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Cover: Dorsal view of Pebble Crab *Seulocia vittata* male from Palk Bay with grey colour pattern. © Sanjeevi Prakash.



## Prey selection and food habits of the Tiger *Panthera tigris* (Mammalia: Carnivora: Felidae) in Kalakkad-Mundanthurai Tiger Reserve, southern Western Ghats, India

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**Abstract:** The Endangered Tiger *Panthera tigris* is the largest felid, distributed over 1.1 million km<sup>2</sup> globally. Conservation of Tigers largely depends on the preservation of its natural prey base and habitats. Therefore, the availability of prey and its selection play a major role in the sustainable future of Tigers in the given landscape. The current study assesses the prey selection patterns by Tigers in tropical evergreen forest of the Kalakkad-Mundanthurai Tiger Reserve (KMTR), southern Western Ghats, India. Density of ungulates was assessed by distance sampling (line transect, N = 21) and diet composition of Tigers was evaluated by analysing their faecal samples (N = 66). The study estimated very low ungulate density (26.87 ± 7.41 individuals km<sup>-2</sup>) with highest density of Gaur *Bos gaurus* (9.04 individuals km<sup>-2</sup>) followed by Wild Boar *Sus scrofa* (8.79 ± 2.73 individuals km<sup>-2</sup>), whereas, primate density was quite high (45.89 ± 12.48 individuals km<sup>-2</sup>), with Nilgiri Langur *Semnopithecus johnii* having the highest density (38.05 ± 10.22 individuals km<sup>-2</sup>). About 74.62% of the biomass of Gaur constituted in the Tiger's diet, consumed lesser than its availability, whereas Sambar constituted 16.73% of the Tiger diet consumed proportionally to its availability. Chital *Axis axis*, Muntjac *Muntiacus muntjak*, and Indian Chevrotain *Moschiola indica* were not represented in the Tiger's diet. The current study is the first scientific information on prey selection of the Tiger in KMTR landscape, which will serve as a baseline for its conservation planning and management.

**Keywords:** Faecal analysis, food habits, line transect, prey abundance, prey selection.

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## INTRODUCTION

The Tiger *Panthera tigris*, is the largest among five big cats in the genus (Sunquist 2010), distributed across the heterogeneous habitats of Asia (Hayward et al. 2012). Globally, Tiger population has precipitously declined, and its range has extensively diminished over the past century (Kerley et al. 2015). Poaching for Tiger body parts, habitat loss, and degradation and depletion of prey base have been the major causes for its decline (Karanth et al. 2004; Miquelle et al. 2010). Despite existence of large tracts of suitable habitats across Asia, Tigers are absent in many of the areas, probably due to lack of adequate prey base (Rabinowitz 1993; Check 2006), however, previous studies have emphasised that Tigers are flexible and recover when their habitat and adequate prey species are well protected (O'Brien et al. 2003).

Tigers are obligate terrestrial carnivores, generally preying upon ungulates (Seidensticker 1997), including diverse ranges of species that differ in size such as cervids, bovids, and suids (Andheria et al. 2007; Miquelle et al. 2010; Hayward et al. 2012). Prey availability, season, topography, and forest types are some of the significant ecological variables that influence the dietary habits of Tigers (Sunquist & Sunquist 1999). Studies have also suggested that predators play a major role in regulating the abundance of herbivore population in an environment of tropical forest (Karanth et al. 2004), which further results in the cascading effect at each trophic level (Polis & Strong 1996). Therefore, understanding of the dietary habits of the Tiger in relation to its prey base availability is essential for efficient management of wildlife and natural habitats (Biswas & Sankar 2002; Bagchi et al. 2003). Most of the information on prey selection of Tiger comes from studies carried out in semi-arid dry thorn and dry deciduous forests of central India (Bagchi et al. 2003; Biswas & Sankar 2002; Sankar et al. 2010) and tropical moist deciduous forests of southern India (Karanth & Suquist 1995; Ramesh et al. 2012a; Kumaraguru et al. 2011). In those areas, Chital was the dominant prey species in the Tiger's diet (Johnsingh 1992; Karanth & Sunquist 1995; Venkataraman et al. 1995; Andheria et al. 2007), however, no comprehensive study has been conducted to estimate the abundance of prey and its selection by Tigers in their distribution range in the southern Western Ghats. There is scanty information about predator-prey selection at Kalakkad-Mundanthurai Tiger Reserve (KMTR) and the lack of such information can be a major limitation in designing and implementing site-specific conservation measures

(Karanth et al. 2003). Understanding the principal constituents of the Tiger diet is essential for planning effective conservation policies (Kerley et al. 2015). Thus, the current research aims to assess the prey selection patterns by the Tiger in the tropical evergreen forest of KMTR.

## STUDY AREA

The current study was carried out between July 2015 and May 2018 in four administrative ranges, namely, Mundanthurai, Papanasam, Ambasamudram, and Upper Kodhayar (Intensive study area, henceforth ISA) of 588km<sup>2</sup> in KMTR (900km<sup>2</sup>), located in the southern Western Ghats (8.357–8.883 °N & 77.169–77.574 °E) in Tamil Nadu, India (Figure 1). The terrain KMTR is mountainous (the elevation ranges 100–1,866 m), and the vegetation ranges from dry thorn scrub to montane wet tropical forest and grassland at high altitudes (Ramesh et al. 2012b). KMTR receives rainfall from both the south-west (June to September) and the north-east (October to January) monsoons (Sarkar 2012). The annual rainfall is about 3,000mm, and the temperature fluctuates between 17°C and 37°C during the year. This reserve is bordered by agricultural lands with human settlements (about 145 villages) in the east (Arjunan et al. 2006), and with forest tracts of the Neyyar, Peppara, and Shendurni wildlife sanctuaries in the Ashambu Hill range (Naniwadekar & Vasudevan 2006) in the west. The rivers Peyar, Karaiyar, Kavuthalaiyar, Servalar, Chithar, and Pambar and their tributaries drain into a perennial river called Tamiraparani. The sympatric carnivore species found here are the Tiger, the Leopard *Panthera pardus*, and the Wild Dog *Cuon alpinus*. Sambar, Gaur, Chital, Wild Boar, Barking Deer, and Indian Chevrotain are some of the major prey species that occur in this reserve. In addition, Asian Elephant *Elephas maximus*, Indian Hare *Lepus nigricollis*, Bonnet Macaque *Macaca radiata*, Tufted Grey Langur *Semnopithecus priam*, Lion-tailed Macaque *Macaca silenus*, Nilgiri Tahr *Hemitragus hylocrius*, Indian Crested Porcupine *Hystrix indica*, Indian Giant Squirrel *Ratufa indica*, Grey Jungle Fowl *Gallus sonneratii*, Red Spurrow *Galloperdix spadicea*, and Indian Peafowl *Pavo cristatus* are also found in the reserve.

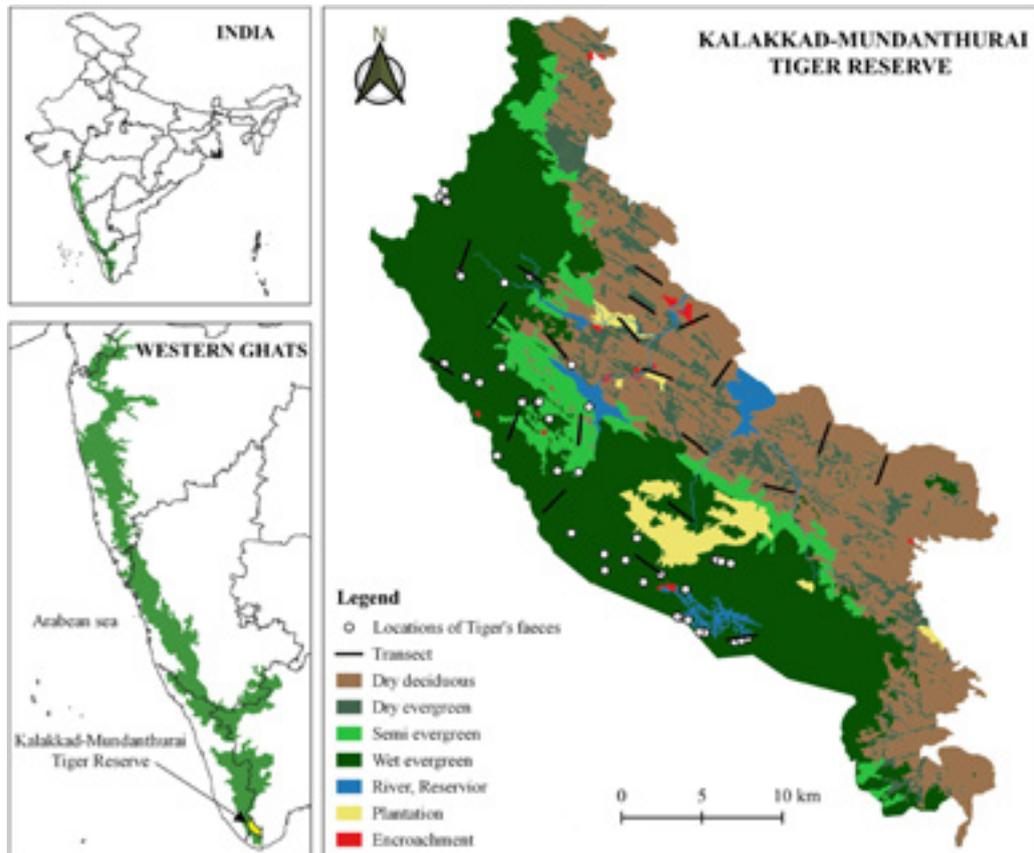


Figure 1. Study area depicting the locations of the line transects and Tiger faeces in Kalakkad-Mundanthurai Tiger Reserve.

