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Registered Office: 3A2 Varadarajulu Nagar, FCI Road, Ganapathy, Coimbatore, Tamil Nadu 641006, India

Ph: +91 9385339863 | [www.threatenedtaxa.org](http://www.threatenedtaxa.org)

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Cover: Golden-headed Lion Tamarin *Leontopithecus chrysomelas*. Watercolor and acrylics by P. Kritika.



## INTRODUCTION

Hormones are chemical messengers that regulate a wide range of bodily functions to maintain homeostasis in the face of environmental change (McEwen & Wingfield 2003; Nelson & Kriegsfeld 2017). They coordinate a series of physiological and behavioral adjustments through which animals appropriately respond to environmental and social cues, resulting in certain fitness outcomes (Higham 2016). Given their central role, hormones have been the focus of research across various fields (Palme 2005; Schwarzenberger 2007), including primatology. With the development of non-invasive and field-based techniques, researchers can now measure endocrine markers in matrices like feces or urine from wild primates, without altering their behavior or hormonal status (Schwarzenberger 2007). Moreover, these advances allow field observations to be combined with non-invasive endocrine monitoring, providing valuable insights into the adaptive aspects of hormone secretion and behavior (Cooke et al. 2014; Fiorini-Torrico et al. 2024) as well as impacts of human activities on the physiology of wild primates (Hodges & Heistermann 2011).

Glucocorticoids (GCs) are widely used in conservation research due to their mediation of the physiological stress response (Busch & Hayward 2009). This process initiates with the activation of the vertebrate hypothalamic-pituitary-adrenal (HPA) axis triggered by predictable or unpredictable environmental perturbations, ultimately leading to the secretion of GCs (Dantzer et al. 2014). These GC elevations then modulate energy allocation to cope with a variety of physical, metabolic, and psychological stressors (Emery-Thompson 2017). If GC increases are short-term, they enhance survival by promoting escape from noxious stimuli (Wingfield et al. 1998). Chronic elevations entail reductions in individual health and longevity (Sheriff et al. 2011; Beehner & Bergman 2017).

Thyroid hormones (THs) are also involved in vertebrate energy balance, but their non-invasive study in wildlife is more recent (Wasser et al. 2010). The synthesis of the two major forms of THs, thyroxine (T4) and triiodothyronine (T3), is controlled by the hypothalamic-pituitary-thyroid (HPT) axis. While the latter form is biologically more active, the former serves as a peripheral reservoir for T3 production via conversion (Behringer et al. 2018; Deschner et al. 2020). Given THs' particular sensitivity to nutritional deficits, which results in a lower metabolic rate (Eales 1988), it has been suggested that measuring both GCs and THs can help

differentiate energetic from psychological influences (Emery-Thompson 2017), as well as distinct strategies to maintain energy balance (Wasser et al. 2010; Dias et al. 2017; Touitou et al. 2021).

To ensure the reliability of hormone measurements, it is essential to validate the hormonal assay for the species and matrix being investigated (Sheriff et al. 2011). Once GCs and THs are released into the bloodstream, they are metabolized by the liver and subsequently excreted into urine or the gut via the kidneys and bile ducts, respectively (Palme 2005; Behringer & Deschner 2017; Visser et al. 2017). Metabolites that reach the intestine are further broken down; as a result, most of the substances found in feces are conjugated forms of native GCs and THs (Touma & Palme 2005; Palme 2019). Consequently, all immunoassays, typically chosen to measure fecal metabolites, must be analytically validated for precision, accuracy, sensitivity, and specificity (Higham 2016). This not only guarantees that potential effects of storage, extraction, and laboratory protocols are accounted for, but also ensures that antibodies cross-react with target metabolites without major interference from other substances in the sample matrix (Hodges & Heistermann 2011).

In addition to analytical validation, studies should determine whether assays show biologically meaningful variations in the species physiology (Touma & Palme 2005). This can be done either through a physiological or a biological approach (Touma & Palme 2005). Physiological validation normally involves inducing changes in circulating hormone levels by administering a specific drug to later evaluate whether such changes are reflected in the excreted metabolites (Behringer & Deschner 2017). Furthermore, if repeated sample collection is possible, this type of experiment provides an opportunity to explore the lag-time between the stimulation of hormone secretion and its excretion in feces or urine (Hodges & Heistermann 2011; Behringer & Deschner 2017). Physiological validation can be very invasive, which is a concern when studying threatened species or wild individuals. In that case, biological validation may be more appropriate (Behringer & Deschner 2017). This alternative examines the levels of non-invasive markers in relation to a state or event known to alter the secretion of target hormones (Touma & Palme 2005). For GC metabolites, specifically, biological validation can be performed through procedures like capture, confinement, translocation, new housing conditions, disturbances by human presence or natural diurnal fluctuations in excreted GCs (reviewed by Touma & Palme 2005 and Higham 2016). For TH metabolites,



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**Image 1.** Golden-headed Lion Tamarin *Leontopithecus chrysomelas* in the study area of Project BioBrasil at Colônia de Una, Bahia.

this has been achieved by assessing the influence of caloric restriction or low body mass (reviewed by Behringer et al. 2018), as well as the impact of infectious diseases (Dias et al. 2017).

In this study, we explore the effect of a series of intrinsic and stress-related factors on the levels of fecal GC and T3 metabolites, hereafter referred to as fGCs and ft3. We do so to validate the quantification of these markers in wild Golden-headed Lion Tamarins (*Leontopithecus chrysomelas*; GHLTs; Image 1), an endangered primate species restricted to the Southern Bahian Atlantic Forest in Brazil. More specifically, we evaluate the stress response of GHLTs to temporary capture and handling, predicting that fGC levels in samples collected during capture events will be higher than fGC levels during group monitoring days. We further examine the impact of body condition, predicting that higher body mass and

better nutritional status (reflected by a qualitative body score) will both correspond with higher ft3 levels. We also assess the differences in fGCs and ft3 in relation to GHLTs' group identity, sex, and dominance status. Currently, there is no published study on the levels of T3 in Lion Tamarins, and only a few studies have used GCs in wild and captive populations of Lion Tamarins to address different hypotheses (e.g., Bales et al. 2002, 2005, 2006; Henry et al. 2013; Costa et al. 2020; Kaisin et al. 2023), including those that focused exclusively on the validation of the techniques (Wark et al. 2016; Bertoli et al. 2019). Therefore, this study adds to the literature showing the potential of non-invasive hormone analyses to understand the physiological responses of wild primates and may serve to better inform conservation actions for this and other threatened species.

## MATERIALS AND METHODS

### Study subjects

We studied 32 individuals belonging to four habituated groups of GHLTs (named ELI, MRO, OZA and RIB), all of which are monitored with radiotelemetry and captured routinely as part of the ongoing long-term research project BioBrasil (De Vleeschouwer & Oliveira 2017). The GHLT groups move freely in an area that comprises various privately owned farms located in the municipality of Una in South Bahia (see map in Image 2, geographic coordinates of field base: -15.285° S, -39.134° W). The study area is a mosaic of disturbed forest fragments of various sizes and an agricultural matrix that includes crop plantations (mainly cocoa, rubber, coffee, banana, and cassava), pastures, open fields, and unpaved roads (De Vleeschouwer & Oliveira 2017). The predominant natural vegetation in this region is classified as the Southern Bahian Moist Forest (Gouvêa et al. 1976) and the climate is characterized by an annual average temperature of 24 °C and precipitation of 2,500 mm, with no marked seasonality (Mori et al. 1983).

Following Miller et al. (2003), we categorized age of individuals into infants (<3 months), juveniles (3–12 months), subadults (12–18 months) and adults (>18 months). Dominance hierarchy and age of adult and subadult GHLTs were assessed based on historical group composition data from Project BioBrasil, behavioral observations, and information provided by BioBrasil's field assistants, who were able to recount the breeding history and relatedness of GHLTs over a longer period. Studies on Golden Lion Tamarins *Leontopithecus rosalia* (GLTs) show that dominant and subordinate breeders (males or females), within the same group, rarely display aggressive interactions and, instead, frequently engage in mutual affiliation, which is something typical of cooperative-breeding social structures (Baker et al. 1993, 2002). Despite that, dominance relationships of both males and females can be classified by examining a set of behaviors that indicate their status and roles within their social context (Baker et al. 2002). Therefore, to determine the dominance of adults and subadults, we considered their involvement in chases during intergroup encounters and intragroup aggression, their access to mates when females were expected to be fertile, their participation in carrying and nursing infants, as well as the frequency of mounts, copulations and arch-walks (Baker et al. 2002; Bales et al. 2005, 2006).

All activities described here involving captures, sample collection and monitoring were ethically approved by the International Committee for the

Conservation and Management of the Lion Tamarins and the Brazilian Environmental Agency (ICMBio/SISBIO permit no. 23457-6).

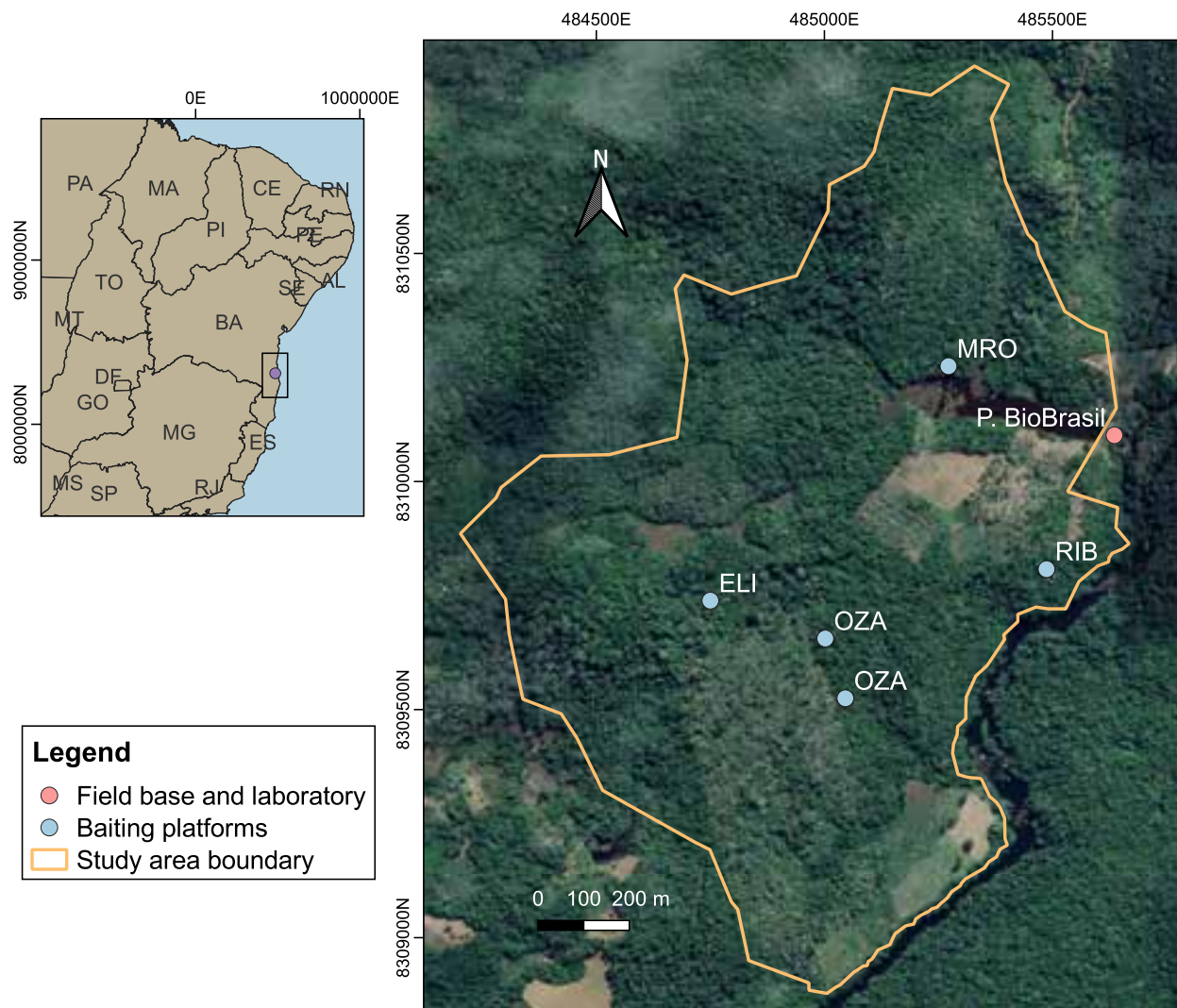
### Capture procedure

Study groups are captured twice a year by a multidisciplinary and trained team of biologists, veterinarians, and field assistants to replace radio-collars (model RI-2D, Holohil Systems Ltd., Ontario) on one or two adult individuals per group and to provide all individuals with a tattoo number and a unique dye mark (Nyanzol Dye). In this way, captures not only allow for the use of radiotelemetry to locate groups in the field but also facilitate identifying individuals during subsequent behavioral observations and sample collections.

Before capturing the GHLTs, platforms baited with banana were assembled in an area regularly used by the group (see Image 2), and Tomahawk traps were set up on these platforms gradually. The traps were activated once there was evidence the groups frequently visited the platform (De Vleeschouwer & Oliveira 2017; Catenacci et al. 2022). During capture days, traps were opened at 0500 h and monitored at regular intervals (0800, 1000, 1200, 1400, & 1630) to verify whether animals had been caught. Trapped GHLTs were taken to a nearby field laboratory and, following a fasting period of two to three hours, anesthetized with a combination of ketamine hydrochloride (dose 8–10 mg/kg) and midazolam (dose 0.25–0.5 mg/kg) to perform examinations (Catenacci et al. 2016; Costa et al. 2022). No infant GHLT was anesthetized or kept separate from its mother or caregiver (Catenacci et al. 2022). Due to the COVID-19 pandemic, which coincided with the present study, and the risk of spreading this disease to the animals (Fedigan 2010), we reinforced biosafety measures for all team members and shortened the handling time by limiting the collection of biometric data. Nonetheless, key variables such as body mass, measured with a one-gram digital scale after containment, and body condition score (see Table 1), determined based on Clingerman & Summers (2005), were still collected. All recovered animals were released either the same day before sunset or the following morning at the location where they were caught (Costa et al. 2020). For a more comprehensive description of the methods used during captures and examinations of GHLTs consult Catenacci et al. (2022).

### Sample collection and preservation

Fresh, uncontaminated fecal samples were collected under two different circumstances: 1) during two capture



**Image 2.** Map of the study area and locations of the baiting platforms used during captures in 2021. The study area boundary was obtained based on GPS coordinates recorded every 20 min during whole-day follows of four habituated Golden-headed Lion Tamarins groups (geographic data provided by Project BioBrasil). Satellite image from July 2021 obtained from Google Earth (© Maxar Technologies, © CNES/Airbus).

events, one in April and the other in November 2021, including all four GHLT groups – capture samples; and 2) over 11 months of full-day field observations between December 2020 and October 2021, involving only MRO, OZA and RIB – monitoring samples. In both cases, we focused our sampling effort on adult and subadult individuals (see Table 2), as GC production increases and GC negative feedback becomes less responsive with age (Sapolsky & Altmann 1991; Gust et al. 2000). Samples were incidentally taken from juvenile and infant GHLTs, which represented 6.25% of capture and 4.67% of monitoring samples. During group monitoring days, feces were collected any time we observed an individual defecate. During captures, samples were taken opportunistically at the field lab following the fasting

period. All samples were labelled with the date, the time of collection, and the individual's identity.

The time from when an individual was found in a trap (between 0800 and 1630) until it defecated and feces were collected was, on average,  $4.80 \pm 2.78$  h. Importantly, before TH and GC metabolites are excreted in feces, their circulating forms are metabolized and pass through the intestinal tract (Behringer et al. 2018; Palme 2019). This introduces a delay time in the appearance of the hormonal signals in feces which more or less corresponds to the species' gut transit time (Palme et al. 1996, 2005; Palme 2005; Touma & Palme 2005). For some callitrichids, including GLTs, gut transit times range from 2.65–6.30 h (Power & Oftedal 1996; reviewed by Lambert 1998). This interval nearly coincides with the

time elapsed before fecal samples were taken from trapped individuals (as mentioned above), increasing the likelihood of detecting hormonal changes caused by capture-related procedures.

Samples were stored inside 15 ml polypropylene screw-cap tubes prefilled with 4 ml of 80% ethanol (Hodges & Heistermann 2011), which is the preferred field preservation technique to prevent microbial degradation when immediate freezing is not available (Schwarzenberger 2007). Care was taken to ensure that stored samples were completely submerged in ethanol before tightly closing the lid (Hunt & Wasser 2003) and then transferred the tubes the same day of collection to a freezer at  $-20^{\circ}\text{C}$ , where samples remained for 30–149 days until oven dried (Khan et al. 2002). To dry samples, we first let the alcohol evaporate overnight for about 12 hours (Terio et al. 2002) and then placed samples inside a laboratory oven at  $50^{\circ}\text{C}$  for 4 h (Gholib et al. 2018), at which point feces became crumbly suggesting complete water loss. Finally, samples were transferred to small, labelled plastic bags containing oxygen absorbers, sealed them, and took them back to a freezer at  $-20^{\circ}\text{C}$  until all samples were shipped to the lab for further processing.

#### Hormone extraction and assay

Extraction of fecal metabolites and quantification of fGCs and ft3 were conducted in the Interdisciplinary Laboratory of Clinical Analyses at Murcia University (Interlab-UMU), Spain, between April and June 2022. To extract the metabolites, we followed a method similar to that described in Wasser et al. (2000). First, large seeds, insect parts and plant debris from crushed dried feces were removed (Foerster & Monfort 2010), and weighed an aliquot of approximately 0.06 g (95.7% of the aliquots had an average weight of  $0.0599 \text{ g} \pm \text{SD } 0.0023$ ). All aliquots were pulverized and shaken for 15 h in 1 ml of analytical-grade methanol (Gómez-Espinosa et al. 2014; Rangel-Negrín et al. 2015). Extracts were then centrifuged at 3,500 rpm for 5 min, and 0.6 ml of supernatant was transferred to a separate tube. Supernatants were then evaporated inside a vacuum concentrator for 2 h, reconstituted with 0.15 ml of a PBS buffer, vortexed, and stored at  $-80^{\circ}\text{C}$  until analysis.

Commercial enzyme immunoassays (EIA) kits from IBL International GmbH for the determination of cortisol (RE52061) and total triiodothyronine (RE55251) to respectively measure fGCs and ft3 in our sample extracts. While the chosen T3 kit has already been validated for T3 metabolites excreted in feces and urine of non-human primates (Behringer et al. 2014; Cristóbal-Azkarate et al. 2016; Deschner et al. 2020; Sadoughi et al. 2021; Touitou

et al. 2021), the cortisol kit has thus far been tested in teleosts (e.g., Nilsson et al. 2012; Cerqueira et al. 2017; Mazzoni et al. 2020) and some mammals (e.g., Brain et al. 2015; Almoosavi et al. 2021; Kaiser et al. 2023) but not in non-human primates. To prevent alterations in the assays, manufacturer's instructions of use were strictly followed. The standard curves for calibration of all 10 plates tested (5 for fGCs & 5 for ft3) exhibited an accuracy of  $R^2 = 0.98\text{--}1$ . Besides the coefficients of variation (CV) for repeatability already provided by the manufacturer (cortisol: intra-assay CV = 2.5–3.5 % and inter-assay CV = 2.1–5.2 %; total T3: intra-assay CV = 3.59–6.61 % and inter-assay CV = 5.23–6.73 %), we performed, prior to the analysis of main samples, an analytical validation on a small set of fecal samples collected from captive GHLTs from Terra Natura in Benidorm (Spain), following the same sample processing previously described. For both the fGCs and ft3 quantification, it was found that intra and inter-assay CVs were less than 15% and displacement curves obtained from serial dilutions of fecal samples ran parallel to the standard hormone curves with a  $R^2$  close to 1.

#### Data analysis

The levels of fGCs and ft3 were compared between groups of the predictor variables by applying ANOVA to multiple mixed-effect models fitted with the 'lmer' function from the R package lme4 (Bates et al. 2015). Because data contained repeated measures for the same individual within and, in some cases, between groups, we consistently defined the individual identity as a random factor throughout this analysis. Both fGC and ft3 levels were transformed to logarithm with base 10 to conform with assumptions of normality of residuals and homogeneity of variance, verified each time data was reorganized. When dealing with ft3 levels measured in monitoring samples, ANOVAs were used with aligned rank transformed data from package ARTool (Wobbrock et al. 2011). If a significant effect was detected for a certain variable, a post-hoc analysis was performed by least-square means from package emmeans (Searle et al. 1980) or a contrast test provided by ARTool. We began evaluating the effect of the stress of capture on both metabolites considering the total number of samples ( $n = 289$ ). We then split up the dataset between capture and monitoring collections and removed data from juveniles and infants to test the influence of sex, dominance, and dominance in interaction with sex (dominance\*sex) on fGCs and ft3 levels. Body condition score and body weight were assessed only with capture samples, for the latter variable we excluded

**Table 1. Description of the body condition scores used during two capture events to categorize nutritional status (body fat and muscle) of captured Golden-headed Lion Tamarins.**

Body condition score	Definition
1	<b>Emaciated:</b> very prominent and easily palpable bones (hips, ribs, and spinal processes), very low to no palpable muscle mass over the ilium or ischium, subcutaneous fat layer is absent, very angular body, sunken anus between ischial callosities, and protruding facial bones.
2	<b>Thin:</b> prominent and palpable bones, low muscle mass over the hips and back, low fat reserves and subcutaneous fat layer, and angular body.
3	<b>Optimum:</b> bones are generally not visible but palpable to soft pressure, both muscle mass and fat layer are well-developed giving the spine and hips a firm but smooth touch.
4	<b>Overweight:</b> bones are not visible and only palpable to firm pressure, abundant subcutaneous fat layer, and smooth and less defined body contour.
5	<b>Obese:</b> bones are not visible at all and difficult to palpate, abundant fat deposits (abdominal, axillary, and inguinal region), difficult abdominal palpation due to large amount of mesenteric fat, and body contour without definition.

Adapted from Clingerman & Summers (2005).

**Table 2. Number of fecal samples per sex and dominance status collected during capture events and regular monitoring days.**

Collection type	Number of groups	Number of individuals	Number of fecal samples						
			♀ D	♀ S	♂ D	♂ S	J & I	♂ U	Total
Capture	4	22	10	10	3	5	2	2	32
Monitoring	3	25	39	54	68	83	12	1	257

Where adults and subadult Golden-headed Lion Tamarins are differentiated in dominant females (♀ D) and males (♂ D), subordinate females (♀ S) and males (♂ S) or males with undetermined dominance status (♂ U). Juveniles and infants are represented by "J & I".

juveniles from the dataset as body weight would not be comparable. To explore the differences in group identity, we solely considered samples from regular monitoring including all age categories. All statistical tests were run in R version 4.3.2. (R Core Team 2023) and considered significant at  $p < 0.05$ .

Since diurnal variation in hormone secretion may potentially confound the excretion of GCs and T3 metabolites (Sousa & Ziegler 1998; Foerster & Monfort 2010; Pizzutto et al. 2015; Behringer et al. 2023), especially in species with rapid gut transit time (Touma & Palme 2005; Rimbach et al. 2013) like GHLTs, we examined the effect of collection time on the fGC and ft3 levels within the complete dataset (all samples), as well as the separate datasets: monitoring and capture samples. To conduct this preliminary analysis, we used linear mixed-effect models ('lmer') for fGCs and generalized linear mixed-effect models ('glmer') for ft3, with individual identity as random term and time of collection as predictor. If collection time had a significant effect, we incorporated it as an additional random factor in the formula of our main analysis to account for the natural circadian fluctuations in the response variables.

## RESULTS

We found that diurnal variation of metabolite excretion affected the levels of ft3 in the complete dataset ( $p = 0.022$ ), and fGCs in the monitoring ( $p = 0.009$ ) and capture ( $p = 0.048$ ) datasets. Therefore, in addition to individual identity, we defined collection time as a random factor when using these datasets with the respective fecal metabolite. As this was beyond the scope of our main research questions, we do not discuss further how fGCs and ft3 reflect the GHLT's circadian rhythms.

### Effects of the stress of capture and body condition

Concentrations of fGCs in capture samples were significantly higher than in monitoring samples ( $F_{1, 267.92} = 36.81$ ,  $p < 0.001$ ) with mean levels of  $6462.14 \pm \text{SEM } 921.76 \text{ ng/g}$  and  $2712.3 \pm \text{SEM } 197.88 \text{ ng/g}$ , respectively, for each collection type. Although the range of fGC levels observed in monitoring samples ( $181.81\text{--}22065.65 \text{ ng/g}$ ) was wider than that in capture samples ( $1020.81\text{--}21712.85 \text{ ng/g}$ ), the range of variation of both collection types nearly overlapped (Figure 1A). On the contrary, ft3 values measured in capture samples were significantly lower than in monitoring samples ( $F_{1, 263.18} = 12.27$ ,  $p < 0.001$ ) with mean levels of  $29.7 \pm \text{SEM } 2.73 \text{ ng/g}$  and

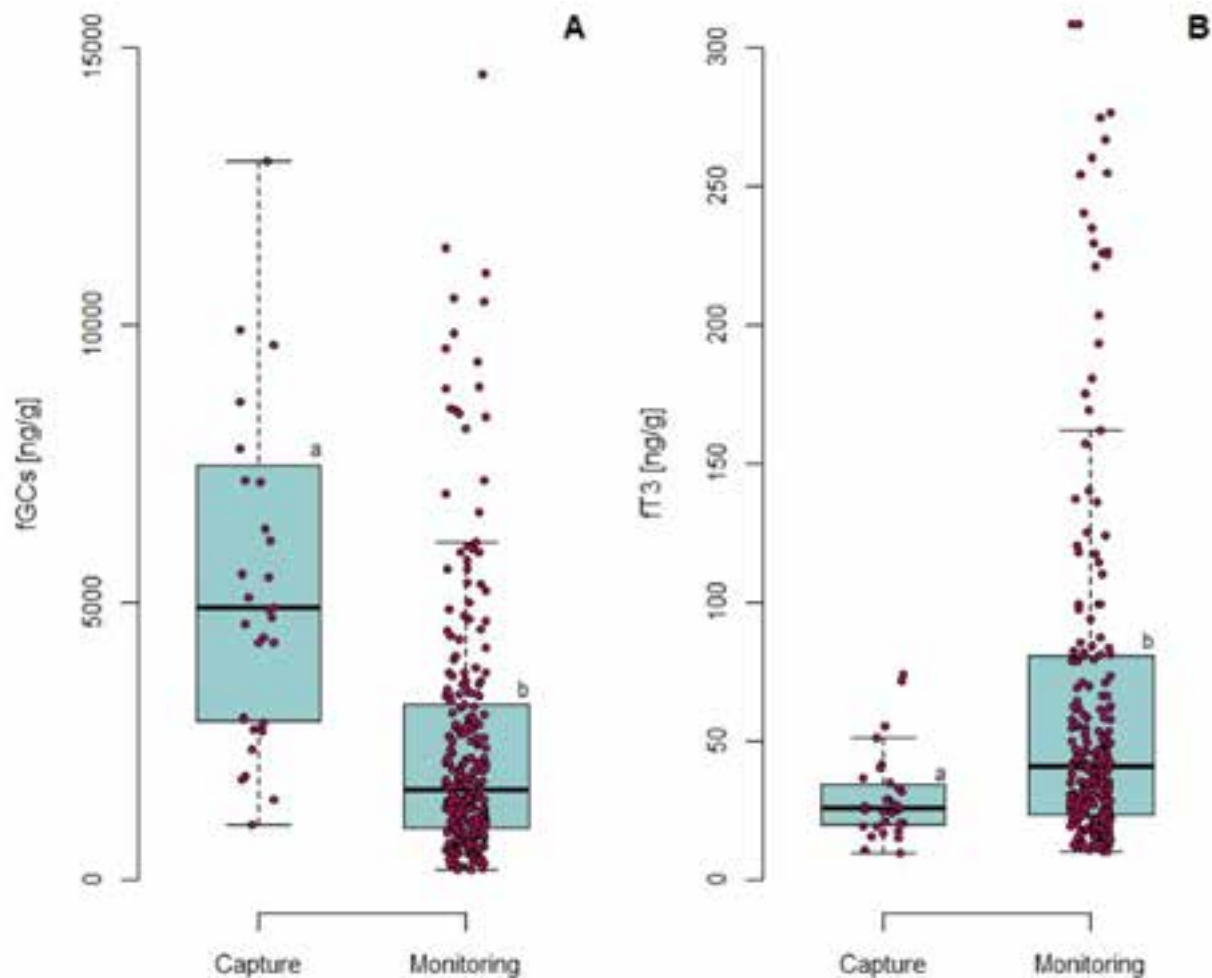


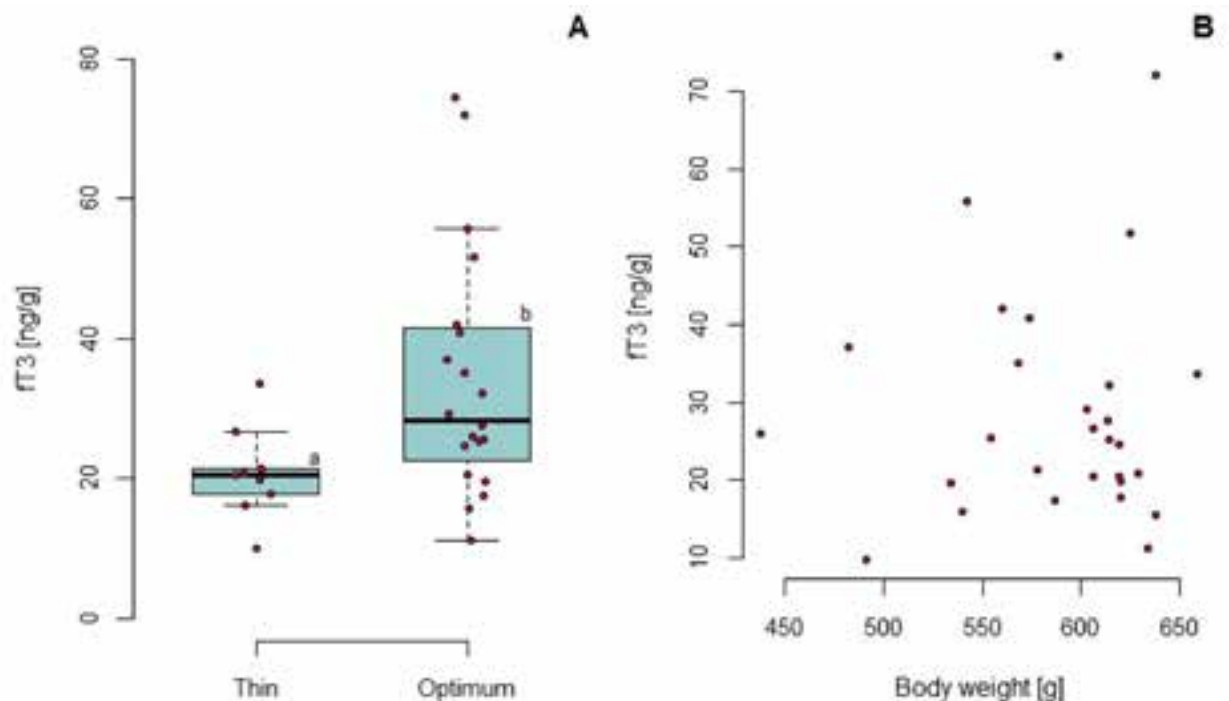
Figure 1. Levels of fecal metabolites of: A—glucocorticoids – fGCs | B—triiodothyronine – ft3 measured in samples collected in capture events and group-monitoring days. Data points are plotted as dots. Boxplots display the median at the center line, the 75<sup>th</sup> and 25<sup>th</sup> percentiles at the box limits, and 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles at the whiskers. Different lowercase letters indicate significant differences among groups.

89.55 ± SEM 7.98 ng/g, respectively. Also, in contrast to fGCs, the range of ft3 levels was much narrower in capture (9.86–74.44 ng/g) than in monitoring samplings (10.76–848.61 ng/g), as shown in Figure 1B. We found no significant effect of body weight on either metabolite. Body condition score of captured individuals was significantly associated with variation in ft3 levels ( $F_{1,27} = 5.54$ ,  $p = 0.026$ ), this association linked higher ft3 concentrations to individuals with a better nutritional state (optimum versus thin), see Figure 2A.

#### The effect of sex, dominance, and group identity

Neither sex nor dominance was associated with significant differences in the levels of fGCs or ft3 for any collection type. Similarly, the interaction between sex and dominance was not significant for any of the metabolites measured in the monitoring samples (see

Figure 3). There was a marginally significant effect of this interaction on the fGCs levels in capture samples ( $F_{1,17.28} = 3.58$ ,  $p = 0.075$ ). Regarding only monitoring samples, group identity had a marginally significant effect on fGC values ( $F_{2,30} = 3.08$ ,  $p = 0.061$ ), whereas for ft3 this effect was significant ( $F_{2,26.6} = 6.22$ ,  $p = 0.006$ ) with ft3 levels in MRO being higher than in OZA and RIB (Figure 4). Given that the variation in ft3 among GHLT groups could potentially bias the previous results regarding stress of capture, we ran an additional test for ft3 and type of collection with individual identity nested within group membership as a random factor in the model configuration. This test confirmed that the procedure of capture has a significant effect on ft3 levels ( $F_{1,120} = 9.81$ ,  $p = 0.002$ ) regardless of group identity.



**Figure 2.** Levels of fecal triiodothyronine metabolites (fT3) measured in capture samples as a function of A—body condition score and B—weight in grams. Data points are plotted as dots. Boxplots displaying the median at the center line, the 75<sup>th</sup> and 25<sup>th</sup> percentiles at the box limits, and 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles at the whiskers. Different lowercase letters indicate significant differences among groups.

## DISCUSSION

Here we demonstrated, through a simple biological approach, that fGCs and fT3 can be reliably quantified in dried feces of wild GHLTs using commercially available cortisol and total triiodothyronine EIA kits. To do so, we evaluated the physiological response of individuals to the stress of capture and compared it to a natural situation. Additionally, we assessed the relationship between body condition and the metabolites of interest. Importantly, the present study offers a different validation approach from methodologies such as physiological validations, which normally allow consecutive sampling of tested subjects after induced stress and are intended to measure peak metabolite levels.

As expected, GC excretion in individuals during capture and handling procedures was, on average, higher than levels measured during the GHLT's daily activity throughout 11 months of sampling. This finding confirms the notion that the activation of the HPA axis during procedures such as physical restraints, captures, and transportation allow detecting GC alterations that show the capacity of individuals to mount their stress response (Touma & Palme 2005; Wikelski & Cooke 2006). Typically, fGC measurements in non-human primates are

validated by obtaining multiple samples from captive individuals before and after a short-term stressor (e.g., capture, isolation, veterinary exam, anaesthesia) or a procedure to pharmacologically stimulate the HPA-axis, such as the adrenocorticotrophic hormone (ACTH) challenge test (e.g., Heistermann et al. 2006; Rangel-Negrín et al. 2009; Rimbach et al. 2013). With this, researchers can delineate the fGC excretion profiles for each individual and identify the peak values, defined as the fGC concentrations exceeding two standard deviations above baseline (Gómez-Espinosa et al. 2014; Pizzutto et al. 2015; Wark et al. 2016). The latter value can correspond to the mean pre-capture concentration (Gómez-Espinosa et al. 2014), or the mean concentration calculated by iteratively excluding values greater than 2 SDs from the mean (Pizzutto et al. 2015; Wark et al. 2016). Studies using this approach have reported a lag time of 22.3–49.3 h in GLTs ( $n = 7$ ; Wark et al. 2016) and 20–25 h in Black Lion Tamarins *Leontopithecus chrysopygus* (BLTs) ( $n = 6$ ; Bertoli et al. 2019) between the stressful event or the ACTH injection and the first observed peak. Given such lag times, we presume that the timeframe considered in this study ( $4.80 \pm \text{SD } 2.78$  h) is not long enough to detect a peak in fGC excretion. The results simply indicate how the stress response of GHLTs,

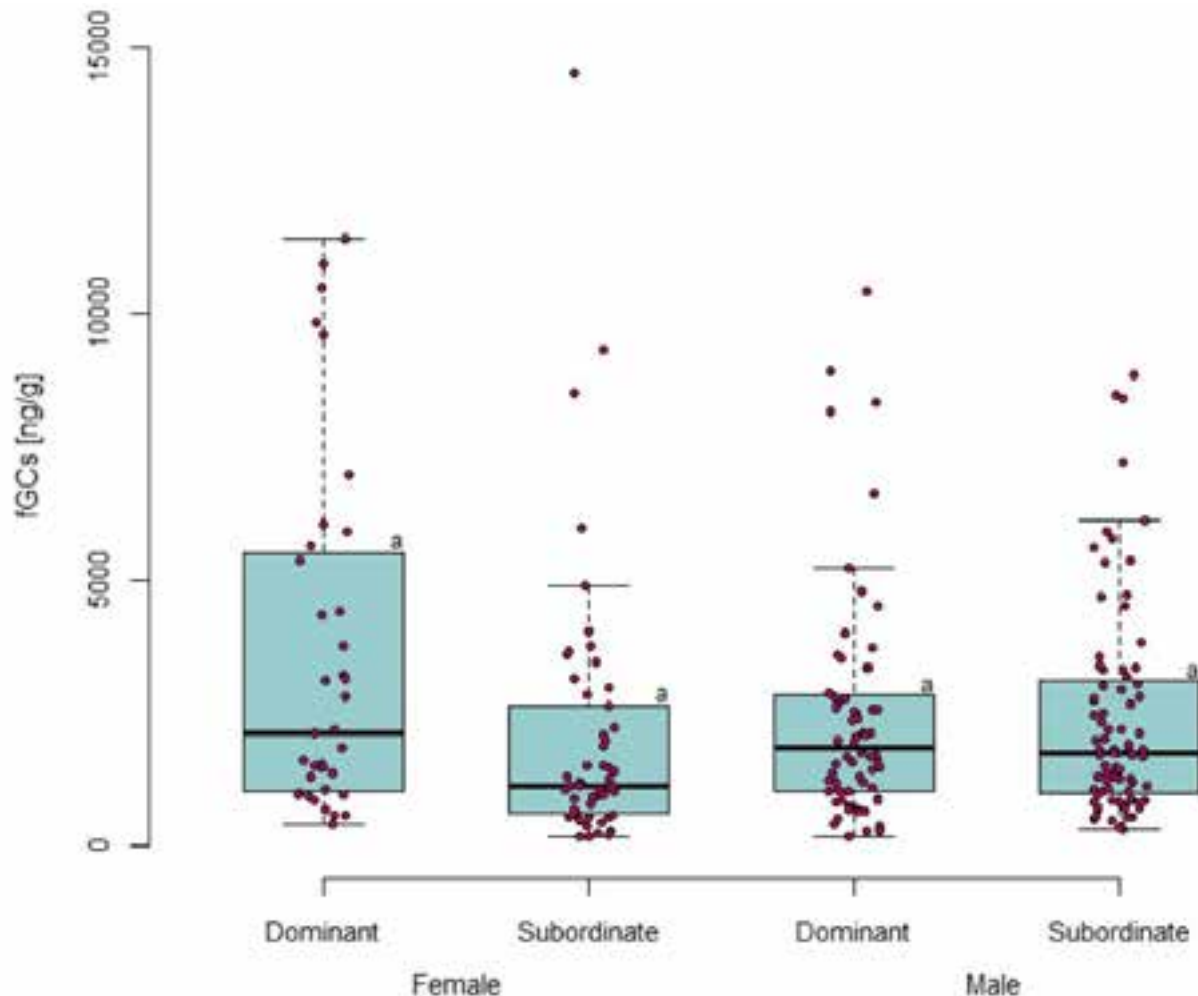


Figure 3. Levels of fecal glucocorticoid metabolites (fGCs) were measured in monitoring samples across different sex and dominance status of adult and subadult Golden-headed Lion Tamarins. Data points are plotted as dots. Boxplots displaying the median at the center line, the 75<sup>th</sup> and 25<sup>th</sup> percentiles at the box limits, and 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles at the whiskers. Different lowercase letters indicate significant differences among groups.

as measured by fGCs, differs between capture events and regular monitoring days, when habituated individuals move freely and interact with their environment. It is possible that lag time before fGC peaks appear in GHLTs will be similar to those found in congeneric species. To verify that, a different experimental design needs to be used which would likely require captive populations or free-ranging individuals to be retained in captivity to ensure repeated fecal sampling for at least three days following an induced stressor (Gómez-Espinosa et al. 2014; Wark et al. 2016; Bertoli et al. 2019).

Notably, the amplitude of fGC response to capture was similar to the one detected during monitoring days, especially regarding upper fGC concentrations. This may indicate that GHLTs in our study area face environmental stressors that trigger a stress response as intense as the one induced by capture and containment (Johnstone

et al. 2012). The study groups occupy fragments of disturbed forest interspersed with agricultural areas (De Vleeschouwer & Oliveira 2017), a landscape that probably presents particular environmental challenges, such as high predation risk (Oliveira & Dietz 2011) or low opportunities to find food and shelter (Kalbitzer & Chapman 2018), which could stimulate or even sustain a GC elevation in the long term (Kaisin et al. 2021). It would be interesting to compare our results with those from GHLTs in different landscapes and explore the possible health consequences linked to an over-stimulation of the stress responses (Romero et al. 2009) in GHLTs occupying disturbed forests.

