogon flexuosus* (Nees ex Steud.) W.Watson*, Themeda cymbaria* Hack.*, Chrysopogon hackellii* (Hook.f.) C.E.C.Fischer*, Arundinella purpurea* Hochst. ex Steud.

Notes: Only one collection (R.H. Beddome's collection from Peerumedu in 1880) has been cited in the protologue of *Vernonia multibracteata* Gamble (Gamble 1920, 1921). Uniyal (1995) categorized it as possibly extinct since it is not recollected after the type collection. Sasidharan (2004) assessed the species as endangered. The detailed analysis of specimens with identification label as *V. multibracteata* (= *U. multibracteata*) at TBGT confirmed that all are misidentifications. The species identification in this genus is based on narrow differences only, which might have been the reason for such misidentifications. From our study we noted that characters of involucral bracts, degree of variations in the leaf margin and pubescence on various plant parts are considerably stable traits to delimit the taxa within, but the terms ribbed, ridged and winged nature of achenes are confusing. Two specimens of *U. bourdillonii* (Coll. No. 15166, 58025), one specimen of *U. ramaswamii* (Coll. No. 20284), and six specimens of *U. comorinensis* (Coll. No. 39230, 72908, 69206 (2 specimens), 67942 (2 specimens) are at TBGT with misidentified label as V. *multibracteata* (= *U. multibracteata*). We studied the voucher specimens cited by Kumar (2012). All the specimens cited in this publication (Kumar et al. 2012) are specimens of *U. comorinensis* only. Interestingly, Kumar et al. (2012) cited a collection of N. Mohanan from Athirumala (Coll. No. 10462) housed at TBGT. One of its duplicates was found at CALI, in which the collector has rightly identified specimen as *V. comorinensis*. Since the rediscovery report of *U. multibracteata* has not yet been made properly. We report it here based on the collections from Memala, Urumbikkara, Vagomon and Odamala of Idukki district, Kerala. Both *Uniyala multibracteata* and

*U. comorinensis* are closely similar in their morphology. But the shape, apex and pubescence of bracts are stable and fit to the protologue and type specimens. The bract characters are enough to distinguish both the taxa.

The type locality of *U. comorinensis* is from Tinnevelly (presently Tirunelvely of Tamil Nadu), and all the recorded collection are from Tirunelveli and Agasthyamala and this species is narrow endemic. Similarly, *U. multibracteata* is narrow endemic to Peerumedu (type locality) and surrounding hills (Memala, Odamala, Vagamon, and Urumbikkara).

Specimens examined: *Uniyala multibracteata* - Peerumedu, Idukki district, India, coll. R.H.Beddome*s. n.* (K, K000372520 [image!]; CAL, CAL0000016750 [image!]), xii. 1880 (Lectotypified, Kumar & Senniappan 2021); Odamala, Idukki District*,* coll. *Jomy Augustine* 17785 (CALI), 15.iii.1997.

## Key

1a. Leaves 7–20 x 3–7 cm; heads 8–15 mm broad, 32–45-flowered; bracts 35–48, outer one 12 mm long, lanceolate, mucronate ......... *U. multibraceata*

1b. Leaves 5–10 x 1–2.5 cm; heads 3.5–7 mm broad, 20–30-flowered; bracts 25–35 outer most bract up to 7.5 mm long, oblong, obtuse at apex .............. *U. comorinensis*

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NOTE

# Addition of two wild jasmines (*Jasminum caudatum* and *J. grandiflorum*) to Sikkim Himalaya, India

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*Jasminum* Tourn. ex L. is the largest genus of Oleaceae with 210 accepted species worldwide, distributed mostly in tropical and subtropical regions (Kiew & Tan 2020; POWO 2023). Southern and southeastern Asia are the centres of diversity of the genus (Kiew 1994). In India, the genus is represented by 48 species, three subspecies and four varieties, of which 17 taxa are endemic (Sabeena et al. 2007).