## FIELD METHODS

### Density and biomass estimation of prey species

The densities of wild prey were estimated by using the line transect sampling technique (Burnham et al. 1980; Buckland et al. 1993, 2001). The line transect method has been extensively applied to estimate animal densities in the tropical forests of southern Asia (Karanth & Sunquist 1992, 1995; Biswas & Sankar 2002; Jathanna et al. 2003; Bagchi et al. 2003; Edgaonkar 2008; Paliwal 2008; Malla 2009). Permanent transect lines ( $n=21$ ) were randomly laid across different habitat types of KMTR by the Tamil Nadu Forest Department. The transect length vary from 1.5 ( $n=3$ ) to 2 ( $n=18$ ) km. The total length and sampling effort was 40.50 and 243km, respectively. Six replicates of 21 transects were walked at dawn (06.30–08.30 h) between January and May 2016 and at dusk (16.30–18.30 h) between January and May 2017 within the ISA area. Data were collected by a researcher and two trained observers on every transect walk. For each detection, the animal bearings were recorded using a look through compass (KB 20, SUNNTO, Vantaa, Finland), while angular sighting distance were recorded using a laser range finder (Yardage Pro 850,

Bushnell, Overland Park, Kansas USA). Group size was also recorded during the transect sampling. Necessary care was taken while walking on transects to maximize detectability of animals before they disappeared from sight.

### Faecal sample collection

As cryptic and nocturnal behaviour of the carnivores limit the direct observation of their predatory behaviour in the wild, faecal samples were collected to determine their food habits. Large carnivores generally prefer to travel along forest roads and trails, and as they travel they defecate to mark their presence and passage (Sunquist 1981; Johnsingh 1983; Smith et al. 1989; Karanth & Sunquist 2000). Therefore, faecal samples of Tiger were collected by intensively searching along such trails, river beds, and open glades from July 2015 to May 2017. All trails were revisited after about two months for consecutive collection. Faeces of Tigers were collected only when they were associated with scraps and tracks. We distinguished faecal samples between Leopard and Tiger by their diameter and supplementary evidence such as pugmarks and scrapes (Karanth & Sunquist

1995). Leopard faeces are much larger, twisted, more coiled between constriction and deposited on the grassy stripes at the centre or the edges of forest road (Andheria et al. 2007), whereas, Tiger faeces appear to be less coiled and have larger distance between two successive constrictions within a single piece of a faeces (Ramesh 2010). Once a faeces was encountered, a large portion was collected in a paper envelope for diet analysis. One-fourth of the faeces was left uncollected to avoid disturbances in Tigers' territorial marking. The collected faecal samples were washed in running water through a nylon mesh (<1mm), later sun-dried in thin paper pages (Andheria et al. 2007). Following that, the dried faecal samples were stored in airtight bags individually labelled with date and location for further identification.

## ANALYTICAL METHODS

### Density and biomass estimation of prey species

The density of major prey species of Tiger was estimated using the program 'DISTANCE' version 7.2 (Thomas et al. 2010). To maximise the number of the sighting, the temporal replicates of each of the line transects were pooled together and were considered as a single spatial sample (n=21). Different detection functions were fitted to the observed data and the appropriate model was selected based on the lowest Akaike information criterion (AIC) values (Burnham et al. 1980; Buckland et al. 1996). Parameters such as effective strip width (ESW), cluster density ( $D_g$ ), cluster size ( $G_s$ ), and animal prey individual density ( $D_i$ ) were also estimated using program DISTANCE 7.2 (Burnham et al. 1980; Buckland et al. 1993).

The density of ungulate commonly represented as the biomass of ungulates available in the ecosystem. The biomass ( $\text{kg km}^{-2}$ ) of major prey species was calculated by multiplying the individual density ( $D_i$ ) of prey species by its average estimated unit weight (Tamang 1982; Wegge et al. 2009) from the available information for major prey species (Karanth & Sunquist 1992, 1995) (see Appendix 1).

### Identification of prey species

Examination of indigestible parts of animals and plants found in a predator's faeces is the primary source of information about its food habits (Andheria et al. 2007). The prey species were identified by microscopic examination of the medullary pattern (colour, length, and thickness of the medulla) in 20 hairs, collected randomly from each faecal sample (Mukherjee et al. 1994), and later corroborated with reference guides of Bahuguna et al. (2010) and Chakraborty & De (2010).

### Estimation of frequency of occurrence and relative biomass of prey consumed

A most commonly used measure of the frequency of occurrence (henceforth FO) for each prey type was to estimate the prey intake and composition (Andheria et al. 2007). The FO, however, does not provide the best approximation of the true dietary patterns of a predator, as the biomass consumed to faeces excreted is not alike for all prey species due to their variation in surface area: volume ratio, described by Floyd et al. (1978) and Ackerman et al. (1984). To preclude such bias, we have used the biomass calculation model recently developed for obligate carnivores by Chakrabarti et al. (2016).

$$Y = ((0.033 - (0.025 \times \exp(-4.284(X/PBM)))) \times PBM$$

Where, Y is the mass of prey consumed per collectable faecal sample, X is the prey body mass, and PBM is the predator body mass. The mean body weight of each prey consumed by Tiger was based on Karanth & Sunquist (1995).

The adequacy of the sample size was calculated using the Brillouin diversity index (Brillouin 1956).

$$HB = \ln N! - \sum \ln n_i! / N$$

Where HB is diversity, N is the number of the prey taxa in all the samples, and  $n_i$  is the number of individual prey taxa in the  $i$ th category.

### Analysis of prey selection

To assess the prey selection patterns of Tigers for different prey species in KMTR, Jacobs' index (1974) of preference (D) was used:

$$D = (r_i - p_i) / (r_i + p_i - 2r_i p_i)$$

Where,  $r_i$  is the proportion of a prey remains in faecal sample, and  $p_i$  is the proportional density of prey species in the population. The resulting values ranges from +1 (strongly selected) to -1 (strongly avoided). Prey selection assessment was restricted to those prey species whose density information was available.

## RESULTS

### Density and biomass of prey species

The overall densities of ungulates and primates were  $26.87 \pm 7.41 \text{ km}^{-2}$  and  $45.89 \pm 12.48 \text{ km}^{-2}$ , respectively, whereas, densities of Indian Giant Squirrel and Grey Jungle Fowl were  $3.20 \pm 1.32 \text{ km}^{-2}$  and  $25.32 \pm 5.09 \text{ km}^{-2}$ , respectively. The estimated individual and cluster density for potential prey species of a large carnivore is given in Table 1 along with cluster size and their percentage of the coefficient variation, and effective stripe width (Appendix 2). Half-normal-cosine was the

**Table 1. Estimated density of major prey species of large carnivore in Kalakkad-Mundanthurai Tiger Reserve. Total sampling effort was 243km.**