Contrary to our expectations, ft3 levels were affected by the stress of capture, but exhibited a response pattern opposite to that of fGCs. This significant effect suggests that, similarly to fGCs, the lag time for the appearance

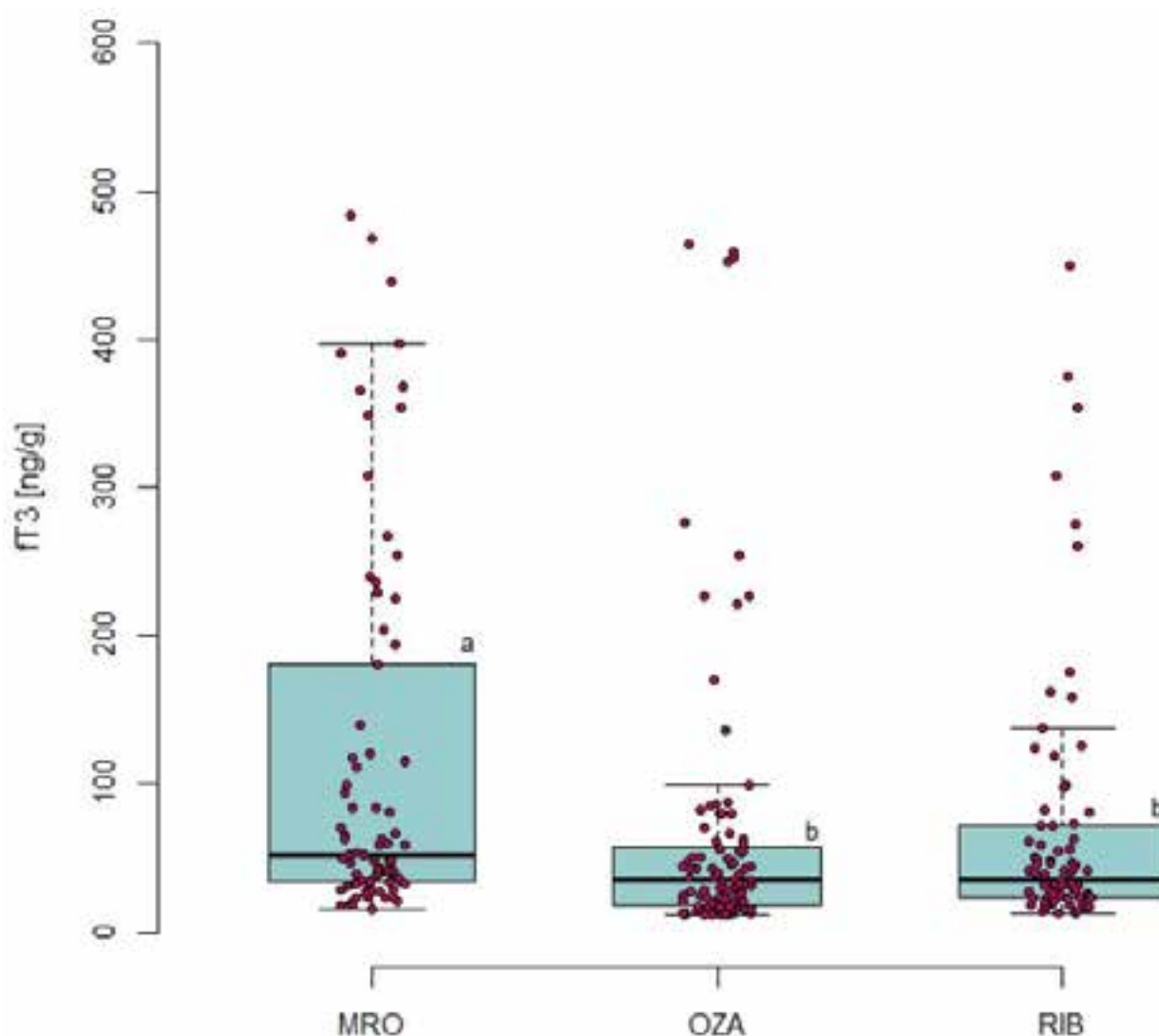


Figure 4. Levels of fecal triiodothyronine metabolites (fT3) as a function of group identity were evaluated for monitoring samples. Data points are plotted as dots. Boxplots display the median at the center line, the 75<sup>th</sup> and 25<sup>th</sup> percentiles at the box limits, and 1.5 times the interquartile range from the 25<sup>th</sup> and 75<sup>th</sup> percentiles at the whiskers. Different lowercase letters indicate significant differences among groups.

of T3 metabolites in feces is comparable to the species' gut passage time (Schaebs et al. 2016; Behringer & Deschner 2017). Furthermore, the lower fT3 but higher fGC levels in capture compared to monitoring samples raises the possibility of a cross-talk or interaction between the HPA- and HPT-axis (Behringer et al. 2018; Touitou et al. 2021), although levels of fGCs and fT3 in capture samples were not correlated, probably due to small sample size. It is frequently suggested that TH levels are downregulated by the release of GCs, associated with stressful situations (Burr et al. 1976; Behringer et al. 2018). Physiological pathways leading to this TH suppression include the inhibition of the thyroid stimulating hormone and the reduced conversion of T4 to T3 (Charmandari et al. 2005). For instance, Helmreich

et al. (2005) found in male Sprague-Dawley rats that mild-electric foot-shocks led to significantly lower levels of serum T3 and, although corticosterone levels were not altered by this experiment, certain HPA-axis components may participate in TH regulation. This relationship may not be so straightforward, even when an acute stressor is involved, as demonstrated for Guadalupe fur seals by DeRango et al. (2019), who associated a capture event to a simultaneous reduction in T3 and an integrated stress response comprising cortisol and corticosterone levels. Furthermore, the potential cross-talk between T3 and GCs may have been confounded by food restriction, performed in order to anesthetize trapped individuals. In fact, various studies have successfully validated the measurement of T3 in primates by linking reduced food

intake to lower levels of T3 in urine and feces (Wasser et al. 2010; Schaebis et al. 2016; Sadoughi et al. 2021). Regardless of the possible explanations, our results for the stress of capture indicate that under fearful situations, GC levels in GHLTs increase in order to promote alertness and a freezing response (Charmandari et al. 2005; Korte et al. 2005) while T3 decreases as a mechanism to modulate the metabolic rate and save energy (Behringer & Deschner 2017; Gesquiere et al. 2018). Both changes likely occur in preparation to future demands or additional stressors (Sapolsky et al. 2000).

Another aspect that links THs' secretion to metabolic activity in relation to energy balance is their response to weight gain or loss (Chatzitomaritis et al. 2017). Specifically, when high food quantity and quality is accompanied by high T4 and T3 levels, bodyweight will normally increase (Behringer et al. 2018). In this study, no association was found between adults and subadults' body mass and fT3 levels, which could be attributed to the fact that weight values were not scaled to specific body length of each individual (DeRango et al. 2019). We measured this value (knee-to-heel distance) in some but not all sampled individuals because of the limitation in handling time during capture imposed by pandemic restrictions. Nonetheless, by using a semiquantitative body condition score, a tendency of lower fT3 levels was identified to be related with individuals presenting a bonier structure and a lesser amount of palpable muscle and fat (Clingerman & Summers 2005). This is in line with evidence that THs play a direct role in regulating the metabolism of brown adipose tissues and skeletal muscles (López et al. 2013).

Primate males and females normally differ in their strategies to achieve and maintain social dominance which, as reviewed by Cavigelli & Caruso (2015), results in dominant males having elevated metabolic demands due to the costs of competition over access to mates, especially during periods of social instability, whereas exclusion of quality food sources entails reduced energy intake, particularly for subordinate females. Accordingly, one may predict that dominant males and subordinate females will probably exhibit high GC but low T3 levels. Fecal glucocorticoid and fT3 concentrations across sex and dominance status in adult and subadult GHLTs were statistically indistinguishable. Such an absence of a dominance status effect is consistent with studies on free-ranging male and female GLTs, at least with respect to fGC metabolites (Bales et al. 2005, 2006). Furthermore, the pattern for fGCs coincides with a hierarchy system, commonly attributed to cooperatively breeding species, where subordinates are not subjected to high rates of aggression and usually rely on close kin

support (Abbott et al. 2003). On the other hand, although not investigated here, it is likely that differences in fGCs in relation to female reproductive status may be present in wild GHLTs, as observed in several other primate species (e.g., Bales et al. 2005; Rimbach et al. 2013; Dias et al. 2017). To address such question and more accurately define reproductive condition in females, GC measures should be accompanied with the determination of estrogen conjugates and pregnanediol glucuronide (De Vleeschouwer et al. 2000; French et al. 2003).

During group monitoring, it was observed that the MRO group was going through various changes in composition which initiated with the death of the eldest dominant male, then the emigration of females and finally, the disintegration of the group with a single subordinate male using the original home range and attempting to enter a neighboring group. Considering such a dramatic and likely stressful group dynamic, one may presume higher fGC levels in MRO than in the other two groups. This was not the case for fGCs, but it was for fT3 levels. The significantly higher level of fT3 in MRO may point to the potential influence of ecological factors, such as the availability of space and associated access to food and shelter. Previous studies on GHLTs have reported differences in home range and feeding behavior among groups using distinct habitats (Oliveira et al. 2011; Costa et al. 2020), as may be generally expected. Coutinho (2018) who worked with three of our study groups during an earlier period, showed that groups have a substantial proportion of overlapping home range and differ in the time devoted to feeding on plants and animals. This requires additional investigation into the extrinsic factors that may potentially lead to constraints in energy intake and explain this variation in metabolic rate between groups.

## CONCLUSION

This study validates the measurement of GC and T3 metabolites in wild GHLTs' fecal samples, employing two commercial EIA kits. It also provides further evidence that biological validation of hormonal measurements in wild individuals is an effective alternative to traditional pharmacological challenges, provided the tested factors are carefully considered. Taken together, the results show that fGCs respond to the stress of capture and possibly, under acute stressors, downregulate fT3 levels. While fT3 responded to changes in body condition and therefore may have a more direct connection to energetic challenges.

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**Author details:** R. FIORINI-TORRICO was a PhD student in the Postgraduate Program in Ecology and Conservation Biology (PPGECB) at the State University of Santa Cruz. He is currently a postdoctoral researcher at the same institution and the research coordinator of Project BioBrasil. L.C. OLIVEIRA is an adjunct professor in the Science Department at the State University of Rio de Janeiro and an associate professor in PPGECB. D. ESCRIBANO is a postdoctoral researcher in the Animal Production Department and the Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU) at Murcia University. J.J. CERÓN is a full professor in the Animal Medicine and Surgery Department at Murcia University and the leader of Interlab-UMU. K.M. DE VLEESCHOUWER is a senior research coordinator at the Antwerp ZOO Centre for Research and Conservation (CRC) and the director of Project BioBrasil.

**Author contributions:** R. Fiorini-Torrico conceptualized the research goals, collected the samples and data, conducted the formal analysis, and wrote and edited the manuscript. L.C. Oliveira supervised the project execution and provided critical reviews and commentary. D. Escribano contributed to lab analysis, supervised sample collection and lab techniques, and reviewed and commented on the manuscript. J.J. Cerón supervised lab work, provided essential lab resources, and offered critical reviews and suggestions. K.M. De Vleeschouwer supervised research activities, provided necessary field resources, and reviewed and commented on the manuscript.

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## Tricho-taxonomic prey identifications from faeces of Indian Rock Python *Python molurus* (Linnaeus, 1758) (Reptilia: Squamata: Pythonidae) in Moyar River Valley, Tamil Nadu, India

Jyoti Nagarkoti<sup>1</sup> , C.S. Vishnu<sup>2</sup> , Chinnasamy Ramesh<sup>3</sup> & Archana Bahuguna<sup>4</sup>

<sup>1–3</sup> Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand 248002, India.

<sup>4</sup> Zoological Survey of India, Northern Regional Centre, Kaulagarh, Dehradun, Uttarakhand 248003, India.

<sup>1</sup> [jjyotinagarkoti6@gmail.com](mailto:jjyotinagarkoti6@gmail.com), <sup>2</sup> [vishnusreedharannair@gmail.com](mailto:vishnusreedharannair@gmail.com), <sup>3</sup> [ramesh.czoo@gmail.com](mailto:ramesh.czoo@gmail.com) (corresponding author),

<sup>4</sup> [archana.bahuguna65@gmail.com](mailto:archana.bahuguna65@gmail.com)

**Abstract:** Identifying the prey species is crucial for successful conservation and landscape-level management of large predators whose feeding ecology is incompletely known. Assessment of faecal samples is a viable method for achieving this goal. The Indian Rock Python *Python molurus*, one of India's largest snakes, is an ambush predator trophically-equated to apex predator in its ecosystem. Yet, compared to trophically-similar big cats and canids there is a notable lack of research on its prey items in southern Indian regions. In this study, 31 faecal samples from radio-tagged pythons and other pythons inhabiting the Moyar River Valley were studied. These samples were dried and washed to obtain the hairs to identify the species. Hairs were obtained from 11 faecal samples, and prey species were identified using the tricho-taxonomy. Our findings revealed that mammals constitute the majority of consumed prey. Pythons preferred the palm squirrel *Funambulus palmarum* as the most desirable prey. Other large prey species include the Sloth Bear *Melursus ursinus*, the Sambar Deer *Rusa unicolor*, and the Spotted Deer *Axis axis*. Seasonal variation in prey species intake peaked during September–November, while no prey species were recorded during April–August.

**Keywords:** Behavior, diet, ecology, feeding, giant snake, hair, mammals, predation, prey, reptile.

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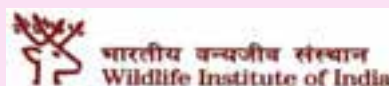
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**Author details:** JYOTI NAGARKOTI is a researcher at the Wildlife Institute of India. C.S. VISHNU works as a researcher at Wildlife Institute of India. CHINNASAMY RAMESH, PhD, serves as a scientist-E at Wildlife Institute of India with an interest in ecology, human-wildlife interactions, invasive species, climate change, biodiversity conservation and marine research. ARCHANA BAHUGUNA serves as additional director, Zoological Survey of India, MoEFCC. Expert of herpetofauna, trichotaxonomy, molecular systematics and Wildlife forensics. Presently she is the founder and director of the Himalayan Centre for Art, Culture and science, Dehradun, Uttarakhand, India.

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## INTRODUCTION

Food is central to all animals' biology and life history, and recognising, locating, capturing, ingesting, and digesting prey are critical activities for their survival and reproductive success (Slip & Shine 1988). Diet studies provide knowledge about species trophic ecology and population dynamics necessary for species management and conservation policies (Morrison et al. 1990; Sutherland et al. 2004; Marti et al. 2007). The feeding habits of giant snakes are of particular interest due to their remarkable adaptations for locating, capturing, subduing, and ingesting large prey (Slip & Shine 1988). They rely on several sources of sensory input to locate food, including vision, vibration, heat, and colour (Slip & Shine 1988). Since large-bodied snakes such as pythons are mostly restricted to tropical areas, presumably due to thermal constraints in temperate climates, detailed information on thermal biology, diet, and other ecological factors for giant snakes inhabiting tropical regions is scarce (Shine & Madsen 1996; Shine et al. 1998). The Indian rock python *Python molurus* is a giant-bodied non-venomous and habitat generalist snake species with a mean adult snout-vent length of 3–4.5 m, with some individuals exceeding 6 m (Bhupathy 1990). It is widely distributed in India, Sri Lanka, Nepal, and parts of Pakistan (Smith 1943; Whitaker 1993; Daniel 2002; Whittaker et al. 2004) inhabiting a wide range of habitats, including wetlands, open forests, scrublands, harsh deserts, rainforests, woodlands, grassy marshes, river valleys, rocky slopes, and savannas (Murphy & Henderson 1997).

Its home range size in the Moyar River Valley Landscape, southern India, is 4.2 km<sup>2</sup> (Vishnu et al. 2023a). Python often functions as a top-level predator that may influence local prey populations, including reptiles, birds, and small to medium-sized mammals, particularly in ecosystems where they are abundant (Bhupathy et al. 2014; Gangaiamaran et al. 2023). Avian prey has been routinely recorded in the diets of giant snakes, such as pythons and anacondas, many times, indicating that almost all of the giants do eat birds (Murphy & Henderson 1997). Six species of birds were found in the diet of *P. molurus* in Keoladeo National Park, Rajasthan (Bhupathy & Vijayan 1989), often including domestic birds (Murphy & Henderson 1997). Studies in Pakistan and Sri Lanka have also reported reptiles such as monitor lizards *Varanus* sp. as prey species of *P. molurus* (Deraniyagala 1955; Minton 1966). Mammals most likely comprise the bulk of the diet of giant snakes (Ernst & Zug 1996; Bhupathy et al. 2014). Mammals of

suitable size that overlap spatially and temporally with *P. molurus* may be vulnerable to predation (Murphy & Henderson 1997). Pythons' diet also frequently include domesticated cats and dogs, rodents, ungulates, and monkeys (Murphy & Henderson 1997). Unusual prey like horned mammals with the potential to cause serious injuries when swallowed have also been recorded in the diet of *P. molurus*, *P. sebae*, and *Malayopython reticulatus* (Duarte 2003). These prey include small to medium-sized bovids such as *Kobus kob* (Hay & Martin 1966), *Aepyceros melampus* (Gasc 1994), *Axis porcinus*, *Gazella thomsoni*, *Muntiacus muntjac* (Greene 1997), and antelopes (Spawls & Branch 1995).

Several methods have been used to study the food habits of reptiles, especially the gut content analysis (Delany & Abercrombie 1986; Lobo et al. 2005; Dove et al. 2011), including stomach flushing (Fitzgerald 1989; Rivas et al. 1996), and faecal sample analysis (Sylber 1988; Bhupathy et al. 2014). Passive and non-abrasive approaches, such as faecal analysis, can be used to determine the feeding behaviour of the study of animals without disrupting their habitat ecology. In snakes, bone and tooth fragments are often poorly preserved in faecal matter due to digestive efficiency, whereas, hair remains relatively undigested and can serve as a reliable tool for prey identification (Quadros & Monteiro-Filho 1998). Data on the diet of *P. molurus* in northern India were reported by Bhupathy et al. (2014) from Keoladeo National Park. Therefore, to provide more information on the dietary patterns of *P. molurus* from southern region of India, we studied the prey items of *P. molurus*, using tricho-taxonomic analysis on faecal samples collected from the Moyar River Valley, Tamil Nadu.

## MATERIALS AND METHODS

### Study area and field method

The study was conducted in Moyar River Valley, between two protected areas, Sathyamangalam and Mudumalai Tiger Reserves in Tamil Nadu (Figure 1). The region is known for its rich biodiversity assemblage (Thirumurugan et al. 2021). During radio-tracking field surveys, faecal samples (n = 31) from pythons were opportunistically collected between 2018 and 2020. The samples (n = 20) were collected from 11 tagged python individuals, while the remaining samples were collected from other individuals in the wild. The samples were collected in zip-lock bags and labelled with markers. Subsequently, the samples were dried in the shade and then stored in a refrigerator at temperatures below

30°C for almost a year with proper packaging to prevent contamination.

### Laboratory method

Hair analyses were done as per Souza & Azevedo (2021). Evidence of mammalian prey species found in the faecal samples was identified following tricho-taxonomy techniques (Bahuguna et al. 2010). The dried faecal samples ( $n = 31$ ) were at least one year old and were broken down with the help of a mortar and pestle and then cleaned with the help of the sieving method. The hairs were extracted using the forceps. The hair samples obtained from the faecal samples were washed in acetone to remove any dirt present in them. Microslides were prepared to identify the hair of the potential prey species of the Indian Rock Python. The medulla was identified, and a cross-section was done to determine the shape of the medulla, which is visible through cross-section (Bahuguna et al. 2010). All photographs used in the identification were captured using a digital micro

camera. The nomenclature of the hair medulla type was adopted from Wildman (1954), and the cuticular and cross-section types were followed as given by Brunner & Coman (1974); Medulla: to identify the medulla type, the whole amount of cleaned hair samples was mounted in DPX (Dibutylphthalate Polystyrene Xylene) without staining, allowing visualisation of internal structures. It was ensured that the hairs were well separated, and individual hairs could be observed. The medulla was observed using a light microscope at 40x magnification. Cross Section: for the present study, hair cross-sections were obtained by simple hand sectioning after mounting the hair in paraffin wax and sectioning the wax block by using surgical blades. The cross-sections were selected based on the clarity of the structure. This method was successfully followed by Bahuguna et al. (2010). No microtome was used for cross-sections. These sections were placed on slides coated with egg albumen and observed at 100x. Scale Casts: to study the scale pattern, 3% gelatin as a special medium was used to obtain

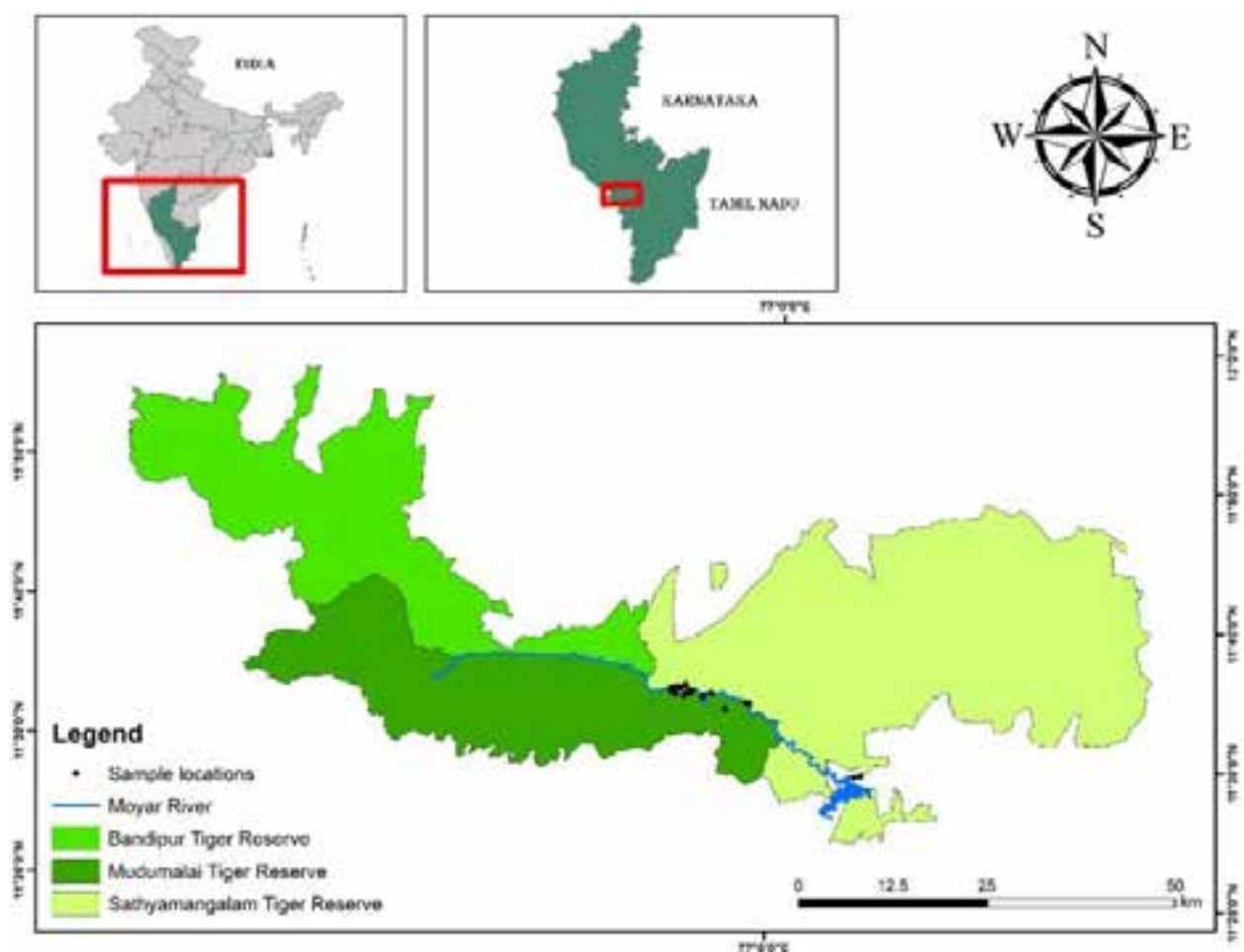


Figure 1. Sampling sites along the Moyar River Valley, India, from where python faecal samples were studied.

the hair impressions. The gelatin is a special medium prepared by mixing the gelatin powder in lukewarm water at a temperature of 50°C. Eosin or hematoxylin was mixed directly into the gelatin mixture to observe the impressions properly. For getting the hair impression or cast, the method by Brunner & Coman (1974) was followed. A thin film of gelatin medium was made. The cleaned hair samples were then directly placed into the glass slide using fine forceps. After drying of gelatin, the hair was removed gently using forceps. This left the hair impression on the glass slide. Different regions (distal, mid and proximal) of hair were examined through 40x microscope to study cuticle patterns.

## RESULTS

Hairs were examined from 11 faecal samples out of 31 collected samples because the remaining samples ( $n = 20$ ) either lacked identifiable remains or were too degraded to analyse. Only the mid-portion of the hair was considered, along with other major factors for scale pattern and cuticular surface. To differentiate the hairs obtained from faecal samples, the hair microstructure (cuticle and medulla) and hair macrostructure (thickness and colour), were taken into consideration (Table 1). Eight mammals were found in the faecal samples of *P. molurus* (Table 2). Of the prey species identified from the samples, the Three-striped Palm-squirrel *Funambulus palmarum* was identified in eight out of the 11 samples contributing to at least 22% of prey individuals, followed by the Grey Mongoose *Herpestes edwardsii* as 20% ( $n = 7$ ), the Sambar Deer *Rusa unicolor* as 17.14% ( $n = 6$ ), the Spotted Deer *Axis axis* 14.28% ( $n = 5$ ), the Black-footed Gray Langur *Semnopithecus hypoleucos* 5.71% ( $n = 2$ ), the Jungle Cat *Felis chaus* 8.57% ( $n = 3$ ), the Sloth Bear *Melursus ursinus* 8.57% ( $n = 3$ ), and the Wild Boar *Sus*

*scrofa* 2.85% ( $n = 1$ ) in terms of percentage composition of individuals ingested by *P. molurus*. Feathers were present in one sample, which could not be identified to the genus or species level because of severe digestion. Feather types can be identified through DNA extraction; since this study is limited to morphology, the feather types were not identified up to the species level.

The variation in feeding of *P. molurus* shows higher prey species in September–November ( $n = 25$ ) as more (Table 2) faecal samples with identifiable prey remains were also found in these months (Figure 2), suggesting higher feeding activity during the post-monsoon period. A few instances of feeding were also observed from January–March. No feeding records were found for April–August and December.

## DISCUSSION

The predominance of mammals in the diet of *P. molurus* in the Moyar River Valley of southern India aligns with findings from other ecosystems, such as Keoladeo National Park in northern India (Bhupathy et al. 2014), for *P. bivitatus* in Everglades National Park in Florida (Snow et al. 2007), and the carpet python *Morelia spilota* in Australia (Slip & Shine 1988). Although prey composition varies based on regional faunal availability and habitat structure, mammals consistently form an important component of the diets of other pythons, with large individuals capable of taking large prey (Pope 1961; Branch & Hacke 1980). Avian prey has always been a part of the diet of giant snakes, including pythons (Snow et al. 2007; Bhupathy et al. 2014). In the present study, the lower occurrence of avian prey may reflect either feeding preference, habitat use or the greater digestibility of feathers relative to hairs. One python was observed feeding on poultry (quail &

**Table 1.** Prey species and their detailed hair characteristics, analysed from faecal samples of *Python molurus* in Moyar River Valley, India.

Prey species (Scientific names)	Prey species (Common names)	Medulla pattern	Scale pattern (mid)	Colour of hair	Cuticular surface (medial margins)
<i>Axis axis</i>	Spotted Deer	wide simple	regular wave	light brown	smooth
<i>Rusa unicolor</i>	Sambar Deer	wide simple	irregular wave	brown	crenate
<i>Semnopithecus hypoleucos</i>	Black-footed Gray Langur	simple fragmented	irregular wave	white	crenate
<i>Felis chaus</i>	Jungle Cat	Simple	irregular wave	grey brown	crenate
<i>Herpestes edwardsii</i>	Grey Mongoose	simple	irregular wave	banded (black and white bands)	crenate
<i>Melursus ursinus</i>	Sloth Bear	narrow simple	irregular wave	black	crenate
<i>Sus scrofa</i>	Wild Boar	narrow simple	irregular wave	brown	rippled
<i>Funambulus palmarum</i>	Three-striped Palm Squirrel	multiseriate	regular wave	brown	crenate

chicken) before faecal collection, suggesting potential anthropogenic feeding opportunities. The low incidence of birds in the diet may also reflect the frequency of arboreal activity as reported by Slip & Shine (1988) in their study on the Australian carpet pythons *Morelia spilota* complex. Use of microhabitats such as burrows, dry bushes, rock crevices, and water bodies by pythons inhabiting the Moyar River valley region (Vishnu et al. 2023b) may facilitate opportunistic predation and access to a broader prey base.

Typical food items consumed by the *P. molurus* include fishes, amphibians, reptiles like lizards and snakes, birds, and mammals (Ernst & Zug 1996). In the Moyar River valley region, *Funambulus palmarum* was found to be the principal prey species of *P. molurus*, having a maximum share. The frequent occurrence of *F. palmarum* in the faecal samples may reflect significant spatial overlap in microhabitats shared by the python and this species. The abundance of small to medium-sized rodents and lagomorphs makes them prime candidates for prey (Murphy & Henderson 1997). *Herpestes edwardsii* was the second most consumed species, which could be attributed to the burrows commonly used by Pythons as well. Pythons have been observed sharing the same burrows with porcupines

*Hystrix* sp. and other small mammals (Bhupathy et al. 2014). The presence of hairs of large mammalian species such as Sloth Bear Sambar Deer, and Spotted Deer in the samples suggests possible predation or scavenging, though confirmation of active predation requires further evidence such as kill observations or telemetry data. Similar predation on large mammals by pythons has been reported from studies in India, USA (non-native), and Indonesia (Fredriksson 2005; Snow et al. 2007; Bhupathy et al. 2014). Occurrence of species such as *M. ursinus*, *F. chaus*, and *S. scrofa* is almost similar to that reported by Bhupathy et al. (2014) from northern India.

Prey intake peaked between September–November, consistent with post-monsoon prey availability (Figure 2). These results are consistent with the seasonal variation reported in northern India at Keoladeo National Park, Rajasthan, by Bhupathy et al. (2014). No feeding incidents occurred in the colder month of December, corresponding to the start of mating season when snakes are found near ground burrows, basking in the sun (Ramesh & Bhupathy 2010). Reproductively active snakes typically stop feeding during the breeding season, as feeding is inconsistent with reproductive activities, a pattern seen in colubrids (Shine et al. 2003), viperids, and pythonids (Aldridge & Brown 1995; Madsen & Shine

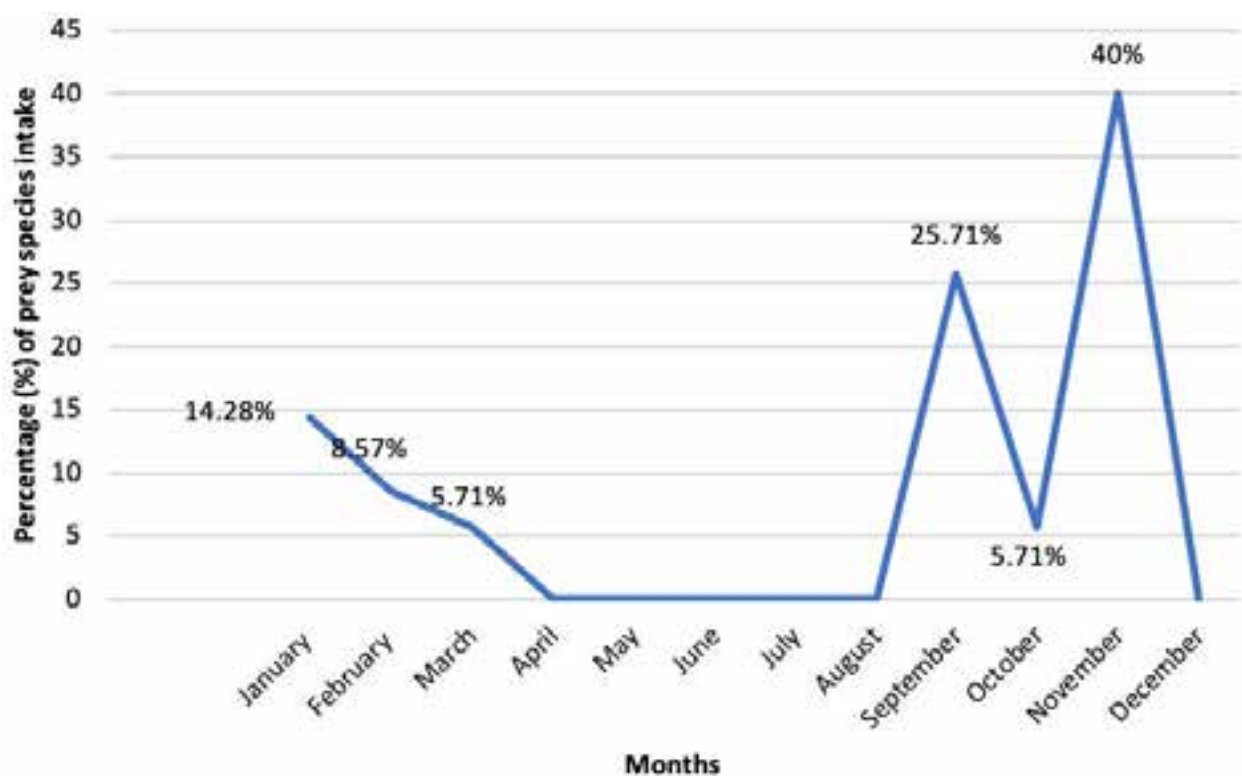


Figure 2. Monthly feeding trends of *P. molurus* as noted through frequency of faecal samples.

**Table 2. Month- and species-wise break-up of prey intake of *Python molurus* studied in Moyar River Valley, southern India.**

Month	No. of samples	Counts / sample	<i>Axis axis</i>	<i>Rusa unicolor</i>	<i>Semnopithecus hypoleucos</i>	<i>Felis chaus</i>	<i>Herpestes edwardsii</i>	<i>Melursus ursinus</i>	<i>Sus scrofa</i>	<i>Funambulus palmarum</i>
January	2	5	1	1	0	0	1	0	0	2
February	1	3	0	1	0	0	1	0	0	1
March	1	2	0	1	0	0	1	0	0	0
April	0	0	0	0	0	0	0	0	0	0
May	0	0	0	0	0	0	0	0	0	0
June	0	0	0	0	0	0	0	0	0	0
July	0	0	0	0	0	0	0	0	0	0
August	0	0	0	0	0	0	0	0	0	0
September	2	9	1	1	1	2	2	1	0	1
October	1	2	1	0	0	0	1	0	0	0
November	4	14	2	2	1	1	1	2	1	4
December	0	0	0	0	0	0	0	0	0	0
<b>Total</b>	<b>11</b>	<b>35</b>	<b>5</b>	<b>6</b>	<b>2</b>	<b>3</b>	<b>7</b>	<b>3</b>	<b>1</b>	<b>8</b>

1993). Feeding may resume to a limited extent during the mating months of January and February (Ramesh et al. 2019), as few faecal samples were recorded during these months, suggesting some individual variation in reproductive or foraging behaviour (Figure 2). The absence of samples during the summer months (April–June) is consistent with reduced feeding, possibly due to warmer and drier conditions, partial aestivation, and breeding activities (Ramesh & Bhupathy 2010).

Cessation of feeding is recorded in different python species during the breeding season, demonstrating a behavioural trade-off, as foraging is incompatible with incubation and egg development (Slip & Shine 1988; Ramesh & Bhupathy 2010). These activities may also reflect adaptive strategies for temperature regulation and reproductive success. Occasional reports of fruit remains (e.g., Indian Jujube *Ziziphus jujuba*) in python's stomach contents may result from secondary ingestion via herbivorous prey and not direct frugivory (Wall 1912; Pope 1961; Bhupathy et al. 2014). Our data shows that mammals are the key prey of the Indian Rock Python in southern India, which is consistent with similar studies by Ernst & Zug (1966) and Snow et al. (2007). Pythons here prefer mammals of different sizes, from Sambar Deer to Three-striped Palm Squirrels. This variation could be due to different prey distributions in the different habitat types, seasonal variations and population abundance of the prey species (Smith & Vrieze 1993; Madsen & Shine 1999; Snow et al. 2007). Studies on the feeding habits of large snakes like pythons are crucial to determine their preying technique, energy intake, feeding habits, prey ecology and ecological value. Thus, detailed studies on

the foraging ecology and prey size range for the different python species are important.

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## INTRODUCTION

*Tanygnathus* and *Prioniturus* (Psittaculidae) are genera of Old World parrots restricted to the Philippines and Indonesia. The genus *Tanygnathus* is currently represented by five species, with three occurring in the Philippines. Meanwhile, *Prioniturus* consists of 10 species, seven of which are endemic to the Philippines (Billerman et al. 2022). Despite their charismatic appeal, information on the breeding and diet for both genera remains limited, as they are particularly challenging to study due to their inconspicuous and highly elusive nature, along with their rarity and cavity-nesting behaviour — with even large parrots now absent from virtually intact habitats (Española et al. 2013). Breeding and diet are fundamental components of a species' ecological niche. Together, they offer valuable insights into a species' natural history that can be of significant conservation importance, especially for threatened and poorly known taxa. Unfortunately, knowledge of breeding biology remains severely limited for tropical forest bird species in Southeast Asia (Xiao et al. 2016). This gap is particularly pronounced among specialized breeders such as cavity-nesting birds in Asia (Cornelius et al. 2008; Cockle et al. 2012; Lammertink 2014), especially in the Philippines (Gicaraya & Española 2024a; Gicaraya et al. 2025). Similarly, avian diet remains poorly documented in the Philippines, mirroring a broader trend across tropical Asia (Sankamethawee et al. 2011). Dietary observations exist for a few species such as those compiled in Birds of the World (Billerman et al. 2022), with fewer species having dedicated field-based observations as in the case of the threatened Philippine Cockatoo *Cacatua haematuropygia* (Widmann et al. 2001) and hornbills (Gonzalez 2007; Widmann et al. 2015; Gicaraya & Española 2024b). Nonetheless, comprehensive studies are still lacking.

Luzon Island, located in northern Philippines, is home to several large parrots, including the endangered Green Racquet-tail *Prioniturus luconensis* and the near-threatened Blue-naped Parrot *Tanygnathus lucionensis* (Española et al. 2013; Allen 2020). The Green Racquet-tail (GRT), endemic to the island, appears monotypic but is sexually dimorphic, with males appearing yellowish whereas females are uniformly greener. Most information about its genus is based on a few species (Billerman et al. 2022): Blue-crowned Racquet-tail *P. discurus* with breeding observations in central (Negros-Panay) and southern (Greater Mindanao) Philippines, Yellow-breasted Racquet-tail *P. flavicans* in Sulawesi (Walker & Seroji 2000), and Buru Racquet-tail *P. mada* in

Buru, South Moluccas, Indonesia.

The Blue-naped Parrot (BNP), formerly widespread across the Philippines, is now limited to a few islands in the archipelago. This large, green parrot is distinguished by its red beak and yellow-and-blue wing feathers. It has been reported to breed from April to June in tree cavities (Collar et al. 2020) with further breeding data remaining scarce. As with most *Tanygnathus* parrots, information on the genus is largely limited to a handful of breeding observations in the wild and in captivity (Billerman et al. 2020).

In this paper, we aim to provide insights on the biology and ecology of the above-mentioned parrots in Luzon and acquire baseline information for their conservation through field observations and citizen science. Citizen science serves as a useful tool for studying diverse taxa, offering opportunities to acquire data on specific species across broad temporal and spatial scales—efforts that would otherwise be challenging to achieve through traditional field observations alone (Hall et al. 2021). Citizen science has been instrumental in elucidating the natural history of lesser-known species and in understanding aspects of a species' ecology (Lees & Martin 2015; Zeng et al. 2018; de Souza et al. 2022; Díaz et al. 2024). In recent years, social media platforms such as Facebook have emerged as a valuable platform for citizen science. Through this social media platform, researchers were able to improve our knowledge of cryptic species distributions (Tabeta & Bejar 2025), enable the crowdsourcing of specimens to collect historical records and biological data (O'Connell et al. 2025), and establish an early warning system for potential new species invasions (Marcenò et al. 2021), demonstrating the potential of the platform as a data-rich hub that can be tapped for biodiversity research. Here, we present the breeding behavior of the parrots through field observations, ascertain their diet through citizen science, and determine their nesting phenology by piecing together data from field observations and citizen science to arrive at a comprehensive picture of the two parrots' breeding cycle.

## MATERIALS AND METHODS

### Study Site

Breeding observations were conducted at the Subic Watershed Forest Reserve (SWFR), in the provinces of Bataan and Zambales, Luzon Island, Philippines. SWFR is a semi-evergreen lowland forest situated west of Luzon Island. It is characterized by vegetation dominated by

dipterocarps primarily *Shorea contorta* and deciduous tree species *Parkia timoriana*, *Pterocymbium tinctorium*, and *Dracontomelon dao* (Fernando et al. 2008; Gicaraya et al. 2025). Wherever the canopy is open, thick bamboo thickets emerge. Subic Watershed Forest Reserve is a mosaic of open and closed canopy forests, interspersed with industrial zones, agricultural, and residential areas. The area has rugged terrain with elevations ranging 0–300 m. West Luzon has distinct dry and wet seasons occurring in January–May and June–December of every year, respectively. Precipitation typically ranges 50–100 mm in the dry season and 400–800 mm in the wet season (Corporal-Lodangco & Leslie 2017).

### Fieldwork Procedures

A thorough nest hole search was undertaken in all accessible areas of the SWFR, along pre-existing and off-trail routes, for two consecutive breeding seasons—February–June 2022 and March–August 2023—with efforts made to survey the site as comprehensively as conditions allowed. Following Gicaraya & Española (2023) and Gicaraya et al. (2025), trees were inspected for cavities either by tracking flying and calling parrots or by observing potential nest trees for breeding-related activities through a pair of 10 × 32 Solognac binoculars. In addition, we interviewed local people and wildlife photographers for previously active nests that we could monitor for possible occupancy. We confirmed active breeding from sightings of parrots staying inside the cavity for prolonged periods of time during the day. Focal nests per species were observed for breeding-related activities in a blind of trees at least 50 m from the nest tree using a KOWA spotting scope (TSN-60). Per observation period, we took note of the general diurnal breeding behaviour, including visible activities within the nest cavity, activities surrounding the nest tree, and when possible, sex roles and parental care strategies. We categorized activities into six types: peeking, preening, out of the nest, feeding, calling, and no visible activity. We then recorded the number of hours spent on each activity per observation day and calculated their respective proportions to understand how these activities vary and change throughout one nesting period. Unfortunately, only the BNP proceeded with breeding between the two parrot species among nests located. Most breeding-related data for the GRT, as well as diet information for both species, were obtained through citizen science.