During 2021–2022, the authors jointly, as well as independently explored several regions of Namchi and Soreng districts of Sikkim for the floristic studies. Two interesting *Jasminum* species in flowering and fruiting stages were encountered and collected for the taxonomical studies. Photographs were taken along with field notes for each species. Specimens were compared with several vouchers deposited in different herbaria (digitally [CAL and K] and physically [Lloyd Botanic Garden, Darjeeling, West Bengal]), literature (Clarke 1882; Watson 1999; Green 2003; Kiew & Tan 2020; Gogoi et al. 2021 and their identities revealed. On checking their distributions, it was also found that two taxa were not recorded in the state. Herbarium sheets were prepared for each species by conventional techniques (Jain & Rao 1977) and deposited at BSHC.

## Taxonomic treatments

***Jasminum caudatum*** Wall. Ex Lindl. In Edward's Bot. Reg. 28: t. 26. (1842). *J. ovatum* Wall., Numer. List [Wallich] n. 2882 (1831).

Type: cult. Ex India (holotype CGE, n.v.).

Description: Scrambling shrub to 4 m long. Stem woody and rigid. Leaves opposite, trifoliate, terminal one always largest; petioles 0.8–2 cm long, glabrous and woody, petiolules of lateral leaflets 3–5 mm long, petiolules of terminal leaflets sub-equaling petiole, lateral leaflets ovate or oblong-ovate, cuneate at base, sometimes oblique, undulate at margin, acuminate at apex, 3–5 × 1.5–2cm, glabrous both sides; terminal leaflets ovate or oblong-ovate, cuneate at base, undulate at margin, acuminate at apex, 5–8 × 2–2.5 cm, glabrous both sides. Inflorescence a terminal or axillary cyme with solitary or 3–5 flowers; peduncle 1–2 cm long, slender, rigid with 1–2 nodes with opposite or sub-opposite acuminate bracts, each bract 1–3 mm long, glabrous; pedicel shorter than peduncle, slender, 0.5–0.8 cm long, both peduncle and pedicel glabrous. Calyx glabrous, teeth 5, each tooth triangular, c. 1 mm long. Flowers faintly scented, white. Corolla tube narrow to 2.5 cm, glabrous both surfaces, lobes 4–5, 9–12 × 3–5 mm. Stamens 2, included in corolla tube; filament 1–2

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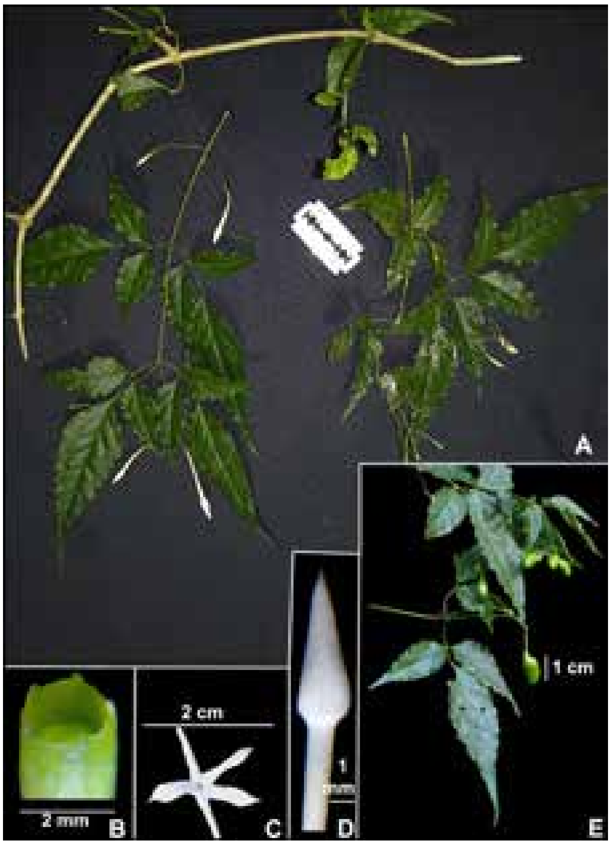

**Image 1. *Jasminum caudatum* Wall.: A—Habit showing stem | B—Calyx | C—Flower top view | D—Flower bud. E-Habit showing fruiting. © Pramod Rai**

**Image 2. *Jasminum grandiflorum* L.: A—Habit and habitat | B—Flower | C—fruiting. © Pramod Rai.**

mm long, slightly twisted, glabrous; anthers oblong, 4–5 mm long, yellow. Pistil 2–5 cm long, glabrous; ovary 1–2 mm across; style glabrous; stigma oblong, 2–3 mm long, undivided. Fruits globose, paired or rarely solitary, c. 0.7 cm across, glabrous.