Species	Model (AIC)	Min AIC	Cluster size (SE)	ESW in meter (SE)	D <sub>i</sub> km <sup>-2</sup> (SE)	%CV (D) (km <sup>-2</sup> ) 95%	95% CI	Dg km <sup>-2</sup> (SE)	Biomass kg km <sup>-2</sup>
Bonnet Macaque	Half-normal / Cosine	15.913	7.88 (2.79)	48.77 (8.46)	1.70 (0.53)	21.15	0.50 – 5.81	0.22 (0.11)	6.8
Tufted Grey Langur	Half-normal / Cosine	9.272	30.74 (10.22)	31.52 (6.77)	6.14 (1.73)	23.34	1.12 – 33.74	0.20 (0.01)	55.26
Nilgiri Langur	Half-normal / Cosine	212.77	7.82 (0.97)	34.93 (2.86)	38.05 (10.22)	26.82	22.33 – 64.87	4.86 (1.15)	342.45
Total primates					45.89				404.51
Chital	Half-normal / Cosine	15.982	2.65 (0.70)	15.61 (4.37)	2.50 (0.92)	18.44	0.65 – 9.60	0.94 (0.32)	117.5
Sambar	Half-normal / Cosine	40.157	1.72 (0.24)	15.03 (2.50)	4.80 (1.04)	21.70	2.06 – 11.17	2.80 (0.57)	643.2
Mouse Deer	Half-normal / Cosine	7.79	*	(8.42) (2.65)	1.74 (0.69)	19.82	0.42 – 7.35	*	5.22
Gaur	Half-normal / Cosine	37.98	4.79 (1.31)	25.55 (4.08)	9.04 (2.03)	28.55	3.08 – 26.52	1.88 (0.47)	4068
Wild Boar	Half-normal / Cosine	33.95	2.70 (0.43)	12.87 (2.18)	8.79 (2.73)	31.07	2.72 – 28.42	3.26 (1.29)	281.28
Total ungulates					26.87				5115.20
Indian Giant Squirrel	Half-normal / Cosine	46.82	1.57 (0.19)	19.33 (3.27)	3.19 (1.32)	20.8	1.42 – 7.20	2.03 (0.81)	
Grey Jungle Fowl	Half-normal / Cosine	201.98	1.6 (0.43)	15.38 (0.79)	25.32 (5.09)	20.12	16.82 – 38.11	15.82 (3.07)	

CV—Coefficient of Variation | Dg—Density of cluster size | D—Density of individuals | ESW—Effective Stripe Width | Min AIC—Minimum Akaike information criterion | SE—Standard Error | CI—95% Confident Interval | \*—data not analysed.

best fit model that had resulted in the lowest AIC value for all the species. The major prey species of Tigers are classified into groups such as ungulates (Chital, Sambar, Mouse Deer, Gaur, Wild Boar) and primates (Tufted Grey Langur, Nilgiri Langur, Bonnet Macaque), while Grey Jungle Fowl was also consumed by them. In terms of density of clusters in ungulates, Wild Boar ( $3.26 \pm 1.29$  km<sup>-2</sup>) were most abundant, followed by Sambar ( $2.79 \pm 0.57$  km<sup>-2</sup>), Gaur ( $1.88 \pm 0.47$  km<sup>-2</sup>), and Chital ( $0.94 \pm 0.32$  km<sup>-2</sup>), whereas density of individual Gaur ( $9.04 \pm 2.03$  km<sup>-2</sup>) was the highest among all the ungulates, followed by Wild Boar ( $8.79 \pm 2.73$  km<sup>-2</sup>), Sambar ( $4.80 \pm 1.04$  km<sup>-2</sup>), Chital ( $2.50 \pm 0.92$  km<sup>-2</sup>) and Mouse Deer ( $1.74 \pm 0.69$  km<sup>-2</sup>). The number of detections for elephants was too low to permit useful analysis. Total estimated biomass for ungulates and primates in KMTR was 5,115.20 kg km<sup>-2</sup> and 404.51 kg km<sup>-2</sup>, respectively.

KMTR harboured high density of primates as individual densities for Nilgiri Langur, Tufted Grey Langur and Bonnet Macaque were  $38.05 \pm 10.22$  individuals km<sup>-2</sup>,  $6.14 \pm 1.73$  individuals km<sup>-2</sup>, and  $1.70 \pm 0.53$  individuals km<sup>-2</sup>, where the density of cluster was  $4.86 \pm 1.15$ ,  $0.20 \pm 0.01$ , and  $0.22 \pm 0.11$  clusters km<sup>-2</sup>, respectively. Substantial observations of Indian Giant Squirrel ( $3.20 \pm 1.32$  km<sup>-2</sup>) and Grey Jungle Fowl ( $25.32 \pm 5.09$  km<sup>-2</sup>) were obtained on transects during the study period.

### Prey composition and selection

After excluding faecal samples ( $n = 6$ ) which had an unidentifiable object and were loose/viscous in consistency, we had a total of 66 Tiger faecal samples. The Brillouin diversity index value for the estimation of adequacy of the sample size reached 15<sup>th</sup> faecal, indicating that we had sampled adequately (Figure 2). Four species of mammals were identified in the Tiger faecal sample (Table 2). All faecal samples contained single prey items. Out of the prey species identified in the Tiger faeces, Gaur constituted 74.2% followed by Sambar (16.6%), Sloth Bear (6.06%), and Nilgiri Tahr (3.0%). No remains of Chital, Muntjac, Mouse Deer, Wild Boar, and primates were found in the Tiger faeces.

The prey selectivity of a Tiger was tested by comparing with the individual density of the prey species. Prey selection analysis was restricted to seven prey species (Gaur, Sambar, Chital, Mouse Deer, Wild Boar, Nilgiri Langur, and Tufted Grey Langur), whose density information was available. The Jacobs' index value showed that Tigers displayed strongest selection of Gaur followed by Sambar (Figure 3) and apparently avoided other prey in KMTR.

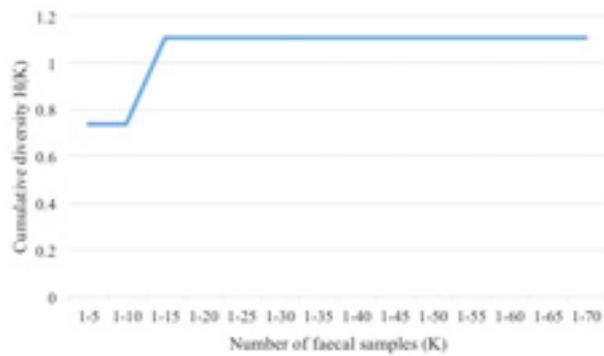


Figure 2. Cumulative diversity, H(k), of Tiger prey items with increased sample size (k).

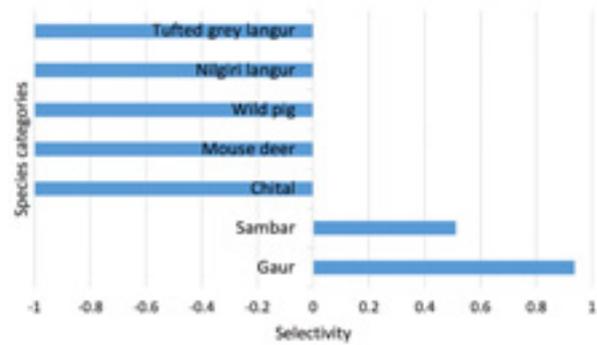


Figure 3. Prey selectivity index of Tiger in Kalakkad-Mundanthurai Tiger Reserve, as assessed by Jacob's index.

Table 2. Food habit of the Tiger in Kalakkad-Mundanthurai Tiger Reserve based on faecal analysis (n=66).

Prey species	Relative frequency of occurrence % (RFO)	Mean body weight (kg)	Biomass consumed/ faeces	Biomass consumed (kg)	Relative biomass consumed
Gaur	74.24	287 (Karanth & Sunquist 1995)	4.95	242.50	74.62
Sambar	16.67	212 (Karanth & Sunquist 1995)	4.94	54.35	16.73
Nilgiri Tahr	3.03	100 (Kumaraguru et al. 2011)	4.73	9.47	2.91
Sloth Bear	6.03	90 (Biswas & Sankar 2002)	4.66	18.65	5.74

## DISCUSSION

### Density and prey biomass

Comparative account of total ungulate densities estimated in the present study (Table 3) with that of other tropical forests in southern Asia revealed that KMTR harboured lower density of ungulates than most of them but higher than the Tiger reserves such as Bori-Satpura, Pakke, and Bhadra. The possible reason for the low density of ungulates might be the majority rocky outcrops and highly precipitous terrain. Mid-elevation forest is dry in most of the place coupled with contiguous tracts (c. 440km<sup>2</sup>) of tropical rainforest in KMTR which is unfavourable for ungulates (Johnsingh 2001). Gaur was found to be most abundant species in the ISA and was comparable with other Tiger reserves of Western Ghats such as Mudumalai (Ramesh 2010) and Nagarhole (Karanth & Sunquist 1992). Nevertheless, most observation of Gaur were in grassland due to increased visibility compared to heavily vegetated habitat types in KMTR, therefore, we presume that this might have influenced the overall density of Gaur. Therefore, we speculated that true density of Gaur would be closer to the lower confidence limit of 3.08km<sup>-2</sup> and it is similar to the previous study in KMTR by Ramesh et al. (2012b). Gaurs were mostly recorded in the morning within the wet grasslands of higher

altitude, whilst they were observed in the dry thorn and teak forest during the dusk hours. The density of Wild Boar appears to be closely comparable to Ranthambore (Bagchi et al. 2003), Barida (Stoen & Wegge 1996), and Katka-Kochikahali of Sundarbans (Reza et al. 2002) but different from Anamalai Tiger Reserve (Kumaraguru et al. 2011). Estimated density of Sambar in the current study was comparable to tropical dry moist deciduous (Bori-Satpura, Badhra, Nagarhole) and tropical dry thorn, dry deciduous, and evergreen forest habitat of Mudumalai and Bandipur (Table 3). Sambar density, however, was quite low compared to Anamalai (Kumaraguru et al. 2011) and Pench (Acharya 2007).