### Citizen Science Data Collection

To gather ecological data on the parrots, citizen

science data was used. Photographs and videos of GRT and BNP were sourced from two citizen science platforms—eBird and Facebook. In these platforms, we filtered available media from January 2010 to January 2025. In eBird, we clicked on the ‘Explore’ bar and typed in ‘*Prioniturus luconensis*’ and ‘*Tanygnathus lucionensis*’. We filtered the location to “Philippines” and manually searched for any media of feeding parrots located. In Facebook, we clicked on the search bar and typed ‘Green Racquet-tail/Green Racket-tail’, ‘Blue-Naped Parrot’, ‘*Prioniturus luconensis*’, ‘*Tanygnathus lucionensis*’ and ‘*loro/pikoy/kalangay*’ and manually examined photos of feeding parrots posted as public by bird enthusiasts or photographers. We also examined photos from Facebook groups dedicated to birdwatching and wildlife photography: ‘Wildlife Photographers of the Philippines’, ‘Philippine Bird Photography Forum-FB Group’, ‘Birdwatch Philippines Community’, and ‘Wild Bird Photographers of the Philippines’. Administrators of these groups require members to post photographs with date taken and general location, nonetheless, we validated the said information from the content owner through Facebook Messenger. We also asked each content owner for additional observations, further fruit/tree descriptions, and possible identity of the fruit sample in their photo. Similarly, for photos with a parrot perched on a fruiting tree, we asked the content owner if the bird was feeding on it before or after the photo was taken. All plants were verified and identified to the lowest taxonomic level by an expert botanist from the University of the Philippines Diliman.

Similarly, we tried to construct the nesting phenology of both parrots using similar methodology as stated above. We searched for photos of parrots in any stages of breeding: copulation, nest building, activity surrounding a nest, feeding of nestlings in a cavity, adults feeding fledglings, or sightings of fledglings. We took note of the date when the photos were taken and had the media owners verify their authenticity and asked for any other observations regarding their record.

## RESULTS

### BREEDING BEHAVIOR

#### Green Racquet-tail (GRT)

We observed a pair of GRT visiting Nest A on 28 May 2022 (Image 1a) and Nest B on 6 March 2023 (Image 1b). Both nests were woodpecker-made cavities excavated on dead trees. In both nests, we observed cavity

inspection behaviour in which the female entered and exited the cavity nest multiple times while maintaining vocal contact with the male. The male parrot did not enter the cavity and was perched only in a nearby tree. Nest inspection lasted 3–20 minutes after which the pair left the site together. A summary of the key behaviours is presented in Table 1. Unfortunately, both pairs did not proceed with nesting.

Copulation attempts of a different pair were observed on 02 March 2023. Fledglings with short spatules were also observed within the site on 3 April and 23 April. For both dates, two juveniles were seen with the adults, suggesting a brood size of two. During these dates, it was noticeable that the juveniles were more vocal than the

adults. We also observed an adult racquet-tail feeding a juvenile on 23 April 2023.

#### Blue-Naped Parrot (BNP)

We located and observed two nests of actively breeding BNP, both in woodpecker-excavated cavities on dead trees. On 27 March 2022, we observed a BNP occupying Nest C. The parrot remained inside the cavity for most of the day and only left upon the arrival of one or two other adult parrots. During these interactions, the pair or group flew to a nearby tree, where the nesting parrot was fed through regurgitation. Copulation was also observed, during which the nesting parrot was mounted, thereby confirming it as the female.



**Image 1.** Green Racquet-tail was seen inspecting a woodpecker-excavated cavity on a—28 May 2022 | b—06 March 2023. © Vince Angelo G. Gicaraya.

**Table 1.** Key behavioural observations in two nests of GRT. Effort in hours (h) pertains to observation effort per day.

Nest code	Date	Effort (h)	Key behaviors	Breeding stage
A	28 May 2022	6	Female inspected cavity for 20 minutes; entered/exited multiple times; male perched on a nearby tree; both birds vocalizing. The pair departed together after inspection.	Nest establishment, Pre-egg-laying
A	29 May, 03 June 2022	6	No activity observed.	Nesting did not proceed
B	6 March 2023	2	Female inspected cavity for 15 minutes; entered/exited; both birds vocalizing. The pair departed together after inspection.	Nest establishment, Pre-egg-laying
B	3 April 2023	2	Female inspected cavity for 3 minutes; both birds vocalizing. The pair departed together after inspection.	Nest establishment, Pre-egg-laying
B	18 May 2023	2	No activity observed.	Nesting did not proceed
B	June 2023	0.5	Nest was found felled.	



**Image 2.** Blue-naped Parrot activities in and surrounding an active nest: a—parrots copulating on a nearby tree | b—male parrot (top) feeding the female parrot (below) after copulation | c—female parrot peeking through the cavity entrance after the arrival of the male. © Vince Angelo G. Gicaraya.

**Table 2.** Key behavioural observations in two nests of BNP. Effort in hours (h) pertains to observation effort per day. Code: U = undetermined.

Week	Nest code	Date	Effort (h)	Key observations	Breeding stage
U	C	27 March 2022	8	Parrot stayed most of the time throughout the day. Nesting parrot flew to a nearby tree and was met by an adult parrot which fed it through regurgitation. Feeding lasted 5 minutes.	Courtship, Pre-egg-laying
U	C	31 March 2022	8	Two adult parrots visited the nesting parrot. The small group flew to a nearby tree. One parrot copulated with the nesting parrot twice lasting 3 minutes. Male fed the female afterwards through regurgitation.	Copulation, Pre-egg-laying
1	D	02 & 03 March 2023	24	Six parrots socializing on the nest tree. Cavity inspection by an adult parrot. Two adult parrots attempted to copulate but failed. Multiple nest visitations by the male in the afternoons. Successful copulation (3-4 minutes) in two consecutive days. Post-copulatory feeding (2-10 minutes).	Courtship, Nest establishment, Copulation, Pre-egg-laying
4	D	22 March 2023	12	Female parrot mostly stayed inside the cavity nest. Left only thrice that day, usually accompanied by the male. Female parrot was seen peeking through the cavity opening with either a fruit or leaf.	Onset of incubation; high nest cavity attendance
6	D	3 April 2023	12	Female had ruffled belly feathers. Nesting female was only out twice, joined by male. Male fed the female. Male visited twice briefly.	Brooding behavior evident; high nest cavity attendance
12	D	18 May 2023	12	Female returned with messy beak. Head movements inside the cavity nest suggest feeding of nestlings. Coleto <i>Sarcops calvus</i> briefly inspected the cavity then left.	Nestling stage; nestling provisioning behavior evident
18	D	15 June 2023	12	Two feeding events. Feeding was done by the female while perched at cavity rim. The nest was left unattended for 5.8 hours that day.	Nestling stage; nestlings likely older; reduced cavity nest attendance
22	D	3 July 2023	12	Female peeked through the cavity opening during Dollarbird <i>Eurystomus orientalis</i> visit (no usurpation). Regurgitation inside the cavity nest. Rapid exit-return movements (3x).	Late nestling to fledging stage; reduced cavity nest attendance
28	D	19 August 2023	12	No visible activity	Presumed fledging completed

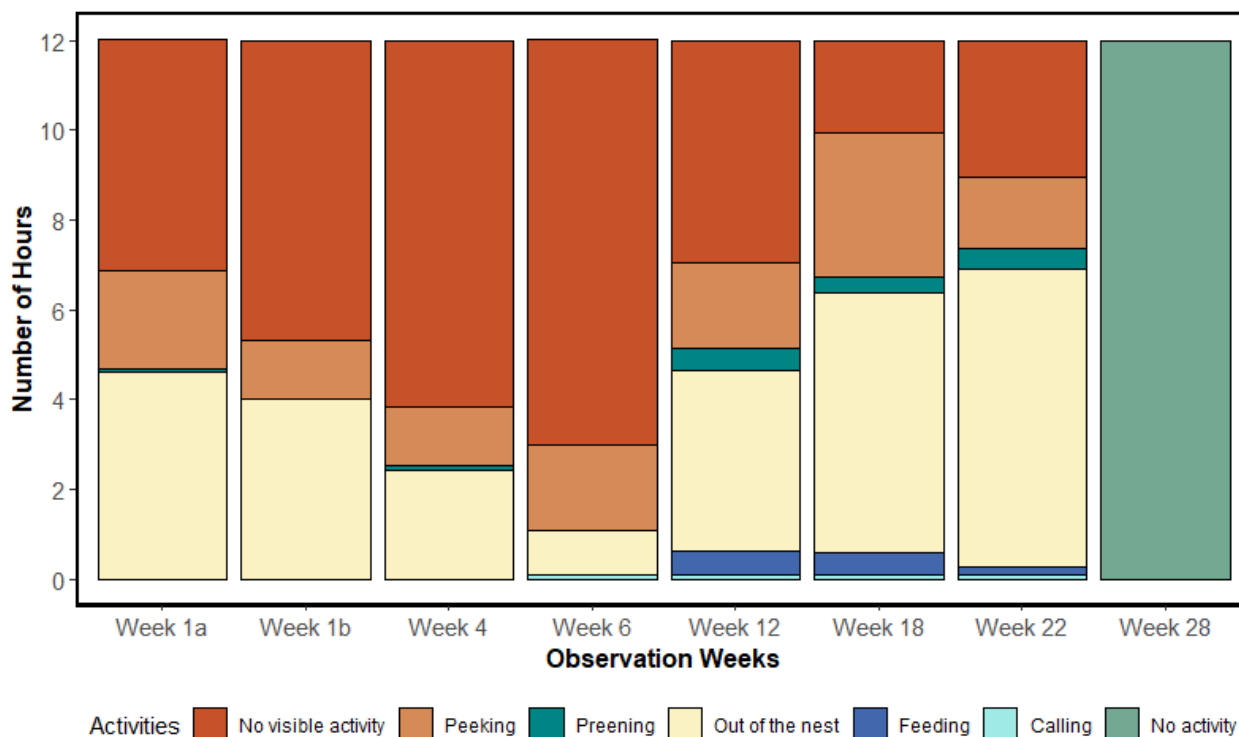


Figure 1. Diurnal activities of the female Blue-naped Parrot inside and surrounding an active nest observed from 0600 h to 1800 h.

The second nest, Nest D, was observed from March to August 2023, which we have observed from cavity inspection and courtship to fledging. Early observations were consistent with those previously recorded on Nest C, that is social behavior with a group of parrots, post-copulatory feeding, and male to female feeding through regurgitation (Image 2b). We also noted multiple copulation events in which the nesting parrot was mounted (Image 2a), thereby confirming it as the female. Nest attendance of the female parrot was highest during the first six weeks of observation (Image 2c, Figure 1), consistent with the onset of incubation and brooding periods. Later observations indicated chick provisioning by the female, evident from its messy mandibles, regurgitation behaviour, and increased time spent outside the nest (Figure 1). The male parrot was not observed provisioning for the brood but was consistently feeding the female and likely foraging with her in the later weeks of observation. By 19 August 2023, the nest had been completely vacated with the brood likely fledging between 4 July (Week 22) and 18 August (Week 28). A summary of the key behaviours is presented in Table 2.

#### Diet

We assessed 1,281 photos of parrots ( $n = 403$  for

GRT,  $n = 878$  for BNP) dating from 2012–2025 across two citizen science platforms, eBird and Facebook. eBird had the highest number of records but Facebook had the greatest number of unique photos (Table 3). Many eBird entries were deemed unusable in analyzing the nesting phenology as they do not bear dates, unlike records in human-moderated Facebook groups that have strict posting rules. Records of GRT only came from a single locality (SWFR) whereas records for BNP came from three (SWFR, Palawan, and Zamboanga). Nonetheless, despite having records from three localities, the majority of BNP photos (86%) analyzed came from SWFR.

We identified 33 plant species from 21 families consumed by both parrot species, 73% of which were native to the Philippines (Table 4). The GRT consumed 14 plant species, of which eight were exclusively recorded in its diet. BNP fed on 25 species, with 19 recorded solely in its diet. Both parrots were observed feeding on six shared plant species: *Mangifera altissima*, *M. indica*, *Commersonia bartramia*, *Macaranga tanarius*, *Mezoneuron latisiliquum*, and *Cratoxylum sumatranum*. Fruits were the most documented plant part used for consumption, followed by flower/flower buds, leaves/leaf buds, seeds, and bark (Image 3). Between the two parrots, only the GRT was recorded to consume leaf buds and seeds.

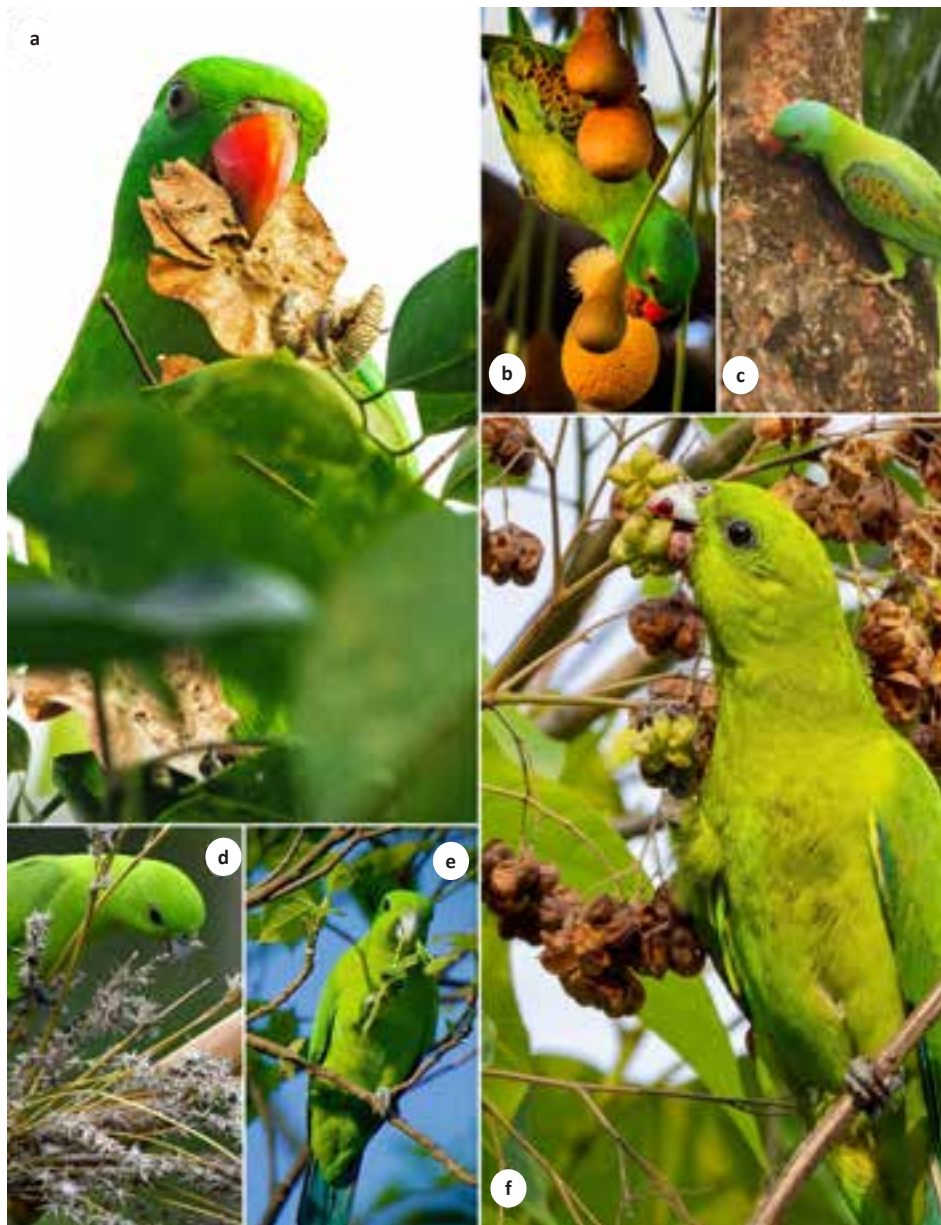


Image 3. Feeding records of parrots obtained through citizen science. Blue-Naped Parrot consuming: a—*Pterocarpus indicus* fruit | b—*Parkia timoriana* inflorescence | c—*Intsia bijuga* bark; Green Racquet-tail feeding on: d—bamboo seeds | e—*Ficus* sp. leaves | f—*Kleinhovia hospita* fruits. © a—Vinz Pascua | b—Caloy Dionela | c—Jorge De Ramos | d—Chris Chafer | e—Fidel Sy | f—Marilyn Gates.

### Nesting Phenology

We analyzed 24 photos of GRT and 23 photos of BNP in various breeding-related activities or stages of development to construct the nesting phenology of the parrots. Photos were classified into five categories: Adult in/inspecting nest, copulating, nesting, juvenile spotted, and adult feeding young. GRT has been recorded inspecting or in nests during both the dry and wet seasons (Figure 2a). Consequently, juveniles have been observed in almost all months of the year. In contrast, breeding activities of the BNP were recorded

only in the first half of the year, with copulation records coinciding with the dry season and sightings of juveniles recorded mostly towards or during the wet season (Figure 2a). These citizen science data agree with our field observations, which we have combined in Figure 2a.

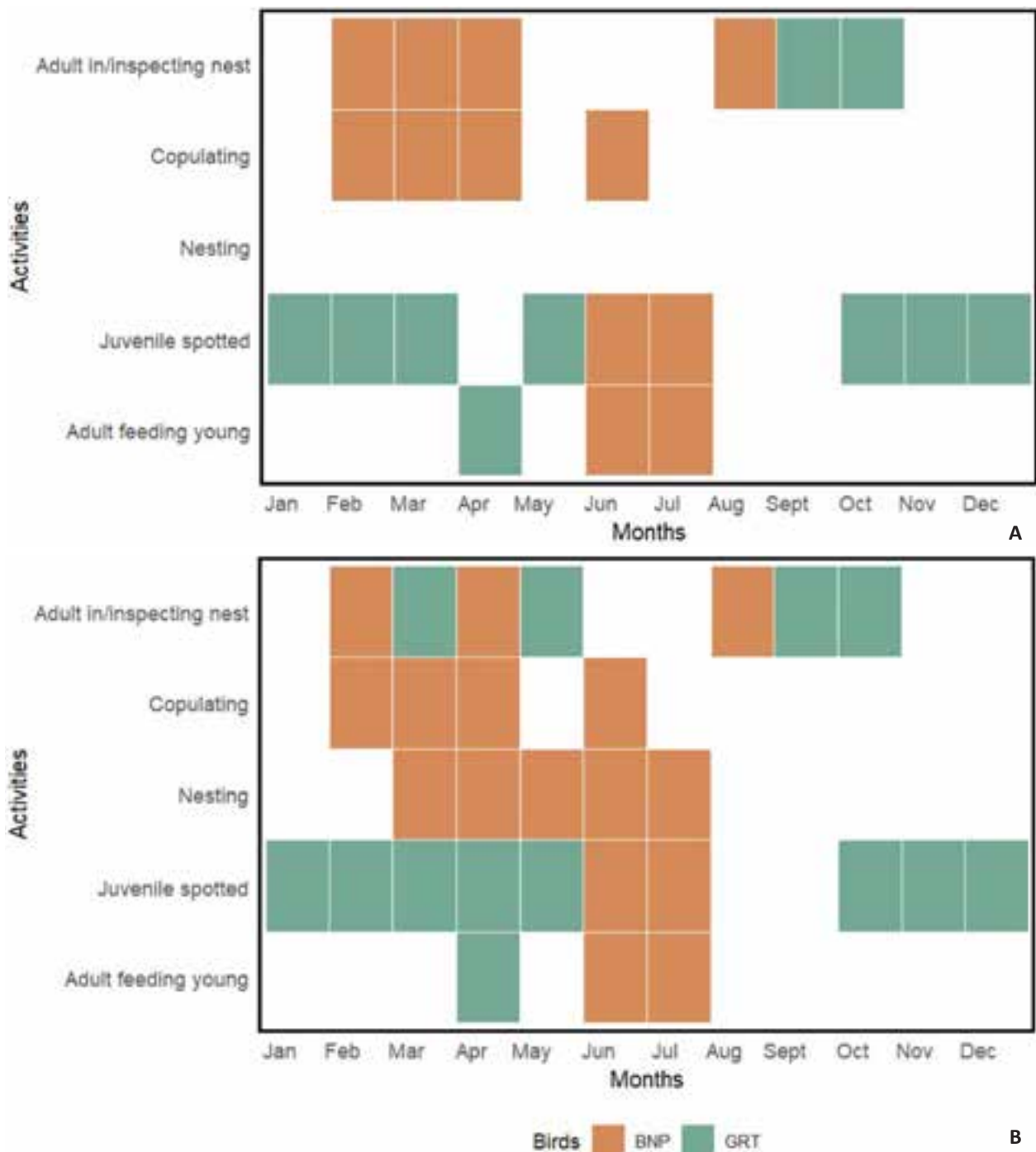


Figure 2. Nesting phenology of parrots constructed using: a—citizen science data | b—citizen science + field data.

## DISCUSSION

Old World parrots are generally monogamous, with both parents contributing to chick-rearing activities (Billerman et al. 2020); however, some species exhibit complex breeding systems like polygynandry for the Greater Vasa Parrot *Coracopsis vasa* (Ekstrom et al. 2007)

and polygynandry-polyandry for the Eclectus Parrot *Eclectus roratus* (Heinsohn 2008). Our findings suggest that BNP may potentially have a complex mating system, evident from observations of several visiting parrots that could either be mates or helpers. Parrots copulating with nesting females could also be different individuals; regrettably, our field methods did not allow for precise

**Table 3. Feeding and breeding records of parrots obtained in different citizen science platforms.**

Citizen science platform	GRT			BNP		
	Number of photos	Foraging	Breeding-related activities	Number of photos	Foraging	Breeding-related activities
eBird	195	3	0	512	12	0
Facebook- Birdwatch Community Philippines	83	8	9	118	11	5
Facebook- Wild Bird Photographers of the Philippines	79	8	8	95	22	8
Facebook- Philippine Bird Photography Forum-FB Group	19	1	7	48	13	6
Facebook- Wildlife Photographers of the Philippines	20	0	0	91	7	4
Facebook- Personal Public Post	1	1	0	2	2	0
Requested from Enthusiasts	6	6	0	12	12	0
Total	403	27	24	878	79	23

identification of individuals, despite our efforts to look for distinguishing marks or features from each visiting parrot. Future studies employing individual identification techniques are necessary to validate the species' mating system. Nonetheless, our observations of parrots partaking in multiple copulation events, males feeding the females through regurgitation, and biparental care are consistent with the typical reproductive behaviour of Old World parrots (Billerman et al. 2020). Sex roles appeared to be unequal, with most of the direct chick-rearing responsibilities undertaken by the female BNP. In addition, BNP exhibited an extended nesting period lasting  $\geq 4$  months, predominantly during the dry season. This observation agrees with the behaviour of other well-studied parrots such as the Lilac-Crowned Parrot *Amazona finsch* ( $\geq 3$  months, Renton & Salinas-Melgoza 2004) and Cape Parrot *Poicephalus robustus* (3–4 months, Wirminghaus et al. 2001), possibly as a response to suitability in cavity-nesting conditions and resource availability (Ekstrom et al. 2007).

In contrast, the GRT appears to breed all year-round, with an observed brood size of two. The species may be reliant on woodpecker-excavated cavities in dead trees as nesting sites, natural cavities in live trees (Realubit et al. 2022; Gicaraya et al. 2025), or even cavities in arboreal epiphytic ferns (Walker & Seroji 2000). Both our field and citizen science data failed to locate nests of actively breeding racquet-tails, underscoring their highly elusive and inconspicuous nature, as well as their aversion to human disturbance (Vince Angelo G. Gicaraya pers. obs. 2022). They are also likely susceptible to the presence of more aggressive cavity users, such as the Coledo *Sarcops calvus*, Dollarbird *Eurystomus orientalis*, or even congeners, as evidenced by their displacement by

Blue-crowned Racquet-tails *Prioniturus discurus* in parts of their range (BirdLife International 2024). Year-round nest surveys and telemetry studies may prove fruitful in elucidating the breeding biology of this species.

In terms of diet, our findings indicate that both parrots are mixed plant resource feeders, with BNP exhibiting a broader dietary range compared to GRT. Leaf and seed consumption by BNP was not recorded but may have occurred, possibly overlooked due to citizen scientists' limited ability to detect subtle feeding behaviours. Bark consumption was observed for both species but has not been reported yet for members of either genus. For well-studied parrots, this behaviour is often associated with foraging for invertebrates, sap, and cambium (O'Donnell & Dilks 1989; De Paula et al. 2017) or as a means to detoxify, digest, or absorb nutrients (de Araújo & Marcondes-Machado 2011). Moreover, it is worth noting that these parrots only consumed native plants (73% of records) from various plant families, underscoring the importance of native vegetation as food sources for native parrots. Nonetheless, both species were recorded consuming naturalized plants indicating a degree of dietary plasticity.

There are several caveats that should be considered when interpreting the results of this study. First, there is a geographic bias towards SWFR, as it is a popular and easily accessible birdwatching site for many enthusiasts. Second, although eBird contains numerous records, many lacked dates and were, therefore, unusable, particularly for analyzing nesting phenology. Third, although many of our findings agree with the behaviour of Old World parrots, increasing the number of nests for observation, along with the use of camera traps, could provide a sounder understanding of the species'

Table 4. Plant species consumed by parrots identified using citizen science.

Plant species	Conservation status	Family	Plant part	GRT	BNP		
				SWFR	SWFR	Palawan	Zamboanga
<i>Buchanania arborescens</i>	Native	Anacardiaceae	Fruit		x		
<i>Mangifera indica</i>	Native	Anacardiaceae	Fruit	x	x		
<i>Mangifera altissima</i>	Native	Anacardiaceae	Fruit	x	x		
<i>Polyscias nodosa</i>	Native	Araliaceae	Flower/peduncle		x		
<i>Spathodea campanulata</i>	Introduced	Bignoniaceae	Flower Bud		x		x
<i>Garuga floribunda</i>	Native	Burseraceae	Fruit			x	
<i>Commersonia bartramia</i>	Native	Byttneriaceae	Fruit	x	x		
<i>Kleinhovia hospita</i>	Native	Byttneriaceae	Fruit	x			
<i>Calophyllum inophyllum</i>	Native	Calophyllaceae	Fruit			x	
<i>Trema orientale</i>	Native	Cannabaceae	Fruit		x		
<i>Casuarina equisetifolia</i>	Native	Casuarinaceae	Fruit		x		
<i>Terminalia catappa</i>	Native	Combretaceae	Fruit				x
<i>Coccinia grandis</i>	Cryptogenic	Cucurbitaceae	Fruit		x		
<i>Tetracera</i> sp.	Native	Dilleniaceae	Flower Buds	x			
<i>Endospermum peltatum</i>	Native	Euphorbiaceae	Fruit		x		
<i>Macaranga tanarius</i>	Native	Euphorbiaceae	Fruit	x	x	x	
<i>Melanolepis multiglandulosa</i>	Native	Euphorbiaceae	Fruit		x		
<i>Intsia bijuga</i>	Native	Fabaceae	Bark		x		
<i>Leucaena leucocephala</i>	Introduced	Fabaceae	Flower Buds	x			
<i>Mezoneuron latisiliquum</i>	Native	Fabaceae	Fruit	x	x		
<i>Parkia timoriana</i>	Native	Fabaceae	Flower		x		
<i>Pterocarpus indicus</i>	Native	Fabaceae	Fruit		x		
<i>Tamarindus indica</i>	Introduced	Fabaceae	Fruit	x			
<i>Cratogeomys sumatranum</i>	Native	Hypericaceae	Fruit	x	x		
<i>Gmelina arborea</i>	Introduced	Lamiaceae	Fruit	x		x	
Unidentified	NA	Lamiaceae	Fruit		x		
<i>Ficus</i> sp.	Native	Moraceae	Leaf buds	x			
<i>Psidium guajava</i>	Introduced	Myrtaceae	Fruit		x		
<i>Syzygium cumini</i>	Introduced	Myrtaceae	Fruit	x			
<i>Bambusa</i> sp.	NA	Poaceae	Seeds	x			
<i>Guioa</i> sp.	Native	Sapindaceae	Fruit		x		
<i>Pterocymbium tinctorium</i>	Native	Sterculiaceae	Flower/Fruit		x	x	
<i>Cissus repens</i>	Native	Vitaceae	Fruit		x		
Unidentified	NA	NA	Bark	x			

breeding biology, particularly in estimating the nesting period and defining parental roles. Nonetheless, as a preliminary investigation, the current study was successful in four respects, in that it; 1) acquired a detailed account of the breeding biology of the BNP and its behaviour within and surrounding an active nest through field observations; 2) acquired insights on diet that could inform parrot-targeted habitat management;

3) constructed the nesting phenology of parrots using field and citizen science data; and 4) demonstrated how citizen science could supplement data from field observations and help acquire information on rare species, particularly when field observations are limited by time and resources. Our findings offer a valuable foundation for future research and conservation initiatives, particularly those aimed at enhancing feeding

opportunities for these rare and threatened parrots through habitat enrichment, development of nest protection measures, establishment of nest monitoring activities, and the meaningful engagement of citizen scientists in biodiversity research and conservation efforts.

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**Filipino:** Sa kabila ng kanilang popularidad at nanganganib na “conservation status”, ang mga loro sa ilalim ng genus *Tanygnathus* at *Prioniturus* ay nananatiling kulang sa pag-aaral. Ang kanilang pagiging madalang at mailap ay balakid sa pagkalap ng komprehensibong datos tungkol sa kanilang ekolohiya. Sa pananaliksik na ito, aming inilalahad sa unang pagkakataon ang “breeding behavior”, “diet”, at “nesting phenology” ng Blue-naped Parrot *Tanygnathus lucionensis* at Green Racquet-tail *Prioniturus luconensis* gamit ang mga nakalap na obserbasyon sa ilang at mga citizen science databases. Isinagawa ang mga obserbasyon sa Subic Watershed Forest Reserve mula Pebrero-Hunyo 2022 at Marso-Agosto 2023. Batay sa aming mga datos, ang *T. lucionensis* ay may mahabang breeding period, paulit-ulit o maraming beses na pagtatalik, pagpapakain ng lalaki sa babae sa pamamagitan ng regurgitation, at pag-aalaga ng parehong magulang sa mga supling—mga katangiang naaayon sa gawi ng mga Old World parrots. Gayunpaman, walang natagpuang aktibong pugad ng *P. luconensis* sa panahon ng pag-aaral sa field. Bilang suplemento, sinuri namin ang 1,281 rekord ng mga lorong ito sa eBird at Facebook mula Enero 2012 hanggang Enero 2025. Aming napag alaman na ang *P. luconensis* ay nagpaparami buong taon, samantalang ang *T. lucionensis* naman ay sa mga buwan ng tag-init. Ang parehong loro ay kumpirmadong kumakain ng iba’t ibang bahagi ng halaman, partikular na ng mga prutas, bulaklak, dahon, at maging balat ng puno. Kapansin-pansin na 73% ng mga rekord ay mula sa mga katutubong puno, pagpapatunay ng kahalagahan ng pagtatanim ng mga katutubong puno bilang mapagkukunan ng pagkain ng mga loro. Ang aming mga natuklasan ay nagbibigay ng mahalagang batayang impormasyon na maaring gamiting gabay para sa mga susunod na pananaliksik at mga programang pangkonserbasyon para sa mga ibong ito.



## INTRODUCTION

Tropical forests are among the most biodiverse and ecologically significant ecosystems, yet they are increasingly threatened by land-use change and fragmentation. One major driver of this fragmentation is the expansion of linear infrastructure (LI), which traverses landscapes in elongated forms, often bisecting habitats. This includes roads, railways, transmission lines, pipelines, and canals (Geist & Lambin 2002; Geneletti 2004; Laurance et al. 2014; Nayak et al. 2020). While LI play a vital role in economic development and connectivity (van der Grift et al. 2015), they also contribute to environmental degradation through habitat loss, increased wildlife mortality, and pollution (Forman & Alexander 1998; De Jonge et al. 2022; Ashwin et al. 2023). Avifauna are particularly vulnerable to LI through electrocution, collisions, and displacement (Bevanger 1998; Loss et al. 2014; van der Grift et al. 2015; Manigandan et al. 2022). While several studies have linked LIs to declines in biodiversity, including bird populations, some studies have also indicated that certain bird species may exploit LI corridors for foraging or perching (van der Grift et al. 2015) and nesting (Byju et al. 2023), highlighting the complexity of ecological responses to the LIs.

People's perceptions of such impacts are critical in shaping conservation and development strategies. Perceived risks and benefits are influenced by individual opinions, environmental knowledge, and sociodemographic factors such as age, education, and occupation (Kaczensky et al. 2004; Viklund 2004; Manigandan et al. 2024). People's perceptions, defined as how individuals interpret and evaluate environmental issues, can provide insights into local ecological knowledge and guide effective conservation interventions (Berkes et al. 2000; Huntington 2011; Bennett 2016) and identify knowledge gaps, plan awareness programs, and guide participatory approaches to conservation (Caily-Arnulphi et al. 2017; Champness et al. 2023).

Despite the recognized importance of perception studies in conservation, the views of local communities regarding LI impacts, especially on avifauna, remain underexplored in India. Particularly in Chhattisgarh, driven by the energy and mining sectors, little is known about how local communities perceive LI impacts on birds (Gajera et al. 2013). Projects such as thermal power plants, transmission lines, and railway corridors are transforming landscapes, raising concerns about ecological consequences and social acceptance. Such rapid development and intrusion of several LI

can have potential impacts on both people and the environment. Understanding LI's impacts on people and the surrounding environment is crucial for scientifically managing these impacts. There are very few systematic studies on birds in this region, and research on avifaunal responses to infrastructure expansion in Chhattisgarh is especially limited. This study, therefore, represents one of the first structured attempts to document community perceptions of bird impacts associated with major LI corridors in the state. Avifauna are particularly relevant in this context because birds are highly sensitive to habitat alteration, fragmentation, and electrocution or collision risks, making them strong ecological indicators of infrastructure impacts. Several stretches of the studied LI corridors pass through forest patches, agricultural landscapes, and open woodlands, where canopy removal, vegetation clearing, and disturbance have been reported. The heightened public awareness will lead to more effective conversation efforts geared towards lessening adverse consequences for both sides. Knowing more about people's views of the influence of LI could lead to better landscape and regional design and management. However, public perception alone cannot guide conservation or infrastructure planning and must be complemented with ecological assessments to ensure scientifically sound decisions.

## Study area

Four selected linear infrastructures in the state of Chhattisgarh, India, were surveyed for the cause: the Ranchi–Dharamjaigarh Transmission Line (765 kV S/C Power Grid Transmission Line), Champa–Kurukshehra (800 kV S/C Power Grid Transmission Line), Korba–Jabalpur (765 kV S/C Power Grid Transmission Line), and the East Rail corridor (Figure 1). These linear



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**Image 1.** Study area showing the Champa–Kurukshehra 800 kV single-circuit (S/C) power grid transmission line.

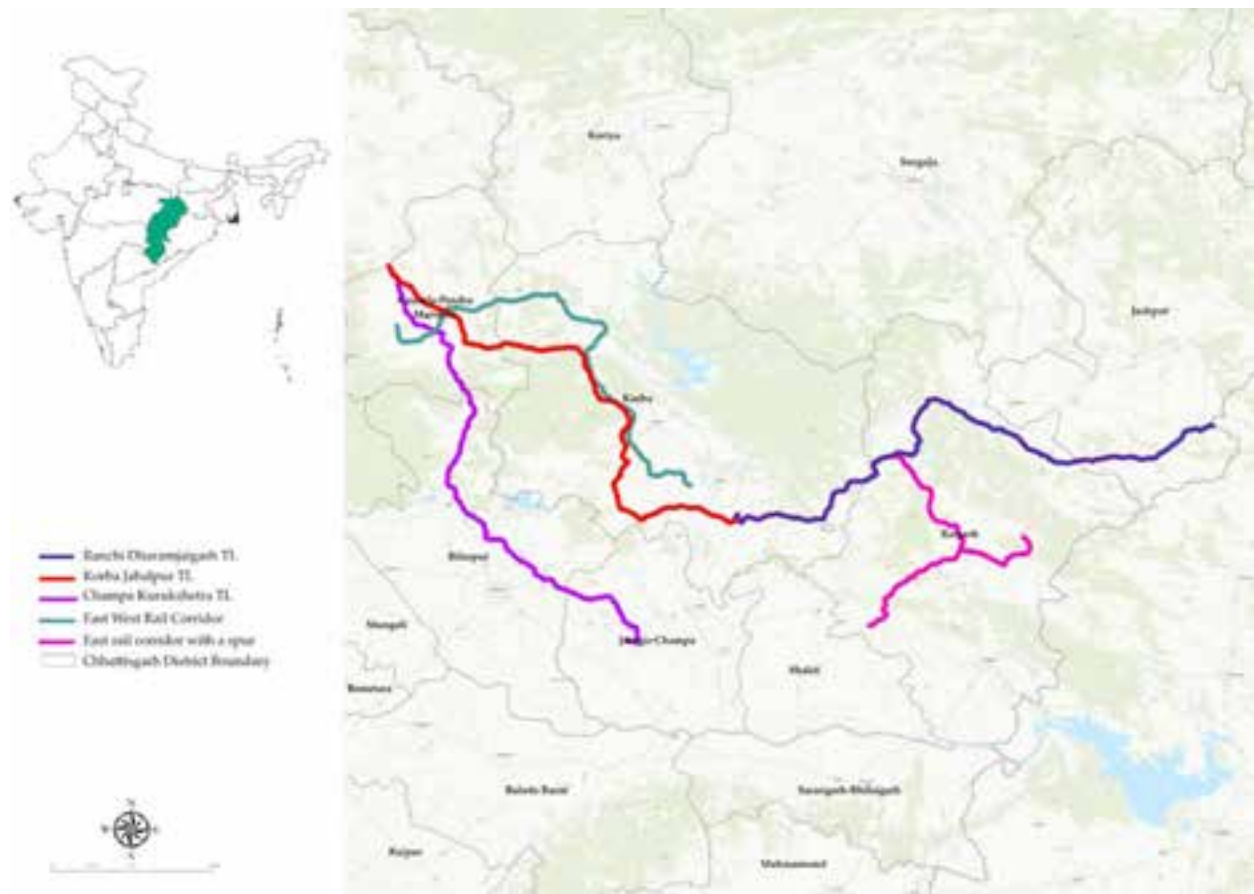


Figure 1. Study area map.

infrastructures intersect six districts in Chhattisgarh, namely Korba, Bilaspur, Gaurela-Pendra-Marwahi, Raigarh, Jangir-Champa, and Jashpur, with an approximate length of 711 km in total.

Chhattisgarh state covers 1,35,191 km<sup>2</sup>, accounting for 4.1% of the country's total area. The LI routes cut across predominantly tropical dry deciduous forests, characterised by *Sal Shorea robusta* and associated mixed deciduous species, classified as northern tropical dry mixed deciduous forests (5B/C2; Champion & Seth 1968) (Forest Survey 2021). Chhattisgarh is home to a varied population with diverse ethnic, social, and religious backgrounds. It has the highest tribal population among all Indian states; one-third of the people in the state are officially categorized as scheduled castes or scheduled tribes (Dixit et al. 2023). Chhattisgarh has a total population of 2,55,45,198 people, with 12,832,895 males and 12,712,303 females. The literacy rate in Chhattisgarh is 70.28%. Male literacy rates are 80.27%, while female literacy rates are 60.24% (Census 2011). Rural areas are home to 76.76% of the total population, and most of them are farmers who primarily depend on

paddy cultivation.

## Methods

A structured, close-ended questionnaire was designed to assess public perceptions of linear infrastructure (LI) impacts on avifauna, based on established guidelines, and expert review. The finalized survey comprised ten simple questions administered through face-to-face interviews, following Patton's (2002). Interviews, lasting 5–10 minutes, were conducted with 868 willing participants between October 2021 and July 2023 across 166 villages near selected LI routes in Korba, Bilaspur, Gaurela-Pendra-Marwahi, Raigarh, Janjgir-Champa, and Jashpur. Villages were selected based on proximity to LI to ensure locally grounded responses. Participants included a diverse group: farmers, students, government employees, housewives, business owners, and daily wage workers. Prior to interviews, participants were briefed on the study's objectives and verbal consent was obtained.

The questionnaire had two sections: (1) socio-demographic data (gender, age, education, occupation,

tribal affiliation, proximity to LI, and duration of residence) (Naha et al. 2014; Chin et al. 2019) and (2) perception of LI impacts on avifauna. In this study, the term 'perception' refers specifically to respondents' views on the impact of LI on avifauna, including perceived effects on bird mortality, behaviour, and habitat. While the questionnaire was developed in English and Hindi, most interviews were conducted in local dialects with field support. Close-ended formats were preferred for efficiency and analytical clarity.

To help participants accurately identify bird species, a photo-elicitation approach was used during interviews. Photographs of commonly occurring birds from the region were shown to respondents. In addition, the Merlin Bird ID application (Cornell Lab of Ornithology) was used to display high-resolution images and, when required, to play bird calls to aid recall and confirmation. Responses were categorized as positive, neutral, or negative based on participants' observations and opinions. Perception was quantified using a binary scoring system: "Yes" = 1 and "No" = 0, resulting in a cumulative score from 0–10 (Darawsheh 2020; Ruan et al. 2022). Scores were categorized into three groups for multinomial logistic regression: negative (0–3), neutral (4–6), and positive (7–10). Data categorization followed standard practices, and all the ethical guidelines were strictly adhered to throughout the study (Gubbi 2006).