Flowering and fruiting: September–November.

Habitat: Scrambling over other shrubs like *Chromolaena odorata, Phlogacanthus thyrsiformis* and *Boehmeria macrophylla* on a cool shaded forest margin.

Distribution: Andaman Is., Assam, Bangladesh, East Himalaya, India, Nepal.

Specimens examined: INDIA. Sikkim, Namchi District, Sirisay forest, 656 m elevation, 27.17584°N, 88.3378°E, 27.xi.2021, P.Rai P0343a (BSHC; P.Rai PO343b, Sikkim University Herbarium, Gangtok, Sikkim).

Notes: The plant is easily distinguished by its rigid stems, trifoliate leaves and conspicuously visible undulate leaf margins (Image 1). Sometimes other shrubs may be suppressing it beneath them. The plant poses narrower and more caudate leaves, due to which it can be distinguishes from its closely related species, *Jasminum flexile* Vahl. (Clarke 1882; Green 2003). The plant was growing in a single population with more than 90 individuals spread over 500 m$^2$. No apparent threat to the population was observed.

***Jasminum grandiflorum*** L., Sp. Pl., ed. 2. 1:9 (1762). *J. officinale* var. *grandiflorum* (L.) Stokes, Bot. Comm. 1:21(1830). *J. officinale* ssp. *grandiflorum* (L.) E.Laguna, Toll Negre 8: 12 (2006). *J. officinale* f. *grandiflorum* (L.) Kobuski, J. Arnold Arbor. 12:161 (1932).

Type: Linn 17.2 (Lectotype, n.v.).

Description: Scrambling shrub to 5 m long, stems spreading. Leaves large, opposite, odd-pinnate with 5–10 leaflets; petioles short of highly reduced, 0.3–1.5 cm long, glabrescent. Leaflets elliptic or oblong elliptic, sessile or sub-sessile, terminal one bigger and narrower than laterals, cuneate at base, acute or acuminate at apex, terminal leaflets 1–3 x 0.5–1.5 cm. Inflorescence an open cyme, 1–10 flowered, peduncle 1–5 cm long, pedicels 0.5–2 cm long, peripheral ones longer than central. Flowers white, gently fragrant; corolla tube 1–2 cm long, lobes 4–6, ovate, 0.5–1.5 x 0.5–0.7 cm, acute, acuminate or abruptly acuminate at apex. Fruits globose, paired, c. 0.5 cm across, glabrous.

Flowering and fruiting: July–November.

Habitat: Growing in open subtropical forest, in association with *Berberis napaulensis, Himalrandia tetrasperma, Rubia sikkimensis, Liparis deflexa, Luculia gratissima, Coelogyne fuscescence,* and *Corralodiscus*.

Distribution: Bangladesh, south-central China, Djibouti, Eritrea, Ethiopia, Kenya, Nepal, Oman, Pakistan, Rwanda, Saudi Arabia, Somalia, Sudan, Uganda, western Himalaya.

Specimens examined: India, Sikkim, Geyzing district, Lingchom, 27.2981$^0$N,88.2147$^0$E, 1,500 m, 11.ix.2022, P. Rai, P0344a (BSHC; P0344b, Sikkim University Herbarium, Gangtok, Sikkim).