The density of chital estimated (2.5 individuals km<sup>2</sup>) was very low compared to other tropical forests in southern Asia. In ISA Chital distribution was restricted to 60km<sup>2</sup> of the Mundanthurai plateau (Sathyakumar 2000), which was covered with dry thorny and deciduous vegetation interspersed with the overgrown teak plantation. Plateau is dominated by unpalatable tall-grass species *Cymbopogon flexuosus* (Sankaran 2005) and invasive thickets, such as Lantana *Lantana camara* and Eupatorium *Eupatorium glandulosum* (Uma et al. 1999). Though cattle grazing has been prohibited in KMTR since 2000 (Venkatesh et al. 2017), there were substantial number of cattle grazing in the reservoir (Karaiyar and Manimuthar) and Mundanthurai Plateau.

**Table 3. Comparison of ungulate densities and their biomass (Individuals km<sup>-2</sup>) from different protected areas in southern Asia.**

Study area	Chital	Gaur	Sambar	Wild Boar	Muntjac	Mouse Deer	Nilgiri Tahr	Total ungulate density	Total ungulate biomass	Source
Current Study	2.5	9.04	4.8	8.79	...	1.74	-	26.87	5115.20	
Mudumalai Tiger Reserve	25.4	9.4	4.8	1.3	1.2	...	NP	42.1	6133.8	Ramesh (2010)
Keoladeo National Park	52.37	NP	0.32	3.21	NP	...	NP	69.58	5069.39	Aakrithi et al. (2017)
Nagarahole National Park	50.6	9.6	5.5	4.2	4.2	...	NP	74.1	7657.8	Karanth & Sunquist (1992)
Anamalai Tiger Reserve	20.54	12.34	6.54	20.61	0.28	...	13.67	73.98	9181.08	Kumaraguru et al. (2011)
Bilgiri Rangasamy Tiger Reserve	13.96	5.08	6.01	5.33	3.7	...	NP	34.08	3995.72	Kumara et al. (2012)
Kalakkad-Mundanthurai Tiger Reserve	...	3.6	7.0	1.3	...	...	NP	11.9	2599.6	Ramesh et al. (2012b)
Bandipur Tiger Reserve	20.1	7	5.6	...	0.7	...	NP	33.4	4859.8	Karanth & Nichols (1998)
Bhadra Tiger Reserve	8.88	3.86	4.4	2.46	4.35	...	NP	23.95	2914.03	Gopaldaswamy et al. (2012)
Pench National Park	115.6	0.4	12.2	20.3	-	...	NP	149.4	8059.6	Acharya (2007)
Kanha National Park	469.7	...	1.5	2.5	0.6	...	NP	57.3	3103.5	Karanth & Nichols (1998)
Bardia National Park	77.7	NP	...	8.8	1.7	NP	NP	99.2	4786.5	Stoen & Wegge (1996)
Bori-Satpura Tiger Reserve	5.4	...	4	1.8	0.8	NP	NP	13.6	1152.2	Edganokar (2008)
Ranthambore National Park	31	...	17.1	9.7	...	NP	NP	74.8	6228.4	Bagchi et al. (2003)
Gir National Park	50.8	NP	2	-	...	NP	NP	56.2	2819.22	Khan et al. (1996)
Sariska Tiger Reserve	33.88	NP	26.38	54.12	NP	NP	NP	157.1	14548.72	Mondal et al. (2011)
Chitwan National Park	61.8	...	20	3.6	...	NP	NP	85.4	5699.8	Sunquist (1981)
Kaziranga National Park	NP	...	...	2.6	...	NP	NP	58.1	4815.6	Karanth & Nichols (1998)
Rajaji National Park	49.9	NP	14.6	1.9	NP	NP	NP	68.8	4794.5	Harihar et al. (2009)
Pakke Tiger Reserve	NP	3.5	3.8	6.7	3.9	NP	NP	17.9	2380.5	Selvan et al. (2013a)
Sundarbans	70.4	NP	NP	7.9	NP	NP	NP	78.3	3561.6	Reza et al. (2002)

Notes: NP - The respective species was not found in the respective area; ... - Data were not reported

Thus, the cattle grazing and lack of suitable grassland might be a potential factor explaining the low density of Chital. Despite being nocturnal in nature, we sighted Mouse Deer on transect line, however, no further analysis could be done as it was a solitary sighting.

We compared the density of Bonnet Macaque with the estimates available from other tropical forests in India (Table 4). The density of Bonnet Macaque was available only for Mudumalai (Ramesh 2010), Nagarahole (Karanth & Sunquist 1992), Bilgiri Rangaswamy Tiger Reserve (Kumara et al. 2012), and Srisi-Honnar (Babureddy et al. 2015). Bonnet Macaque density in KMTR was lower than that of the aforesaid parks. The specialist folivore Tufted Grey Langur was in low densities but their density was found to be comparable with Bilgiri Rangasamy Tiger Reserve. In terms of density amongst ungulates and primates, Nilgiri Langur was found in high density

(38.05 individuals km<sup>-2</sup>) in ISA. The present study has reported that the densities have increased as compared to a previous study (Ramesh et al. 2012b).

**Prey composition and selection of tiger**

In the current study, the Tiger preyed on three large ungulates, including Gaur, Sambar, and Nilgiri Tahr. We did not find multiple prey species in a single sample which is contrary to the prediction of Bekoff et al. (1984). Gaur accounted for 74.6% of the Tiger diet by biomass. Such selective predation towards large body mass was also reported in Anamalai Tiger Reserve (Kumaraguru et al. 2011), Nagarahole (Karanth & Sunquist 1995), Bandipur Tiger Reserve (Andheria et al. 2007), and Pakke Tiger Reserve (Selvan et al. 2013a). Carnivores tend to prefer the most abundant prey (Breuer 2005). Tiger’s selective predation for Gaur in the present study area indicates

**Table 4. Comparison of arboreal prey densities (individuals km<sup>-2</sup>) and biomass from protected areas in Indian subcontinent.**

Study area	Tufted Grey Langur (previously known as common langur)	Nilgiri Langur	Bonnet Macaque	Total primate density	Total primate biomass	Reference
KMTR (Present study)	6.14	38.05	1.7	45.89	404.51	
Mudumalai Tiger Reserve	35.4	...	1.9	37.3	340.6	Ramesh (2010)
Nagarahole National Park	23.8	...	5.5	29.3	236.2	Karanth & Sunquist (1992)
Bilgiri Rangasamy Tiger reserve	6.34	NP	6.56	12.9	83.3	Kumara et al. (2012)
Kalakkad–Mundanthurai Tiger Reserve	...	9.9	...	9.9	89.1	Ramesh et al. (2012)
Sirsi-Honnavaar	25.06	NP	12.4	37.46	275.14	Babureddy et al. (2015)
Badhra Tiger Reserve	22.6	NP	...	22.6	203.4	Jathanna et al. (2003)
Pench Tiger Reserve	65.8	NP	...	65.8	592.2	Acharya (2007)
Bori – Satpura	28.3	NP	NP	28.3	254.7	Edganokar (2008)
Melghat	42.92	NP	NP	42.92	386.28	Narasimmarajan et al. (2014)
Bardia National Park	2.3	NP	NP	2.3	20.7	Stoen & Wegge (1996)
Ranthambore National Park	21.75	NP	NP	21.75	195.75	Bagchi et al. (2004)
Sariska Tiger Reserve	50.67	NP	NP	50.67	456.03	Mondal et al. (2011)
Chitawan National Park	3.6	NP	NP	3.6	32.4	Sunquist (1981)
Chilla range of Rajaji National Park	14.1	NP	NP	14.1	126.9	Harihar et al. (2009)

Notes: NP - The respective species was not found in the respective area; ... - Data were not reported

selection for a large ungulate. Thus, in ISA of KMTR, Gaur occurred in higher densities (9.04km<sup>-2</sup>) at wet grassland in high altitudes interspersed with reed brakes (*Ochlandra* sp.), majority of collected faecal samples were found from such habitat, which suggests that the Tiger prefers habitat where Gaur occur more commonly. Such spatial correlation might have increased their encounter with the predator. Crepuscular and poor eyesight of Gaur could have enabled the Tiger to stalk Gaur easily (Karanth 1993). On the other hand, this selective predation could also be related to optimal foraging theory (Stephens & Krebs 1987), which suggests that the selected prey could provide higher benefits in terms of net biomass intake whilst reduce the cost of handling (stalking, subduing, and disemboweling prey) and injury risks (Scheel 1993). Hence, the predator must shift to profitable species, which may be either medium-size or high density that make them easier to be captured (Lamichhane & Jha 2015).