### Data analysis

Analysis of qualitative data was done through content analysis (coding) or thematic analysis by categorizing themes according to the way they relate to research objectives and building relationships and implications as provided by Patton (2002). After data collection in the field, the data were organised, coded, classified, and tabulated using Microsoft Excel and descriptive statistics. In SPSS 23.0, data were cross-tabulated, and a chi-square test (notation:  $\chi^2$  df) was applied to all combinations of independent and dependent variables. To determine the factors that could predict the perceptions of people, a multinomial logistic regression model was fitted to the responses and was used to predict the probabilities of the different possible outcomes (Umaña-Hermosilla et al. 2020). Multinomial logistic regression utilizes maximum likelihood estimation to assess the likelihood of belonging to a specific category, allowing us to characterize the probability of a respondent's decision for a particular multinomial discrete choice, conditional on the values of the explanatory variables (Clark 2009; Umaña-Hermosilla et al. 2020). We use the multinomial function from the net package to estimate a multinomial

**Table 1. Respondent demographics.**

Demographic variables (M $\pm$ SD)	Categories	Frequency (Percentage) n = 868
Age (1.13 $\pm$ 0.86)	15–30 years	244 (28.1)
	31–45 years	295 (34)
	46–70 years	299 (34.4)
	> 71	30 (3.5)
Gender (0.22 $\pm$ 0.42)	Male	674 (77.6)
	Female	194 (22.4)
Tribe/non-tribe (0.52 $\pm$ 0.50)	Tribe	418 (48.2)
	Non-tribe	450 (51.8)
Education level (1.56 $\pm$ 0.78)	Uneducated	35 (4)
	Primary	434 (50)
	High school	281 (32.4)
	Graduate and above	118 (13.6)
Occupation (3.35 $\pm$ 1.58)	Business	16 (1.8)
	Farmer	436 (50.2)
	Government staff	34 (3.9)
	Homemaker	100 (11.5)
	Labour	174 (20)
	Students	108 (12.4)
Proximity to the LI (0.44 $\pm$ 0.52)	0–300 m	502 (57.8)
	>300–600 m	354 (40.8)
	>600–900 m	12 (1.4)
Years of residency (0.66 $\pm$ 0.58)	0–30 years	343 (39.5)
	>30–60 years	476 (54.8)
	>60–90 years	49 (5.6)

logistic regression model in R.

### Respondent demographics

Most of the respondents (34.4%) were in the age group of 46–70, followed by 31–45 years (34%), 15–30 years (28.1%), and more than 70 years old (3.5%). Occupation-wise, 50% were farmers. Respondents were predominantly male (77.6%) since most of the female participants were reluctant to respond. In terms of tribal affiliation, 51.8% were non-tribal and 48.2% tribal. Education levels varied: 50% had primary education, 32.4% high school, 13.6% graduate or above, and 4% were uneducated. Regarding proximity to LI, 57.8% lived or owned land within 0–300 m, and 40.8% within 301–600 m. A majority (54.8%) had resided in the area for 31–60 years (Table 1).

## RESULTS

### Participant's response – summary

The study assessed public perceptions of LI impacts on avifauna. Overall, 56.6% of respondents perceived LI as having a negative effect on local bird populations, while 43.4% did not. A decline in common bird species post-installation was noted by 51.7%, whereas 48.3% reported no such change. Regarding migratory birds, 41.5% observed a decline, while 58.5% did not. Concerns about bird electrocution or collision were raised by 43.5% of respondents. Only 23.3% reported birds avoiding LI structures during flight, and 34.2% noted an increase in human–bird negative interactions after installation; 65.8% did not. A vast majority (91.6%) did not observe invasive plant proliferation post-installation. While 80.8% did not believe LI had positive effects on birds, 19.2% perceived some benefits. Increased sightings of birds of prey were reported by 10.7%, and 30.8% observed birds using LI pylons for perching, nesting, roosting, or foraging (Table 2).

### PEOPLE PERCEPTION

#### People's perception on the impact of LI on avifauna

Chi-square tests revealed significant associations between perception of LI impacts on avifauna and multiple socio-demographic variables (Table 3). Age was significantly associated with perception ( $p < 0.001$ ), with younger respondents (15–45 years) tending to be more neutral, while older groups (46+ years) expressed a mix

of views. Education level also influenced perceptions ( $p < 0.001$ ); uneducated individuals more frequently expressed negative views, whereas those with formal education showed more neutral or varied responses. Tribal affiliation was strongly associated with perception ( $p < 0.001$ ), with tribal respondents mostly neutral and non-tribal respondents more evenly distributed across categories. Occupation significantly affected perception ( $p < 0.001$ ), with labourers showing a slightly more positive outlook. Proximity to LI was also significant ( $p = 0.040$ ), with those living nearer expressing greater concern, though neutral views still dominated. Gender ( $p = 0.188$ ) and years of residency ( $p = 0.084$ ) were not significantly associated with perception.

### FACTORS DETERMINING THE PEOPLE'S PERCEPTION OF LI.

#### Multinomial logistic regression results for people's perception on the impact of LI on avifauna (Reference category: Neutral)

Multinomial logistic regression analysis (Table 4) revealed several significant predictors of perception. Individuals aged 30–45 had slightly lower odds of negative perception compared to neutral ( $\beta = -0.636$ ,  $p < 0.1$ ). Males were not significantly associated with negative perception responses but showed a significant negative association with positive responses ( $\beta = -0.544$ ,  $p < 0.1$ ), indicating that males were less likely to report positive perceptions. Non-tribal respondents had significantly higher odds of both negative ( $\beta = 1.212$ ,  $p < 0.01$ ) and positive ( $\beta = 0.858$ ,  $p < 0.01$ ) perceptions, suggesting that non-tribal individuals were more likely to express stronger opinions in either direction. High school-educated individuals had slightly lower odds of negative perception ( $\beta = -0.799$ ,  $p < 0.1$ ), while graduates and above had significantly lower odds ( $\beta = -1.163$ ,  $p < 0.01$ ). Labourers had increased odds of negative perception ( $\beta = 1.551$ ,  $p < 0.01$ ) and suggesting that labourers were more likely to express negative views. Proximity to LI was a strong predictor; individuals living closer to the LI (0–900 m) were significantly more likely to express negative views, with extremely high coefficients ( $\beta = 11.515$ ,  $p < 0.01$ ). Residency of 31–60 years showed slightly lower odds of negative perception ( $\beta = -0.493$ ), while those residing for 61–90 years had significantly higher odds of positive perception ( $\beta = -1.377$ ,  $p < 0.05$ ), suggesting that very long-term residents were less likely to express positive views.

**Table 2. Participant's response summary.**

	Variables	Yes	No
<b>People's perception on the impact of LI on avifauna</b>			
1	There is a negative impact of LI on the local Avifauna	491 (56.6%)	377 (43.4%)
2	Absence of regular/common bird species after the LI installation	449 (51.7%)	419 (48.3%)
3	Reduction in migratory birds after the LI installation?	360 (41.5%)	508 (58.5%)
4	LI is imposing significant threats to birds by Electrocution/Collision	378 (43.5%)	490 (56.5%)
5	Birds avoid LI during their flight	202 (23.3%)	666 (76.7%)
6	Human-wildlife conflict (birds) increased after the installation	297 (34.2%)	571 (65.8%)
7	Invasive plant species proliferation increased after the installation of LI	73 (8.4%)	795 (91.6%)
8	LI can positively affect the birds	167 (19.2%)	701 (80.8%)
9	Increased number of birds of prey after the installations	93 (10.7%)	775 (89.3%)
10	Birds utilising the LI pylon for perch, nest, roost, & foraging	267 (30.8%)	601 (69.2%)

**Table 3. Peoples' perception on the impact of LI on avifauna.**

People's perception on the impact of LI on avifauna		Negative (n)	Neutral (n)	Positive (n)	
Age	15–30 years	75 (30.7%)	95 (38.9%)	74 (30.3%)	$\chi^2 = 25.569$ , df = 6, p = 0.000
	31–45 years	57 (19.3%)	142 (48.1%)	96 (32.5%)	
	46–70 years	75 (25.1%)	102 (34.1%)	122 (40.8%)	
	> 71	11 (36.7%)	15 (50.0%)	4 (13.3%)	
Gender	Male	58 (29.9%)	77 (39.7%)	59 (30.4%)	$\chi^2 = 3.345$ , df = 2, p = 0.188
	Female	160 (23.7%)	277 (41.1%)	237 (35.2%)	
Tribe/non-tribe	Tribe	69 (16.5%)	224 (53.6%)	125 (29.9%)	$\chi^2 = 60.369$ , df = 2, p = 0.000
	Non-tribe	149 (33.1%)	130 (28.9%)	171 (38.0%)	
Education level	Uneducated	17 (48.6%)	10 (28.6%)	8 (22.9%)	$\chi^2 = 25.696$ , df = 6, p = 0.000
	Primary	102 (23.5%)	168 (38.7%)	164 (37.8%)	
	High school	57 (20.3%)	133 (47.3%)	91 (32.4%)	
	Graduate and above	42 (35.6%)	43 (36.4%)	33 (28.0%)	
Occupation	Business	2 (12.5%)	8 (50.0%)	6 (37.5%)	$\chi^2 = 38.216$ , df = 10, p = 0.000
	Farmer	86 (19.7%)	183 (42.0%)	167 (38.3%)	
	Government staff	11 (32.4%)	12 (35.3%)	11 (32.4%)	
	Homemaker	31 (31.0%)	46 (46.0%)	23 (23.0%)	
	Labour	57 (32.8%)	49 (28.2%)	68 (39.1%)	
	Students	31 (28.7%)	56 (51.9%)	21 (19.4%)	
Proximity to the LI	0–300 m	95 (21.4%)	184 (41.5%)	164 (37.0%)	$\chi^2 = 10.038$ , df = 4, p = 0.040
	301–600 m	122 (29.5%)	164 (39.7%)	127 (30.8%)	
	601–900 m	1 (8.3%)	6 (50.0%)	5 (41.7%)	
Years of living in the locality	0–30	65 (19.0%)	53 (15.5%)	225 (65.6%)	$\chi^2 = 8.228$ , df = 4, p = 0.084
	31–60	102 (21.4%)	57 (12.0%)	317 (66.6%)	
	61–90	13 (26.5%)	1 (2%)	35 (71.4%)	

**Table 4. Multinomial logistic regression results for people's perception on the impact of LI on avifauna.**

Dependent variable	Negative (Odds Ratio)	Positive (Odds Ratio)
Age (31–45)	-0.636* (-0.334)	-0.495 (-0.304)
Gender (Male)	-0.4 (-0.306)	-0.544* (-0.288)
Non-tribe	1.212*** (-0.198)	0.858*** (-0.176)
Education (High school)	-0.799* (-0.446)	0.334 (-0.51)
Education (Graduate and above)	-1.163** (-0.47)	0.03 (-0.524)
Occupation (labour)	1.551* (-0.834)	0.725 (-0.591)
Proximity to the LI (0–300 m)	11.306*** (-0.385)	-0.359 (-1.317)
Proximity to the LI (301–600 m)	11.515*** (-0.384)	-0.599 (-1.319)
Proximity to the LI (601–900 m)	10.296*** (-0.882)	-0.493 (-1.454)
Years of living in the locality (31–60)	-0.493* (-0.267)	-0.295 (-0.235)
Years of living in the locality (61–90)	0.452 (-0.51)	-1.377** (-0.6)
Constant	-12.041*** (-0.794)	0.195 (-1.557)

AIC (Akaike information criterion) value—1,786.93 | \*— $p < 0.1$  | \*\*— $p < 0.05$  | \*\*\*— $p < 0.01$ .

## DISCUSSION

This study reveals the multifaceted impacts of LI on avifauna, with respondents expressing mixed but predominantly neutral to negative perceptions. Key concerns include bird mortality from collisions and electrocutions, consistent with earlier studies (Bevanger 1998; Raman 2011; Loss et al. 2014; Serratos et al. 2024). Environmentally conscious respondents emphasize the need for ecological integration in infrastructure planning (Kaltenborn & Bjerke 2002). Socio-demographic factors significantly influence perceptions. Younger individuals tend to be neutral, likely due to limited experience (Milfont et al. 2010), while tribal affiliation correlates with more neutral or positive views, reflecting cultural influences (Shelley et al. 2011; Bain 2017). Higher education corresponds to fewer negative perceptions, highlighting education's role in environmental awareness (Harris et al. 2016). Proximity to LI and occupation also affect attitudes, with those living closer and in labour-intensive jobs showing more negativity (Batel et al. 2015).

Multinomial logistic regression confirms that proximity to the LI had a very strong and significant association with negative responses across all distance categories. This indicates that individuals residing closer to the LI were substantially more likely to report negative responses, likely reflecting direct exposure to environmental, social, or economic externalities, and this supports the prior findings of spatial proximity to infrastructure often intensifying perceptions of risk (Dear 1992; Devine-Wright & Batel 2013). Non-tribal respondents showed higher odds of both negative and positive responses, suggesting greater polarization and engagement within this group. This contrasts with tribal populations, who may be structurally marginalized or less empowered to express dissent—a pattern noted in participatory governance literature (Cornwall 2008). Lower education increases the odds of negative perceptions, whereas both high school and graduate-level education significantly reduce the likelihood of negative responses. This finding may reflect greater resilience, access to information, or broader worldview among more educated individuals, allowing them to contextualize or mitigate concerns (Dietz et al. 2007). Similarly, long-term residents showed more positive views, indicating perceptual shifts linked to socioeconomic change (Manfredo et al. 2009; George et al. 2016). Local ecological knowledge accrued through experience remains vital for conservation (Ruan et al. 2022). Integrating avian conservation into LI planning supports critical ecosystem services like pollination, seed dispersal, pest control, enhancing biodiversity, ecosystem resilience, and community well-being.

## CONCLUSION

This study reveals varied community perceptions on the impacts of LI on birds. Many of the respondents recognized negative effects like electrocution and collisions, but neutral views were common, indicating gaps in awareness and the influence of multiple socio-demographic factors. Perceptions varied by age, education, culture, occupation, and proximity to LI. Younger and tribal individuals tend to be more neutral in their perception of impacts, while uneducated and non-tribal respondents are likely to perceive more negative impacts. Those living closer to LI show greater concern about the impacts, whereas long-term residents are relatively less concerned, possibly suggesting shifting attitudes over time, and acclimatization.

These perception patterns do not necessarily reflect

the full ecological impacts, as several bird groups—particularly raptors, hornbills, storks, and owls—are known from existing literature to be highly vulnerable to electrocution and collision. Strengthening environmental awareness among local communities, especially in areas undergoing rapid infrastructure expansion, will help bridge these gaps. The prevalence of neutral views points to a need for improved environmental education and awareness. Measures such as insulating power lines, installing bird diverters, and maintaining habitat buffers can substantially reduce risks. Incorporating bird conservation concerns into infrastructure development and involving local communities are essential to harmonize development with biodiversity conservation and overall ecosystem health.

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## Habitat associations and feeding ecology of adult Tamdil Leaf-litter Frog *Leptobrachella tamdil* (Amphibia: Megophryidae) from the type locality – the Tamdil wetland, Mizoram, India

Malsawmdawngliana<sup>1</sup> , Esther Lalhmingliani<sup>2</sup> , Samuel Lalronunga<sup>3</sup> , Lalrinmawia<sup>4</sup>   
& Lalnuntluanga<sup>5</sup>

<sup>1,5</sup> Department of Environmental Science, Mizoram University, Aizawl, Mizoram 796004, India.

<sup>2</sup> Systematic and Toxicology Laboratory, Department of Zoology, Mizoram University, Aizawl, Mizoram 796004, India.

<sup>3</sup> House No. B-10/B, YMA Road, Chawnpui, Aizawl, Mizoram 796009, India.

<sup>4</sup> Department of Zoology, Govt. Zirtiri Residential Science College, Durtlang Leitan, Mizoram 796025, India.

<sup>1</sup> valpuia17@gmail.com, <sup>2</sup> es\_ralte@yahoo.in (corresponding author), <sup>3</sup> samuellrna@gmail.com, <sup>4</sup> lrmawia@gmail.com,

<sup>5</sup> tluanga\_249@rediffmail.com

**Abstract:** We conducted a study on the habitat use, food, and feeding ecology of the lesser known Tamdil Leaf-litter Frog *Leptobrachella tamdil* at its type locality. The places occupied by *L. tamdil* in the wild are characterised by 13–25 °C night temperature, 10–20 °C water temperature, 54–86% humidity, 6.84–7.15 pH, and 5–7 mg/L dissolved oxygen. We observed eight orders of prey in the gut contents of *L. tamdil*, examined by stomach-flushing live frogs among which the order Diptera was the most abundant, followed by Hymenoptera, while Hemiptera and Coleoptera were the least abundant.

**Keywords:** Anuran, biodiversity hotspot, Chhawl-chang, food, gut contents, Indo-Burma, microhabitat, natural history.

**Mizo:** Tamdil Chhawl-chang (*L. tamdil*) khawsak phung leh an chaw ei zirchianna Tamdil-ah neih a ni a. Kan zir hun chhung hian zan lam boruak chu 13-25° C, tui vawh zawng 10-20° C, boruak uap zawng 54-86% a ni. Heng uchang kawchhung atanga rannung chi hrang hrang order chi 8 hmuh a ni a, chung zingah chuan Diptera (tho/thosi lam chi) an tam ber a, Hymenoptera (fanghmir lam chi) ten an dawt a, chutih lain Hemiptera (khuangbai lam chi) leh Coleoptera (Rawmung lam chi) te an tlem ber a ni.

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**Author details:** MALSAWMDAWNGLIANA: He is currently enrolled as a Phd scholar in the department of Environmental Science, Mizoram University. His interest lies in the ecology of lesser known herpetofauna with special reference to northeastern India. ESTHER LALHMINGLIANI: She currently works as a professor in the Department of Zoology, Mizoram University. Her main works lies in the systematics and toxicology of insects and herpetofauna. SAMUEL LALRONUNGA: Former research associate and DBT-National Post-doctoral fellow, his main interest lies in the systematics and ecology of Herpetofauna and Ichthyofauna with special preference to northeastern India. LALRINMAWIA: He currently works as associate professor in the Department of Zoology, Govt. Zirtiri Residential Science College, Aizawl. He specialise in parasitic helminthology & soil bio-engineering. LALNUNTLUANGA: He currently works as professor in the Department of Environmental Science, Mizoram University. His specialize in social and agroforestry and biodiversity.

**Author contributions:** Malsawmdawngliana did field work and data collection, laboratory works and wrote the manuscript. Esther Lalhmingliani co-supervised the work and provided the financial support, laboratory support and manuscript writing. Samuel Lalronunga helps in designing the work, data collection and editing the manuscripts. Lalrinmawia helps in manuscript writing and editing. Lalnuntluanga supervised the work, preparation and editing manuscript.

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## INTRODUCTION

Understanding the habitat selection of an organism is essential in determining its distribution and behaviour. For this, we need to understand the behavioural interaction with its surrounding environments and quality of habitats (Figuera & Crowder 2006). Amphibians begin their lives in water, and terrestrial dwellers later transition to moist terrestrial habitats (Wells 2007). They remain closely linked to specific microhabitats and their precincts. Amphibians are endothermic animals, and are sensitive to rising temperatures. They depend on an external source to raise body temperature by absorbing solar radiation (Carey 1978). Climate change is one factor threatening amphibian populations, driving them into decline (Stuart et al. 2004). Amphibian assemblages in natural forests are always higher than in areas disturbed by anthropogenic activities, viz., jhum (slash-&-burn) cultivation land, plantation, and logging (Pawar 1999; Krishnamurthy 2003), and the presence of leaf litter and canopy cover are predictors for their assemblage (Balaji et al. 2013).

Leaf litter presence and depth are essential for the occurrence of amphibians (Fauth et al. 1989; Allmon 1991). Vegetation, sunlight, temperature, and other environmental factors strongly influence the distribution and activities of amphibians (Halverson et al. 2003). The ecological organisation of a community and its assemblage, the phylogenetic relationship among species, their behaviour, and their physiology can all be used to understand to a large extent the species' feeding ecology (Lima & Magnuson 1998; Grant et al. 2006; Arroyo et al. 2008). The diet of amphibians may depend on seasonal availability of prey, presence of competitors, and other relevant factors (Isaach & Barg 2002). Amphibians also play an important role in the biological control of mosquitoes to control diseases like malaria and dengue (Raghavendra et al. 2008; Bowatte et al. 2013).

The megophryiid frog genus *Leptobrachella* Smith, 1925 consists of 104 species, out of which four species, viz., *L. khasiorum* (Das, Tron, Rangad & Hooroo, 2010), *L. lateralis* (Anderson, 1971), *L. nokrekensis* (Matthew & Sen, 2010), and *L. tamdil* (Sengupta, Sailo, Lalremsanga, Das & Das, 2010), are found in India, all restricted to the northeast of the country (Frost 2025). The Tamdil Leaf-litter Frog (*L. tamdil*) was described from Tamdil wetland, Saitual District, Mizoram, in 2010 (Sengupta et al. 2010). Later on, additional records were made from Dampa Tiger Reserve, Hmuifang Community Reserve Forest, Sihzau Lake from Zotlang, Champhai District (Vanlalsiammawii et al. 2020; Muansanga et al. 2021) and also from Manipur (Decemson et al. 2021). However, the natural history of

the frog is still largely unknown (Vanlalsiammawii et al. 2020). Muansanga et al. (2021) partially reported the diet of *L. tamdil* to be insects of four orders based on the study of three individuals of *L. tamdil*. Herein, we present the habitat association and diet of *L. tamdil* studied at the type locality, based on in-situ field observations of wild frogs.

## METHODS

### Study area

Tamdil wetland was enlisted as a National Wetland in 2006–2007 by the National Wetland Conservation Programme, Government of India. It is located in the Saitual District of Mizoram (23.71° E & 92.95° N), about 100 km from the capital district, Aizawl and it covers about 13.7 km<sup>2</sup> with a mean elevation of 760 m (Image 1). The area falls under the Indo-Burma biodiversity hotspot and the vegetation type falls under Cachar Tropical Semi-evergreen Forest (2B/2C) category of Champion & Seth (1968).

### Data Collection

Studies were conducted from February 2023 to May 2024 along the streams in Tamdil Wetland. The species were found to be active during the pre-monsoon months before the onset of peak monsoon. Visual encounter survey (Crump & Scott 1994), audio encounter survey, and opportunistic search were deployed to assess the diet during the survey period. Microhabitats in which the individuals were found are recorded along with relative humidity and temperature. The ecological parameters were recorded in the field using portable devices. We photographed the targeted species in its natural habitat with minimal disturbances.

### Species confirmation

Genomic DNA was extracted from the collected tissue samples of one tadpole and one adult using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. A partial fragment (~570 base pairs) of the 16S rRNA was amplified and sequenced using previously published primers in Simon et al. (1994). Polymerase chain reaction (PCR) conditions were as follows: initial denaturation at 95 °C for 3 minutes, followed by 39 cycles of denaturation at 94 °C for 45 seconds, annealing at 52 °C for 45 seconds, and extension at 72 °C for 2 minutes. Final extension was at 72 °C for 10 minutes. Amplified PCR products were run on a 2% agarose gel and viewed under UV transilluminator. Purified PCR product was sequenced

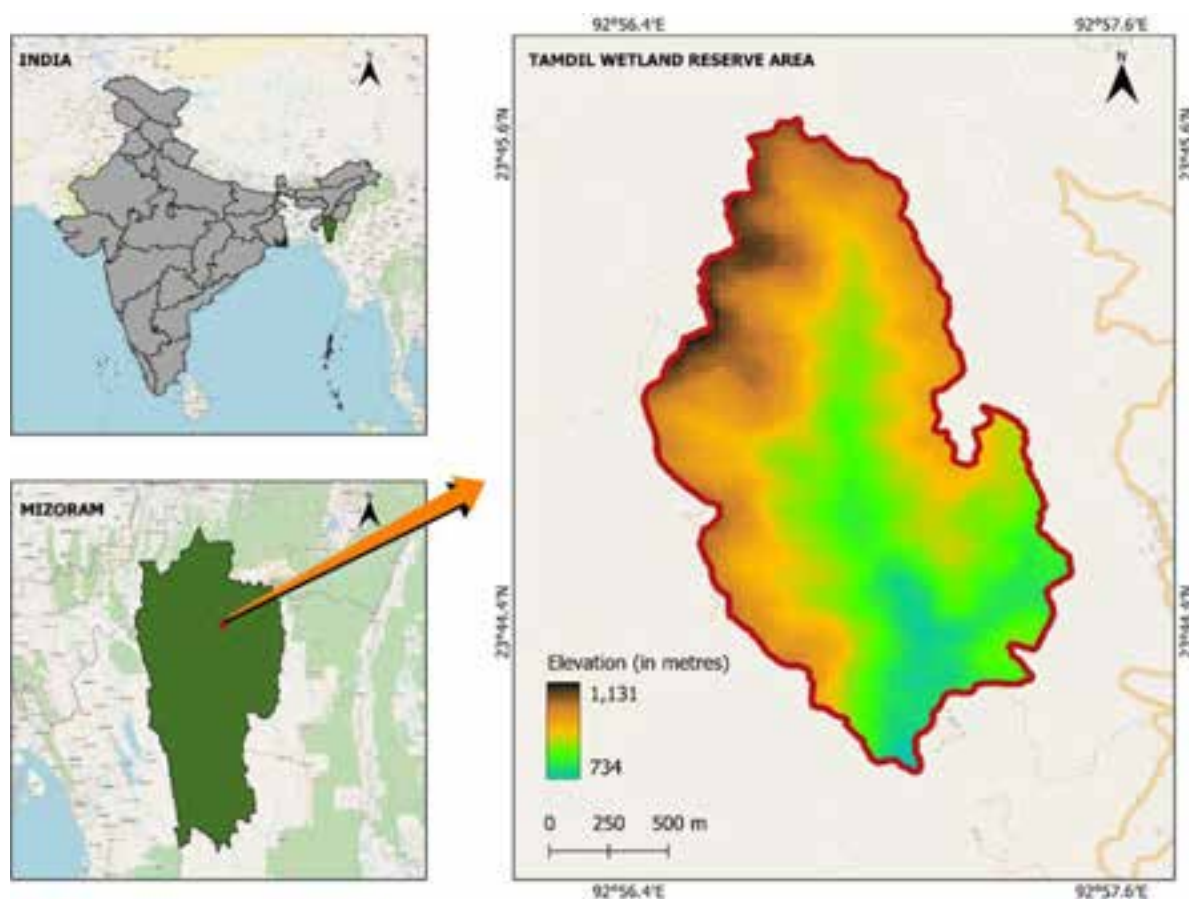


Image 1. Map of Tamdil Wetland (Inset: Elevation map), in Mizoram, northeastern India.

directly in an Applied Biosystems Genetic Analyzer 3500 XL in both directions using BigDye v3.1. The tadpole we studied was identified as *Leptobranchella tamdil* based on genetic congruence (0.11% in 16S rRNA) of our sample's sequence with published (also see Vanlalsiammawil et al. 2020; Decemson et al. 2021; Muansanga et al. 2021) sequences of *L. tamdil* in GenBank (NCBI # ON500517.1, generated from MZMU 2675; [ncbi.nlm.nih.gov/nuccore/ON500517.1](https://ncbi.nlm.nih.gov/nuccore/ON500517.1)).

#### Gut content analysis

Specimens were collected by hand and gut contents of the collected specimens were obtained by flushing the stomach using standardised protocol (Solé et al. 2005). The flushed contents are fixed in 10% formalin solution for laboratory analysis. The snout-vent length (SVL) of the specimens were taken using a calliper to the nearest 0.1 mm and they were released on the spot. The flushed gut contents obtained from the field were analysed in the laboratory using a Motic SMZ - 161 microscope and identified to the level of order. The abundance of various prey was estimated from the stomach contents

(percentage of the total number of individual prey/total number of all prey). Frequency of occurrence was determined by dividing the number of stomachs that contained a particular prey by the total number of stomachs with prey (Hyslops 1980). The rate of feeding activity was estimated as the percentage of stomach containing food with respect to the total number of stomachs examined (Sala & Ballesteros 1997). Rate of feeding activity =  $100 \times n/N$  (where  $n$  is the number of stomachs with food,  $N$  is the total number of stomachs examined). Diet diversity was estimated using the Shannon-Weiner diversity Index ( $H'$ ), where  $H' = -\sum P_i (\ln P_i)$  and  $P_i$  = proportion of each taxa in the sample.

#### Food availability of the study area

The habitat where the species were most frequently encountered was selected for food availability estimation. Prey availability was estimated by making 10 plots of 1 x 1 m in the stream bank and inside the stream. The different types of potential prey (such as insects, spiders) were identified to the level of order and are left in the habitat. The potential preys were then compared with the actual

food items obtained from the gut of adult frogs.

## RESULTS

We encountered different stages of *Leptobrachella tamdil* during the dry months when there is little precipitation and low stream flow (Image 2). Adults of *L. tamdil* were encountered under rocks, above rocks, in water-logged puddles, and on fallen leaves and twigs in and around the streambed. Encounters of tadpoles were usually in small water puddles, but upon approach, they bury themselves in mud quickly. The surrounding vegetation includes *Melocanna baccifera*, *Laurocerasus jenkinsii*, *Ficus fistulosa*, *Terminalia myriocarpa*, *Duabanga grandiflora*, *Macropanax* sp., *Pilea symmeria*, *Leea compactiflora*, *Drypetes indica*, *Aglaiia spectabilis*, *Homalium ceylanicum*, *Phrynium capitatum*, and *Ensete* sp. (Image 3).

The air temperature during the study ranged from 13–25 °C at night; the water temperature ranged from 10–20 °C; the relative humidity ranged 54–86 %; the pH of the water ranged 6.84–7.15; the dissolved oxygen ranged 5–7 mg/L. The species emerges mainly during the dry pre-monsoon months (January–May) when the water level is low, and pockets of small, waterlogged pools are formed in the depressions of the streambanks.

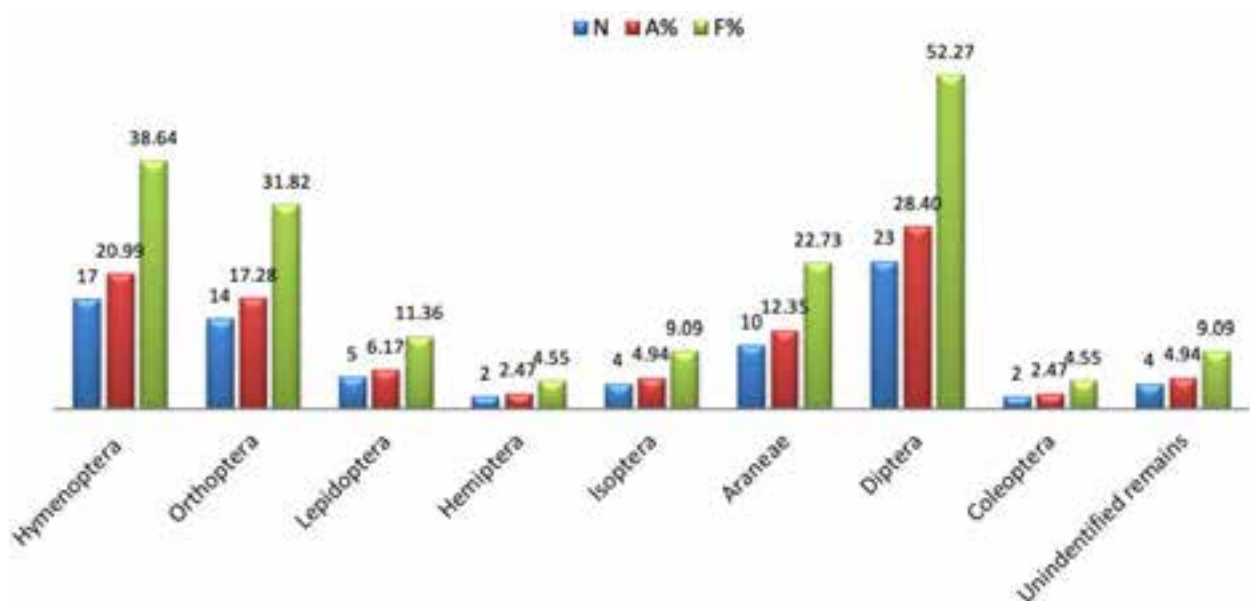
A total of 50 adult individuals of *L. tamdil* (SVL 25.2–35.9 mm) were examined for diet content. We obtained

**Table 1. Prey availability of the Tamdil Leaf-litter Frog in the study area.**

Hymenoptera	Ants
Orthoptera	Grasshoppers & crickets
Lepidoptera	Moths & butterflies
Hemiptera	Water striders, water bugs, & leafhopper
Isoptera	Termites
Diptera	Flies
Araneae	Spiders
Coleoptera	Beetles
Odonata (larvae)	Dragonflies & damselflies

81 prey items from 44 frogs, belonging to eight orders, viz., Hymenoptera (ants), Orthoptera (grasshoppers, crickets), Lepidoptera (moths), Hemiptera (leafhoppers), Isoptera (termites), Araneae (spiders), Diptera (flies), Coleoptera (beetles), and unidentified objects (Image 4). The remaining six individuals had empty stomachs. The rate of feeding was found to be 88% and the diversity of prey, measured with Shannon-Weiner diversity Index, is 1.90.

The order Diptera (28.40%) was the most abundant food item followed by Hymenoptera (20.99%). The least consumed food items were Coleoptera and Hemiptera (both at 2.47%) (Image 4). The frequency of occurrence was also calculated and it shows that Diptera (52.27%) and Hymenoptera (38.64%) were the most frequent



**Figure 1. Frequency of occurrence and abundance of prey (%) in gut contents of *Leptobrachella tamdil*.**



Image 2. Larvae, subadults, and adult *Leptobrachella tamdil* in its microhabitat. © Malsawmdawngliana.



Image 3. Habitat of *Leptobrachella tamdil* at the type locality – Tamdil wetland (Images A–D indicate different parts of the stream banks in the study area). © Malsawmdawngliana.



Image 4. Diet of *Leptobrachella tamdil*: A—Hymenoptera | B—Orthoptera | C—Lepidoptera | D—Hemiptera | E—Isoptera | F—Araneae | G—Diptera | H—Coleoptera | I—Unidentified objects. © Malsawmdawngliana.

contents (Figure 1). We recorded nine orders of potential prey species during the study (Table 1).

## DISCUSSION

After the description of *L. tamdil* based on two individuals by Sengupta et al. (2010), there were no reports of this species for almost a decade. Vanlalsiammawii et al. (2020) recorded the third individual from Dampa Tiger Reserve (~54 km from type locality) and subsequent records were made from Hmuifang Community Reserve Forest (~39 km from type locality), Zotlang (~52 km from type locality), and Chakpi stream (~122 km from type locality) (Decemson et al. 2021; Muansanga et al. 2021). These, however, are inventory studies that were at the alpha taxonomic level, and the information on the natural history and its ecology is still at its infant stage. This study found that *Leptobrachella tamdil* inhabits forest floors and hill streams with rocks, similar to reports made in other studies on its congeners, viz., Lathrop et al. (1997)

(*L. ailaonicum*, *L. sungi*), Matsui (2006) (*L. fuliginosa*), Jiang et al. (2013) (*L. zhangyapingi*), Rowley et al. (2013) (*L. botsfordi*), and Tron et al. (2015) (*L. khasiorum*).

This study found eight orders of prey in the gut of *L. tamdil* of which the most abundant prey were Diptera and Hymenoptera, and also the most frequent food items obtained. Muansanga et al. (2021) reported four orders of insects, i.e., Orthoptera, Hemiptera, Hymenoptera, and Coleoptera from the gut contents of three individuals of *L. tamdil*. The gut contents observed by Muansanga et al. (2021) were also found in our studies with the addition of Lepidoptera, Isoptera, Araneae, and Diptera. Although studies have recorded that body size and prey size have a positive relation in amphibians (Quigora et al. 2009), *L. tamdil* is a medium-sized frog species, and the size of male and female individuals do not vary much, as deduced during our study.

Available prey of *L. tamdil* is represented in Table 1, and most of the available food items (except for Odonata) were found in the gut of the examined frogs. We cannot rule out the possibility that *L. tamdil* feeds on odonates, as

most amphibians are opportunistic feeders. The present study demonstrated that the diet of *L. tamdil* is largely composed of the available food items in their habitat. The peak active season of the frog was before the onset of the monsoon (Sengupta et al. 2010; Vanlalsiammawii et al. 2020; Decemson et al. 2021; Muansanga et al. 2021) when insect activity is low, which may influence the availability of potential food in the area. Additionally, plants, vegetation remains, sand, rocks, and other particles may be accidentally ingested and are therefore not counted as part of the diet. We have a few caveats in our study as this is the first attempt to study the ecology and natural history of *L. tamdil*. This study will, nevertheless, further help in the conservation of this endemic species and documenting its natural history.

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## INTRODUCTION

Hymenoptera is one of the fourth largest orders of insects after Coleoptera, Lepidoptera, and Diptera but is the most important order due to its economic value, ecological role as pollinators, biological pest control agents, and scavengers of the environment. It is well known that the increasing anthropogenic activities have shown detrimental effects on the biodiversity loss. There are many studies which provide us the direct evidence of the potentially drastic effect of this biodiversity loss on human health and nutrition (Martens et al. 1997; Sala et al. 2009; Eilers et al. 2011; Myers et al. 2013). The decline of pollinating species can lead to a parallel decline of plant species as 35% crops which account for global food production and supply up to 40% of the world's supply of nutrients depend entirely on animal pollinators (Williams et al. 1991; Klein et al. 2007; Eilers et al. 2011). Bees and wasps are the most economically valuable pollinators of crop plants worldwide, and the yields of some fruit, seed, and nut crops decrease by more than 90% without these pollinators (McGregor 1976; Southwick 1992; Roubik 2002). Populations of wild pollinators can enhance production of many crops, but many of them are still awaiting similar comparative pollinator studies. Even the ants are ecologically very important as they turn and aerate the soil, allowing water and oxygen to reach plant roots. Ants also act as decomposers in environment by feeding on organic waste, insects and other dead animals and help in cleaning the environment. Some wasps are important predators of crop pests and can be used as biological control agents as substitutes for chemical pesticides, which have adverse effects on crop yield. The evidence of successful use of the species as biological control agents is already documented (Donovan 2003). The social paper wasp *Polistes satam* is a successful predator on the larvae of two economically important crop pests, the sugarcane borer *Diatraea saccharalis* (a predator of Sugarcane *Saccharum* spp.) and the fall armyworm *Spodoptera frugiperda* (a predator of Maize *Zea mays*) (Southon et al. 2019). Similarly, the predatory activity of *Polistes canadensis* on *Plutella xylostella*, a predator of *Brassica oleracea* has been observed (Montefusco et al. 2017). Keeping in view the importance of this aspect of biodiversity, a preliminary taxonomic survey has been conducted on the hymenopteran insects of Jammu region. The species are catalogued systematically under the respective superfamilies, families, and subfamilies. The approach will prove useful in the conservation of the species as the first step of conservation is to identify

them and then develop detailed conservation plans.

## MATERIAL AND METHODS

### Study area

The present study was undertaken at various geographical locations in different districts of Jammu region for a period of four years from 2019–2022. The different areas including crop fields, gardens, grasslands, forest areas, and household places, were explored for the collection of insects belonging to Hymenoptera. The areas covered for the collection of specimens are depicted in Table 1 and Figure 1.

### Methods

Collection of the insects was done with the use of collection nets and hand picking. The collected samples were killed with ethyl acetate vapours. The specimens were stretched and properly preserved in fumigated insect boxes. Keys given by Bingham (1897), Goulet & Huber (1993), Carpenter & Nguyen (2003), Michener (2007, 2010), Saini et al. (2011), and online identification platforms like Antwiki, iNaturalists were used to identify the collected specimens. The collected species after dry or wet preservation were submitted in the museum of Department of Zoology, University of Jammu and ZSI Kerala.

## RESULTS AND DISCUSSION

A total of 27 geographical locations from different districts of Jammu region were surveyed from January 2019 to September 2022 and a total of 165 insect samples were collected. They were found to belong to 51 different species, 37 genera, and 10 families with maximum 22 species of the family Formicidae, 13 species of the family Vespidae, six species of Apidae, two species of Crabronidae & Sphecidae each, and the families Evaniidae, Halictidae, Megachillidae, Mutillidae, & Pompilidae are represented by one species each (Image 1–51). A total of 14 species were found to be new records from the region. All the identified species were submitted in the depository and their respective accession number were generated.

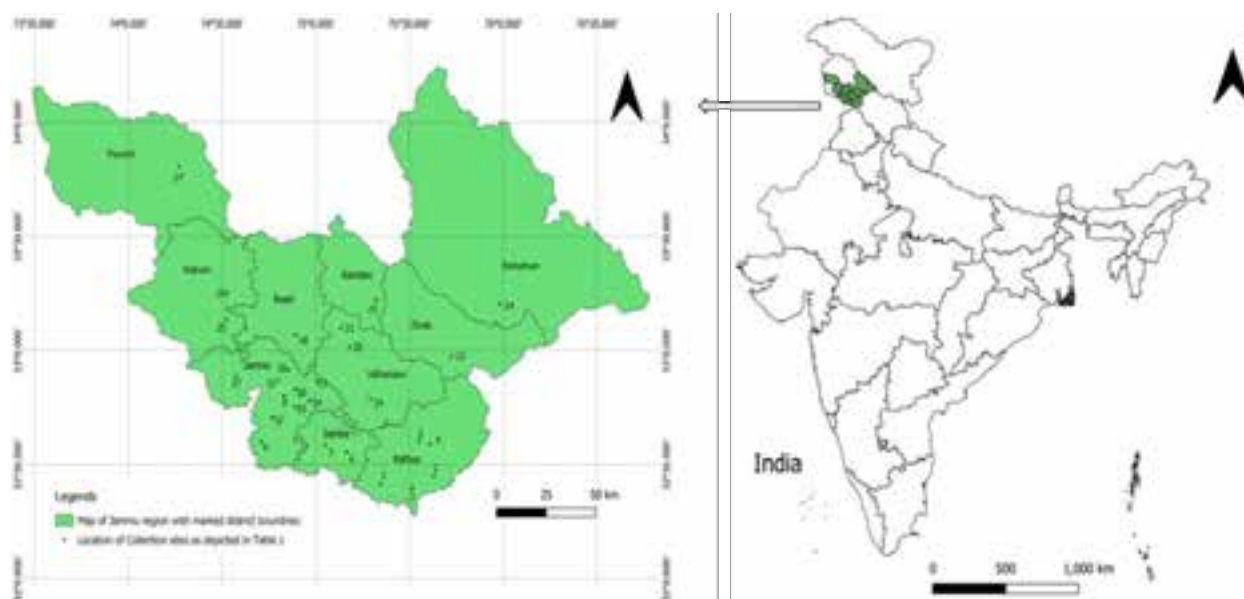


Figure 1. Map depicting collection sites for the present study.