Notes: It is a widely cultivated species in France, Italy, China, Japan, India, Morocco and Egypt and used to extract 'oil of Jasmine', used in perfume industries (Watson 1999). The plant is documented to possess beneficial effects as odontalgic, thermogenic, aphrodisiac, antiseptic, emollient, anthelmintic, deobstruent, suppurative, tonic, in fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, otorrhea, otalgia, wounds, corns and aroma therapy (Sandeep & Paarakh 2009). *J. grandiflorum* ssp. *floribundum* (R.Br. ex Fresen.) P.S.Green occurs in Saudi Arabia, Oman and Southernmost Sudan south to Kenya (Green 2003). The current report is from a population consisting of more than 70 individuals distributed vertically, spread over an area more than 1 km$^2$. Fodder collection was posing a threat to its population from lower region of population.

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NOTE

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# Extended distribution of *Ceropegia bhatii* S.R.Yadav & Shendage (Apocynaceae)—an endemic plant from Haveri District, Karnataka, India

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The genus *Ceropegia* L. (Apocynaceae: Asclepidoideae: Ceropegieae) comprises 450 species in the world, distributed in Africa, Canary Islands, Arabia, India, China, Madagascar, New Guinea, and Australia (Bruyns 2014; Mabberley 2017; POWO 2024). The majority of *Ceropegia* species in India are distributed in the peninsular part where they occur along steep hill slopes, rock crevices at low to high-elevation lateritic plateaus, along with bushes, forest margins, grasslands of dry deciduous forests, shola forest margins and still others prefer to grow at drier habitats. Out of 61 Indian taxa, 44 (72%) are endemic (Kambale & Yadav 2019). In Karnataka, the genus was represented with 16 species, out of which 11 are endemic (Singh et al. 2015; Sanjappa & Sringeswara 2019).

*Ceropegia bhatii* S.R.Yadav & Shendage, an endemic plant was first time reported from its type locality Davangere-Malebennur Ghat (14.3112$^0$N, 75.7251$^0$E) in the year 2008, Shendage 2550 & 2551, holotype CAL0000006905; isotypes BSI0000000171 (Yadav & Shendage 2010). The species is now recollected after 14 years for the first time outside its type location from the dry deciduous forest of Kanavisiddageri hillock of Jokanal beat of Hirekerur forest range in Haveri district, Karnataka state, and herbarium was submitted to the Herbarium of Karnatak College Dharwad (HKCD).

## Taxonomic Treatment

***Ceropegia bhatii*** S.R.Yadav & Shendage, Kew Bull. 65(1): 107 (2010); S.S. Kambale & S.R. Yadav, Rheedea 29(1): 01–115 (2019) (Image 1).

Holotype: India, Karnataka, Davangere district, Malebennur Ghat, 14.3112$^0$N, 75.7251$^0$E 15.ix.2008, Shendage 2550. CAL0000006905.

Perennial twining herbs with tuberous rootstock. Roots a few, fibrous. Stem 1–2 mm diam., twining, terete, slightly pubescent towards base, glabrous at tip. Leaf linear-lanceolate, 3.0–7.5 × 0.3–0.8 cm, dark green above, pale below, hairy entirely, petioles ciliated 5 mm long, channeled above. Flowers solitary or 2-flowered cymes; peduncles c. 6 mm long, glabrous; bracts c. 4 mm, linear-subulate, glabrous; pedicels c. 1.0 cm long, glabrous, terete. Sepals c. 5 mm, subulate. Corolla c. 4.2 cm long; tube c. 2.5 cm long, slightly curved, gradually dilated at base, throat with funnel-shaped, narrow at middle, lower portion with purple blotches within, upper portion striated with dark purple lines up to the throat within (at the mouth of tube blotching is deep purple otherwise tube is yellowish-green within); lobes c. 2.2 cm long, linear, glabrous, slightly reflexed to their