In the current study, Sambar biomass constituted relatively lesser (16.73%) proportion in the Tiger diet than other tropical forests of India such as Nagarhole (Karanth & Sunquist 1992), Sariska (Sankar & Johnsingh 2002), Ranthambhore (Bagchi et al. 2003), Bandipur (Andheria et al. 2007), Satpura (Edgaongar 2008), and Mudumalai (Ramesh 2010). This may be due to spatial

distance from the Tiger, as Sambar mostly forage around tea plantation (personal observations), near human habitation, and dry deciduous and thorn forest of low elevation. Such spatial segregation between them might have strengthened the predation on Gaur. Chital, being a common prey for the Tiger in other protected areas (McDougal 1977; Sunquist 1981; Johnsingh 1983; Karanth & Sunquist 1995; Stoen & Wegge 1996; Biswas & Sankar 2002) was absent in the faecal samples of Tigers in KMTR. This is due to scarce and restricted distribution of Chital in Mundanthurai Plateau with low density (Selvan et al. 2013b). This spatial segregation has compelled the Tiger to depend on Gaur. During the current study, we did not see any sign of Tigers in Mundanthurai Plateau, which also corroborates a previous study by Uma et al. (1999).

Presence of Sloth Bear remains in the Tiger’s faeces reflected the occasional predation on this species. Predation on bear is not a new phenomenon, as other investigators also reported the same (Biswas & Sankar 2002; Swaminathan et al. 2002; Harsha et al. 2004; Andheria et al. 2007). Though the bear remains a relatively minor component of the Tiger diet relative to Gaur and Sambar, this was more than Nilgiri Tahr in the current study. One possible explanation is the density of Sloth Bear and Nilgiri Tahr in the study area. In addition,

Nilgiri Tahr occur only in restricted cliffs in the present study area (Hopeland et al. 2016). Conversely, bears are spread across the study area and are mostly nocturnal and crepuscular (Chauhan et al. 2004; Yoganand et al. 2005). Such spatial segregation between the Tiger and the Nilgiri Tahr, while spatial and temporal overlap between the Tiger and the Sloth Bear, could have increased encounter rate and led to high predation on Sloth Bear compared to Nilgiri Tahr in our study area. Unfortunately, we could not determine density of Sloth Bear, Nilgiri Tahr, and their activity pattern on our study site; therefore, future research is needed to confirm the relationships among density, prey selection, spatial, and temporal overlap.

The present study revealed that the moderate prey availability is enough to preserve the Tiger in the long run in this landscape. Management of relatively few ungulates, primarily Gaur may be critical for Tiger conservation in this region.

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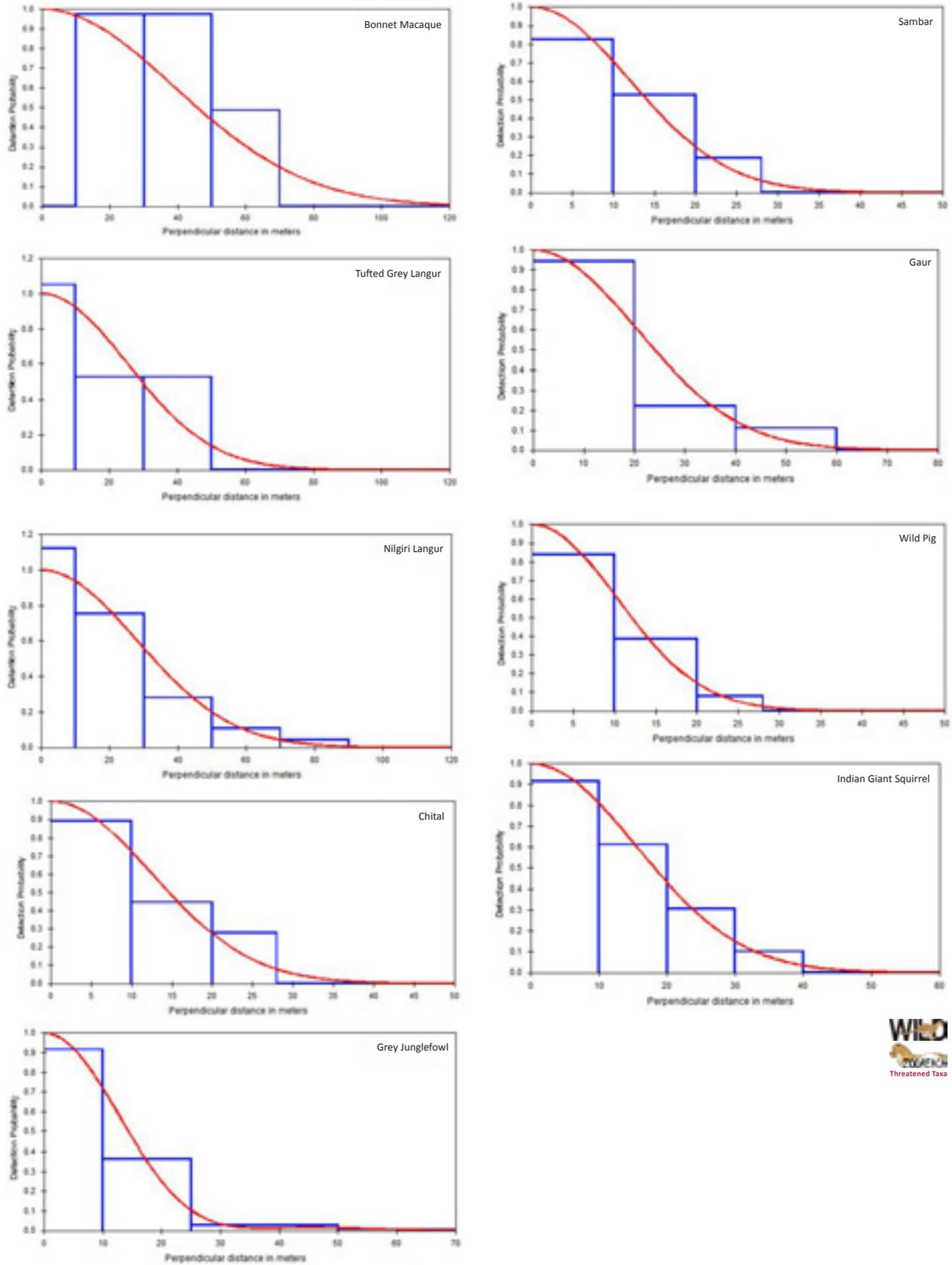
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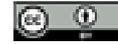
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**Appendix 1. Average estimated unit weight of prey species in Kalakkad-Mundanthurai Tiger Reserve.**

Species	Weight (kg)	Source
Bonnet Macaque	4	Karanth & Sunquist (1992)
Tufted Grey Langur	9	Karanth & Sunquist (1992)
Nilgiri Langur	9	Karanth & Sunquist (1992)
Chital	47	Karanth & Sunquist (1992)
Sambar	134	Karanth & Sunquist (1992)
Mouse Deer	5	Karanth & Sunquist (1995)
Gaur	450	Karanth & Sunquist (1992)
Wild Boar	32	Karanth & Sunquist (1992)

Appendix 2. Detection distances for primates and ungulates in Kalakkad-Mundanthurai Tiger Reserve.





## Community-based study to demonstrate the presence and local perspectives of the Critically Endangered Chinese Pangolin *Manis pentadactyla* in Zhejiang Wuyanling, China

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**Abstract:** Illegal hunting and trading of the Chinese Pangolin *Manis pentadactyla* has pushed this Critically Endangered species close to extinction. While local reports have suggested its continued presence in mainland China, this has not been confirmed by a research group except for a survey of presumed pangolin burrows in 2004. We conducted a six-month field study using infrared camera surveillance and community questionnaire survey in Zhejiang Wuyanling National Nature Reserve in China, to determine the status of Chinese Pangolins and understand local attitudes towards the conservation of this species. Our study details the first verifiable documentation of two visual records of a Chinese Pangolin in the wild, demonstrating the suitability of pangolin habitat in Wuyanling region, and suggests an increasing awareness and strong willingness in local communities to conserve the Chinese Pangolin.

**Keywords:** Community attitude, conservation, infrared camera, *Manis pentadactyla*, Wuyanling National Nature Reserve.