The systematic checklist is as:

**Order Hymenoptera** Linnaeus, 1758

**Suborder Apocrita** Gerstaecker, 1867

**Superfamily Apoidea** Latreille, 1802

**Family Apidae** Latreille, 1802

**Genus *Apis*** Linnaeus, 1758

***Apis cerana*** Fabricius, 1793

**Material examined:** 2 females, India, Jammu & Kashmir, Jammu, Girls Hostel University of Jammu, 32.718° N, 74.870° E, 319 m, 29.viii.2019 (Reg. No. ZOO211.1, ZOO211.2); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.604° N, 75.288° E, 516 m, 29.iv.2022 (Reg. No. ZOO21.3); 1 female, India, Jammu & Kashmir, Kathua, Jasrota Wildlife Sanctuary, 32.459° N, 75.411° E, 364 m, 16.v.2022 (Reg. No. ZOO211.4); 1 female, India, Jammu & Kashmir, Jammu, GCW Parade, 32.737° N, 74.865° E, 370 m, 20. vii.2022 (Reg. No. ZOO211.5).

**Distribution in India:** Central India, Haryana, Himachal Pradesh, Jammu & Kashmir, Punjab.

***Apis dorsata*** Fabricius, 1793

**Material examined:** 2 females, India, Jammu & Kashmir, Jammu, Girls Hostel University of Jammu, 32.718° N, 74.870° E, 319 m, 29.viii.2019 (Reg. No. ZOO212.1, ZOO212.2); 1 female, 1 male, India, Jammu & Kashmir, Kathua, 32.732° N, 74.864° E, 352 m, 26.xii.2020 (Reg. No. ZOO212.3, ZOO212.4).

**Distribution in India:** Throughout India.

***Apis mellifera*** Linnaeus, 1758

**Material examined:** 2 females, India, Jammu & Kashmir, Samba, Vijayapur, 32.705° N, 74.880° E, 341 m, 11.vi.2021 (Reg. No. ZOO213.1, ZOO213.2); 1 female, India, Jammu & Kashmir, Kathua, Jasrota Wildlife Sanctuary, 32.459° N, 75.411° E, 364 m, 16.v.2022 (Reg. No. ZOO213.4).

**Distribution in India:** Throughout India.

**Tribe Bombini** Latreille, 1802

**Genus *Bombus*** Latreille, 1802

***Bombus haemorrhoidalis*** Smith, 1852

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.282° E, 505 m, 23.vi.2019 (Reg. No. ZOO214.1, ZOO214.2); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.604° N, 75.288° E, 516 m, 25.xi.2019 (Reg. No. ZOO214.3); 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.278° E, 449 m, 24.x.2019 (Reg. No. ZOO214.4, ZOO214.5); 1 male, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 25.xii.2019 (Reg. No. ZOO214.6); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.349° E, 669 m, 7.vii.2020 (Reg. No. ZOO214.7).

**Distribution in India:** Jammu & Kashmir, Punjab, Uttarakhand, West Bengal.

Table 1. The geographical information of the collection sites.

Country	State/UT	District	Name of collection site	Latitude (°N)	Longitude (°E)	Height (m)
India	Jammu & Kashmir	Doda	Doda	33.1493	75.5477	1,200
		Jammu	Akhnoor	32.8995	74.7425	330
			Arnia	32.5192	74.7995	271
			Bantalab	32.7843	74.8237	375
			Botany Department, JU	32.7189	74.8680	313
			Botanical Garden, JU	32.7194	74.8681	314
			Department of Zoology	32.7242	74.8666	316
			Gandhinagar, Jammu	32.7014	74.8596	302
			GCW Parade	32.7377	74.8656	370
			Hostel, JU	32.7180	74.8702	319
			Janipur	32.7553	74.8497	385
			Kot Balwal	32.7334	74.8760	309
			Manda Zoo	32.7497	74.8673	414
			Manda Zoo	32.7503	74.8713	412
			R.S. Pura	32.6049	74.7315	272
			Talab Tillo	32.7226	74.8376	287
		Kathua	GDC Kathua	32.3863	75.5300	344
			Jasrota Mata	32.4717	75.4259	383
			Jasrota Wildlife Sanctuary	32.4596	75.4113	364
			Jatwal	32.5297	75.1689	395
			Kathua Station 1	32.3857	75.5186	348
			Kathua Station 2	32.4510	75.3705	359
			Kathua Station 3	32.7329	74.8642	352
			Nagrota Gujroo	32.6430	75.3911	768
			Ramkot Station 1	32.6042	75.2881	516
			Ramkot Station 2	32.6081	75.2786	449
			Ramkot Station 3	32.6101	75.2822	505
			Ramkot Station 4	32.6103	75.2823	500
			Ramkot Station 5	32.6418	75.3436	666
			Ramkot Station 6	32.6418	75.3494	669
		Kishtwar	Kishtwar	33.3223	75.7765	1,801
		Poonch	Mendhar	33.6051	74.1431	938
		Rajouri	Rajouri	33.3818	74.3865	1,035
			Sunderbani	33.0493	74.4907	576
		Ramban	Ramban	33.2464	75.1939	935
			Sanasar	33.0653	75.1565	1,727
		Reasi	Domael, Katra	32.9395	74.9499	715
		Samba	Ghaghwal	32.5092	75.1863	366
			Vijaypur	32.7053	74.8800	341
		Udhampur	Patnitop	33.0847	75.3354	2,024
			Udhampur	32.9160	75.1416	680

**Subfamily Xylocopinae** Latreille, 1802**Tribe Xylocopini** Latreille, 1802**Genus *Xylocopa*** Latreille, 1802***Xylocopa fenestrata*** Fabricius, 1798

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, Botanical Garden of University of Jammu, 32.719° N, 74.868° E, 314 m, 16.iv.2019 (Reg. No. ZOO215.1); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.2822° E, 505 m, 08.vi.2020.

**Distribution in India:** Arunachal Pradesh, Bihar, Goa, Gujarat, Himachal Pradesh, Jammu & Kashmir, Maharashtra, Odisha, Punjab, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal.

***Xylocopa pubescens*** Spinola, 1838

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, Botanical Garden of University of Jammu, 32.719° N, 74.868° E, 314 m, 18.iv.2019 (Reg. No. ZOO260.1).

**Distribution in India:** Bihar, Jammu & Kashmir, Karnataka, Odisha, Sikkim, Tamil Nadu, West Bengal.

**Family Crabronidae** Latreille, 1802**Subfamily Crabroniidae** Latreille, 1802**Tribe Larrini** Latreille, 1810**Genus *Tachysphex*** Kohl, 1883

**Material examined:** 1 female, India, Jammu & Kashmir, Rajouri, Sunderbani, 33.049° N, 74.490° E, 576 m, 10.x.2022 (Reg. No. ZOO216).

**Genus *Tachytes*** Panzer, 1806

**Material examined:** 1 female, India: Jammu & Kashmir, Kathua, Ramkot, 32.604° N, 75.288° E, 516 m, 25.viii.2019 (Reg. No. ZOO217).

**Family Halictidae** Thomson, 1869**Tribe Halcitini** Thomson, 1869**Genus *Halictus*** Latreille, 1804***Halictus vicinus*** Vachal, 1895

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.278° E, 449 m, 24.x.2019 (Reg. No. ZOO218.1, ZOO218.2); 1 female, India, Jammu & Kashmir, Ramban, 33.246° N, 75.193° E, 935 m, 15.iv.2022 (Reg. No. ZOO218.3); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.6081° N, 75.2786° E, 449 m, 05.ii.2022 (Reg. No. ZOO218.4).

**Distribution in India:** Assam, Jammu & Kashmir, Kerala, Madhya Pradesh, Punjab, Rajasthan, Sikkim, Uttarakhand, West Bengal.

**Family Megachilidae** Latreille, 1802**Genus *Megachile*** Latreille, 1802***Megachile lanata*** Fabricius, 1798

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Nagrota Gujroo, 32.643° N, 75.391° E, 768 m, 15.ix.2019 (Reg. No. ZOO219.1, ZOO219.2).

**Distribution in India:** Andhra Pradesh, Assam, Bihar, Gujarat, Haryana, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Odisha, Uttarakhand, Punjab, West Bengal.

**Family Sphecidae** Latreille, 1802**Subfamily Sceliphrinae** Latreille, 1802**Genus *Chalybion*** Dahlbom, 1843***Chalybion bengalense*** Dahlbom, 1845

**Material examined:** 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 7.x.2020 (Reg. No. ZOO220.1); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 29.iv.2022 (Reg. No. ZOO220.2); 1 male, India, Jammu & Kashmir, Jammu, University of Jammu, 32.724° N, 74.866° E, 316 m, 08.ii.2022 (Reg. No. ZOO220.3).

**Distribution in India:** Andhra Pradesh, Arunachal Pradesh, Chhattisgarh, Gujarat, Himachal Pradesh, Jharkhand, Jammu and Kashmir (new record), Kerala, Madhya Pradesh, Punjab, West Bengal.

**Genus *Sceliphron***, Klug, 1801***Sceliphron madraspatanum*** Fabricius, 1781

**Material examined:** 2 females, India, Jammu and Kashmir, Kathua, Jasrota Wildlife sanctuary, 32.459° N, 75.411° E, 364 m, 30.iv.2022 (Reg. No. ZOO221.1, ZOO221.2); 1 female, India, Jammu and Kashmir, Jammu, University of Jammu, Department of Zoology, 32.724° N, 74.866° E, 316 m, 09.vi.2022 (Reg. No. ZOO221.3).

**Distribution in India:** Assam, Arunachal Pradesh, Delhi, Himachal Pradesh, Jammu & Kashmir (new record), Kerala, Maharashtra, Madhya Pradesh, Punjab, Rajasthan, Tamil Nadu, Odisha, Uttar Pradesh.

**Superfamily Evanioidea** Latreille, 1802**Family Evanidae** Latreille, 1802**Genus *Evania*** Fabricius, 1775***Evania appendigaster*** Linnaeus, 1758

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, R.S. Pura, 32.604° N, 74.731° E, 272 m, 24.iv.2022 (Reg. No. ZOO222.1)

**Distribution in India:** Andhra Pradesh, Assam, Gujarat, Haryana, Jammu and Kashmir, Karnataka, Kerala, Maharashtra, Odisha, Tamil Nadu, Telangana, Uttar Pradesh, West Bengal.

**Superfamily Vespoidea** Latreille, 1802

**Family Formicidae** Latreille, 1809

**Subfamily Dolichoderinae** Forel, 1878

**Genus *Tapinoma*** Foerster, 1850

***Tapinoma melanocephalum*** Fabricius, 1793

**Material examined:** 2 females, India, Jammu & Kashmir, University of Jammu, Girls hostel, 32.718° N, 74.870° E, 319 m, 30.xii.2021 (Reg. No. ZOO223.1, ZOO223.2).

**Distribution in India:** All over India.

**Subfamily Dorylinae** Leach, 1815

**Genus *Aenictus*** Shuckard, 1840

***Aenictus aitkeni*** Foral, 1901

**Material examined:** 1♂ (worker caste), India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 15.x.2021 (Reg. No. ZOO224.1).

**Distribution in India:** Arunachal Pradesh, Assam, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Manipur, Sikkim, and West Bengal.

**Genus *Dorylus*** Fabricius, 1793

***Dorylus laevigatus*** Smith, 1857

**Material examined:** 1 male, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.349° E, 669 m, 07.vii.2020 (Reg. No. ZOO225.1)

**Distribution in India:** Arunachal Pradesh, Jammu & Kashmir (new record).

**Subfamily Formicinae** Latreille, 1809

**Tribe Camponotini** Forel, 1878

**Genus *Camponotus*** Mayr, 1861

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Jasrota wildlife sanctuary, 32.459° N, 75.411° E, 309 m, 14.v.2022 (Reg. No. ZOO255.1).

***Camponotus compressus*** Fabricius, 1787

**Material examined:** 1 female, India, Jammu & Kashmir, Ramban, Sanasar, 33.122° N, 75.262° E, 2048 m, 07.vii.2019 (Reg. No. ZOO226.1); 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 23.iv.2022 (Reg. No. ZOO226.2, ZOO226.3); 2 females, India, Jammu & Kashmir, Kathua, Jasrota wildlife sanctuary, 32.459° N, 75.411° E, 364 m, 14.iv.2022 (Reg. No. ZOO226.4, ZOO226.5); 2 females, India, Jammu & Kashmir, Jammu, Arnia, 32.519° N, 74.799° E, 271 m, 15.v.2022 (Reg. No. ZOO226.6, ZOO226.7); 2 females, India, Jammu & Kashmir, Jammu, University of Jammu, Department of Botany, 32.718° N, 74.868° E, 313 m, 22.vii.2022 (Reg. No. ZOO226.8, ZOO226.9); 1 female, India, Jammu and Kashmir, Jammu, Kot Balwal, 32.733°

N, 74.876° E, 309 m, 05.xii.2022 (Reg. No. ZOO226.10).

**Distribution in India:** All over India.

***Camponotus pennsylvanicus*** de Geer, 1773

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, University of Jammu, Department of Zoology, 32.724° N, 74.866° E, 316 m, 27.xii.2021 (Reg. No. ZOO227.1).

**Distribution in India:** Assam, Jammu & Kashmir (new record).

**Genus *Polyrhachis*** Smith, 1857

***Polyrhachis lacteipennis*** Smith, 1858

**Material examined:** 2♀, India, Jammu & Kashmir, Poonch, Mendhar, 33.605° N, 74.143° E, 939 m, 05.xi.2022 (Reg. No. ZOO228.1, ZOO228.2);

**Distribution in India:** Arunachal Pradesh, Assam, Gujarat, Himachal Pradesh, Jammu & Kashmir, Karnataka, Punjab, Uttarakhand, West Bengal.

**Tribe Formicini** Latreille, 1809

**Genus *Formica*** Linnaeus, 1758

***Formica clara*** Forel, 1886

**Material examined:** 2♀, India, Jammu & Kashmir, Doda, 33.149° N, 75.547° E, 1200 m, 10.x.2021 (Reg. No. ZOO256.1).

**Distribution in India:** Himachal Pradesh, Jammu & Kashmir.

**Tribe Plagiolepidini**

**Genus *Lepisiota*** Santschi, 1926

***Lepisiota integra*** Forel, 1894

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, Manda, 32.749° N, 74.867° E, 414 m, 08.iii.2022 (Reg. No. ZOO229.1); 2 females, India, Jammu & Kashmir, Jammu, Gandhinagar, 32.701° N, 74.859° E, 302 m, 05.iv.2022 (Reg. No. ZOO229.2, ZOO229.3); 1 female, India, Jammu & Kashmir, Jammu, Kathua, Jatwal, 32.529° N, 75.168° E, 395 m, 15.v.2022 (Reg. No. ZOO229.4); 2 females, India, Jammu & Kashmir, Kathua, Jasrota wildlife sanctuary, 32.459° N, 75.411° E, 364 m, 16.v.2022 (Reg. No. ZOO229.5, ZOO229.6); 1 female, India, Jammu & Kashmir, Jammu, Bantalab, 32.784° N, 74.823° E, 375 m, 17.v.2022 (Reg. No. ZOO229.7).

**Distribution in India:** Himachal Pradesh, Jammu & Kashmir, Madhya Pradesh, Meghalaya, Punjab, Uttar Pradesh.

***Lepisiota sericea*** Forel, 1992

**Material examined:** 2♀, India, Jammu & Kashmir, Jammu, Manda Zoo, 32.750° N, 74.871° E, 412 m,

08.iii.2022 (Reg. No. ZOO230.1); 2♀, India, Jammu & Kashmir, Kathua, Ramkot, Government Higher Secondary School, 32.641° N, 75.349° E, 669 m, 23.iv.2022 (Reg. No. ZOO230.2).

**Distribution in India:** Himachal Pradesh, Jammu & Kashmir, Maharashtra.

**Subfamily Myrmicinae** de Saint-Fargeau, 1835

**Tribe Attini**

**Genus *Pheidole*** Westwood, 1839

***Pheidole indica*** Mayr, 1879

**Material examined:** 3♀, India, Jammu & Kashmir, Rajouri, 33.381° N, 74.386° E, 1035 m, 30.xi.2022 (Reg. No. ZOO231.1).

**Distribution in India:** Arunachal Pradesh, Assam, Bihar, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Meghalaya, Nagaland, Orissa, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal.

**Tribe: Crematogastrini** Forel, 1893

**Genus *Tetramorium*** Mayr, 1855

***Tetramorium similimum*** Smith, 1886

**Material examined:** 2 females, India, Jammu & Kashmir, Jammu, Arnia, 32.519° N, 74.799° E, 271 m, 15.v.2022 (Reg. No. ZOO257.1, ZOO258.2).

**Distribution in India:** Himachal Pradesh, Jammu & Kashmir, Karnataka, Maharashtra, Punjab, West Bengal.

**Genus *Trichomyrmex*** Mayr, 1865

***Trichomyrmex aberrans*** Forel, 1902

**Material examined:** 2 females, India, Jammu & Kashmir, Jammu, University of Jammu, Botanical Garden, 32.719° N, 74.868° E, 314 m, 17.vii.2019 (Reg. No. ZOO232.1).

**Distribution in India:** Arunachal Pradesh, Haryana, Himachal Pradesh, Jammu & Kashmir, Kerala, Madhya Pradesh, Meghalaya, Punjab, Uttar Pradesh.

***Trichomyrmex destructor*** Jerdon, 1851

**Material examined:** 3♀, India, Jammu & Kashmir, Kathua, GDC Kathua, 32.386° N, 75.530° E, 344 m, 17.iv.2022 (Reg. No. ZOO233.1, ZOO233.2, ZOO233.3); 2♀, India, Jammu & Kashmir, Jammu, Janipur, 32.755° N, 74.849° E, 385 m, 18.iv.2022 (Reg. No. ZOO233.4, ZOO233.5); 2♀, India, Jammu & Kashmir, Kathua, Jatwal, 32.529° N, 75.168° E, 395 m, 15.v.2022 (Reg. No. ZOO233.6, ZOO233.7).

**Distribution in India:** Andhra Pradesh, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Punjab, Rajasthan,

Tamil Nadu, Uttar Pradesh, West Bengal.

***Trichomyrmex scabriceps*** Mayr, 1879

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Jasrota wildlife sanctuary, 32.459° N, 75.411° E, 364 m, 14.iv.2022 (Reg. No. ZOO234.1).

**Distribution in India:** Arunachal Pradesh, Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Kerala, Maharashtra, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal.

**Tribe Myrmicini** de Saint-Fargeau, 1835

**Genus *Myrmica*** Latreille, 1804

***Myrmica aimonissabaudiae*** Menozzi, 1939

**Material examined:** 2 females, India, Jammu & Kashmir, Udhampur, Patni top, 33.084° N, 75.335° E, 2024 m, 07.vii.2019 (Reg. No. ZOO258.1, ZOO258.2)

**Distribution in India:** Himachal Pradesh, Jammu & Kashmir, Meghalaya, Sikkim, West Bengal.

**Tribe Solenopsidini** Forel, 1893

**Genus *Monomorium*** Mayr, 1855

***Monomorium indicum*** Forel, 1902

**Material examined:** 2 females, 1 male, India, Jammu & Kashmir, Samba, Ghagwal, 32.509° N, 75.186° E, 366 m, 29.ix.2021 (Reg. No. ZOO235.1, ZOO235.2, ZOO235.3); 1 female, India, Jammu & Kashmir, Ramban, 33.246° N, 75.193° E, 935 m, 15.iv.2022 (Reg. No. ZOO235.4); 1 male, India, Jammu & Kashmir, Kathua, 32.451° N, 75.370° E, 359 m, 22.iv.2022 (Reg. No. ZOO235.5); 2 females, India, Jammu & Kashmir, Kathua, 32.385° N, 75.518° E, 348 m, 22.iv.2022 (Reg. No. ZOO235.6).

**Distribution in India:** Andhra Pradesh, Arunachal Pradesh, Assam, Gujarat, Haryana, Himachal Pradesh, Jammu and Kashmir, Karnataka, Maharashtra, Manipur, Nagaland, Orissa, Punjab, Rajasthan, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal.

**Genus *Myrmicaria*** Saunders, 1842

***Myrmicaria brunnea*** Saunders, 1842

**Material examined:** 1 female, 2 males, India, Jammu & Kashmir, Jammu, Manda Zoo, 32.750° N, 74.871° E, 412 m, 08.iii.2022 (Reg. No. ZOO236.1).

**Distribution in India:** Arunachal Pradesh, Assam, Bihar, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Orissa, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal.

**Tribe Stenammini** Ashmead, 1905**Genus *Messor*** Forel, 1890***Messor himalayanus*** Forel, 1902

**Material examined:** 3 females, India, Jammu & Kashmir, Doda, 33.1493° N, 75.5477° E, 1200 m, 01.v.2022 (Reg. No. ZOO237.1, ZOO237.2, ZOO237.3).

**Distribution in India:** Himachal Pradesh, Jammu & Kashmir, Kerala, Punjab, Rajasthan.

***Messor instabilis*** Smith, 1858

**Material examined:** 1 female, 1 male, India, Jammu & Kashmir, Kathua, 32.385° N, 75.518° E, 348 m, 22.iv.2022 (Reg. No. ZOO238.1, ZOO238.2).

**Distribution in India:** Andhra Pradesh, Haryana, Himachal Pradesh, Jammu & Kashmir, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, and Uttar Pradesh.

**Tribe Ponerini** de Saint-Fargear, 1835**Genus *Leptogenys*** Roger, 1861***Leptogenys diminuta*** Smith, 1857

**Material examined:** 2♀, India, Jammu & Kashmir, Kathua, Jasrota wildlife sanctuary, 32.459° N, 75.411° E, 364 m, 17.iv.2022 (Reg. No. ZOO239.1, ZOO239.2); 2♀, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 12.vi.2022 (Reg. No. ZOO239.3).

**Distribution in India:** Arunachal Pradesh, Assam, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Maharashtra, Meghalaya, Orissa, Sikkim, Tamil Nadu, and West Bengal.

**Genus *Odontoponera*** Mayr, 1862***Odontoponera denticulate*** Smith, 1857

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Jasrota, Jasrotsa mata temple, 32.471° N, 75.425° E, 383 m, 15.iv.2022 (Reg. No. ZOO240.1, ZOO240.2).

**Distribution in India:** Arunachal Pradesh, Assam, Haryana, Himachal Pradesh, Jammu & Kashmir, Karnataka, Kerala, Meghalaya, Nagaland, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, and West Bengal.

**Subfamily Pseudomyrmecinae****Genus *Tetraponera*** Smith, 1852***Tetraponera rufonigra*** Jerdon, 1851

**Material examined:** 2♀, India, Jammu & Kashmir, Kathua, Ramkot, Govt: Higher Secondary School, 3.641° N, 75.343° E, 666 m, 23.iv.2022 (Reg. No. ZOO241.1, ZOO241.2).

**Distribution in India:** Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Goa, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka,

Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Punjab, Rajasthan, Sikkim, Tamil Nadu, Telangana, Tripura, Uttar Pradesh, Uttarakhand, and West Bengal.

**Family Vespidae** Latreille, 1802**Genus *Indodynerus*** Gusenleitner, 2008***Indodynerus capitatus*** Gusenleitner, 2008

**Material examined:** 1 female, India, Jammu & Kashmir, Kathua, 32.451° N, 75.370° E, 359 m, 24.iv.2022; 1 female, India, Jammu & Kashmir, Jammu, University of Jammu, Botanical Garden, 32.719° N, 74.868° E, 314 m, 15.ix.2022 (Reg. No. ZOO243.1).

**Distribution in India:** Chhattisgarh, Goa, Jammu & Kashmir (new record), Karnataka, Kerala, and Madhya Pradesh.

**Genus *Vespa*** Linnaeus, 1758***Vespa basalis*** Smith, 1852

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, Jammu university Hostel, 32.718° N, 74.870° E, 319 m, 04.vii.2019 (Reg. No. ZOO244.1); 3 females, India, Jammu & Kashmir, Reasi, Domael, 32.939° N, 74.949° E, 715 m, 04.xi.2019 (Reg. No. ZOO244.2, ZOO244.3, ZOO244.4); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.278° E, 449 m, 22.iii.2020 ((Reg. No. ZOO244.5); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.278° E, 449 m, 03.iv.2022 (Reg. No. ZOO244.6).

**Distribution in India:** Arunachal Pradesh, Assam, Himachal Pradesh, Jammu & Kashmir (new record), Meghalaya, Mizoram, Orissa, Sikkim, Uttarakhand, Uttar Pradesh, and West Bengal.

***Vespa tropica*** Linnaeus, 1758

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.282° E, 500 m, 06.viii.2019 (Reg. No. ZOO245.1, ZOO245.2); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.282° E, 500 m, 03.iv.2020 (Reg. No. ZOO245.3); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.282° E, 449 m, 07.vii.2022 (Reg. No. ZOO245.4).

**Distribution in India:** Andaman & Nicobar Islands, Arunachal Pradesh, Assam, Bihar, Haryana, Himachal Pradesh, Jammu and Kashmir (new record), Karnataka, Kerala, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Orissa, Pondicherry, Sikkim, Tamil Nadu, Uttarakhand, and West Bengal.

***Vespa velutina*** Lepeletier, 1836

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Nagrota Gujroo, 32.643° N, 75.391° E, 768 m, 15.ix.2019 (Reg. No. ZOO246.1, ZOO246.2); 1 female, India, Jammu & Kashmir, Jammu, Talab Tillo, 32.722° N, 74.837° E, 287 m, 22.ix.2021 (Reg. No. ZOO246.3); 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.288° E, 449 m, 4.xi.2021 (Reg. No. ZOO246.4).

**Distribution in India:** Arunachal Pradesh, Assam, Jammu & Kashmir, Sikkim, and West Bengal.

**Subfamily Eumeninae** Latreille, 1802**Genus *Antepipona*** de Saussure, 1855***Antepiporia bipustulata*** de Saussure, 1856

**Material examined:** 1 male, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 30.x.2019 (Reg. No. ZSI/WGRC/IR/INV.23171).; 2 males, India, Jammu & Kashmir, Rajouri, Sunderbani, 33.049° N, 74.490° E, 576 m, 08.ix.2022. (Reg. No. ZOO261.1, ZOO261.2).

**Distribution in India:** Arunachal Pradesh, Chhattisgarh, Jammu & Kashmir (new record), Meghalaya, Nagaland.

**Genus *Antodynerus*** de Saussure, 1855***Antodynerus limbatus*** de Saussure, 1852

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.278° E, 449 m, 24.x.2019 (Reg. No. ZOO247.1, ZOO247.2).; 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.610° N, 75.278° E, 500 m, 06.viii.2020 (Reg. No. ZOO247.3); 2 females, India, Jammu & Kashmir, Rajouri, Sunderbani, 33.049° N, 74.490° E, 576 m, 01.ix.2022 (Reg. No. ZOO247.4).

**Distribution in India:** Andhra Pradesh, Assam, Himachal Pradesh, Jammu & Kashmir (new record), Gujarat, Kerala, Maharashtra, Tamil Nadu, and West Bengal.

**Genus *Delta*** de Saussure, 1855***Delta pyriforme pyriforme*** Fabricius, 1775

**Material examined:** 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.278° E, 449 m, 24.x.2019 (Reg. No. ZOO248.1).

**Distribution in India:** Andaman & Nicobar Islands, Andhra Pradesh, Arunachal Pradesh, Assam, Chhattisgarh, Delhi, Gujarat, Himachal Pradesh, Jammu & Kashmir (new record), Jharkhand, Karnataka, Kerala, Maharashtra, Manipur, Meghalaya, Mizoram, Nagaland, Odisha, Pondicherry, Rajasthan, Sikkim, Tamil Nadu,

Tripura, Uttarakhand, Uttar Pradesh, and West Bengal.

**Genus *Rhynchium*** Spinola, 1806***Rhynchium brunneum*** Spinola, 1808

**Material examined:** 2 females, India, Jammu & Kashmir, Kathua, Ramkot, 32.608° N, 75.278° E, 449 m, 20.viii.2020 (Reg. No. ZOO249.1, ZOO249.2); 1 female, India, Jammu & Kashmir, Jammu, R.S. Pura, 32.604° N, 74.731° E, 272 m, 20.xii.2020 (Reg. No. ZOO249.3).

**Distribution in India:** Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Chhattisgarh, Himachal Pradesh, Jammu and Kashmir (new record), Karnataka, Maharashtra, Madhya Pradesh, Odisha, Tamil Nadu, and West Bengal.

**Subfamily Polistinae** Latreille, 1802**Tribe Polistini** Latreille, 1802**Genus *Polistes*** Latreille, 1802***Polistes indicus*** Stolf, 1934

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, Gandhi Nagar, 32.701° N, 74.859° E, 302 m, 30.v.2022 (Reg. No. ZOO250.1).

**Distribution in India:** Jammu & Kashmir (new record).

***Polistes olivaceus*** de Geer, 1773

**Material examined:** 2 females, India, Jammu & Kashmir, Ramkot, 32.604° N, 75.288° E, 516 m, 25.viii.2019 (Reg. No. ZOO251.1); 1 female, India, Jammu & Kashmir, Jammu, Akhnoor, 32.899° N, 74.742° E, 330 m, 28.viii.2020 (Reg. No. ZOO251.2); 1 female, India, Jammu & Kashmir, Jammu University, Botanical Garden, 32.719° N, 74.868° E, 314 m, 28.ix.2020 (Reg. No. ZOO251.3).

**Distribution in India:** Andhra Pradesh, Arunachal Pradesh, Assam, Gujarat, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Karnataka, Madhya Pradesh, Manipur, Meghalaya, Nagaland, Odisha, Rajasthan, Sikkim, Tripura, Uttarakhand, Uttar Pradesh, and West Bengal.

***Polistes quadricingulatus*** Gusenleitner, 2006

**Material examined:** 2 females, India, Jammu & Kashmir, Kishatwar, 33.322° N, 75.776° E, 1801 m, 22.vii.2022 (Reg. No. ZOO252.1, ZOO252.2).

**Distribution in India:** Jammu & Kashmir, Uttar Pradesh.

***Polistes watti*** Cameron 1900

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, University of Jammu, Botanical garden, 32.719° N, 74.868° E, 314 m, 24.xii.2020 (Reg. No.

ZOO253.1).; 2 females, India, Jammu & Kashmir, Samba, Vijaypur, 32.705° N, 75.888° E, 341 m, 06.xi.2021 (Reg. No. ZOO253.2, ZOO253.3); 1 female, India, Jammu & Kashmir, Rajouri, Sunderbani, 33.049° N, 74.490° E, 576 m, 05.v.2022 (Reg. No. ZOO253.4); 2 females, India, Jammu & Kashmir, Jammu, R.S. Pura, 32.604° N, 74.731° E, 272 m, 07.v.2022 (Reg. No. ZOO253.5, ZOO254.6); 2 females, 1 male, Jammu & Kashmir, Jammu, Arnia, 32.519° N, 74.799° E, 271 m, 08.v.2022 (Reg. No. ZOO253.7, ZOO253.8, ZOO253.9); 1 female, Jammu & Kashmir, Jammu, Kotbalwal, 32.733° N, 74.876° E, 309 m, 10.v.2022 (Reg. No. ZOO253.10); 1 female, Jammu & Kashmir, Kathua, 32.385° N, 75.518° E, 348 m, 24.iv.2022 (Reg. No. ZOO253.11).

**Distribution in India:** Bihar, Chhattisgarh, Delhi, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Jharkhand, Madhya Pradesh, Meghalaya, Orissa, Punjab, Rajasthan, Uttar Pradesh, Uttarakhand, West Bengal.

**Tribe Ropalidiini** Guerin-Meneville, 1831

**Genus Ropalidia** Guerin-Meneville, 1831

**Ropalidia brevita** Das & Gupta, 1989

**Material examined:** 2 females, 1 male, India, Jammu & Kashmir, Kathua, Ramkot, 32.604° N, 75.288° E, 516 m, 25.viii.2019 (Reg. No. ZOO254.1, ZOO254.2); 1 female, Jammu & Kashmir, Kathua, Ramkot, 32.604° N, 75.288° E, 516 m, 17.v.2022 (Reg. No. ZOO254.3); 2 females, Jammu & Kashmir, Punch, Mendhar, 33.605° N, 74.143° E, 938 m, 01.v.2022 (Reg. No. ZOO254.4, ZOO254.5).

**Distribution in India:** Assam, Delhi, Goa, Haryana, Himachal Pradesh, Jammu & Kashmir (new record), Orissa, Rajasthan, Sikkim, Uttarakhand, Uttar Pradesh, and West Bengal.

#### Unidentified Species:

**Family Mutillidae** Latreille, 1802

**Subfamily Mutillinae** Latreille, 1802

**Genus Promecilla** Andre, 1902

**Material examined:** 2 males, India, Jammu & Kashmir, Udhampur, 32.641° N, 75.141° E, 680 m, 07.vii.2022; 1 female, India, Jammu & Kashmir, Kathua, Ramkot, 32.641° N, 75.343° E, 666 m, 12.ix.2022 (Reg. No. ZOO259.1).

**Family Pompilidae** Latreille, 1804

**Material examined:** 1 female, India, Jammu & Kashmir, Jammu, University of Jammu, Department of Zoology, 32.724° N, 74.866° E, 316 m, 08.iv.2022 (Reg. No. ZOO242.1).

There are 14 species which are first records from

Jammu region: *Aenictus aitkeni*, *Antepipona bipustulata*, *Antodynerus limbatus*, *Camponotus pennsylvanicus*, *Chalybion bengalense*, *Delta pyrifforme pyrifforme*, *Dorylus laevigatus*, *Indodynerus capitatus*, *Polistes indicus*, *Rhynchium brunneum*, *Ropalidia brevita*, *Sceliphron madraspatanum*, *Vespa basalis*, and *Vespa tropica*.

## DISCUSSION

Similar studies were conducted by various works in different parts of Jammu & Kashmir. Saini et al. (2012) investigated the species diversity of bumblebees from Jammu, Kashmir, and Ladakh provinces. During the study, they found across various species of bumble bees with four first records from the region namely, *Bombus cornutus*, *B. partthenius*, *B. miniatus*, and *B. morawitzianus*. The species *Bombus morawitzianus* was also the first record from the nation during their study. studied the insect community of Hirpora Wildlife Sanctuary, Jammu and Kashmir and found that the Hymenoptera is the second most diverse insect order in the region after Lepidoptera. Rajmohana et al. (2018) recorded 3,054 species of Hymenoptera from the Himalayan biogeographic zone and the ZSI published their findings. Wachkoo et al. (2020) was the first to record the inventory of ants' species from Jammu & Kashmir based on the museum collections and scientific literature. Their studies recorded 198 ants belonging to 54 genera from the region. Rameshkumar et al. (2024) has also made the inventory of Hymenoptera species from Jammu & Kashmir and provided a list of 764 species from the state.

Various workers studied the diversity of the order Hymenoptera from different regions of the country. The region-specific studies collectively establish that Hymenoptera diversity varies significantly across biogeographic zones, land-use types, and states in India.

Badwaik (2022) prepared a comprehensive list of ant species from Wardha District of Maharashtra and recorded 57 species belonging to 30 genera and six subfamilies. Gawas & Gupta (2022) compared the diversity of Vespidae wasps in the forest and agroecosystem of Western Ghats region of Goa. They found that the forest ecosystem is rich in diversity of Vespidae with 125 individuals belonging to 19 species and 12 genera while 106 individuals belonging to 13 species and seven genera were recorded from agroecosystems, showing the influence of habitat type on species richness.

The studies are notable for their coverage of semi-

1. *Apis cerana*2. *Apis dorsata*3. *Apis mellifera*4. *Bombus haemorrhoidalis*5. *Xylocopa fenestrata*6. *Xylocopa pubescens*7. *Tachysphex* species8. *Tachytes* species9. *Halictus vicinus*10. *Megachile lanata*11. *Chalybion bengalense*12. *Sceliphron madraspatanum*13. *Evania appendigaster*14. *Promecilla* species15. *Pompilidae* species

Image 1–15. Pictures of the observed hymenopteran species. © Authors.

16. *Tapinoma melanocephalum*17. *Aenictus aitkeni*18. *Dorylus laevigatus*19. *Camponotus compressus*20. *Camponotus pennsylvanicus*21. *Camponotus* species22. *Polyrhachis lacteipennis*23. *Formica clara*24. *Pheidole indica*25. *Tetramorium similimum*26. *Trichomyrmex aberrans*27. *Trichomyrmex destructor*28. *Trichomyrmex scabriceps*29. *Myrmica aimonissabaudiae*30. *Lepisiota integra*31. *Lepisiota sericea*32. *Monomorium indicum*33. *Myrmecaria brunnea*34. *Messor himalayanus*

Image 16–34. Pictures of the observed hymenopteran species. © Authors.

35. *Messor instabilis*36. *Leptogenys diminuta*37. *Odontoponera denticulate*38. *Tetraponera rufonigra*39. *Indodynerus capitatus*40. *Vespa basalis*41. *Vespa tropica*42. *Vespa velutina*43. *Antodynerus limbatus*44. *Delta pyriforme pyriforme*45. *Antepipona bipustulata*46. *Rhynchium brunneum*47. *Polites indicus*48. *Polistes olivaceus*49. *Polistes quadricingulatus*50. *Polistes watti*51. *Ropalidia brevita*

Image 35–51. Pictures of the observed hymenopteran species. © Authors.

evergreen forest ecosystems, arid & semi-arid landscapes and highlights the species richness of hymenopterans in western India.

The study documented 50 species across 21 families and 42 genera, indicating a rich and diverse hymenopteran fauna in the northeastern plains. Minz et al. (2024) prepared an annotated checklist from the Chattisgarh region and documented the diversity of Chalcididae in the region, contributed to the understanding of parasitoid wasp distribution. In this way, the studies well explained the diversity of Hymenopterans in the Eastern and Northeastern region of India.

Jangra et al. (2022) conducted a study on the semi-arid Green park area of Hisar region in Haryana and documented nine ant species representing seven genera and three subfamilies, highlighting the adaptability of ants in diverse habitats. The studies cover the Southern and Northern regions of the India.

The analysis of different region-specific studies provides a more informed and comparative analysis of hymenopteran diversity and underscores the importance of localized surveys in understanding the distribution patterns and ecological roles of hymenopteran insects. The findings of the current study from the Jammu region contribute to this growing knowledge and highlight the need for continued research in the region.

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## Four new additions to the angiosperm flora of Manipur, India

**Bimolkumar Singh Sadokpam<sup>1</sup>**, **Sanatombi Devi Yumkham<sup>2</sup>**, **Dhaneshwor Waikhom<sup>3</sup>**  
& **Sorokhaibam Sureshkumar Singh<sup>4</sup>**

<sup>1-4</sup> Department of Botany, School of Life Sciences, Manipur University, Canchipur, Manipur 795003, India.

<sup>1</sup>bimolkrsadokpam1@gmail.com, <sup>2</sup>rifle\_yumkham@rediffmail.com, <sup>3</sup>waikhomdhaneshwor0@gmail.com,

<sup>4</sup> suresh@manipuruniv.ac.in (corresponding author)

**Abstract:** Field surveys and exploration studies in natural forest vegetations in the hill and the central valley areas of Manipur led to documentation of four species under four genera and four families of Angiosperms. The species reported as new records to the flora of Manipur are *Epithema carnosum* Benth. (Gesneriaceae), *Flemingia latifolia* Benth. (Fabaceae), *Persicaria chinensis* var. *hispida* (Hook.f.) Kantachot (Polygonaceae), and *Solanum diphyllum* L. (Solanaceae). Detailed taxonomic descriptions, illustrations, nomenclature, phenology, and conservation status of the four species are described. Based on the findings of the present study, it is concluded that further floristic exploratory studies of the natural vegetation in the valley and hill regions of the state could lead to documentation of several new records, including the discovery of species previously unknown to the world.

**Keywords:** Conservation, *Epithema*, *Flemingia*, floristic diversity, habitats, Indo-Burma, inventory, *Persicaria*, *Solanum*, Spermatophytes.

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**Author details:** BIMOLKUMAR SINGH SADOKPAM is a research scholar working in the fields of plant systematics and ethnobotany in the Department of Life Sciences (Botany), Manipur University, Canchipur, Impha.. DR. SANATOMBI DEVI YUMKHAM is as guest faculty and working in the fields of plant systematics, ethnobotany, anatomy and cryptogam in the Department of Life Sciences (Botany), Manipur University, Canchipur, Imphal. DHANESHWOR WAIKHOH is a research scholar working in the field of soil microbiology in the Department of Life Sciences (Botany), Manipur University, Canchipur, Imphal. DR. S. SURESHKUMAR SINGH is associate professor in Department of Life Sciences (Botany), Manipur University, Canchipur, Imphal and working in the field of diversity and conservation of wild *Musa* species

**Author contributions:** BSS—carried out field survey, initial manuscript drafting, photo preparation; SDY—final manuscript drafting, photo preparation, designing experiment; DW—assisting in field survey, manuscript drafting; SSS—supervised the research, editing, conceptualization, revision and finalization of the manuscript

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## INTRODUCTION

Manipur, a northeastern state of India, lies between 92°59'–94°46' E & 23°50'–25°42' N under the Indo-Burma Biodiversity Hotspot region of the world, harbors a rich floristic diversity and shows a high degree of endemism. The phytogeographical features and varying climatic regimes provide luxuriant growth of diverse flora. Recently, Botanical Survey of India reported a total of 22,214 angiosperm species from India (BSI 2024). Several exploration works have been reported on floristic explorations and discovery of new species from this state since the later part of the 19<sup>th</sup> Century (Clarke 1889; Kaith 1936; Deb 1961a,b; Singh 1990). Deb (1961a,b) reported a total of 1,961 species and 48 varieties of angiosperms in two important published works, “Dicotyledonous Plants of Manipur Territory” and “Monocotyledonous Plants of Manipur Territory”. Shukla & Baishya (1979) reported 53 species of flowering plants under 49 genera and 13 species of cryptogams to the flora of Manipur. Singh et al. (2000) recorded 2,380 species of plants under 1,052 genera belonging to 205 families from Manipur. A total of 27 species of flowering plants belonging to 26 genera under 17 families have been reported as new records from Manipur (Sharma et al. 2000). Fifteen angiosperm taxa of the family Cyperaceae and Poaceae from different parts of the valley districts of Manipur were reported by Khan et al. (2007). Balachandran & Ravikumar (2014) reported 14 species of flowering medicinal plants as new distribution records during exploratory studies in different parts of the state. Dhatchanamoorthy et al. (2018) added 11 angiosperm taxa to the flora of Manipur. Chanu & Bhattacharyya (2020) reported the occurrence of five species of woody climbers (Lianas) as new records to the flora of Manipur. A total of 4,012 species of plants under 1,405 genera belonging to 225 families were reported from the state (Agrawala et al. 2023).