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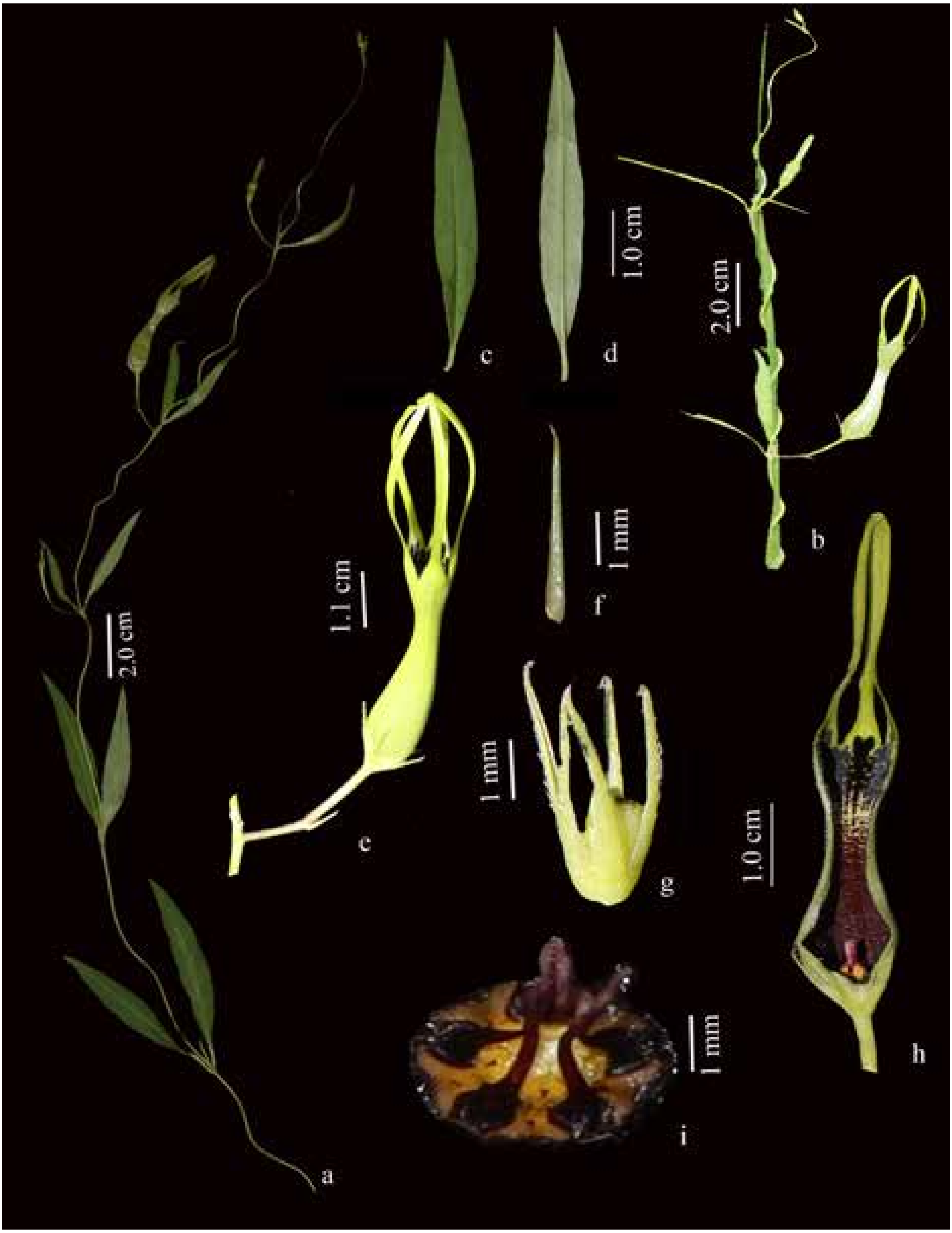

Image 1. *Ceropegia bhatii* S.R.Yadav & Shendage: a—flowering twig | b—enlarged twig | c & d—dorsal and ventral view of leaf | e—enlarged flower | f—bract | g—calyx | h—longitudinal section of flower | i—corona. © Ningaraj S. Makanur.

Image 2. Herbarium sheet of *Ceropegia bhatii* [HKCD 20907].

Image 3. Herbarium sheet of *Ceropegia bhatii* [HKCD 20908].

back, connate at the tip forming an ovoid cage. corona 2-seriate, stipitate, c. 5 mm long; outer lobes 5-bifid, c. 2.3 × 3 mm long, saucer-shaped, green, ciliate along margins, yellow; inner lobes 5, c. 2.8 mm long, deep purple, sparsely hairy at the tip.