非法捕猎和贸易将中华穿山甲这一极度濒危的物种推向了灭绝的边缘。虽然一些本地的报道表明中华穿山甲在中国大陆地区一直存在，但除了2004年一项对中华穿山甲洞穴的研究，目前对中华穿山甲个体在自然生境中的存在尚缺乏充分的记录。该研究在中国浙江乌岩岭保护区进行了六个月的红外相机监测和社区问卷调查，来确定该保护区内中华穿山甲存在的状况，并了解当地居民对于保护中华穿山甲的态度。调查首次获得了中华穿山甲在野外环境中的影像记录，表明乌岩岭地区作为中华穿山甲生境的适宜性，问卷调查的结果反映了当地社区对于保护中华穿山甲具有较强的意识和参与行动的意愿。

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**Ethical standards:** This study was approved by the Institutional Review Board at China Jiliang University for the community questionnaire survey.

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## INTRODUCTION

The Chinese Pangolin *Manis pentadactyla* is listed as Critically Endangered by the IUCN primarily due to extensive poaching for their meat and scales (Challender et al. 2019). It receives the highest level of protection from trade in CITES Appendix I (Challender & Waterman 2017). In China, the Chinese Pangolin is listed as a State Category II protected species under the Wildlife Protection Law, with protection from the Regulations on Implementation of Protection of Terrestrial Wild Animals (Zhang 2008). Despite this, the population of the Chinese Pangolin is thought to have declined by 89–94 % since the 1960s (Wu et al. 2004a). Little is known about the current distribution or population of this solitary and nocturnal species, few studies have been conducted in mainland China to detail the population of Chinese Pangolins (Wu et al. 2002). Recent reports suggest that some remnant individuals have been traded in illegal wildlife markets or confiscated from traders, but the provenance of these animals from mainland China has not been verified (Xu et al. 2016).

In 2013, our group recorded two camera-trap images of a pangolin (*Manis* spp.) within the Wuyanling National Nature Reserve (Zhang et al. 2017). The species was not identified due to limited characteristics recognisable in the images. Our current study was designed to further assess the presence of Chinese Pangolins in the reserve and understand local community attitudes towards the species' conservation. Wuyanling National Nature Reserve is located in Taishun County in southern Zhejiang Province (27.706, 119.675) (Figure 1, 2). The reserve was established in 1975 and is an important bird conservation area (BirdLife International 2019) consisting of subtropical evergreen broadleaf forest with highly diverse flora and fauna. Within an area of 18,861.5ha, the reserve contains 4,170 households with 15,444 residents officially registered with the local authorities, including 3,064 people residing in the core area and buffer zone, and 12,380 in the transition area. The number of residents actually living within the reserve is estimated to be less than 5,000, due to a large number having migrated to cities for work, according to the latest population census in 2017 (Taishun Government 2017).

## MATERIALS AND METHODS

### Line transect and quadrat survey

Ten (10) line transects ranging 2–6 km in length (total 39.8km) and six quadrats (1.00 hm<sup>2</sup>/quadrat)

were selected within the reserve extending from the uninhabited regions to the nearby villages. With limited resources, selection of the line transects and quadrats was based on the historical records of pangolin sighting, poaching, and releasing activities, and in an effort to cover the under-researched low-human disturbance areas. Diurnal surveys were conducted in each line transect and quadrat once per month for six months from July to December 2017 to identify and examine potential burrows for Chinese Pangolins. Observational environmental data were collected around each burrow, including estimated duration since burrow excavation, presence of termites and decayed wood, and pangolin faeces or other evidence of active occupation following the methods published in Wu et al. (2004b).

### Camera traps surveillance

Sixty infrared camera traps (LTL Acorn 5210A, LTL Acorn 6210) were placed in three surveillance areas around the identified (potentially active) pangolin burrows from the line transect and quadrat surveys, and in the reserve's core area and its adjacent transition area where was not covered by the line transect and quadrat surveys, to maximize the chance of detection. All camera traps were placed 0.5–1 m above ground, with consideration of the ground slope, height of trees, and the inclination angle between 15°–30° to achieve the maximum diameter and range of camera coverage. The intervals between each camera trap and each surveillance area were >500m and >5,000m, respectively. Corresponding to the altitude range of potentially active burrows, the altitudes of camera traps ranged 313–1,128 m across four different vegetation types. All camera traps were installed on 1 July 2017 and active until 31 December 2017. Burrows presumed to be inactive or confirmed to be occupied by other species were omitted in the subsequent investigations, and all potentially active burrows were investigated by the monthly line transect or quadrat surveys and infrared camera traps for six months (Figure 1).

### Community questionnaire survey

In order to understand local knowledge of and attitudes toward Chinese Pangolins, a standardized questionnaire was designed in Mandarin (Appendix 1) for community survey. We aimed to obtain a sample size of n=3,000 to be statistically representative, covering 60% of the permanent residents within the reserve at the maximum estimation of 5,000 in total. 1) Prior to recruitment and data collection, study staff from local village committee and Wuyanling National Natural

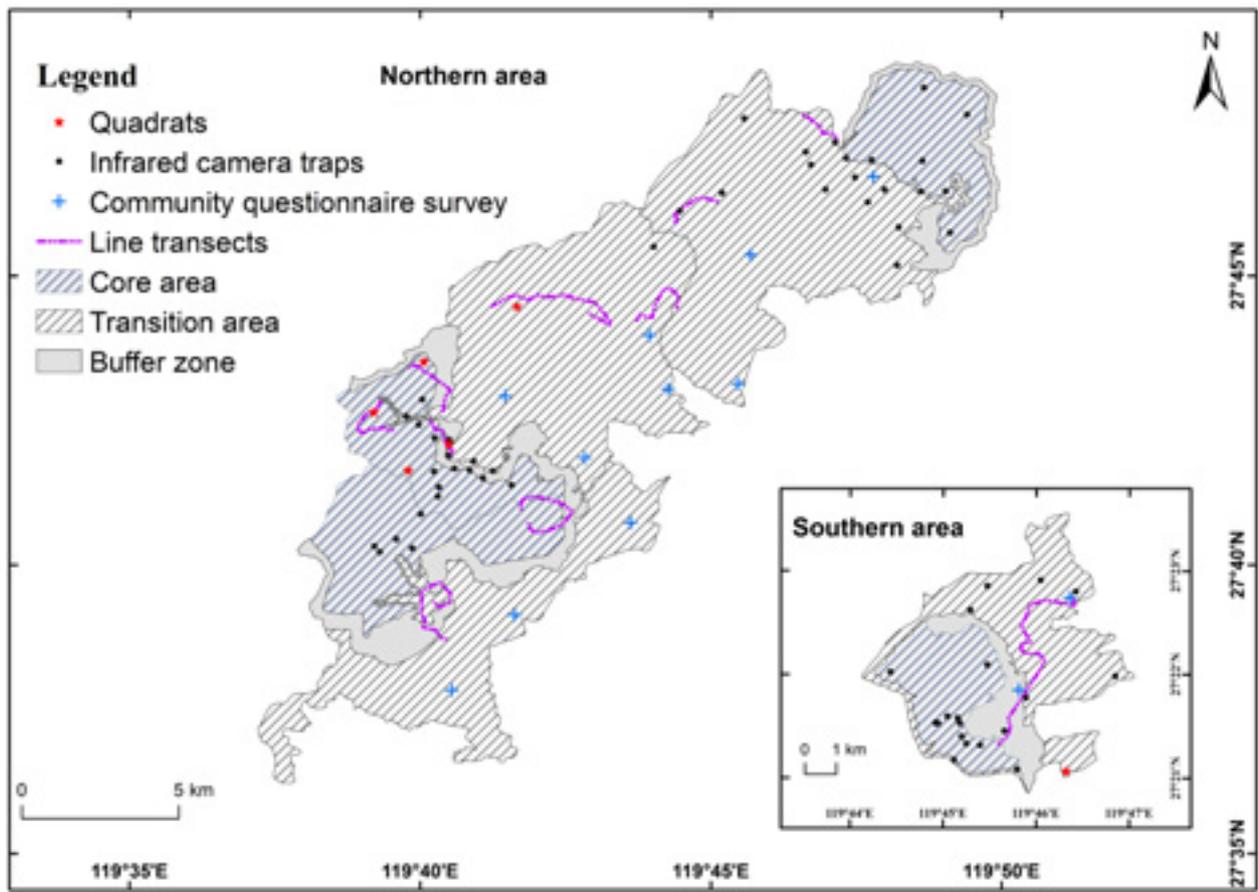


Figure 1. Locations of 10 line transects, six quadrats, 60 infrared camera traps, and community questionnaire surveys among 12 villages in both the northern and southern areas of Wuyanling National Nature Reserve. Surveys were conducted from July to December 2017.