Due to inaccessible hills and mountain habitats, plant exploration works have been slow and preparing a complete floristic inventory challenging. There has been a number of new records of distribution reported from Manipur during the last few decades (Khomdram et al. 2011; Gogoi & Mao 2012; Thongam et al. 2013; Yumkham et al. 2013; Nanda et al. 2014; Devi et al. 2015, 2016, 2021, 2022; Rao & Kumar 2015; Khuraijam et al. 2017; Pradheep et al. 2019; Chowla et al. 2020; Gogoi & Rana 2020; Gogoi et al. 2022; Singh & Mao 2024). Similarly, several new species have been discovered and added to the flora of Manipur, namely, *Roscoeia ngainoi* A.A. Mao & Bhaumik (Mao & Bhaumik

2007); *lone kipgenii* Kishor, Chowlu & Vij (Kishor et al. 2012); *Zingiber kangleipakense* Kishor & Škorničk. (Kishor & Leong–Škorničková 2013); *Nymphaea manipurensis* Asharani & Biseshwori and *Nymphaea manipurensis* var. *versicolor* Asharani & Biseshwori (Devi & Thongam 2014); *Thrixspermum indicum* Vik. Kumar, D. Verma & A.N. Rao (Kumar et al. 2017); *Trichodesma kumarianum* S.D. Yumkham, N.P. Devi, S.D. Khomdram & M.R. Devi (Yumkham et al. 2019); *Argostemma kamjongense* Sadokpam, S.D. Khomdram & S.D. Yumkham (Sadokpam et al. 2023); *Curcuma kakchingense* Bidyaleima, R. Kishor & G.J. Sharma (Laishram et al. 2023).

Studies conducted on limited accessible natural habitats in the state have nonetheless revealed high floristic diversity, with numerous records on species distribution, new records, and description of new species in the past. It is assumed that there could be a few unreported flora including new species and new records yet to be discovered and reported from the unexplored habitats of the state. The geographical features of the larger area (90%) of the state are inaccessible terrains of hills and mountains with poor road and communication facilities which have hindered floristic studies. Therefore, the present study aimed to explore and document floral diversity of angiosperms with special emphasis on unreported taxa from different parts of Manipur.

## MATERIALS AND METHODS

Extensive floristic surveys were conducted in the hill forests of Kamjong District and five valley districts (Imphal East, Imphal West, Thoubal, Kakching and Bishnupur) of Manipur from 2022 to 2024. Preparation of herbarium voucher specimens was done following the standard herbarium techniques (Jain & Rao 1976) and deposited at Botanical Survey of India (BSI), Eastern Regional Centre (ASSAM) & Manipur University Museum of Plants (MUMP), Department of Botany, Manipur University. Identification of specimens was done by referring to available floras and literature. The classification, nomenclature with synonyms is provided based on APG IV (2016), Brummitt & Powell (1992), accepted names were verified from POWO (2024) and IPNI (2024). All the species are listed alphabetically and described briefly with morphological characters, phenology, distribution and conservation Status (IUCN 2024). Microphotographs for analyzing morphological characters were obtained by using Stereo zoom Microscope (BA 210 Digital LED Motic), Sony Cybershot DSC–HX400V, Leica Wild M3Z

Stereo Microscope (Heerbrugg, Switzerland).

The details of each species are described below.

## RESULTS

A total of four taxa under four different families of Spermatophytes were found to be new records and reported as additions to the state flora of Manipur. The reported plant species are *Epithema carnosum* Benth. (Gesneriaceae), *Flemingia latifolia* Benth. (Fabaceae), *Persicaria chinensis* var. *hispida* (Hook.f.) Kantachot (Polygonaceae) and *Solanum diphyllum* L. (Solanaceae).

**1. *Epithema carnosum* Benth.,** *Scroph. Ind.* 57. 1835; C.B.Clarke, Commelyn. Cyrtandr. Bengal 129, t. 90. 1874; Hook.f., Fl. Brit. India 4: 369. 1884; Kanjilal et al., Fl. Assam 3: 399. 1939; Giri & al., Mat. Fl. Arunachal Pradesh 231. 2008. (Gesneriaceae) (Image 1).

**Synonyms:** *Aikinia carnosus* (Benth.) G.Don

Stem herbaceous, 5–36 cm, puberulent; basal leaves unpaired, petiolate, 4.5–7.5 cm long, membranous, cordate, margin undulate-denticulate, obtuse apex, 5–12 × 5–11.5 cm, upper leaves in pair, petiolate or



Image 1. *Epithema carnosum* Benth.: a—Lithophytic habit | b & c—epiphytic habit | d—inflorescence | e—young plant associated with *Argostemma kamjongense*. © Bimolkumar Singh Sadokpam.

subsessile (0–6 cm long), leaf blade subspherical, cordate to ovate-elliptic, acute apex, subcordate base to truncate base, margin subentire to dentate or crenate-sinuate, 2.3–11.5 × 2.2–5.8 cm; inflorescence dense cyme, one-many-flowered, peduncle 0.4–6 cm long, puberulent; bract 1, subcucullate, 1–12 × 2–8 mm, dentate; flowers cleistogamous; pedicels 2–6 mm long; calyx whitish-green, 5-lobed from above middle, 3.5–5.4 × 1–3 mm, lobes triangular, 1.4–2.2 × 0.6–1.2 mm, outer pubescent, 0.25–0.5 mm long, inner glabrous; corolla dark purple with white tinge, tube 3.2–4.2 × 2.1 mm, 2-lipped limb, apex notched, outside glabrous, villous inside; stamen 0.5–0.8 mm long, staminodes 0.7–0.8 mm long, longitudinal dehiscence; ovary ovoid-globose, style puberulent, simple, capsules subglobose, 3–4 mm wide; seeds oblong or ellipsoid

**Flowering & Fruiting:** July–September

**Distribution:** China, Thailand, Taiwan, Nepal, Borneo, Sri Lanka, Bhutan, Myanmar, India (Arunachal Pradesh, Andhra Pradesh, Meghalaya, Orissa, Uttarakhand, West Bengal and now in Manipur).

**Specimen examined:** India, Manipur, Kamjong District, Yeasom Hills, 24.698° N, 94.178° E, 950 m elevation, 19.ix.2022, Bimolkumar 1001.

**Notes:** It is a small annual herb primarily found to grow in tropical and subtropical forests. The taxon prefers to grow in terrestrial habitats but can sometimes adapt to grow as lithophytes and epiphytes.

**Conservation status:** Least Concern (LC)

**2. *Flemingia latifolia*** Benth., Miquel, Pl. Jungh. 2: 246. 1852; Kurz, Forest Fl. Burma 2: 375. 1877; Prain, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 69(2): 441. 1897; Baker, Hook.f., Fl. Brit. India 2: 228. 1876; Kuntze, Revis. Gen. Pl. 1: 199. 1891; Mukerjee, Bull. Bot. Soc. Bengal 6(1): 17. 1953; Gavade et al., Webbia 75(2): 157. 2020; Do & Gao, Phytotaxa 429(1): 28. 2020. (Fabaceae) (Image 2).

**Synonyms:** *Maughania latifolia* (Benth.) Mukerjee; *Flemingia congesta* var. *latifolia* (Benth.) Baker

Shrub, erect; leaves trifoliolate; stipules 1–3 cm, lanceolate; petiole 4–9 cm, sometimes winged narrowly, leaflets papery, glands present, terminal leaflet elliptic–lanceolate, 8–14 × 4–6 cm, pubescent, apex acuminate, lateral leaflets oblique, broadly lanceolate; inflorescence rusty villous, 1–3 flowers per axil or raceme terminal; bracts elliptic, 0.7–1 × 0.5–0.6 cm, peduncle 0.2 cm; flowers 1–1.2 × 0.9–1 cm; calyx 0.9 cm; corolla longer than calyx, purplish-pink, standard obovate-elliptic, auricles obtuse; wings with slender claw, obovate-oblong; keel wider than wings, suborbicular, clawed with

auricle; ovary with brown hairs, elliptic, gland dotted; fruit elliptic, 11–14 × 6–7 mm, villous, inflated, beaked; seeds two, dark brown or black, suborbicular-globose.

**Flowering & Fruiting:** July–December

**Distribution:** China, India (Assam, Arunachal Pradesh, Meghalaya, Mizoram, now in Manipur), Indonesia (Java), Myanmar, Laos and Vietnam.

**Specimen examined:** India, Manipur, Imphal West, Heibok Hill, 24.743° N, 93.931° E, 815 m elevation, 28.x.2024, Bimolkumar 0017.

**Notes:** The taxon is a shrub which grows primarily in tropical forests. The species was found growing mainly along the foothills.

**Conservation status:** Not Evaluated (NE).

**3. *Persicaria chinensis* var. *hispid*** (Hook.f.) Kantachot, Thai Forest Bull. Bot. 38: 135. 2010; Hook.f., Fl. Br. Ind. 5(13): 45. 1886; Sam., Symbolae Sinicae 7(1): 180. 1929; Li et al., Fl. China 5. 301. 2003. (Polygonaceae) (Image 3)

**Synonyms:** *Polygonum chinense* var. *hispidum* Hook.f.; *Polygonum chinense* f. *hispidum* (Hook.f.) Sam.

Herb, perennial; stem erect, 80–84 cm tall, densely hispid internode; petiole 1.9 cm, lobed at base; leaf elliptic-ovate, 3.7–15 × 1.3–9 cm, abaxial and adaxial surfaces hispid, base cordate, entire margin, apex acuminate, ocrea tubular, 1.1 cm long, hairy, membranous, 11–13 parallel veins; inflorescence terminal or axillary, capitate, aggregated, peduncle 5 cm long, hairy; bract broadly ovate, each with 1–3 flowered; perianth white, 5-partite, tepals ovate, stamens 8, 0.2–0.3 cm long, pistil 0.4 cm long, style 3, connate; achenes, black, 0.6 cm wide, opaque, broadly ovoid.

**Flowering & Fruiting:** November–January.

**Distribution:** China, Myanmar, Thailand, Laos, India (Meghalaya, Nagaland, West Bengal and now in Manipur).

**Specimen examined:** India, Manipur, Imphal East (Nongmaiching Hill) 24.805° N, 94.031° E, 891m elevation, and Imphal West (Manipur University) 24.749° N, 93.925° E, 814 m elevation, 26.x.2023 and 31. vii. 2024, Bimolkumar 1020.

**Note:** It is a perennial plant growing primarily in temperate forest along the foothills.

**Conservation status:** Not Evaluated (NE).

**4. *Solanum diphyllum*** L., Sp. Pl. 184. 1753; Zhi Y.Zhang et al., in C.Y.Wu and P.H.Raven, Fl. China 17: 317. 1994; T.K.Paul & M.C.Biswas in Bull. Bot. Surv. India 37: 137. 2000. M.ReemaKumari in Rheede 23(1) 50–51. 2013. (Solanaceae) (Image 4).

**Synonyms:** *Pseudocapsicum diphyllum* (L.) Medik.

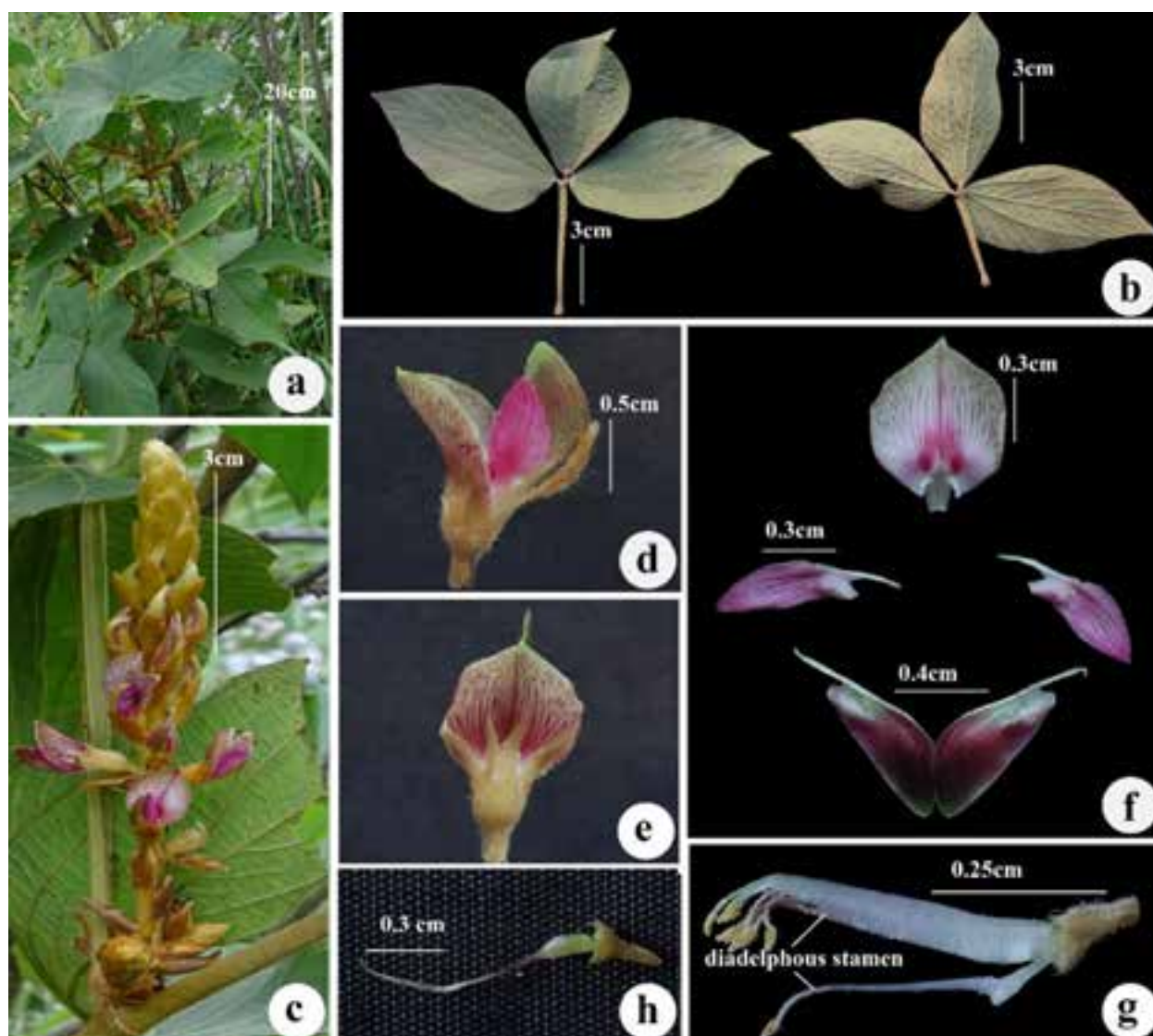


Image 2. *Flemingia latifolia* Benth.: a—habit | b—abaxial & adaxial leaves | c—inflorescence | d & e—single flower | f—dissected flower | g—stamen | h—pistil. © Bimolkumar Singh Sadokpam.

Shrub, perennial, upto 3 m high, minutely pubescent; leaves two at each node, unequal, linear-obovate or oblong-elliptic, margin entire, apex acute, 1.2–10.5 × 0.6–4.3 cm, lateral nerves 5–7 on each side, minutely hairy, petioles 0.5 cm; inflorescence racemose fascicle, up to 10-flowered; calyx cupular, 0.15–0.21 × 0.2–0.24 cm, minutely pubescent, 5-lobed; corolla stellate, 0.6–0.8 cm across, creamy white, 5-lobed, 0.4–0.6 × 0.25–0.3 cm, tube 0.1–0.2 cm long; stamens five, epipetalous, filaments 0.1 cm long, anthers oblong, 0.2 × 0.1 cm, yellow, dehiscing poricidally at first, later longitudinally; ovary globose, 0.1 cm long; style 0.3–0.5 cm long; berries globose, 0.6–1.0 cm in diam., glabrous, yellow or reddish-yellow, seeds reniform or discoid, 0.2 × 0.4 cm,

creamy yellow.

**Flowering & Fruiting:** March–November

**Distribution:** China, Taiwan, United Kingdom; Mexico, Central America, India (West Bengal, Assam and now in Manipur).

**Specimen examined:** India, Manipur, Imphal West, Singjamei Waikhom Leikai 24.773° N, 93.942° E, 803 m elevation, 10.xi.2023, Bimolkumar 1015.

**Notes:** It is easily distinguishable from other species of *Solanum* in having two unequal leaves at each node.

**Conservation Status:** Not Evaluated (NE).

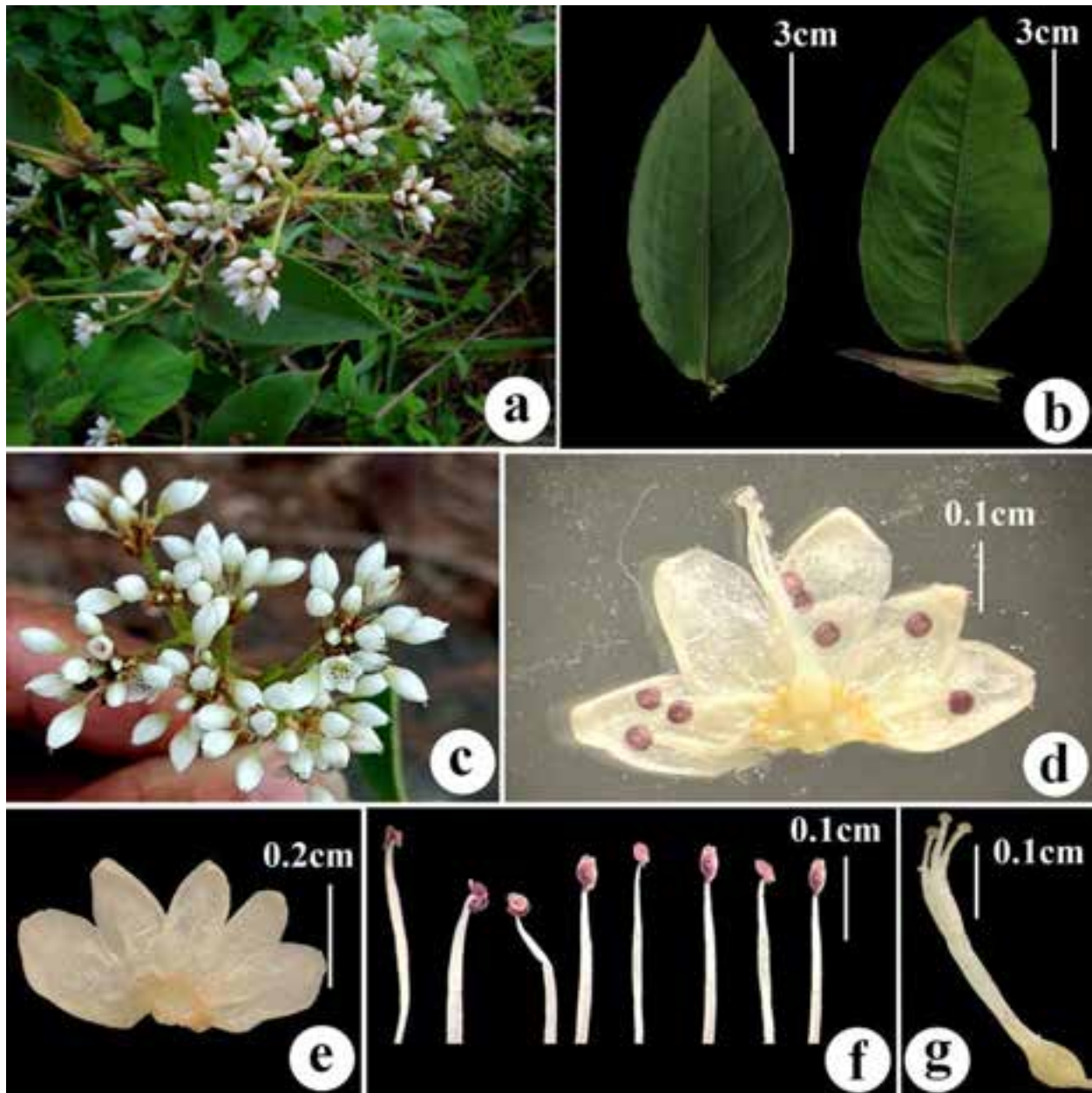


Image 3. *Persicaria chinensis* var. *hispida* (Hook.f.) Kantachot: a—habit | b—ventral leaf comparison between *Persicaria chinensis* var. *chinensis* and *Persicaria chinensis* var. *hispida* | c—inflorescence | d—dissected flower | e—tepals | f—stamens | g—pistil. © Sanatombi Devi Yumkham.

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Image 4. *Solanum diphyllum* L.: a—habit | b—Inflorescence | c—a twig with fruit | d & e—abaxial & adaxial leaves showing unequal pair of leaves | f—fruits on plant | g—single fruit | h—T.S. of fruit. © Sanatombi Devi Yumkham.

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REVIEW

## Historical records of the Jaguar *Panthera onca* (Linnaeus, 1758) (Mammalia: Carnivora: Felidae) in the state of Santa Catarina, Brazil

**Jackson Fábio Preuss<sup>1</sup>** & **Pedro Henrique Amancio Padilha<sup>2</sup>**

<sup>1</sup> Wildlife Studies Center (NEVS), University of the West of Santa Catarina (UNOESC), São Miguel do Oeste, 89900-000, State of Santa Catarina, Brazil.

<sup>2</sup> University of the Educational Society of Santa Catarina (UNISOCIESC), Joinville, 89206-101, State of Santa Catarina, Brazil.

<sup>1</sup>jackson\_preuss@yahoo.com.br (corresponding author), <sup>2</sup>pedroamancio2002@gmail.com

**Abstract:** We compiled and analysed 16 historical photographic records of the Jaguar *Panthera onca* in the state of Santa Catarina before 1985, most of which have not been published in scientific literature. These records obtained from personal collections, newspapers, and public institutions cover more than a century and reveal a broad geographic distribution of the Jaguar in the state prior to its possible local extinction. The greatest concentration of records was registered in the extreme west and west of Santa Catarina (56.25%), followed by Itajaí Valley and northern plateau (25%), northeastern (12.5%), and the southern highlands (6.25%). Eight records are located less than 150 km off the Argentinian border. Thirteen records represent hunted individuals and three individuals were captured alive. The localities with records include Joinville, Corupá, Blumenau, Itapiranga, Fraiburgo, Taió, Sul Brasil, Guaraciaba, Paraíso, Anchieta, Cunha Porã, Urubici, and Campo Erê. The latter, registered in 1984, represents the most recent evidence of the Jaguar in the state. Our compilation provides a new historical base on the distribution of the Jaguar in Santa Catarina, suggesting that hunting, associated with habitat loss and fragmentation and reduction of natural prey, played a significant role in the Jaguar's likely extinction in the state.

**Keywords:** Geographic distribution, photographic records, Itajaí Valley, north-east, west of Santa Catarina.

**Resumo:** Compilamos e analisamos 16 registros fotográficos históricos de onças-pintadas *Panthera onca* no estado de Santa Catarina anteriores a 1985, sendo a maioria inéditos na literatura científica. Esses registros, obtidos por meio de acervos pessoais, jornais e instituições públicas, abrangem mais de um século e revelam uma ampla distribuição geográfica da espécie no estado, antes de sua provável extinção local. A maior concentração de ocorrências foi registrada no extremo oeste e Oeste Catarinense (56,25%), seguido do Vale do Itajaí e Planalto Norte (25%), nordeste Catarinense (12,5%) e Planalto Serrano (6,25%). Oito registros localizam-se a menos de 150 km da fronteira com a Argentina. Treze registros correspondem a animais abatidos, e três a indivíduos capturados vivos. As localidades com registros incluem Joinville, Corupá, Blumenau, Itapiranga, Fraiburgo, Taió, Sul Brasil, Guaraciaba, Paraíso, Anchieta, Cunha Porã, Urubici e Campo Erê. Este último, registrado em 1984, representa o dado mais recente confirmado da espécie no estado. Os resultados fornecem uma base histórica inédita sobre a distribuição da onça-pintada em Santa Catarina e sugerem que a caça predatória, associada à perda e fragmentação de habitat e à redução de presas naturais, desempenhou papel central na possível extinção dessa espécie no estado.

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## INTRODUCTION

The Jaguar *Panthera onca* is the largest felid in the Americas and the third largest in the world (Seymour 1989). As a top predator, it plays a key ecological role in maintaining ecosystem balance by regulating prey populations (Dalerum et al. 2008). Despite its ecological importance and cultural relevance, it faces persistent conservation challenges, largely driven by conflicts with humans (Hofstatter & Oliveira 2020).

Over the last century, the Jaguar has disappeared from extensive portions of its historical range (Sanderson et al. 2002). Historically distributed from the southern United States to northern Argentina, it now occurs in roughly half of its original range, having undergone an estimated 49% population decline over the past 50 years (Seymour 1989; Sanderson et al. 2002; Zeller 2007). The Jaguar is extinct in El Salvador, the United States, and Uruguay, with remaining populations occurring from northern Mexico to northern Argentina (Quigley et al. 2017). It is currently listed as 'Near Threatened' on the IUCN Red List (Quigley et al. 2017) and as Vulnerable in the Brazilian Red List (Ministério do Meio Ambiente 2022).

In Brazil, the Jaguar occurs across all major biomes except the Pampas, where the last known individual was killed in 1952, resulting in the species' extirpation in that region (Peters et al. 2016; Morato et al. 2018). The most viable Brazilian populations today persist in the Pantanal and the Amazon (Silveira & Crawshaw 2008; Cavalcanti et al. 2012; Oliveira et al. 2012). In contrast, the Atlantic Forest, one of the world's most threatened biomes, retains approximately 11% of its original cover (Ribeiro et al. 2009). And only 10.32% of its landscape is considered to provide suitable habitat for the Jaguar. In this biome, it is classified as Endangered, with an effective population size estimated at fewer than 250 individuals (Ferraz et al. 2012; Morato et al. 2013).

Multiple anthropogenic pressures have contributed to the Jaguar's decline in Brazil since the early 1900s, including habitat loss caused by logging, agricultural expansion, and urbanisation (Morato et al. 2016), poaching, retaliatory killing following livestock predation (Hoogesteijn et al. 1993; Nowell & Jackson 1996), reductions in prey availability (Foster et al. 2016), and broader human-wildlife negative interactions (Murray et al. 1999; Zimmermann et al. 2005; Zeller 2007). These stressors, combined with ecological disruption and increased exposure to pathogens, pose additional risks to already vulnerable populations (Murray et al. 1999; Furtado & Filoni 2008).

In the state of Santa Catarina, historical records of the Jaguar are scarce. Cherem et al. (2004) documented records in Brusque, Blumenau, Urubici, Campo Alegre, and Joinville. The last known records from the southernmost portion of the Atlantic Forest date between the 1960s and the 1990s (Mazzolli 2008). More recently, Fusco-Costa et al. (2022), based on extensive surveys in the Serra do Mar region of southern Brazil, expanded the modelled Jaguar range in the Atlantic Forest by 9% and suggested that forested areas in Santa Catarina may represent potential habitat. Nevertheless, its actual presence in the state remains uncertain, relying primarily on sporadic, unconfirmed observations in forest remnants of the Upper Itajaí Valley and Araucárias National Park. Many of these assumptions stem from records near the Paraná and Rio Grande do Sul borders, reinforcing the hypothesis that the Jaguar may have become extinct in Santa Catarina due to habitat loss and intensive hunting. The most recent threatened species list for Santa Catarina categorizes the Jaguar as Critically Endangered in the state (FATMA 2011).

Understanding Jaguar occurrence in Santa Catarina requires a comprehensive evaluation of historical evidence. Therefore, the objective of this study is to compile and review historical photographic records of the Jaguar to clarify its past distribution and assess factors that may have contributed to its decline and possible extirpation in the state.

## Study area

The state of Santa Catarina in southern Brazil (Figure 1) has an approximate area of 95,730 km<sup>2</sup> (IBGE 2024). Santa Catarina's topography is characterized by a diversified relief, with elevated plateaus in the inland and a narrow coastal plain in the east. The western region is marked by hilly terrains and the presence of the Serra Geral Mountain Range, which influences the state's climatic dynamics. These mountainous formations act as natural barriers, regulating airflow distribution and affecting precipitation patterns (Vibrans et al. 2010, 2012). Thus, the most elevated areas register the lowest temperatures and frequent rainfall, whereas the coast, influenced by the Atlantic Ocean humidity, presents a mild and humid climate throughout the year (Alvares et al. 2013). The elevation gradient also reflects in the vegetation, with tropical and subtropical forests in the lowlands, and *Araucaria* formations in the plateaus and highlands (Klein 1978; Vibrans et al. 2010, 2012).

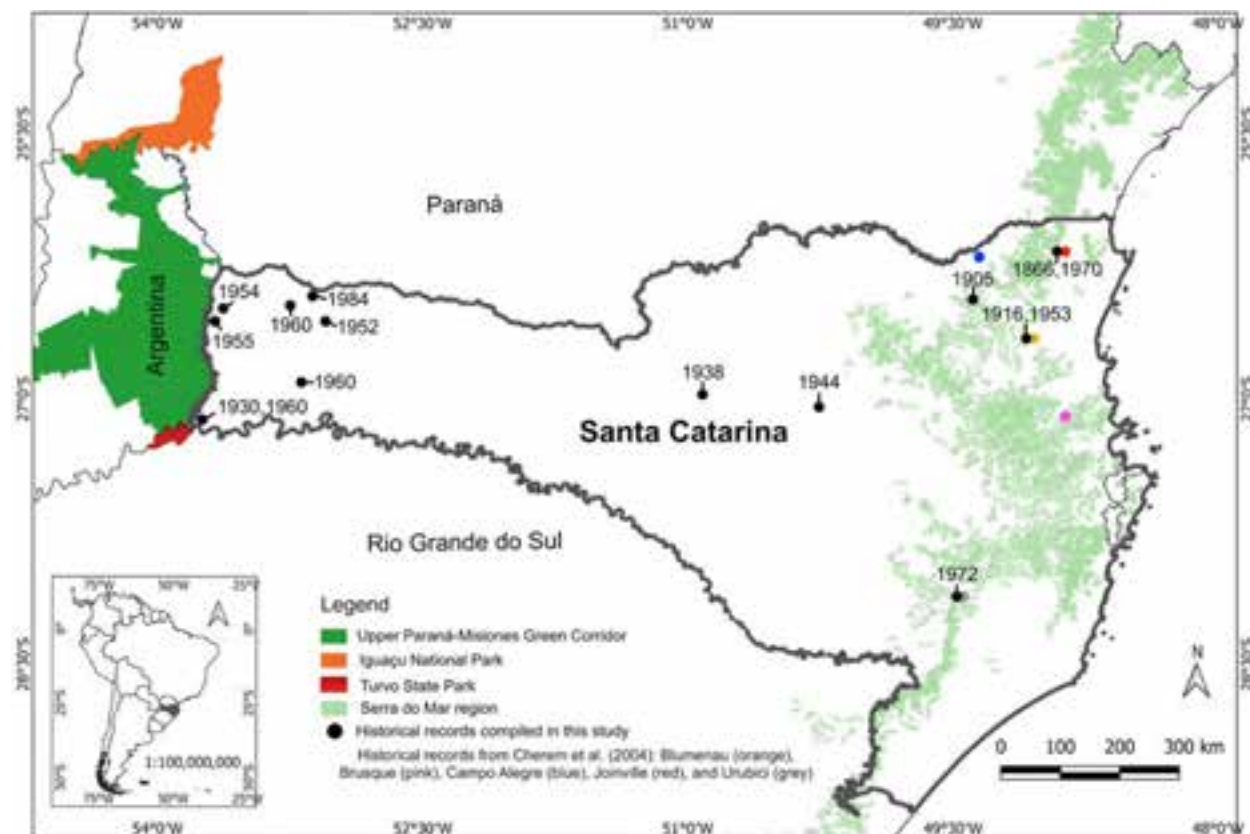


Figure 1. Jaguar records in the state of Santa Catarina between 1866 and 1984. Black dots indicate places and approximate dates.

## MATERIALS AND METHODS

### Historical records

We searched for historical photographs in non-indexed online newspapers and museum collections, and analysed personal reports followed by photos. After that, we elaborated a survey of Jaguars known to have been photographed or killed in the state of Santa Catarina.

The capture of a Jaguar in the state of Santa Catarina was frequently reported by many media outlets and shared as an attraction for long periods, thus helping our assessment. We found that the most useful words of search are: “Onça”, “Onça-pintada” (Jaguar), and “Tigre” (Tiger).

We only included Jaguars photographed in the state and omitted reports of individuals without photographic proof, animals possibly sighted, or rumours that they were wandering through the state, or any implausible records. When two or more records may cover the same individual, we adopted a conservative approach and counted both as a single record. For the historical records, we used the best available data and registered

the location of these Jaguars in Figure 1 and Table 1. When mapping the individuals, we used the location and date of the photographs with a location on the map. We recognize the limitations associated with newspaper reports, verbal testimonies, and other unverified sources. However, we assert that the reports presented here constitute the most complete available survey about the historically documented presence of the Jaguar in the state of Santa Catarina.

All historical records were georeferenced based on the localities described in the primary sources, i.e., photographs, newspapers, and personal accounts. For rural sites or historical place names, we used current administrative boundaries and toponyms to approximate locations. Coordinates were obtained using Google Earth Pro. Because many sources lack precise spatial detail, we estimated an uncertainty range of 1–20 km for each coordinate. These uncertainty levels were considered when interpreting spatial patterns, avoiding over-interpretation of fine-scale distribution.

## RESULTS

In total, we documented 16 photographic records of the Jaguar in the state of Santa Catarina between 1866 and 1984 (Table 1, Images 1–6), most of which have not been published in scientific journals. The historical records available indicate that the Jaguar was widely distributed across the state, with documented evidence throughout the 20<sup>th</sup> Century.

In the extreme west and west of Santa Catarina, nine occurrences were registered (56.25% of all). In the Itajaí Valley and northern plateau, four records were documented (25%). In the north-east, two records were identified (12.5%), and in the highlands, there was only one confirmed record (6.25%).

Of the 16 documented photographic records, 13 (81.25% of all) refer to hunted animals, while only three (18.75%) present captured individuals. Our dataset indicates for the first time that a Jaguar was shot in 1984 in the town Campo Erê (Image 6), located in the extreme west, close to the border with Paraná State. This record represents the most recent documentation in the state, updating the previously available data.

## DISCUSSION

### Reliability and limitations of historical records

Historical records have long been used to describe

the distribution of mammals, especially in regions where contemporary ecological data are scarce or absent (Tyler & Anderson 1990; Timm et al. 1997; Aubry et al. 2007; Díaz 2010; Babb et al. 2022). In Santa Catarina, however, determining these records presents substantial challenges due to the lack of consistent historical documentation. Similar difficulties were highlighted by Babb et al. (2022) in Arizona, where the absence of systematic registers and inconsistencies in sources compromised the reconstruction of temporal distribution patterns.

This challenge is exacerbated by the fact that much of the information concerning the Jaguar in Santa Catarina is dispersed across non-indexed documents, oral accounts, and journalistic records. These limitations reinforce the relevance of integrating different types of historical evidence to improve understanding of the species' past distribution in the state.

Despite these difficulties, the data gathered suggest that the Jaguar once had a broad distribution in Santa Catarina. However, estimating its actual area of occupation with precision remains difficult not only because of gaps and inconsistencies in the documentation (Babb et al. 2022), but also due to intrinsic biological characteristics that hinder detection. The Jaguar is a solitary animal with large home ranges and a naturally low population density (Sanderson et al. 2002; Silver et al. 2004), making both historical and contemporary assessments of distribution inherently challenging.

**Table 1. Confirmed historical records of the Jaguar *Panthera onca* in the state of Santa Catarina, Brazil, between 1866 and 1984.**

Year	Locality	Approximate coordinates	Type of record	Primary source of photograph
1866	Joinville	–26.304760, –48.845871	Killed	Fundação Biblioteca Nacional
1905	Corupá	–26.426010, –49.243450	Killed	Ligmar Raeder
1916	Blumenau	–26.916578, –49.071732	Captured	Museu Frei Miguel
1930	Itapiranga	–27.167940, –53.712952	Killed	Museu Almiro Theobaldo Müller
1938	Fraiburgo	–27.023319, –50.921928	Killed	Walter Neves
1944	Taió	–27.115748, –49.994181	Killed	Maria Vizentainer
1952	Sul Brasil	–26.741123, –52.969914	Killed	Ross family
1953	Blumenau	–26.916578, –49.071732	Killed	Alfredo Kath
1954	Guaraciaba	–26.597978, –53.521480	Captured	Lauro Steffen
1955	Paraíso	–26.619028, –53.673220	Killed	Biazzi family
1960	Anchieta	–26.536889, –53.331465	Killed	Piccoli family
1960	Itapiranga	–27.167940, –53.712952	Captured	Museu Almiro Theobaldo Müller
1960	Cunha Porã	–26.892682, –53.171931	Killed	Fundação e Casa da Cultura
1970	Joinville	–26.304760, –48.845871	Killed	Newspaper <i>A Notícia</i>
1972	Urubici	–28.006726, –49.591561	Killed	Orquiso Rei de Oliveira
1984	Campo Erê	–26.395318, –53.079029	Killed	Goelzer family



Image 1. Historical photographs of Jaguars in Santa Catarina: A—Joinville (1866) © Fundação Biblioteca Nacional | B—Corupá (1905) © Ligmar Raeder | C—Blumenau (1916) © Museu Frei Miguel.

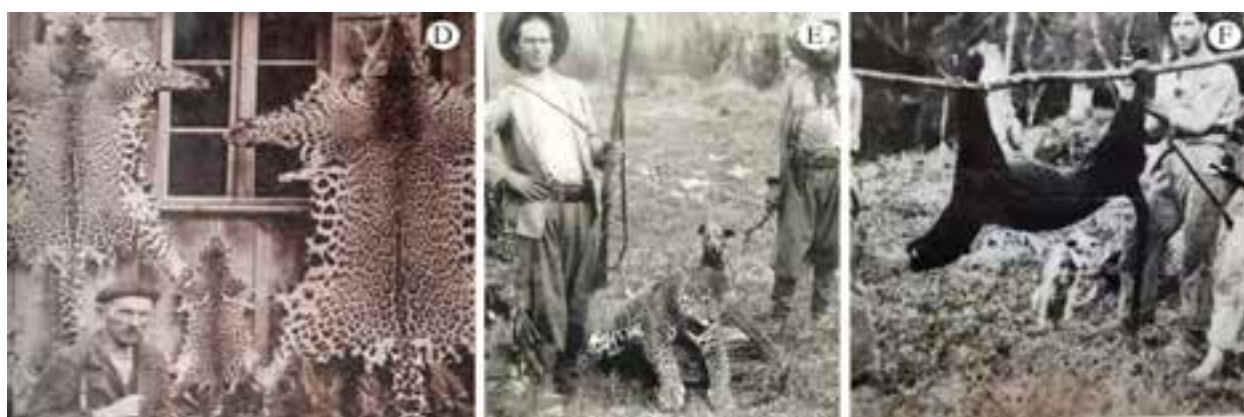


Image 2. Historical photographs of Jaguars in Santa Catarina: D—Itapiranga (1930) © Museu Almiro Theobaldo Müller | E—Fraiburgo (1938) © Walter Neves | F—Taió (1944) © Maria Vizentainer.

### Historical patterns of hunting and human persecution

The relatively high number of Jaguars shot in Santa Catarina over the decades demonstrates that hunting has been a recurrent practice in the state. This pattern converges with global evidence indicating that direct persecution is one of the main factors responsible for the decline of large carnivore populations (Ripple et al. 2014). Although the state of Santa Catarina lacks quantitative historical datasets on hunting pressure or prey abundance, the predominance of hunted specimens among the available photographic records strongly suggests that direct persecution played a central role in the Jaguar's decline. This qualitative pattern is consistent with trends documented, where hunting intensity has been identified as a major predictor of local extirpations (Gittleman et al. 2001; Mazzolli 2008; Nyhus 2016; Paviolo et al. 2016; Franco et al. 2018; Teixeira et al. 2023; Thompson et al. 2023). The combined effects of predatory hunting, retaliatory killing, and recreational hunting for trophies (Franco et al. 2018; Thompson et al.

2023) likely accelerated the disappearance of the Jaguar in Santa Catarina.

Beyond commercial and recreational motivations, human-wildlife negative interactions also played a substantial role. Competition for prey, aggravated by ecological imbalance and resource scarcity, often forced Jaguars into anthropogenic areas, resulting in predation on livestock and consequent retaliatory killing by farmers (Peters et al. 2016; Romero-Muñoz et al. 2019). Even though Jaguar meat is not traditionally consumed and is considered “disgusting” by some traditional communities (Fonseca et al. 2006), retaliatory hunting is widely documented in response to livestock depredation (Marchini & Macdonald 2012).

Cultural perceptions additionally contributed to the Jaguar's decline. Since early colonisation, the Jaguar has occupied a symbolic place of fear in the popular imagination, associated with risks to human life (Adams 2012). Although attacks on humans are rare, they amplify fear and negative attitudes (Dickman 2010; Kelly



Image 3. Historical photographs of Jaguars in Santa Catarina: G—Sul Brasil (1952) © Ross family | H—Blumenau (1953) © Alfredo Kath | I—Guaraciaba (1954) © Lauro Steffen.



Image 4. Historical photographs of Jaguars in Santa Catarina: J—Paraíso (1955) © Biazzi family | K—Anchieta (1960) © Piccoli Family | L—Itapiranga (1960) © Museu Almiro Theobaldo Müller.

2019), often justifying preemptive killing (Neto et al. 2011; Iserson & Francis 2015; Jędrzejewski et al. 2017). Fear-driven persecution is considered one of the major threats to large carnivore populations in Brazil (Inskip & Zimmermann 2009; ICMBio 2013), and has historically contributed to the decline of the Jaguar in Santa Catarina.