Flowering & fruiting: September–November.

Habitat: Grows in slopes of grasslands in dry deciduous forests, close association with *Cymbopogon coloratus* (Hook.f.) Stapf., *Blepharispermum subsessile* DC., *Argyreia cuneata* (Willd.) Ker Gawl., *Terminalia anogeissiana* Gere & Boatwr., *Lagerstroemia parviflora* Roxb. and *Soymida febrifuga* (Roxb.) A. Juss.

Distribution: In Karnataka, Haveri district, around 15–20 individuals were observed (present study) and in Davanagere district.

Specimen examined: 20907 & 20908 (HKCD) (Image 2,3), 12.ix.2022 India, Karnataka, Haveri district, Hirekerur Forest Range, Rattihalli taluka, Kanavisiddanagiri hillock; 718 m, coll. Ningaraj S. Makanur & Kotresha K. 1162.

Threat status: The species was evaluated as critically endangered by Yadav & Shendage (2010). Since it has been recollected again from a locality other than its type location, threat status needs to be assessed again in the light of new data.

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RESPONSE

# Small Paa Frog and Marbled Cascade Frog are not endemic to Nepal: a response to Tachamo-Shah et al. 2023

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Ramaroshan area is one of the prominent biodiversity-rich area outside the protected area network of Nepal and recently the researches on the aspect of biodiversity is gradually increasing (Acharaya et al. 2023; Tachamo-Shah et al. 2023), which is encouraging. We need baseline information on the species' distribution and abundance upon which tailored conservation measures can be initiated. However, this information should be based on the facts and evidence. Recently, Tachamo-Shah et al. 2023 have published an article entitled 'Wetland biodiversity of Ramaroshan Lake complex: a need for conservation' on JoTT 26 December 2023 issue. Much information in the article is promising, highlighting the additional attention the area should receive for conservation. However, there are some aspects, particularly those related to conservation status and endemism of amphibian species which is misleading, making me to realize to write this response.

## Endemism of species

The authors have mentioned two species of herpetofauna namely Small Paa Frog *Nanorana minica* and Marbled Cascade Frog *Amolops marmoratus* as endemic species (Tachamo-Shah et al. 2023). According to the IUCN Red List Assessment of the Small Paa Frog, the species is found in Uttar Pardesh and Arunanchal Pradesh of India and Bhutan (IUCN SSC Amphibian Specialist Group, 2022) which means they are not endemic to Nepal. Furthermore, the presence of *Amolops marmoratus* is mentioned with certainity for Myanmar only while for other regions the presence has been uncertain (IUCN SSC Amphibian Specialist Group, 2023a). Without photographic and other evidence, it is difficult to ascertain that the species has been identified properly.

## Conservation status of the species

The authors have mentioned the worng conservation status for amphibian species. For instances, they have mentioned the Small Paa Frog as globally vulnerable species and the Indian Bull Frog *Hoplobatrachus tigerinus* as near thretened species (Tachamo-Shah et al. 2023). The Small Paa Frog is currently listed as the least concerned species (IUCN SSC Amphibian Specialist Group 2022). The species was listed as vulnerable species in previous assessment conducted in 2004. This could be due to the status being updated recently, possibly after the paper was submitted to the journal.

In the case of the Indian Bull Frog, the species was listed as least concerned species in previous assessment and it holds the same status in the recent assessment as well (IUCN SSC Amphibian Specialist Group 2023b).

## Other issues

The name of the Liebig's Paa Frog is *Nanorana liebigii* which has been mentioned as *Nanorana leibgii* in the manuscript. Furthermore, despite Ramaroshan's potential as site for Red Panda distribution, there is not enough evidence to support the presence of the species. However, they have failed to provide the details information about the means of confirmation.

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## Conclusion

Despite the attempts of the authors to bring the information on the status of important yet neglected wetland of Western Nepal, some information on the journal article needs reassessment and verification.

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