Figure 2. Wuyanling National Nature Reserve in Taishun County, Zhejiang Province

Reserve Management Bureau conducted community meetings, house visits, and broadcasted the information in the village to introduce this study to generate interest

in the community; 2) Recruitment was conducted at the community activity centre or town hall in each village where local residents who were interested in the project visited for further information and became potential participants; 3) Study staff informed all potential participants about the survey for consent before data collection; 4) After the completion of informed consent process, questionnaires were administered and one-on-one interviews were conducted in a private setting to ensure confidentiality. Children aged 12–18 years were interviewed with the permission and presence of a parent or guardian (e.g., school teacher). From September to December 2017, a total of 3,041 questionnaires were distributed in 12 out of the 15 villages within the nature reserve. Three villages were omitted from the surveys because they had few residents (<50) or only a small area of these villages was under the administration of the nature reserve. Collected data were entered into IBM SPSS Statistics software Version 25 and analysed by cross-table and Pearson's chi-squared test. All results were translated into English for reporting.

## RESULTS

### Potentially active burrows for pangolins

A total of 33 burrows were located along nine line transects and in six quadrats in the initial field survey. During the monthly survey, burrows that were estimated to have been created more than 15 days or covered by spider webs and decayed leaves at the entrance were recorded as inactive. Active burrows for Chinese Pangolins were identified based on several environmental factors, including the presence of termitarium and decayed wood (e.g., *Pinus massoniana*, *Cunninghamia lanceolata*) around a burrow (<50m), fresh soil at the entrance, and suspect faeces (Image 1). As the survey was conducted from July to December, seasonal burrow characteristics in regard to the utilization of a burrow, and preferred locations for burrow excavation in summer and winter were considered in identifying the active Chinese Pangolin burrows (Wu et al. 2004b). Active burrows were found at five line transects and two quadrats, further examination confirmed five potentially active burrows for the Chinese Pangolins at two line transects and one quadrat during the six-month survey (Table 1). Some of the active burrows from the initial survey were later confirmed by infrared cameras to be habitats for Chinese Ferret-Badger *Melogale moschata*, MongOOSE *Herpestes urva*, or White-Bellied Rat *Niviventer coninga*.

### Record of the Chinese Pangolin

A Chinese Pangolin was recorded by an infrared camera on 21 December 2017 during the study period, and another image of a Chinese Pangolin was recorded on 21 January 2018 at a different site (23km straight-line distance from the first recording site)

during the preparation of this manuscript (Image 2). The identification of these individuals as the Chinese Pangolin *Manis pentadactyla* was confirmed by notable characteristics, including well-developed external ears with big ear pinna, short heads (neck to snout) covered by small scales, and soft, off-white hair on its underside and face. Their scales are larger than the scales of the Philippine Pangolin *Manis culionensis* but smaller than that of the Indian Pangolin *Manis crassicaudata*, and gradually increase in size behind the ears (Challender et al. 2019; Cota-Larson 2017). While the Chinese Pangolin's geographic range sometimes overlaps with that of the Sunda Pangolin *Manis javanica*, it can also be distinguished by the scales on its flank or behind its ears. Additionally, there are no historic records of the presence of Sunda Pangolins in this region as well (Challender et al. 2014; IUCN SSC Pangolin Specialist Group 2019).

### Demographic characteristics of community survey participants

Out of the 3,041 distributed questionnaires, data were collected from 2,654 anonymous participants (87.3% response rate) in 12 villages. Most of the participants (51.1% male; 48.9% female) were subsistence farmers (59.9%) who raise crops and domestic animals. Other significant groups were migrant workers (20.6%), students (12%) and government employees (2.5%) (Table 2).

### Knowledge and attitude about Chinese Pangolins

About half of the participants (49%) considered the Chinese Pangolin as endangered or critically endangered



**Image 1. Potential active burrows for Chinese Pangolins at Wuyanling: A—an active burrow excavated within 15 days with fresh soil | B—termites and decayed wood around active burrows. © Zhejiang Wuyanling Natinoal Nature Reserve Management Bureau.**

**Table 1. Observation of burrows in 10 line transects and six quadrats. Active burrows were located at five line transects and two quadrats, further examination confirmed five potentially active burrows for Chinese Pangolins at two line transects and one quadrat.**

Line Transect					
	Length (km)	Burrow(s)	Estimated duration of burrow excavation	Termitarium & Decayed wood	Other Information
1	5.5	Inactive	>1 month	X	Release location
2	3.2	Inactive	>15 days	X	
3	2.5	Inactive	>1 month	X	
4	5.0	Inactive	>1 month		
5	4.5	Active*	<15 days	X	Suspect faeces
6	4.0	Active	<15 days		Record of other species
7	6.0	None	n/a	n/a	
8	3.5	Active	<15 days		Record of other species
9	2.0	Active	<15 days		Record of other species
10	3.6	Active*	<15 days	X	Record of a Chinese Pangolin
Quadrat					
	Size (hm <sup>2</sup> )	Burrow(s)	Estimated duration of burrow excavation	Termitarium & Decayed wood	Other Information
1	1	Inactive	>1 month	X	
2	1	Inactive	>1 month	X	
3	1	Inactive	>15 days	X	
4	1	Inactive	>15 days		
5	1	Active*	<15 days	X	Suspect faeces
6	1	Active	<15 days		Record of other species

\* Potentially active burrows for Chinese Pangolins.

based on their experience and knowledge and 11% believed that it had become extinct locally, while some participants didn't think the Chinese Pangolin was endangered (21%) or expressed no knowledge of its current status (19%). For those participants who were aware of the Chinese Pangolin, they acquired the information mostly from social media platforms (31.4%), school teaching (30.2%), or television (24.1%), government campaign appeared to be a minor (7.1%) channel to disseminate the relevant information. More than half (58.6%) of the participants recognized the ecological value of Chinese Pangolins, but many still regarded the species as a valuable economic (12.7%), medical (20.5%), and food (6.6%) source, and 21% of the participants stated that they would support the use of pangolins or pangolin products for traditional Chinese medicine.

#### Pangolin consumption in local community

Majority of the participants who reported having consumed pangolin meat or relevant products (101, 3.8%) in their lifetimes were male (73.3%), subsistence farmers (82.2%), and over 50 years old (97%), which

was significantly associated with age ( $p < 0.001$ ), gender ( $P < 0.001$ ), and occupation ( $p < 0.001$ ) (Table 3). The taste and nutrition (34.1%), perceived medical benefits (25.4%), demonstration of wealth (15.9%), or simply out of curiosity (11.1%) were reported as the reasons of pangolin consumption. Some participants (11.9%) indicated consuming pangolins as a supplemental source of dietary protein a long time ago (the year was not specified). Other participants reported refusing to consume pangolins because of its illegality (39%), the perceived cruelty (38.4%), the cause of population decline (15.9%), or due to its expected bad taste (6.7%).

#### Community willingness for pangolin conservation

Most participants (95.3%) indicated that they would report pangolin hunting or trading activities to the forestry department's public security staff (61.3%) or the general public security staff (38.7%). Some participants, particularly those between 31–50 years old ( $p < 0.001$ ) regardless of their occupation, reported that they would like to reap the economic benefits of trading pangolins (4.7%). Many participants indicated their willingness to contribute to local pangolin conservation (60%) and



**Image 2.** Camera trap images of the Chinese Pangolin *Manis pentadactyla* in Zhejiang Wuyanling National Nature Reserve recorded in 2017 and 2018 (ACDSEE Pro). © Zhejiang Wuyanling National Nature Reserve Management Bureau.

believed that informing the public about the species' protected status would help motivate public action to protect pangolins (60.4%). When asked to provide insights about the specific action that would help protect local Chinese Pangolins, participants emphasized the needs to strengthen law enforcement (21.9%), improve local participation in voluntary work (19.8%), disseminate information (19.7%), refuse to consume pangolin products (19.7%), and actively protect pangolin habitat (16.0%).

## DISCUSSIONS

Our camera trap records of the Chinese Pangolin in 2017 and 2018, and the prior record in 2013 in

Wuyanling (Zhang et al. 2017) suggest that Wuyanling National Nature Reserve contains a viable habitat for Chinese Pangolins. While samples were not collected for DNA analysis, the distinctive features of the Chinese Pangolin are evident in the video and images captured, confirming its presence (Video 1). Greater efforts needed to conduct initial fieldwork to identify potentially active burrows and increase the coverage of camera traps in Wuyanling region and other sites that are suspected to harbour extant populations of Chinese Pangolins to further understand the populations. In addition, camera traps were positioned at 0.5–1.0 m height in our study based on our experience in mammal surveillance, however, positioning camera traps lower around 30cm above ground may better record Chinese Pangolins and other small mammals in future study (Willcox et al.