#### Habitat loss, prey decline, regional context, and extinction dynamics

Habitat loss and fragmentation represent critical factors in the decline of Jaguar populations. The species depends on large, well-connected forest remnants and abundant prey to persist (Cullen et al. 2005; De Angelo et al. 2011). Fragmentation reduces connectivity between habitat patches (Sanderson et al. 2002; Zeller 2007; Galetti et al. 2013; Knox et al. 2019; Romero-Muñoz et al. 2019), leading to rapid reductions in Jaguar occupancy.

In Santa Catarina, historical forest loss and extensive habitat fragmentation (Astete et al. 2008; Rocha et al. 2023) likely undermined the ecological conditions

required to sustain viable Jaguar populations, contributing to the marked depletion of key native prey species such as White-lipped Peccary *Tayassu pecari*, Collared Peccary *Dicotyles tajacu*, and Lowland Tapir *Tapirus terrestris*. The disappearance of these taxa, primarily due to poaching (Mazzolli 2008; Keuroghlian et al. 2012; Whitworth et al. 2022), may have played a decisive role in the Jaguar's extirpation. Reduced prey availability is known to elevate extinction risk for large predators, particularly in fragmented landscapes where recolonisation is limited (Karanth & Chellam 2009; Foster et al. 2016). In contrast, Jaguar populations persisting elsewhere in Brazil and Central America occur in regions with substantially greater prey abundance, reflected in markedly higher population densities, e.g., 6.7 individuals per 100 km<sup>2</sup> in the Pantanal (Soisalo & Cavalcanti 2006), 17.9 individuals per 100 km<sup>2</sup> in the Amazon (Ramalho 2012), and 4.6–8.8 individuals per 100 km<sup>2</sup> in Mexico and Belize (Silver et al. 2004; Torre & Medellín 2011). Even within the Atlantic Forest, regions such as Vale Natural Reserve support



Image 5. Historical photographs of Jaguars in Santa Catarina: M—Cunha Porã (1960) © Fundação e Casa da Cultura | N—Joinville (1970) © Newspaper A Notícia | O—Urubici (1972) © Orquiso Rei de Oliveira.



Image 6. Historical photograph of a Jaguar in Campo Erê, Santa Catarina (1984) © Goelzer family.

Jaguars largely because native prey assemblages remain relatively intact (Facure & Giaretta 1996; Galetti et al. 2009; Srbek-Araujo & Chiarello 2016).

Understanding the Jaguar's disappearance also requires considering the broader regional context of the Atlantic Forest. Across this biome, fewer than 300 Jaguars remain, distributed in small, isolated, and highly vulnerable subpopulations (Paviolo et al. 2016). Within this already precarious scenario, the southern portion of the Atlantic Forest stands out for exhibiting the most severe levels of contraction of the Jaguar range.

When compared with its neighbouring southern states, Santa Catarina presents the scarcest confirmed records of Jaguar occurrence. While Paraná and Rio Grande do Sul still retain recent or occasional confirmations of the species (Kasper et al. 2015; ICMBio 2020; Morato et al. 2023; Marcuzzo et al. 2025), the last verified record in Santa Catarina dates to 1984, with no subsequent evidence of persistence. Since that record, no confirmed occurrences have been documented despite increasing research effort in the region. More recently,

the surroundings of the 1984 record in Campo Erê have been the focus of some ongoing and unpublished wildlife monitoring initiatives, including camera trap surveys conducted by the first author, as well as informal surveys based on transect walks and interviews with forestry professionals, long-term residents, and biologists working in the area (forest engineer Rafael Link pers. comm. 10.xii.2024; biologist Augusto Finco pers. comm. 14.vi.2025; local residents Vilmar Tonatto pers. comm. 11.vi.2025, and Benhur Tonatto pers. comm. 16.xii.2025). These combined sources have not yielded any verifiable evidence of Jaguar presence to date. This temporal and evidential contrast suggests that Jaguar populations declined earlier and more abruptly in Santa Catarina, culminating in local extirpation, whereas remnant populations persisted in adjacent states. This pattern likely reflects a combination of accelerated habitat conversion, collapse of prey populations, and intense persecution throughout the 20<sup>th</sup> Century.

In Paraná, although the Jaguar is classified as Critically Endangered, it persists in two forested nuclei: the Serra

do Mar region and the Upper Paraná-Misiones Green Corridor, both of which have yielded confirmed records since 2016 (Casanova & Bernardo 2017; Nagy-Reis et al. 2020; Subirá et al. 2024). Recent population estimates derived from the 2020 Jaguar Census indicate that the broader bi-national Green Corridor supports an average of 93 individuals with a range of 73–122, representing the largest remaining Jaguar subpopulation in the Atlantic Forest (Paviolo et al. 2008; Subirá et al. 2024). Within the Brazilian portion, 19–33 Jaguars are estimated to be present in an area of 1,852.6 km<sup>2</sup> in Iguaçu National Park and surroundings (ICMBio 2020; Freitas 2021). The situation is even more critical in Rio Grande do Sul, where fewer than five individuals are thought to remain, all of them restricted to Turvo State Park, the last known area of its occurrence in the northeastern portion of the state (Paviolo et al. 2006; Kasper 2007; Kasper et al. 2015; Marcuzzo et al. 2025).

In contrast, Santa Catarina shows a complete absence of confirmed records for over four decades, reinforcing the interpretation that the Jaguar disappeared in the state earlier than in Paraná and Rio Grande do Sul. The 1984 record in Campo Erê updates previous knowledge, surpassing the last record cited by Mazzoli (2008) in Urubici in 1972, and reveals a pattern in which the final occurrences were concentrated near state borders and in regions with a lower human population density. Such spatial clustering suggests that human expansion and fragmentation may have accelerated range contraction, a trend consistent with broader patterns described for the Jaguar across South America (Chávez et al. 2016; Pereira-Garbero 2016).

Finally, distribution models indicate that parts of Santa Catarina retain potential connectivity with ecological corridors extending to Argentina (Rabinowitz & Zeller 2010). This reinforces the hypothesis proposed by Fusco-Costa et al. (2022), according to which certain forest remnants in the state may function as occasional passage zones or receive sporadic dispersers from neighbouring regions. Whether such movements still occur needs to be figured out in dedicated surveys.

Although the lack of recent confirmed records strongly supports the hypothesis of local extirpation, the absence of detection does not necessarily imply true absence. Modern survey techniques, such as large-scale camera trapping, environmental DNA, could reveal whether individuals still disperse into Santa Catarina from neighbouring areas. Integrating such approaches in future research may help clarify whether the Jaguar is definitively extinct or simply undetected at extremely low densities.

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**Author details:** DR. JACKSON FÁBIO PREUSS is a biologist, professor, and researcher at the Universidade do Oeste de Santa Catarina (UNOESC), Brazil. He holds a bachelor's degree in Biological Sciences, a master's degree in environmental sciences with emphasis on biodiversity and sustainability, and a PhD in biology with specialization in wildlife diversity and management. His research focuses on wildlife conservation, biodiversity, and ecosystem health in tropical environments. He leads the Wildlife Studies Center (Núcleo de Estudos em Vida Selvagem - NEVS) at UNOESC, has served as an ad hoc expert for the Environmental Military Police of Santa Catarina since 2010, and is actively involved in environmental advisory boards and science communication initiatives related to biodiversity conservation. PEDRO HENRIQUE AMANCIO PADILHA is a veterinary medicine student at the Universidade Sociedade Educacional de Santa Catarina (UNISOCIESC), Brazil. Presently, he is the vice-president of the Wild Animal Studies Group (Grupo de Estudos de Animais Silvestres - GEAS) at UNISOCIESC university, having worked with exotic pets and wild animals at clinics and rehabilitation centers.

**Author contributions:** JFP: conception of the study; collection and analysis of historical records; photographic survey; preparation of maps and figures; drafting and final revision of the manuscript. PHAP: collection and organization of historical records; photographic survey; preparation of maps and figures; contribution to the drafting and revision of the manuscript.





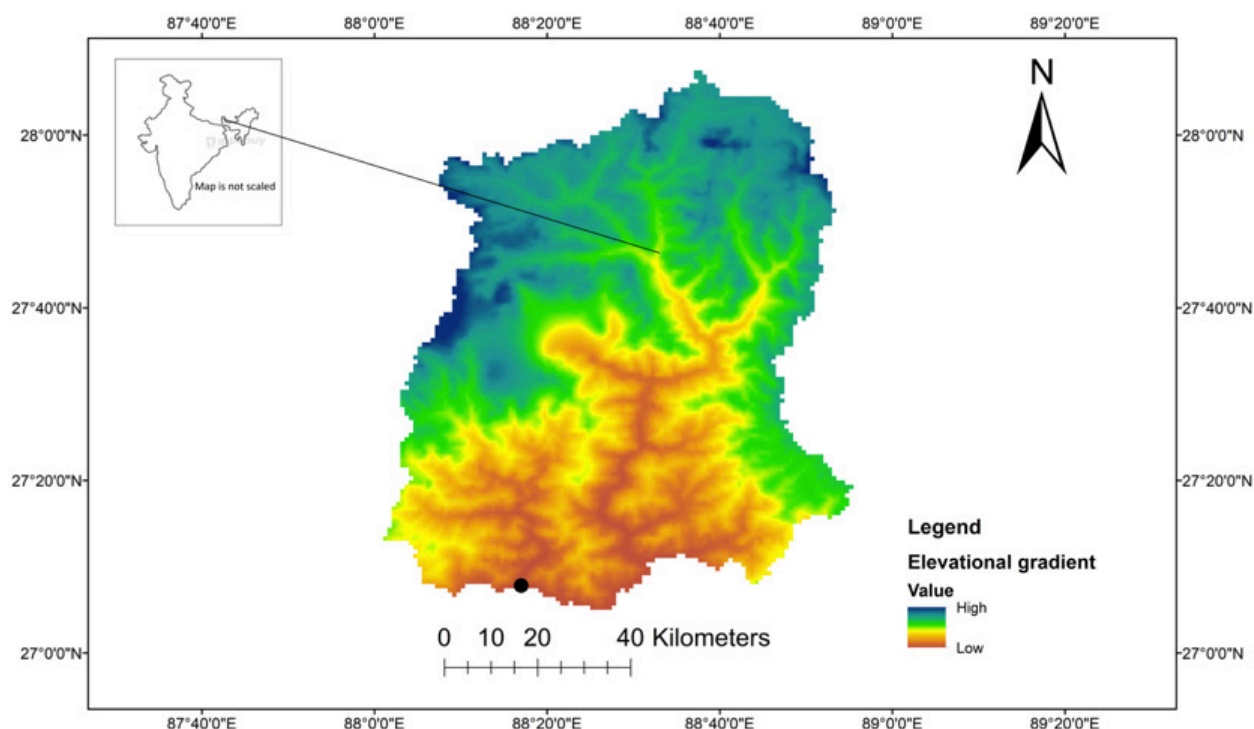


Figure 1. Photographic record of Red-naped Ibis *Pseudibis papillosa* in Jorethang, South Sikkim (black dot).

poles and Vaishnav et al. (2022) recorded them on mobile towers in Rajasthan.

### Feeding

Probing behaviour is the principal feeding mode for this species, frequenting feeds which are often found in municipal garbage dumps and wastewater bodies. The species shows a significant non-nocturnal foraging rhythm and occupies the terrestrial habitats during morning and evening and aquatic habitats during noon; it usually consumes frogs, snakes, insects, fish, earthworms, and carcasses; it is also known to eat grains and seeds in dry agricultural fields (Katuwal & Quan 2022). Sinha (2022) observed the species feeding on the nectar of the Silk Cotton Tree *Bombax ceiba*, which has a single occurrence of ibis feeding on non-carnivore items.

### Methods and Observations

The Red-naped Ibis was observed opportunistically at Jorethang Town, South Sikkim, during a field study at the river Rangeet. The species identification was done with the help of a field guide (Grimmett et al. 2019). The dark brown plumage, greenish wing sheen, long-curved beak, and reddish legs of the medium-sized Red-naped Ibis make it easy to identify. Adolescents lack the red patch on the head that distinguishes the adult bird (Soni & Rana 2023). This species shares a similar

appearance with the Glossy Ibis; however, the Glossy Ibis lacks the red patch and the white shoulder patch. A Nikon DSLR camera (Nikkor Z 28–400mm), Zeiss Terra ED (10 x 42) binoculars, and a Garmin GPS were used to photograph, observe, and record locations, respectively. We generated a digital elevation model (DEM) map of the study area. We observed two adult Red-naped Ibis (male and female) in Jorethang, South Sikkim (27.130° N & 88.283° E, at 332 m; Figure 1). The two birds were perched on the top branches of a Peepal tree *Ficus religiosa*, making loud, squealing calls and sharing their habitat with House Crow *Corvus splendens* and Common Myna *Acridotheres tristis* (Table 1).

The dominant vegetation of the area consisted of *Albizia lebbeck*, *Ficus religiosa*, *Mangifera indica*, *Areca catechu*, *Persea americana*, *Ficus altissima*, *Carica papaya*, and *Cocos nucifera* (Table 2).

On 27 July 2024, at 0640 h, the Red-naped Ibis was sighted by Laxmi Rai at Jorethang. Based on the data collected from the locals & fishermen, our observations indicate that these birds are most active in the early morning, making loud calls before flying across the river Rangeet to forage. The birds were seen rarely foraging in the wetlands.

Table 1. Associate bird species found around the study site.

	Common name	Scientific name	Family
1	House Sparrow	<i>Passer domesticus</i>	Passeridae
2	Common Myna	<i>Acridotheres tristis</i>	Sturnidae
3	White Wagtail	<i>Motacilla alba</i>	Motacillidae
4	Little Egret	<i>Egretta garzetta</i>	Ardeidae
5	Nepal House Martin	<i>Delichon nipalense</i>	Hirundinidae
6	Rock Pigeon	<i>Columba livia</i>	Columbidae
7	House Crow	<i>Corvus splendens</i>	Corvidae
8	Common Sandpiper	<i>Actitis hypoleucos</i>	Scolopacidae
9	Crested Kingfisher	<i>Megaceryle lugubris</i>	Alcedinidae

Image 1. Photographic record of Red-naped Ibis *Pseudibis papillosa* in Jorethang, South Sikkim. © Laxmi Rai.

## DISCUSSION

Sikkim is a small landscape state with many climatic zones that provide a large gene pool of faunal and floral components. According to a checklist published by Acharya & Vijayan (2011), Sikkim is home to 574 different species of birds. In Sikkim, no photographic documentation of Red-naped Ibis existed; also, the checklist provided by ebird shows a total of 537 bird species, yet no record of Red-naped Ibis sightings (Cornell Lab of Ornithology 2024). Thus, our findings on Red-naped Ibis can be useful for the Sikkim Himalayan bird database. The major threats to this species include habitat loss, fragmentation, predators, and pesticides (Anjali & Rana 2022). They are susceptible to anthropogenic pressure and natural disasters, and their population is declining (GreenVerz 2022).

Table 2. Vegetation found around the study site.

	Common tree name	Scientific name	Family
1	Peepal	<i>Ficus religiosa</i>	Moraceae
2	Siris	<i>Albizia lebeck</i>	Fabaceae
3	Papaya	<i>Carica papaya</i>	Caricaceae
4	Coconut	<i>Cocos nucifera</i>	Arecaceae
5	Mango	<i>Mangifera indica</i>	Anacardiaceae
6	Banyan	<i>Ficus benghalensis</i>	Moraceae
7	Betelnut	<i>Areca catechu</i>	Arecaceae

Image 2. A male and a female Red-naped Ibis *Pseudibis papillosa* in Jorethang, South Sikkim. © Laxmi Rai.

## CONCLUSION

Climate change is more prominent in the Himalayas, which is further boosted by anthropogenic pressure like habitat fragmentation due to modernisation. These may have been the driving forces for the range extension of the Red-naped Ibis in the Sikkim Himalaya. This occurrence might be crucial for studying more about biodiversity and its conservation strategies in response to climate change in the Sikkim Himalaya. Therefore, it is essential to consistently monitor this species and its habitat for effective conservation and future prospects.

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under a Zeiss SteREO Discovery.V20 stereomicroscope and microphotographic images were taken by a Axiocam 208 color digital camera attached to the stereomicroscope, enabled with the software Zeiss ZEN 3.3. The left palp was removed, studied and photographed by placing it in a cavity block filled with 70% ethanol, following Parmar & Prajapati (2023). The species was identified based on illustrations in Caleb et al. (2025) and Wesołowska & Tomasiewicz (2008). All measurements are in millimeters (mm). Leg measurements are given in the following order: total (femur, patella, tibia, metatarsus, tarsus). The examined specimen has been deposited in the reference collection of Zoology Laboratory at Department of Zoology, R.R. Lalan College, Bhuj-Kachchh, Gujarat, India.

Family Salticidae Blackwall, 1841

Genus *Sonoita* G.W. Peckham & E.G. Peckham, 1903

Type species: *Sonoita lightfooti* G.W. Peckham & E.G. Peckham, 1903

***Sonoita lightfooti* G.W. Peckham & E.G. Peckham, 1903**  
(Image 1–6)

*Sonoita lightfooti* Peckham & Peckham, 1903: 184, pl. 19, fig. 4

*Sonoita lightfooti* Wesołowska & Tomasiewicz, 2008: 48, figs 189–194, 213–214

*Sonoita* cf. *lightfooti* Caleb et al., 2025: 79, figs 1A–C, 2A–H, 3A–B

For a complete list of taxonomic references refer WSC (2025).

**Materials examined:** RRLC-ZC/SP-09, 28.v.2025, 1 male, India, Gujarat, Kachchh, Sanosara (23.113° N, 69.699° E), 212 m, from leaf litter in arid habitat, leg. S. Parmar.



Images 1–6. *Sonoita lightfooti* G.W. Peckham & E.G. Peckham, 1903: 1—male habitus, dorsal view | 2—male habitus, ventral view | 3—male habitus, frontal view | 4—left pedipalp, ventral view | 5—left pedipalp, retrolateral view | 6—left pedipalp, dorsal view. © Subhash Parmar.



Image 7. Distribution of *Sonoita lightfooti* G.W. Peckham & E.G. Peckham, 1903 in India. Red star indicates previous record & yellow star indicates new record.

**Diagnosis:** The species is easily distinguished by following characters: male pedipalp with long, slender embolus, arising at 6 O'clock position, running prolaterally with tip concealed behind the conductor (Image 4); bulb with a retrolateral median apophysis; retrolateral tibial apophysis (RTA) broad, flattened and spatula-shaped (Images 5 & 6) (Caleb et al. 2025).

For the detailed diagnosis of female see, Wanless (1985).

**Description:** Male (Images 1–6), total length 3.13; carapace length 1.47, width 1.26; abdomen length 1.67, width 1.05. Eye measurements: anterior median eye (AME) 0.29, anterior lateral eye (ALE) 0.16, posterior median eye (PME) 0.12, posterior lateral eye (PLE) 0.16. Leg measurements: I 3.11 (0.96, 0.53, 0.73, 0.53, 0.36); II 2.75 (0.86, 0.45, 0.58, 0.50, 0.37); III 2.78 (0.94, 0.38, 0.58, 0.53, 0.35); IV 3.64 (1.07, 0.54, 0.76, 0.86, 0.41). Leg formula: 4132. Carapace pale brown, clothed with pale hairs. Eyes surrounded with dark patches; all eyes surrounded by white orbital setae. Sternum yellowish-brown and oval; labium brownish. Chelicerae light brown. Abdomen pale brown, oval-shaped, with pairs of lateral white setae on posterior half, chevron-shaped brown markings on posterior part; ventrally brownish with pale brown spots arranged longitudinally. Spinnerets yellowish brown in color. Leg I bearing leaf like setae on

ventral part of femora, patellae and tibiae; tibial setae dense and longer than femoral setae; metatarsi and tarsi of leg I clothed with white setae (Image 3); legs II–IV pale yellowish with annulations and covered with white hairs. Pedipalp yellowish-brown; cymbium and tibia covered with white hairs; cymbium apically covered with black hairs; bulb with a retrolateral median apophysis (Image 4); long and slender embolus arising at 6 O'clock position, running prolaterally with tip concealed behind conductor (Image 4); tibia with broad, flattened and spatula-shaped RTA (Images 5 & 6).

**Natural history:** The present collection of *S. lightfooti* was made in arid thorn-forest habitats, where the species was observed occupying leaf litter during daylight hours, indicating diurnal activity and a preference for ground-associated microhabitats.

**Distribution:** Parts of Africa (Cape Colony, Ethiopia, Ivory Coast) and India (WSC 2025).

**Distribution in India:** Karnataka (Caleb et al. 2025) and Gujarat (new record) (Image 7).

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## *Garcinia pedunculata* (Clusiaceae), a new record for Bhutan and its ethnopharmacological potential

Jigme Wangchuk<sup>1</sup> , Ugyen Dorji<sup>2</sup> , Sherab Dorji<sup>3</sup> , Yograj Chhetri<sup>4</sup> & Tsethup Tshering<sup>5</sup>

<sup>1,4,5</sup> Ugyen Wangchuck Institute for Forestry Research and Training, Lamai Goempa, Bumthang 34005, Bhutan.

<sup>2,3</sup> Panbang Forest Range, Divisional Forest Office, Zhemgang, 34103, Bhutan.

<sup>1</sup>jickmew@gmail.com (corresponding author), <sup>2</sup>ugyendorji551@gmail.com, <sup>3</sup>sgandhi6655@gmail.com, <sup>4</sup>chhetriy@gmail.com,

<sup>5</sup>tsethup2015@gmail.com

**Abstract:** *Garcinia pedunculata* Roxb. ex Buch.-Ham. is reported here for the first time in Bhutan, specifically from Zhemgang District. The species holds significant local value; its fruits are consumed as food and used as dye mordants, as well as in traditional remedies for various ailments. These uses exemplify the deep-rooted ethnobotanical knowledge maintained by local communities.

**Keywords:** Angiosperm, bioactive constituents, conservation status, digital herbaria, dioecy, eastern Himalaya, ethnobotanical uses, least concern, taxonomy, Taikor, Zhemgang District.

The genus *Garcinia* L. of the Angiosperm family Clusiaceae comprises over 416 species of trees and shrubs, occurring mainly in tropical and subtropical regions of Asia, Africa, Central & South America, Australia, and Polynesia (Lin et al. 2021; POWO 2025). Members of this genus are renowned for their economic and medicinal significance, notably providing edible fruits and phytochemicals with pharmaceutical potential (Santo et al. 2020; Kazmierczak et al. 2023). Species of *Garcinia* are ethnobotanically important and have been utilized in traditional medicine, culinary practices, dyeing, and cultural rituals since ancient times (Lin et al. 2021). Because of the ethnobotanical and phyto medicinal

properties, in regions such as Bangladesh, this species, though found in the wild, has also been occasionally cultivated by local communities for fruit, vegetables, and local therapy (Islam et al. 2015).

*Garcinia pedunculata* Roxb. ex Buch.-Ham., commonly known as ‘Amlavetasa’ in India and ‘Taikor’ in Bangladesh, is native to the Indo-Burma biodiversity hotspot. The species produces large, yellowish-green, acidic fruits. Local communities use these fruits in cooking and traditional medicine, particularly to ease gastrointestinal issues, as a cardi tonic, and as a skin softener (Santo et al. 2020; Bhattacharjee & Devi 2021). Phytochemical analysis has identified important compounds in ripe fruits, such as hydroxycitric acid, benzophenones, garcinol, and pedunculol. These compounds are known for their antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, hypolipidemic, and antidiabetic activities (Santo et al. 2020). Despite its recognized value in ethnobotanical uses, which has led to its population decline elsewhere, the species has been listed as Least Concern on the IUCN Red List (IUCN SSC GTSG & BGCI 2025).

In Bhutan, three *Garcinia* species have been recorded

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so far, *G. stipulata* T.Anderson, *G. sopsopia* (Buch.-Ham.) Mabb., and *G. xanthochymus* Hook.f. ex T.Anderson (Grierson & Long 1984). The presence of *G. pedunculata* had long been recognized by local ancestral communities in the current study areas; however, it had not been documented in the literature until our study. This may be because the area was not covered in earlier studies (Wangchuk et al. 2023). The species was discovered during the biomass sample collection for the development of an allometric equation. The herbarium specimen was collected along with the photographic evidence. We compared our specimen to the previously documented genus *Garcinia* in Bhutan by Grierson & Long (1984) and found no match. We then examined digital herbaria, including GBIF (2025), POWO (2025), WFO (2025), eFlora of India (2025), Singh (1993), and Li et al (2007), and confirmed that the species is *G. pedunculata* based on morphological correspondence.

#### TAXONOMIC TREATMENT

*Garcinia pedunculata* Roxb. ex Buch.-Ham., Edinburgh J. Sci. 7:45. 1827 (Image 1).

Type (Lectotype: INDIA—Sylhet, Wallich Cat. 4860C (K-W [K001104084], designated by Ridsdale (1978), Blumea 24:141); Isolectotype: INDIA—Assam, Goalpara, 10.x.1808, Wallich Cat. 4860A (CAL, K-W[K001104082]); cultivated in H.B.C (Calcutta Botanical Garden), s.d., Wight s.n. (K [K000677592]); H.B.C., East Bengal, s.d., Herb. Griffith 853 (K [K000677593]).

Synonym: *Garcinia planchonii* Pierre in Fl. Forest. Cochinch.: t. 61 (1882).

Dioecious trees 10–20 m height. Stem trunk fluted, bark thick, corky, dark greyish-brown; branchlets glabrous, subterete with fine longitudinal striations and lenticels. Leaves opposite, glabrous on both surfaces; petiole 2–2.5 cm long; leaf blade oblong to oblong-lanceolate, occasionally ovate, chartaceous, (12–) 15–25 (–30) × 7–12 (–17) cm; base cuneate, margin narrowly revolute, apex usually rounded, midvein stout, raised abaxially, slightly impressed adaxially; secondary veins regular, oblique, 9–14 pairs, near margin arching and joining together forming loops; tertiary veins nearly parallel and inconspicuous. Flowers 4-merous; male flowers in panicle-like cymes bearing 8–15 flowers, subtended by 3–6 cm long peduncles; pedicels robust, 3–7 cm long; sepals fleshy, broadly ovate or suborbicular, thick, with membranous margins; petals yellow, oblong-lanceolate, 7–8 mm long; stamens arranged in capitate ring ca. 3 mm high, sometimes pistillode bears short filaments, anthers sessile, 2-celled, dehiscent longitudinally; pistillode columnar-cuneate, slightly angular with a peltate stigma,

inconspicuously tuberculate. Female flowers usually in pairs or solitary at the apex of branchlets; pedicels stout, slightly tetragonous, with two suborbicular bracts at the base; staminodes 80–100 and encircling the ovary with 8–10 subglobose locules, and the stigma radiate with 8–10 lobed and papillate. Fruits yellow, oblate, concave on both ends when mature, 10–18 × 11–20 cm, smooth, pedicel 5–6 cm long; seeds 8–10, reniform.

Flowering: August to December.

Fruiting: December to January.

The species most closely resembling *G. pedunculata* is *G. lanceifolia* Roxb. *Garcinia pedunculata* shares several characters with *G. lanceifolia*. Both are small to medium-sized trees with simple, opposite, leathery leaves that are elliptic to lanceolate with prominent veins. Their flowers are unisexual, small, and arranged in axillary clusters, and their fruits are globose to ovoid with a thick rind, a sour taste, edible, and widely used in traditional medicine. Both species are found in tropical and subtropical Asia. They can, however, be distinguished as follows: *G. pedunculata* has large, broad leaves (13–30 cm long and ca. 8–18 cm wide) and large, yellow to yellowish-orange fruits, whereas *G. lanceifolia* has smaller, narrow leaves (6–12.5 cm long by 2–3 cm wide) and smaller, yellow to yellowish-orange fruits.

**Distribution:** Assam, Arunachal Pradesh, Manipur, Meghalaya, Nagaland, West Bengal in northeastern India, Southwest China, Bangladesh (sometimes cultivated), Laos, Myanmar, Thailand, Vietnam, and Bhutan. Usually grown in dense forests on hills 200–400 m, and exceptionally, up to 1,500 m.

**Specimen examined:** Bhutan. Zhemgang District, Nangla, Panbang along the Yumdang farm road, 26.862° N, 90.985° E, 219 m, 12.iv.2025, collected by Tshethup Tshering TT016 (UWIFoRT); voucher specimen deposited at the Bhutan National Herbarium, National Biodiversity Centre, Serbithang (THIM22423) (Image 2).

Note: It is also observed within the same District at several locations along the forest-farmland edges at Thinleygang 26.854° N, 90.980° E; Chakchawa, between Madulung and Prigchang along the footpath 26.890° N, 91.005° E; Amalagang, Labir at the edge of the agricultural land (26.933° N, 90.956° E), Bjoka, Zarkapla 26.882° N, 91.009° E.

**Conservation status:** *G. pedunculata* has been evaluated as Least Concern on the IUCN Red List despite its extensive ethnopharmacological pressure. The baseline surveys in Bhutan to assess current distribution and abundance are crucial. In Arunachal Pradesh, the species occurs at low population densities, frequently limited to a single tree or only a few individuals per

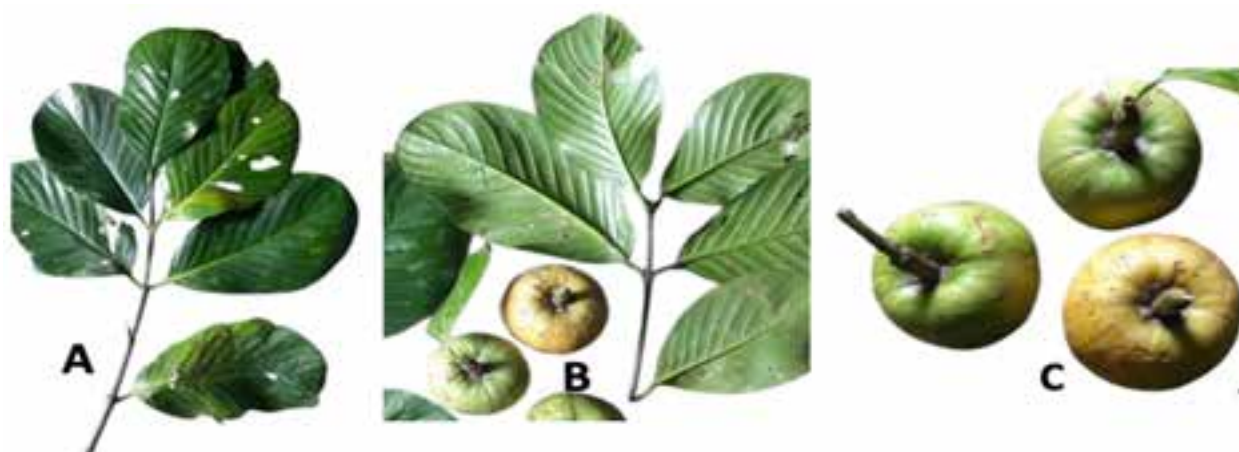


Image 1. *Garcinia pedunculata* Roxb. ex Buch.-Ham: A—Adaxial surface of leaf blade | B—Abaxial surface of leaf | C—Fruits. © Tshethup Tshering.

survey site, raising concerns about its continued survival (Gogoi & Das 2016). To maintain its population while reaping its benefits, the species should be incorporated into agroforestry and afforestation programs, not only for its conservation but also to enhance livelihoods through the sustainable harvest and sale of its products.

#### Ethnobotanical uses and therapeutic potentials

*Garcinia pedunculata* is traditionally known for culinary purposes and has been used as an ascorbic, astringent, cardi tonic, and emollient (Paul et al. 2017), while also being used to treat chronic catarrh, asthma, cough, bronchitis, dysentery, digestive disorder, and fever (Kagyung et al. 2010). In Bangladesh, the plant is cultivated for its fruit. In Assam, it is used in folk medicine to treat gastrointestinal disorders and is also part of ethnic cuisine. The ripe fruit, seldom consumed fresh, is cut into thin slices and sun-dried for preservation (Bhattacharjee & Devi 2021). Many therapeutic values have been scientifically established, including antioxidant (Islam et al. 2015), antimicrobial (Negi et al. 2008), antibacterial, antifungal, anthelmintic, hypolipidemic, antidiabetic, hepatoprotective (Mundugaru et al. 2014), cardioprotective (Mundugaru et al. 2016), neuroprotective, and nephroprotective properties (Bhattacharjee & Devi 2021). Additionally, research has identified polyphenols and flavonoids, along with bioactive compounds such as hydroxycitric acid, garcinol, and cambogin (Bhattacharjee & Devi 2021).

Studies on the nutritional, phytochemical, and antioxidant properties of mature fresh fruits found that the fruit contains significant levels of phenolics and flavonoids, contributing to its antioxidant activity (Devi et al. 2025). Similarly, fruit extract exhibits significant



Image 2. Herbarium sheet of *Garcinia pedunculata* Roxb. ex Buch.-Ham: [# THIM22423].

antihyperglycemic, antidiabetic, and antioxidant effects in rats, suggesting that fruit extract effectively reduces blood glucose levels and enhances antioxidant defenses (Ali et al. 2017). An aqueous extract tested on rats for 14 days protects their hearts from damage caused by a heart attack, harmful blood enzymes, and improves

heart tissue structure, suggesting the fruit's potential as a natural remedy for heart health (Mundugaru et al. 2016). Methanol and ethanol extracts of the fruits demonstrated potent antibacterial activity against multiple clinical bacterial strains, attributed to bioactive constituents such as hydroxycitric acid lactone, garcinone-E, and mangostins (Zoliansanga & Lalfakzuala 2021).

In Bhutan, mature fruits have been used as a dyeing mordant for silk threads from ancestral times until silk became readily available from the Indian market. The ripe fruit has a strong sour taste and is also edible; however, it must be swallowed or chewed without contact with the teeth to avoid enamel erosion. Local people prepare juice from fruits and sell them at markets. Communities have also observed that wildlife, such as ungulates, feed on these fruits, and the area around the base of the trees shows heavy signs of foraging. Enhancing the population of this species in forest is expected to promote human-wildlife co-existence (Tshering, pers. comm. viii.2025). Although a survey of subtropical medicinal plants near the location of this newly recorded species lists 61 species (Wangchuk et al. 2017), this species has not been formally documented in any ethnobotanical study from Bhutan, despite its well-known uses in local medicine. Thus, we report its presence and local medicinal uses in this paper.

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## First record of Indian Fox *Vulpes bengalensis* in Dang, Gujarat, India

Aadil Kazi<sup>1</sup> , Mohmad Navaz Dahya<sup>2</sup> , Rohit Chaudhary<sup>3</sup> & Pravin Chaudhari<sup>4</sup>

<sup>1,4</sup>Department of Wildlife Sciences, College of Forestry, Navsari Agricultural University, Navsari, Gujarat 396450, India.

<sup>2</sup>Department of Biology, BKM Science College, Valsad, Gujarat 396001, India.

<sup>3</sup>Department of Wildlife Sciences, College of Forestry, Banda University of Agriculture and Technology, Banda, Uttar Pradesh 210001, India.

<sup>1</sup>aadilkazi@nau.in, <sup>2</sup>nawazd8@gmail.com (corresponding author), <sup>3</sup>rchaudhary259@gmail.com, <sup>4</sup>chaudharipravin998@gmail.com

The Indian Fox *Vulpes bengalensis* is a small carnivore weighing between 1.8–3 kg, endemic to the Indian subcontinent (Johnsingh 1978). The Indian Fox is distributed from the foothills of the Himalaya down to the southern extremity of the Indian peninsula. Indian Foxes show a strong preference for dry, open areas characterized by sparse tree and shrub coverage and grasslands (Pocock 1936; Prater 1971; Johnsingh & Jhala 2004). They are frequently observed in fields and near human settlements, indicating their high adaptability to human presence (Johnsingh 1978; Manakadan & Rahmani 2000; Vanak 2003; Johnsingh & Jhala 2004).

The Indian Fox is omnivorous, and their diet consists of mammals, reptiles, birds, insects, and fruits (Johnsingh 1978; Manakadan & Rahmani 2000; Vanak 2003; Jhala et al. 2009). Their breeding season spans from December to January, with litters ranging between 2–4 offsprings after a gestation period of 50–53 days (Sheldon 2013). The dens of Indian Foxes consist of a network of interconnected tunnels and a few blind passages (Johnsingh 1978). These dens cover an area of approximately 10 x 8 m, typically featuring 2–7 openings (Manakadan & Rahmani 2000).

The Indian Fox population has faced habitat degradation due to conversion of wastelands and

grasslands into agricultural fields, leading to a significant decline in their population and distribution. The primary threats to Indian Foxes are human-induced, such as killings by humans, habitat destruction, and disease transmission from free-ranging and feral dogs (Jhala et al. 2022). One of the critical concerns in conserving Indian Foxes is the lack of comprehensive information on their exact population distribution (Manakadan & Rahmani 2000).

The present work aims to contribute to the knowledge base on Indian Fox distribution by documenting its presence in the Dang District of Gujarat, an area where it had not been reported previously. During our camera trapping survey for leopards in the village Lavchali, located in the Dang District, on 21 November 2023, at 1124 h, we captured an image of an Indian fox *Vulpes bengalensis* at coordinates 20.842° N, 73.740° E (Figure 1; Image 1). The vegetation around the camera trap locations consisted of a mix of grasses and scattered shrubs, along with some saplings of *Tectona grandis* around den. The broader landscape surrounding the den sites was primarily agricultural, particularly paddy fields, interspersed with small open patches. Camera trap recorded video during both day and night, documenting the routine den-site behaviour of *V. bengalensis*. In the daytime sequence,

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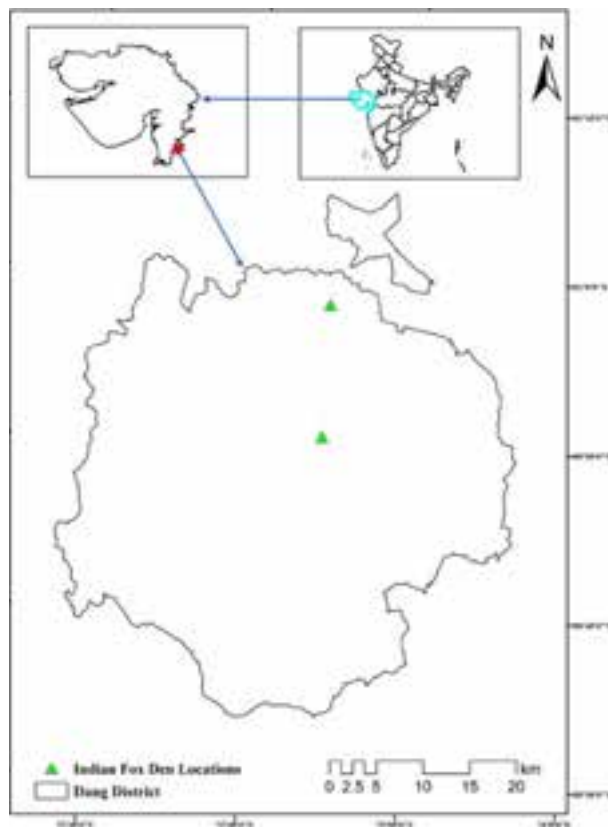


Figure 1. Map of Dang District along with locations of Indian Fox den.

the fox approached the den from outside and engaged in den-inspection behaviour, repeatedly lowering its head into the opening, likely assessing the den's condition or occupancy. The individual then displayed vigilance,

scanning its surroundings before briefly directing its attention toward the camera trap, suggesting curiosity toward the novel object. Night-time images similarly showed investigatory behaviour, with the fox closely approaching and inspecting the camera trap, along with routine movement around the den entrance. No social interactions or den-maintenance activities were observed in any of the videos. This incidental recording prompted further exploration of the area, leading to the discovery of a den of eight interconnected chambers.

To gain deeper insights into the occupancy and behaviour of the species, we strategically placed an infrared camera trap at the den entrance. Over subsequent monitoring, the footage confirmed the presence of at least four individual Indian Foxes using the site. Further, we engaged with local villagers, showing them the photographs of the Indian Fox. The villagers expressed their familiarity with the species, referring to it by its local name 'Kiklla'. Their insights proved invaluable, as they directed us to another den site approximately 15 km away from the first location. This second den, situated at coordinates 20.972° N, 73.743° E, featured six distinct openings.

Upon visiting this second site, we observed the presence of free-ranging domestic dogs in the vicinity, which could pose a potential threat to the fox population (Image 2). Additionally, some of the den openings appeared to have been deliberately sealed with stones, presumably by villagers. This suggests a possible human-fox negative interaction or an effort to manage the presence of the species in the area.

Our findings highlight the importance of incorporating



Image 1. Image of Indian Fox *Vulpes bengalensis*.



Image 2. Image of free-ranging domestic dogs outside Indian Fox den.

local ecological knowledge into conservation efforts and shed light on potential threats faced by Indian fox populations due to human activities and interactions with free ranging or feral dogs. Further research and conservation initiatives could help mitigate these challenges and ensure the protection of Indian Fox in this region. Although the Indian Fox is considered a commonly occurring species in many parts of Gujarat, distribution records remain patchy, particularly for the eastern and southern districts. Most published studies focus on Kutch, Saurashtra, and northern Gujarat, leaving a clear knowledge gap regarding its occurrence in central and southern regions. Previous records in the state come from districts such as Kutch, Bhavnagar, Rajkot, Sabarkantha, Mehsana, and Banaskantha—areas largely situated in the west, north, and south-west—but no prior records exist from the Dang District is in southern Gujarat (Chandrima & Jhala 2009; Gajera & Dharaia 2011; Desai & Dharaia 2023). Our record from Dang helps bridge this gap and highlights the need for systematic surveys in under-studied landscapes to better understand the species' distribution and habitat associations. The landscape of Dang District consists of hilly to undulating terrain, unlike the preferred habitat of the fox, which normally prefers open habitat patches in relatively flat areas. Therefore, an urgent survey is required to ascertain the status of Indian Foxes and identify habitat patches in Dang, allowing for appropriate conservation measures. Across their range, foxes occasionally prey on small domestic animals such as poultry, which can lead to negative perceptions among villagers and may result in retaliatory behaviour. However, during the present study, we did not document any such incidents. Nonetheless, systematic investigations are required to draw stronger conclusions regarding

the extent and nature of human-fox interactions in this region. The present study is significant in augmenting our understanding of the Indian Fox's distribution within its existing range, particularly in Gujarat.

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## First record with photographic evidence of Dhole *Cuon alpinus* (Pallas, 1811) from Panshet, Pune, Maharashtra, India

Sonali Shinde<sup>1</sup> & Chinmay Sonawane<sup>2</sup>

<sup>1</sup> Annasaheb Kulkarni Department of Biodiversity, MES Abasaheb Garware College, Karve Road, Pune, Maharashtra 411004, India.

<sup>2</sup> Ecological Society, Jayanti Apartments, B-2, Senapati Bapat Road, Pune, Maharashtra 411016, India.

<sup>1</sup>sss.agc@mespune.in (corresponding author), <sup>2</sup>chinmayrsonawane@gmail.com

The Dhole *Cuon alpinus* (Pallas, 1811), also known as the Asiatic Wild Dog or Whistling Dog, is an endangered canid distributed across Asia (Kamler et al. 2015). Dholes are widely distributed across the continent but are primarily restricted to protected areas (Sillero-Zubiri et al. 2004; Kamler et al. 2015). The global population of dholes is estimated at 4,500–10,500 individuals, with fewer than 2,215 mature animals (Kamler et al. 2015). India is considered a stronghold for the species, and the Western Ghats may support the largest meta-population worldwide (Srivathsa et al. 2014, 2019; Kamler et al. 2015). As a social top predator, the Dhole plays a crucial role in regulating prey populations, particularly in ecosystems with reduced tiger and leopard densities (Johnsingh 1992; Acharya 2007). Dholes are habitat generalists, utilising a mosaic of evergreen, deciduous, and mixed forests, as well as grasslands, with open or deciduous areas preferred for hunting, and closed or mixed forests used for denning (Kamler et al. 2015, 2020). Prey selection, human disturbance, habitat fragmentation, and competition with domestic dogs influence their distribution and connectivity. Dholes are primarily crepuscular (Ghaskadbi et al. 2016, 2022), and existing ethograms provide key insights into their activity patterns and behavioural ecology, which are critical for

conservation planning.

Given the ecological significance of Dholes, documenting new occurrences is important for understanding their distribution and informing conservation strategies. While the species has been reported from various parts of the Western Ghats, photographic evidence from Pune District has not been recorded previously. Here, we present the first photographic record of the Dhole from Panshet, Pune, Maharashtra, India. This record strengthens evidence of landscape-level connectivity of *Cuon alpinus* across the northern Western Ghats and provides information for future conservation studies in fragmented landscapes.

On 6 September 2025, a single Dhole was opportunistically sighted crossing a forest trail around 1400 h and was photographed (Image 1) near Panshet (18.437° N, 73.580° E), Pune, Maharashtra. Panshet is located near the Western Ghats in Maharashtra, India. The Panshet Dam catchment lies in the Sahyadri range of northern Western Ghats, a region representing one of the most fragile ecosystems (Karandikar et al. 2015).

The individual was identified by its characteristic reddish coat, bushy dark tail, and typical posture. The record was verified by experts Pallavi Ghaskadbi (pers. comm.) and forest officials Mangesh Tate (pers. comm.).

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This represents the first photographic evidence of the Dhole in Panshet, a region outside of any notified protected area in the northern Western Ghats.

Dholes are known to prefer forested landscapes with adequate prey availability (Srivathsa et al. 2014; Punjabi et al. 2017; Pattekar et al. 2024). However, this report documents their occurrence in fragmented patches that

include altered habitats, including human-modified and fragmented landscapes.

Recent Dhole sightings across multiple regions reveal both rediscoveries and new distribution records for this endangered canid (Kazi et al. 2021). However, information from the northern Western Ghats remains scarce. The southern Western Ghats have received more



Image 1 (up and down). Photographic record of Dhole *Cuon alpinus*, captured from both front and back views, taken using a Nikon Z50 on 6 September 2025 at approximately 1400 h.

conservation attention. Studies have highlighted Dhole distribution in the northern Western Ghats (Punjabi et al. 2017), including estimates of approximately nine Dholes per 100 km<sup>2</sup> in areas such as Radhanagari Wildlife Sanctuary (Punjabi et al. 2022). This sighting complements recent reports of Dholes from Bhimashankar Wildlife Sanctuary and Phansad Wildlife Sanctuary (Pardeshi et al. 2020). These records suggest ongoing presence of Dholes in the landscape, although their population status and viability need further investigation. The occurrence in fragmented, human-modified landscapes suggests the potential role of non-protected habitats as corridors or refuges.

Dholes are currently listed as Endangered on the IUCN Red List under criteria C2a(i), however, despite its Endangered status, India supports the largest remaining population of dholes (Kamler et al 2015). Recent communications with field researchers, forest officers, and enthusiasts suggest mixed observations. While some regard sightings as rare, even if the occurrence increases based on the traits of a species. Although camera-trap and systematic surveys have confirmed Dhole presence in regions where they were absent for decades, the contrasting perspectives highlight the need for further research. We recommend employing camera trapping and prey-base assessments to evaluate the current status of Dholes in Pune District and adjoining landscapes. Conservation planning should also account for catchment forests and fragmented habitats, which provide functional connectivity. This region is significant for large predators, which adds further weight to the importance of this record. This sighting is particularly noteworthy as tigers and Dholes are known to coexist where prey densities are sufficient, suggesting that these species do not necessarily compete. Karanth et al. (2017) highlight that with effective prey management; multiple large carnivores can share landscapes sustainably. In this context, the Dhole's role as a top predator, especially in ecosystems with reduced tiger and leopard densities (Johnsingh 1992), underscores the importance of maintaining adequate prey populations for their persistence. Documenting such populations is therefore critical for regional conservation management and may also inform reassessment of the species' IUCN Red List status.

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## First photographic evidence of Spot-bellied Eagle Owl *Ketupa nipalensis* (Strigiformes: Strigidae) in Palamau Tiger Reserve, Jharkhand, India

Arshyaan Shahid<sup>1</sup> , Shahzada Iqbal<sup>2</sup> & Orus Ilyas<sup>3</sup>

<sup>1,2,3</sup>Department of Wildlife Sciences, Aligarh Muslim University, Qila Road, Fort Enclave, Firdaus Nagar, Aligarh, Uttar Pradesh 202001, India.

<sup>1</sup>arshyanshahid@gmail.com (corresponding author), <sup>2</sup>shahzada90@yahoo.com, <sup>3</sup>o.ilyas.wl@amu.ac.in

The Spot-bellied Eagle Owl *Ketupa nipalensis*, also known as the Forest Eagle Owl, is a large species of owl found in parts of southern and southeastern Asia (König & Weick 2008). The Spot-bellied Eagle Owl has a disjunct distribution in India, primarily inhabiting well-forested areas in hilly or mountainous regions. Its range includes the sub-Himalayan belt from Uttarakhand eastward through northeastern India, the Western Ghats from southwestern Maharashtra to its southern tip, and locally in the Eastern Ghats, central Indian highlands, and parts of Gujarat (Jayapal et al. 2005; Grimmer et al. 2011; Majumder et al. 2011; Rasmussen & Anderton 2012). The species is categorized as ‘Least Concern’ under the IUCN Red List due to its large range, although its population trend is decreasing (Birdlife International 2018).

On 27 September 2024, at approximately 2130 h, an individual of Spot-bellied Eagle Owl was observed and photographed perching on a *Ficus religiosa* tree (Image 1) in the mixed deciduous forests of the South Division, within the Palamau Tiger Reserve, Jharkhand (23.599° N, 84.153° E). It was sighted on a clear night with a temperature of approximately 24 °C. The individual was identified as a Spot-bellied Eagle Owl by its large

size (60–70 cm), prominent ear tufts, and characteristic spotting on the underparts, which were clearly visible even in the low light conditions (Grimmett et al. 2011). The deep, booming call often heard at dusk and dawn further confirmed our suspicions (Ali & Ripley 1981). These features distinguish it from other owl species found in the region, such as the Brown Fish Owl *Ketupa zeylonensis* or the Indian Eagle Owl *Bubo bengalensis*.

The nearest known populations are in the Central Indian highlands, though specific recent records from this region are scarce. The closest documented recent sighting is from Similipal Tiger Reserve, Odisha, approximately 400 km south-east, where the first photographic record for the state was obtained in 2018 (Nayak et al. 2019). Additionally, individuals of this species were observed at Satkosia Tiger Reserve, perched on a *Shorea robusta* tree, and at Tamana Reserve Forest roosting on a *Terminalia bellirica* tree (Jena et al. 2025). Recent eBird records have documented the species from several locations in Odisha: Labangi Forest Road, Angul (Nair 2023), Bhajam-Champagarh Forest Road, Mayurbhanj (Rathore 2020), Rissia, Kuldiha, and Baleswar (Chand 2024), all within 250–350 km south-east of our observation. Further north-east, the species has

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been consistently recorded from northern West Bengal, including Buxa Tiger Reserve (Shome 2018), Mahananda Wildlife Sanctuary, and Rongtong, Darjeeling (Mehta 2024; Roy 2024), approximately 450–550 km from the present location. Several records indicate the presence of the Spot-bellied Eagle Owl in central India. Notably, photographic evidence exists from Kanha National Park,

Madhya Pradesh, with sightings reported in February 1994 (D'Cunha & Ali 2001) and May 2007 (Deshmukh 2008). Additional records include observations from Bori Wildlife Sanctuary (Mehta 1998; Jayapal et al. 2005) and Pench Tiger Reserve, where an individual was documented in April 2004 (Jayapal et al. 2005) and again in March 2011 (Majumder et al. 2011). Another notable



Image 1. Spot-bellied Eagle Owl perched on a peepal tree. © Shera Gupta

record comes from Papikonda National Park, Andhra Pradesh, roughly 700 km south, which represented a range extension in the Eastern Ghats (Srinivasan 2013). The observation in Palamau Tiger Reserve extends the known distribution of the Spot-bellied Eagle Owl significantly westward within central India, suggesting a broader but underreported presence in this region. This sighting is particularly noteworthy given the species' association with specific habitat types and its generally patchy distribution.

Several hypotheses could explain this unusual sighting, as intercontinental vagrants are even seen due to climatic changes (Byju & Raveendran 2022): (1) Range Expansion: Climate change and habitat alterations might be driving the species to expand its range. (2) Undocumented Population: A small, previously unrecorded population might exist in or around Palamau Tiger Reserve. (3) Vagrant Individual: The observed owl could be a vagrant that has strayed far from its typical range. (4) Seasonal Movement: Although not known for long-distance migrations, this sighting could indicate previously unknown seasonal movements.

This observation underscores the need for comprehensive avifaunal surveys within Palamau Tiger Reserve and surrounding areas. Such surveys could reveal whether this sighting represents an isolated incident or indicates a broader shift in the species' distribution. The documentation of the Spot-bellied Eagle Owl in Palamau Tiger Reserve highlights the potential for discoveries even in relatively well-studied areas and emphasizes the importance of continued biodiversity monitoring in protected areas.

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## Sighting of Sooty Gull *Ichthyaetus hemprichii* from the salt pans of Nagapattinam Coast, Tamil Nadu, India

S. Babu<sup>1</sup> , Anand Shibu<sup>2</sup> & M. Kishore<sup>3</sup>

<sup>1-3</sup> Sálím Ali Centre for Ornithology & Natural History (South India Centre of Wildlife Institute of India), Anaikatty, Coimbatore, Tamil Nadu 641108, India.

<sup>1</sup> sanbabs@gmail.com (corresponding author), <sup>2</sup> anandshibu12@gmail.com, <sup>3</sup> kishoreak1999@gmail.com

As part of an ongoing project on coastal birds in Tamil Nadu, we visited the coastal areas of Nagapattinam District on 5 September 2025. During the survey, we explored all potential natural (mudflats, mangroves, and sandy beaches) and human-made habitats (aquaculture, salt pans, and jetties) for shorebirds, gulls, and terns. At approximately 1345 h, we surveyed the Akkaraipettai (10.744° N & 79.828° E) salt pan near Kaduvaiyar River. We located a large flock of gulls and terns within a deceptively abandoned saltpan. On closer approach, we observed species of waders, namely Common Redshank *Tringa totanus*, Common Greenshank *Tringa nebularia*, Black-winged Stilts *Himantopus himantopus*, and a few unidentified calidrids, gulls (Lesser Black-backed Gulls *Larus fuscus* in adult and immature plumage stages), and terns (Gull-billed Tern *Gelochelidon nilotica* and Greater Crested Tern *Thalasseus bergii*).

We began systematically counting the individuals using a pair of binoculars (Nikon Monarch M5 8x42). During the process, we spotted a lone, relatively small, and distinctive dark gull (compared to the Lesser Black-

backed Gulls) with a bi-coloured bill. We took a series of photographs using a Nikon P950 digital camera to identify the bird; upon close examination, it was confirmed as a Sooty Gull *Ichthyaetus hemprichii* (Image 1). Key identifying morphological characters—a yellowish bill with a red tip, blackish-brown hood, white collar on the neck sides, dull yellowish legs, and a greyish upper mantle—are all consistent with the description for Sooty Gull (Burger et al. 2020). This maritime species is known to occur in the Red Sea, the Gulf of Aden, the Persian Gulf, the Gulf of Oman, Pakistan, Kenya, and occasionally reported along the coastal areas of peninsular India (Burger et al. 2020). It is known to breed in parts of the Red Sea and Kenya (Kiunga Island).

Excluding our observation, a total of 23 sightings of Sooty Gull have been reported from the Indian coast (Table 1). The species was more often reported from the west coast (20 out of 23 records) (Figure 1) than from the eastern coast. On the western coast, all states, particularly Kerala and Maharashtra, have well-documented their occurrence. Along the eastern coast, it was recorded

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Table 1. Compilation of previously published records of the Sooty Gull in India, along with additional eBird records (NA—Not applicable).

Location	Coordinate (N, E)	Date and time of observation	Number of individuals & major habitat	Associated birds	source
Sundarbans delta, West Bengal	21.70, 88.85	24th January 2005	NA	NA	Sharma & Zöckler (2007)
Government Museum, Chennai, Tamil Nadu	13.033, 80.283	19 <sup>th</sup> Century	Single	NA	Kesavabharati (2014)
Puthalam Estuary, Kanyakumari, Tamil Nadu	8.133, 78.300	28 <sup>th</sup> November 2016	Single bird on salt pans	NA	Sivakumar & Amirtha Balan (2017)
Kothakulam Beach, Thrissur, Kerala	10.379, 76.099	26 <sup>th</sup> February 2024	Single bird on sandy beach	Brown-headed Gull	Nidheesh (2024)
Thiruvathra Beach, Thrissur, Kerala	10.586, 76.000	9 <sup>th</sup> Number 2022	Single bird on sandy beach	NA	Dijumon (2022)
Edakkazhiyur Beach, Thrissur, Kerala	10.608, 75.990	12 <sup>th</sup> November 2022	Single bird on sandy beach	Lesser Black-backed Gull	Sayeer et al., (2022)
Azhikkal Beach, Kannur, Kerala	11.942, 75.296	16 <sup>th</sup> December 2023	Single bird on sandy beach	Slender-billed Gull, Black-headed Gull, Brown-headed Gull, Lesser Black-backed Gull	Nayakkan (2023)
Matool Beach, Kannur, Kerala	11.958, 75.286	17 <sup>th</sup> December 2023	Single bird on sandy beach	Slender-billed Gull, Black-headed Gull, Brown-headed Gull, Lesser Black-backed Gull	Vinod et al., (2023)
Hoode - Kodi Bengare Road - Delta Beach, Udupi, Karnataka	13.433, 74.694	15 <sup>th</sup> October 2014	Single	NA	Doraiswamy (2015)
Tonka Beach, Uttara Kannada, Karnataka	14.282, 74.425	6 <sup>th</sup> February 2025	Single bird on sandy beach	Lesser Black-backed Gull	Sahana (2025)
Handigon Beach, Uttara Kannada, Karnataka	14.390, 74.400	4 <sup>th</sup> February 2025	Single bird on sandy beach	Pallas's Gull, Brown-headed Gull, Lesser Black-backed Gull, Black-headed Gull, Slender-billed Gull, Lesser Crested Tern	Shwetha (2025)
Chapora Estuary, North Goa, Goa	15.600, 73.733	21 <sup>st</sup> April 2005	Single bird at Estuary	NA	Lainer & Alvares (2013)
Morjim Beach, North Goa, Goa	15.619, 73.731	5 <sup>th</sup> November 2013	Single bird on sandy beach	NA	Mark (2013)
Chapora river, North Goa, Goa	15.649, 73.835	8 <sup>th</sup> July 2020	A single bird flying at the Chapora River	NA	Karapurkar et al., (2020)
Near Prongs Lighthouse, Colaba Point, Mumbai, Maharashtra	18.883, 72.800	January 1875	Single bird at Estuary	NA	Hume (1876); Aitken (1903); Prasad (2006)
Coast of Mumbai	18.836, 18.853	22 <sup>nd</sup> March 2009 & 9 <sup>th</sup> November 2011	Single bird & off the coast	NA	Macker (2013)
Bhandup, Mumbai Suburban, Maharashtra	19.138, 72.947	25 <sup>th</sup> January 2017	A single bird flying near the Marsh and saltpan.	NA	Sekar (2017)
Vasai salt pans, Palghar, Maharashtra	19.37547, 72.839	22 <sup>nd</sup> September 2015	Single bird at Saltpan	NA	Dalvi (2015)
Tukda Estuary, Porbandar, Gujarat	21.536, 69.711	13 <sup>th</sup> April 2025	single bird on Estuary sand bar	NA	Alvani et al., (2025)
Tukda Estuary, Porbandar, Gujarat	21.532, 69.709	3 <sup>rd</sup> May 2025	Single bird on Estuary sand bar	Slender-billed Gull, Brown-headed Gull, Pallas's Gull, Lesser Black-backed Gull, Caspian Tern <i>Hydroprogne caspia</i> , Common Tern <i>Sterna hirundo</i> , Sandwich Tern <i>Thalasseus sandvicensis</i> , Lesser Crested Tern, Great Crested Tern, Masked Booby <i>Sula dactylatra</i>	Mansuri et al., (2025)
Mokarsagar Wetland, Porbandar, Gujarat	21.578, 69.747	30 <sup>th</sup> April 2025	Single bird on Wetland	NA	Gharat & Sonalkar (2025)
Porbandar-Veraval Highway, Odedr Beach, Porbandar, Gujarat	21.545, 69.694	1 <sup>st</sup> May 2021	Single bird on sandy beach	Lesser Black-backed Gull, Great Crested Tern, Lesser Crested Tern	Karia & Karia (2021)



Image 1. Sooty Gull *Ichthyaelus hemprichii* recorded from the salt pans of Nagapattinam, Tamil Nadu (red square). © S. Babu.

three times: once each off West Bengal, Chennai, and Kanyakumari. It was most often documented alone amid mixed flocks of other gulls (Lesser Black-backed Gulls, Black-headed Gulls *Chroicocephalus ridibundus*, Brown-headed Gulls *Chroicocephalus brunnicephalus*, Slender-billed Gulls *Chroicocephalus genei*, and Pallas's Gulls *Ichthyaelus ichthyaelus*) and terns (Greater Crested Tern *Thalasseus bergii* and Lesser Crested Terns *Thalasseus bengalensis*). Most sightings occurred between September and February, except for those in Gujarat, which were in April and May. Based on this compilation of observations, the species appears to be rare and irregular along the east coast.

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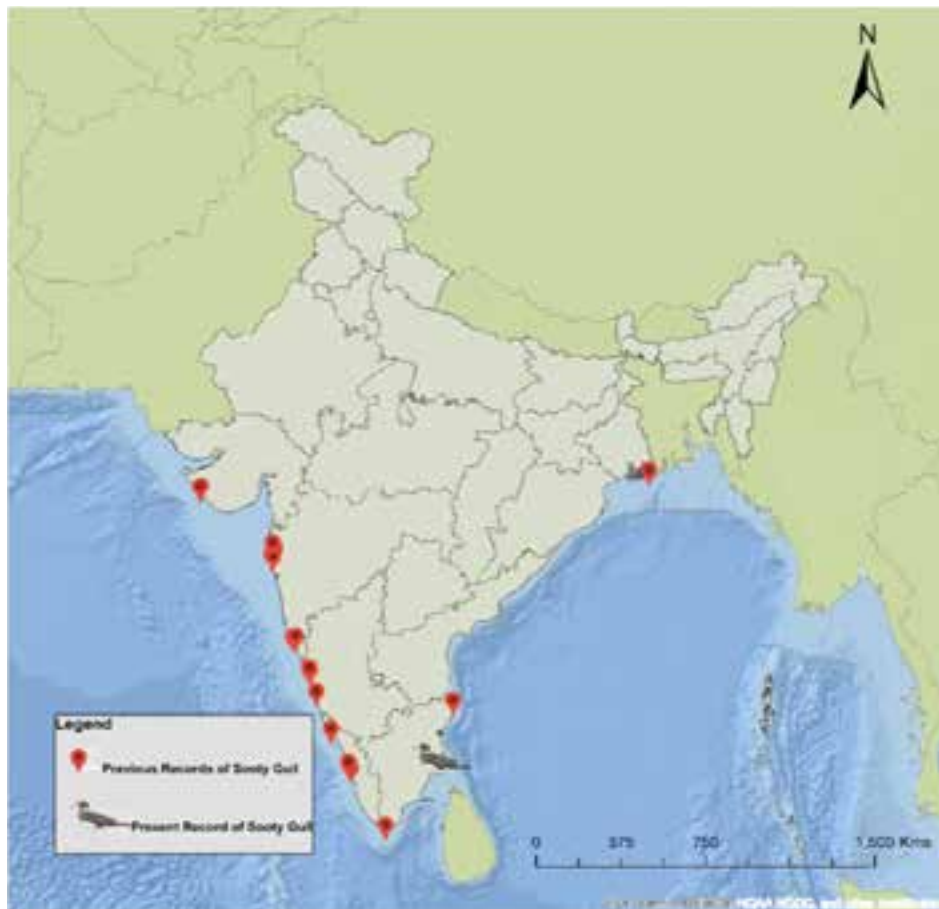


Figure 1. The previous and present records of Sooty Gull *Ichthyaelus hemprichii* in India.

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## First record of *Colyttus bilineatus* Thorell, 1891 (Arachnida: Araneae: Salticidae) from India

Monica Chetry<sup>1</sup> , John T.D. Caleb<sup>2</sup> & Parthankar Choudhury<sup>3</sup>

<sup>1,3</sup>Wildlife Conservation Research Laboratory, Department of Ecology and Environmental Science, Assam University, Silchar, Assam 788011, India.

<sup>2</sup>Department of Anatomy, Saveetha Medical College & Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu 602105, India.

<sup>1</sup>monicachetry17@gmail.com, <sup>2</sup>caleb87woodgate@gmail.com, <sup>3</sup>parthankar@rediffmail.com (corresponding author)

The Jumping Spider genus *Colyttus* Thorell, 1891 currently comprises nine species distributed across the Oriental region (World Spider Catalog 2026). Within India, the genus is represented by two species: *Colyttus nongwar* Sudhin & Sen, 2025 and *Colyttus proszynskii* Caleb et al. 2018 (Caleb & Sankaran 2026).

During recent fieldwork conducted in Assam, India, an ecologically significant part of the Indo-Burma biodiversity hotspot, an additional member of the genus was collected. Subsequent morphological examination identified the specimen as *Colyttus bilineatus* Thorell, 1891, a species previously recorded exclusively from Indonesia (World Spider Catalog 2026).

The present paper documents the first record of *C. bilineatus* from India, thereby extending its known geographic distribution and contributing to the understanding of regional Salticidae diversity.

The specimen was collected by vegetation beating and was preserved in 70% ethanol. Photographs of the specimen in life were captured by a Realme 7 Pro mobile phone attached with a Nikon 16–50 mm prime lens. Morphological examination and imaging were performed by the Leica EZ4 stereomicroscope. All measurements

are in millimetres (mm). The studied specimen has been deposited in the reference collection of Wildlife Conservation Research Laboratory, Department of Ecology and Environmental Science, Assam University, Silchar, India.

### Genus *Colyttus* Thorell, 1891

Type species: *Colyttus bilineatus* Thorell, 1891

### *Colyttus bilineatus* Thorell, 1891

Images 1–5

*Colyttus bilineatus* Thorell, 1891: 132 (original description of female); Simon 1903: 737, figs 848–850 (male, synonym of *Hyllus modestus*); Prószyński 1984: 19 (illustration of male); Prószyński 1987: 17 (illustration of male); Zhang & Maddison 2015: 31, figures 524–530, 884–885 (illustration of male and female); Prószyński, 2017: 74, figs 38Q, 39R (illustration of male and female).

*Hyllus modestus* Simon, 1899: 113 (description of male)

Material examined: 1 male (AUS-WCR-925M), India, Assam, Loharbong (24.585° N, 92.737° E) 47 m, 22.ix.2025, coll. M. Chetry.

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**Diagnosis:** The males of *C. bilineatus* can be distinguished by the stout, finger-like lamella of embolus and the narrow proximal tegular lobe (Images 3–5 and Figures 526–527 in Zhang & Maddison 2015). Females can be recognized by the pair of brown longitudinal thin stripes on the abdomen; vulva with pear-shaped primary and secondary spermathecae (Figures 525 & 530 in Zhang & Maddison 2015).

**Description:** Total length: 6.73, carapace length 3.11, width 2.81; abdomen length 3.62, width 1.85. Carapace oval, reddish-brown; dorsal part with a pentagonal

golden-yellow patch starting from the posterior ocular region to the posterior mid thoracic region; eye field black. Anterior eyes surrounded by white orbital setae (Image 1). Clypeus covered long white setae (Image 2). Chelicerae are dark brown. Endites and labium are yellow-brown. Sternum oval, yellow. Legs brown with yellow coxae, trochanters, and proximal portion of femora (Images 1, 2). Abdomen elongated oval, brown with a broad mid-longitudinal yellow stripe (Image 1); venter yellow. Spinnerets yellow. Pedipalp segments are yellow-brown. Retrolateral tibial apophysis is directed



Images 1–5. *Colyttus bilineatus* Thorell, 1891: 1—habitus of male in life, dorsal view | 2—same, frontal view | 3—male, right pedipalp (image flipped horizontally), pro-ventral view | 4—same, ventral view | 5—same, retrolateral view. Scale bars = 0.2 mm (3–5). © Monica Chetry.

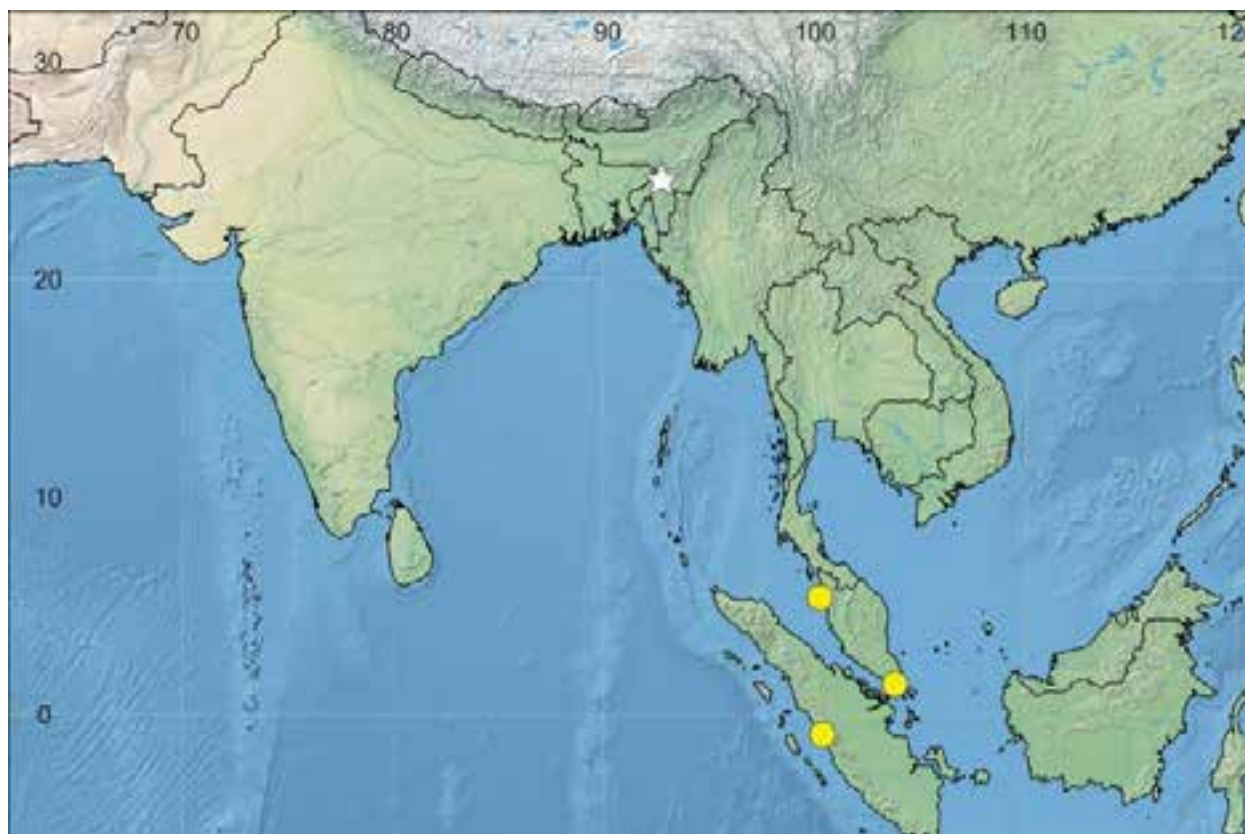


Figure 1. Distribution map of *Colyttus bilineatus*. The white star represents a new location from which it has been recorded; the yellow circle represents records from old literature.

retro laterally in ventral view, elongated with a narrow tip. Proximal tegular lobe along the retrolateral side, nearly oval. Embolus thin and crescent-like; lamella of embolus broad and directed at the 11 o'clock position (Images 3–5).

Distribution: India (present data), Malaysia (Thorell 1891), Singapore (Simon 1903), Indonesia (Simon 1899) (World Spider Catalog 2026) (Figure 1).

Habitat: The specimen was collected from an Inner Line Reserved Forest in Cachar District, Barak Valley, Assam, India, which forms the southern part of the state. The area comprises mixed evergreen and deciduous vegetation, with a closed canopy approximately 20–30 m above ground level (Islam et al. 2013). The forest also supports diverse bamboo and cane species. The region experiences a tropical wet climate, characterized by hot, humid summers and comparatively cool winters.

## Discussion

The salticid tribe Euophryini is currently represented in India by 16 species belonging to 11 genera: *Bathippus* (1 species), *Chalcoscirtus* (2), *Chalcotropis* (1), *Colyttus* (3, including the new national record reported herein),

*Cytaea* (1), *Euophrys* (1), *Foliabitus* (1), *Laufeia* (1), *Saitis* (1), *Tanzania* (1), and *Thiania* (3) (Caleb & Sankaran 2026).

The discovery of *C. bilineatus* in Assam significantly extends the species' known range beyond Indonesia, suggesting either a wider natural distribution than previously documented or under sampling across its potential habitats. The Indo-Burma biodiversity hotspot, in particular, remains poorly surveyed for Salticidae, and several genera traditionally associated with southeastern Asia have been reported from this region (Caleb et al. 2018; Basumatary et al. 2020).

Recent studies emphasize that biodiversity-rich regions such as the Western Ghats and Indo-Burma continue to yield new taxonomic records and undescribed species when systematically explored (Asima et al. 2023; Sudhin & Sen 2025). The present finding reinforces this trend and highlights the need for more comprehensive surveys in northeastern India, particularly within forested and topographically complex landscapes like those of the Barak Valley.

Overall, the present record contributes to the growing understanding of Indian euophryine diversity

and underscores the importance of targeted field studies in bridging existing biogeographic knowledge gaps.

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Mr. Jatishwor Singh Irungbam, Biology Centre CAS, Branišovská, Czech Republic.  
Dr. Ian J. Kitching, Natural History Museum, Cromwell Road, UK  
Dr. George Mathew, Kerala Forest Research Institute, Peechi, India  
Dr. John Noyes, Natural History Museum, London, UK  
Dr. Albert G. Orr, Griffith University, Nathan, Australia  
Dr. Sameer Padhye, Katholieke Universiteit Leuven, Belgium  
Dr. Nancy van der Poorten, Toronto, Canada  
Dr. Kareen Schnabel, NIWA, Wellington, New Zealand  
Dr. R.M. Sharma, (Retd.) Scientist, Zoological Survey of India, Pune, India  
Dr. Manju Siliwal, WILD, Coimbatore, Tamil Nadu, India  
Dr. G.P. Sinha, Botanical Survey of India, Allahabad, India  
Dr. K.A. Subramanian, Zoological Survey of India, New Alipore, Kolkata, India  
Dr. P.M. Sureshan, Zoological Survey of India, Kozhikode, Kerala, India  
Dr. R. Varatharajan, Manipur University, Imphal, Manipur, India  
Dr. Eduard Vives, Museu de Ciències Naturals de Barcelona, Terrassa, Spain  
Dr. James Young, Hong Kong Lepidopterists' Society, Hong Kong  
Dr. R. Sundararaj, Institute of Wood Science & Technology, Bengaluru, India  
Dr. M. Nithyanandan, Environmental Department, La Ala Al Kuwait Real Estate. Co. K.S.C., Kuwait  
Dr. Himender Bharti, Punjabi University, Punjab, India  
Mr. Purnendu Roy, London, UK  
Mr. Saito Motoki, The Butterfly Society of Japan, Tokyo, Japan  
Dr. Sanjay Sondhi, TITLI TRUST, Kalpavriksh, Dehradun, India  
Dr. Nguyen Thi Phuong Lien, Vietnam Academy of Science and Technology, Hanoi, Vietnam  
Dr. Nitin Kulkarni, Tropical Research Institute, Jabalpur, India  
Dr. Robin Wen Jiang Ngiam, National Parks Board, Singapore  
Dr. Lionel Monod, Natural History Museum of Geneva, Genève, Switzerland.  
Dr. Asheesh Shivam, Nehru Gram Bharti University, Allahabad, India  
Dr. Rosana Moreira da Rocha, Universidade Federal do Paraná, Curitiba, Brasil  
Dr. Kurt R. Arnold, North Dakota State University, Saxony, Germany  
Dr. James M. Carpenter, American Museum of Natural History, New York, USA  
Dr. David M. Claborn, Missouri State University, Springfield, USA  
Dr. Kareen Schnabel, Marine Biologist, Wellington, New Zealand  
Dr. Amazonas Chagas Júnior, Universidade Federal de Mato Grosso, Cuiabá, Brasil  
Mr. Monsoon Jyoti Gogoi, Assam University, Silchar, Assam, India  
Dr. Heo Chong Chin, Universiti Teknologi MARA (UiTM), Selangor, Malaysia  
Dr. R.J. Shiel, University of Adelaide, SA 5005, Australia  
Dr. Siddharth Kulkarni, The George Washington University, Washington, USA  
Dr. Priyadarsanan Dharma Rajan, ATREE, Bengaluru, India  
Dr. Phil Alderslade, CSIRO Marine And Atmospheric Research, Hobart, Australia  
Dr. John E.N. Veron, Coral Reef Research, Townsville, Australia  
Dr. Daniel Whitmore, State Museum of Natural History Stuttgart, Rosenstein, Germany.  
Dr. Yu-Feng Hsu, National Taiwan Normal University, Taipei City, Taiwan  
Dr. Keith V. Wolfe, Antioch, California, USA  
Dr. Siddharth Kulkarni, The Hormiga Lab, The George Washington University, Washington, D.C., USA  
Dr. Tomas Ditrich, Faculty of Education, University of South Bohemia in Ceske Budejovice, Czech Republic  
Dr. Mihaly Foldvari, Natural History Museum, University of Oslo, Norway  
Dr. V.P. Uniyal, Wildlife Institute of India, Dehradun, Uttarakhand 248001, India  
Dr. John D. Caleb, Zoological Survey of India, Kolkata, West Bengal, India  
Dr. Priyadarsanan Dharma Rajan, Ashoka Trust for Research in Ecology and the Environment (ATREE), Royal Enclave, Bangalore, Karnataka, India

Fishes

Dr. Topiltzin Contreras MacBeath, Universidad Autónoma del estado de Morelos, México  
Dr. Heok Hee Ng, National University of Singapore, Science Drive, Singapore  
Dr. Rajeev Raghavan, St. Albert's College, Kochi, Kerala, India  
Dr. Robert D. Sluka, Chiltern Gateway Project, A Rocha UK, Southall, Middlesex, UK  
Dr. E. Vivekanandan, Central Marine Fisheries Research Institute, Chennai, India  
Dr. Davor Zanella, University of Zagreb, Zagreb, Croatia  
Dr. A. Biju Kumar, University of Kerala, Thiruvananthapuram, Kerala, India  
Dr. Akhilesh K.V., ICAR-Central Marine Fisheries Research Institute, Mumbai Research Centre, Mumbai, Maharashtra, India  
Dr. J.A. Johnson, Wildlife Institute of India, Dehradun, Uttarakhand, India  
Dr. R. Ravinesh, Gujarat Institute of Desert Ecology, Gujarat, India

Amphibians

Dr. Sushil K. Dutta, Indian Institute of Science, Bengaluru, Karnataka, India  
Dr. Annemarie Ohler, Muséum national d'Histoire naturelle, Paris, France

Reptiles

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Dr. Raju Vyas, Vadodara, Gujarat, India  
Dr. Pritpal S. Soorae, Environment Agency, Abu Dubai, UAE.  
Prof. Dr. Wayne J. Fuller, Near East University, Mersin, Turkey  
Prof. Chandrashekher U. Rivonker, Goa University, Taleigao Plateau, Goa. India  
Dr. S.R. Ganesh, Kalinga Foundation, Agumbe, India.  
Dr. Himansu Sekhar Das, Terrestrial & Marine Biodiversity, Abu Dhabi, UAE

Birds

Dr. Hem Sagar Baral, Charles Sturt University, NSW Australia  
Mr. H. Byju, Coimbatore, Tamil Nadu, India  
Dr. Chris Bowden, Royal Society for the Protection of Birds, Sandy, UK  
Dr. Priya Davidar, Pondicherry University, Kalapet, Puducherry, India  
Dr. J.W. Duckworth, IUCN SSC, Bath, UK  
Dr. Rajah Jayapal, SAGON, Coimbatore, Tamil Nadu, India  
Dr. Rajiv S. Kalsi, M.L.N. College, Yamuna Nagar, Haryana, India  
Dr. V. Santharam, Rishi Valley Education Centre, Chittoor Dt., Andhra Pradesh, India  
Dr. S. Balachandran, Bombay Natural History Society, Mumbai, India  
Mr. J. Praveen, Bengaluru, India  
Dr. C. Srinivasulu, Osmania University, Hyderabad, India  
Dr. K.S. Gopi Sundar, International Crane Foundation, Baraboo, USA  
Dr. Gombobaatar Sunde, Professor of Ornithology, Ulaanbaatar, Mongolia  
Prof. Reuven Yosef, International Birding & Research Centre, Eilat, Israel  
Dr. Taej Mundkur, Wetlands International, Wageningen, The Netherlands  
Dr. Carol Inskipp, Bishop Auckland Co., Durham, UK  
Dr. Tim Inskipp, Bishop Auckland Co., Durham, UK  
Dr. V. Gokula, National College, Tiruchirappalli, Tamil Nadu, India  
Dr. Arkady Lelej, Russian Academy of Sciences, Vladivostok, Russia  
Dr. Simon Dowell, Science Director, Chester Zoo, UK  
Dr. Mário Gabriel Santiago dos Santos, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, Vila Real, Portugal  
Dr. Grant Connette, Smithsonian Institution, Royal, VA, USA  
Dr. P.A. Azeez, Coimbatore, Tamil Nadu, India

Mammals

Dr. Giovanni Amori, CNR - Institute of Ecosystem Studies, Rome, Italy  
Dr. Anwaruddin Chowdhury, Guwahati, India  
Dr. David Mallon, Zoological Society of London, UK  
Dr. Shomita Mukherjee, SAGON, Coimbatore, Tamil Nadu, India  
Dr. Angie Appel, Wild Cat Network, Germany  
Dr. P.O. Nameer, Kerala Agricultural University, Thrissur, Kerala, India  
Dr. Ian Redmond, UNEP Convention on Migratory Species, Lansdown, UK  
Dr. Heidi S. Riddle, Riddle's Elephant and Wildlife Sanctuary, Arkansas, USA  
Dr. Karin Schwartz, George Mason University, Fairfax, Virginia.  
Dr. Lala A.K. Singh, Bhubaneswar, Orissa, India  
Dr. Mewa Singh, Mysore University, Mysore, India  
Dr. Paul Racey, University of Exeter, Devon, UK  
Dr. Honnavalli N. Kumara, SAGON, Anaikatty P.O., Coimbatore, Tamil Nadu, India  
Dr. Nishith Dharaiya, HNG University, Patan, Gujarat, India  
Dr. Spartaco Gippoliti, Socio Onorario Società Italiana per la Storia della Fauna "Giuseppe Altobello", Rome, Italy  
Dr. Justus Joshua, Green Future Foundation, Tiruchirappalli, Tamil Nadu, India  
Dr. H. Raghuram, Sri S. Ramasamy Naidu Memorial College, Virudhunagar, Tamil Nadu, India  
Dr. Paul Bates, Harison Institute, Kent, UK  
Dr. Jim Sanderson, Small Wild Cat Conservation Foundation, Hartford, USA  
Dr. Dan Challender, University of Kent, Canterbury, UK  
Dr. David Mallon, Manchester Metropolitan University, Derbyshire, UK  
Dr. Brian L. Cypher, California State University-Stanislaus, Bakersfield, CA  
Dr. S.S. Talmale, Zoological Survey of India, Pune, Maharashtra, India  
Prof. Karan Bahadur Shah, Budhanilakantha Municipality, Kathmandu, Nepal  
Dr. Susan Cheyne, Borneo Nature Foundation International, Palangkaraja, Indonesia  
Dr. Hemanta Kafley, Wildlife Sciences, Tarleton State University, Texas, USA

Other Disciplines

Dr. Aniruddha Belsare, Columbia MO 65203, USA (Veterinary)  
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Dr. Jack Tordoff, Critical Ecosystem Partnership Fund, Arlington, USA (Communities)  
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Tamil Nadu 641006, India  
ravi@threatenedtaxa.org & ravi@zooreach.org

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