**Table 2. Demographics of community questionnaire survey participants.**

Characteristics	Participants (n=2,654)	
	Frequency N	Percent %
<b>Gender</b>		
Male	1,356	51%
Female	1,298	49%
<b>Age (years)</b>		
<18	383	14%
18–30	262	10%
31–50	1,247	47%
>50	762	29%
<b>Occupation</b>		
Government employee	67	3%
Peasant	1,590	60%
Student	319	12%
Migrant worker	546	21%
Others	132	5%
<b>Village</b>		
Bai Hai	72	3%
Cha Shi	366	14%
Wu Dou	62	2%
Ma Lian	223	8%
Huang Qiao	557	21%
Zhu Li	264	10%
Yang Bian	109	4%
Dao Jun Yang	285	11%
Wen Yang	58	2%
Xin Bei	256	10%
Ye Shan	186	7%
Shang Di	216	8%

2019).

We noted the existence of an estimated 5–10 burrow entrances grouped within an area of 300m<sup>2</sup>, suggesting Chinese Pangolins possibly create burrows in a cluster, which needs further study to confirm. Future field surveys should include collection of faeces or scales for DNA analysis, improved surveillance methods (e.g., distance sampling combined with confirmation of burrow occupancy, proper camera setting), and further understanding of the burrow ecology of Chinese Pangolins. With enhanced methodology, similar research can be conducted at other sites in China and across Asia. The characteristics of the habitat in Wuyanling could be used to identify other potential habitats where extant populations may be identified, as well as for the site selection to release confiscated Chinese Pangolins from the authorities in China. DNA testing of confiscated pangolins will be needed to ensure that only native species are released and the IUCN protocols on the appropriate release of animals into the wild would be followed (IUCN/SSC 2013).

The community survey identified male subsistence farmers over 50 years old as the main population in Wuyanling to have had consumed pangolins. The fact that younger population do not report consumption of pangolins (Nash et al. 2016) may be explained by the improved livelihoods over the past 40+ years leading to improved nutrition and reduced dependence on wildlife as an alternate source of protein. Significant social change has also occurred during this time, including the migration of people into cities for work which may have decreased exposure of younger people to the custom of wildlife consumption. The teaching of wildlife conservation principles in schools and opportunities

**Table 3. Questionnaire results from local population in Wuyanling region (n = 2,654): consumption of pangolin products and its associations with the age, gender, and occupation.**

Pangolin Products Consumption	Yes	No	Pearson chi-square		
			Value	df	P (2-sided)
<b>Gender</b>					
Female	27 (26.7%)	1,271 (49.8%)	20.661	1	<.001
Male	74 (73.3%)	1,282 (50.2%)			
<b>Age</b>					
<18 yr	2 (2.0%)	381 (14.9%)	239.686	3	<.001
18–30 yr	1 (1.0%)	261 (10.2%)			
31–50 yr	0 (0.0%)	1,247 (48.8%)			
>50 yr	98 (97.0%)	664 (26.0%)			
<b>Occupation</b>					
Government employee	1 (1.0%)	66 (2.6%)	26.146	4	<.001
Subsistence farmers	83 (82.2%)	1,507 (59.0%)			
Student	0 (0.0%)	319 (12.5%)			
Migrant worker	12 (11.9%)	534 (20.9%)			
Others	5 (5.0%)	127 (5.0%)			

for volunteering in conservation work may also contribute to the changing attitude and behaviour towards conservation of Chinese Pangolins. While the time frame of pangolin consumption among Wuyanling residents was not identified in the questionnaire, local knowledge about the current status of Chinese Pangolins as well as the frequent consumption reported by older participants suggest most consumption may have been historic. Further study will be conducted to understand the context of pangolin consumption behaviours to develop evidence-based behavioural change programs.

Our findings demonstrate an overall positive public attitude towards the ecological value of the Chinese Pangolin and its conservation. There is a significant awareness of the illegality of pangolin hunting and consumption in the communities, prompting the communities to report illegal hunting and refuse consumption. Constant education about the illegality of consumption and reinforced conservation needs of Chinese Pangolins via social media, television, and school programs is recommended to keep raising the awareness and motivate action in the communities for pangolin conservation. In addition, the marked community willingness to participate in pangolin conservation work suggests a potential for positive behavioural changes when effective programs implemented. With a remnant viable Chinese Pangolin population present in the wild in mainland China and a potential generational transformation in public perception of its conservation status, we believe there may still be an opportunity to avoid extinction of this Critically Endangered species.

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**Video 1.** Camera trap video of a Chinese Pangolin in Zhejiang Wuyanling National Nature Reserve, 2017.

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## Appendix 1. Community questionnaire

### Community Pangolin Conservation Survey in Wuyanling National Nature Reserve

Thank you very much for taking time to participate in this study, all your response will be kept confidential and only used for this study. Please fill in according to your honest thought.

Date : \_\_\_\_\_ Village : \_\_\_\_\_ Gender : \_\_\_\_\_

#### **1. Your Age**

- 1.1 1–18 years old
- 1.2 18–30 years old
- 1.3 30–50 years old
- 1.4 > 50 years old

#### **2. Your occupation**

- 2.1 Migrant worker
- 2.2 Peasant
- 2.3 Government employee
- 2.4 Student
- 2.5 Others

#### **3. How many Chinese Pangolins you think still exist in Wuyanling?**

- 3.1 Extinct
- 3.2 Critically endangered
- 3.3 Endangered
- 3.4 Not endangered
- 3.5 I don't know

#### **4. Have you or your relatives ever eaten pangolins?**

- 4.1 Yes
- 4.2 No

#### **5. What are the reasons you (want to) consumed pangolins? (choose all that apply)**

- 5.1 Out of curiosity
- 5.2 Source of protein as regular meat
- 5.3 To show off
- 5.4 For medical function
- 5.5 Tasty and nutritious food
- 5.6 Others

#### **6. What are the reasons that you refuse(d) to consume pangolins (choose all that apply)**

- 6.1 It violates the law
- 6.2 It's cruel
- 6.3 Effects on the population and environment
- 6.4 It doesn't taste good
- 6.5 They carry diseases
- 6.6 Others

#### **7. What do you think is the biggest value of pangolin?**

- 7.1 Economic value
- 7.2 Medicine
- 7.3 Food
- 7.4 Ecological value
- 7.5 Fur and skin
- 7.6 Ornamental or exhibiting animal
- 7.7 Others

#### **8. Do you support using pangolin as medicine**

- 8.1 Yes
- 8.1 No
- 8.2 I don't know

#### **9. How do you learn about pangolin (choose all that apply)**

- 9.1 Books
- 9.2 The Internet through computer
- 9.3 WeChat and other social media platforms
- 9.4 School teaching
- 9.5 News
- 9.6 Government promotion
- 9.7 Television
- 9.8 Others

#### **10. Would you like to help pangolin protection work at in Wuyanling?**

- 10.1 Yes, I'd love to very much
- 10.2 Yes, I'd like to
- 10.3 No, I don't want to
- 10.4 I don't care

#### **11. What do you think we can do to protect pangolins (choose all that apply)**

- 11.1 Strengthen law enforcement
- 11.2 Participate in voluntary protection work
- 11.3 Refuse to consume wildlife
- 11.4 Protect the habitat
- 11.5 Tell friends not to consume
- 11.6 Others

#### **12. What do you think we can do to motivate local community to protect pangolins? (choose all that apply)**

- 12.1 Promotion and spread the message
- 12.2 Public education events
- 12.3 Develop relevant products (e.g. App)
- 12.4 Voluntary protection activities
- 12.5 Make documentary about pangolins
- 12.6 Others

#### **13. What would you do if you find someone hunting or eating pangolins?**

- 13.1 None of my business
- 13.2 Try to get involve to share the benefits
- 13.3 Report them
- 13.4 Ask someone else to report

#### **14. Do you know where to report?**

- 14.1 Forestry public security
- 14.2 General public security 110
- 14.3 120 (medical emergency)
- 14.4 119 (fire department)
- 14.5 Other

## Appendix 1. Community questionnaire (Chinese)

## 乌岩岭保护区穿山甲社区保护研究调查问卷

非常感谢您在紧张的工作学习之余做这份调查, 此份问卷的内容只用于学术研究, 对您的回答将给以保密, 请放心, 希望您根据真实想法填写。祝您工作学习进步, 生活愉快!

日期: \_\_\_\_\_ 所在行政村: \_\_\_\_\_ 性别: \_\_\_\_\_

**1. 您的年龄是?**

- 1.1 1-18岁
- 1.2 18-30岁
- 1.3 30-50岁
- 1.4 大于50岁

**2. 您的职业是?**

- 2.1 外出打工者
- 2.2 农民或林民
- 2.3 政府工作人员
- 2.4 学生
- 2.5 其他人员

**3. 您知道乌岩岭保护区穿山甲多不多?**

- 3.1 已灭绝
- 3.2 极度濒危
- 3.3 濒危
- 3.4 不濒危
- 3.5 不知道

**4. 您或您亲戚朋友的餐桌是否出现过穿山甲?**

- 4.1 是
- 4.2 否

**5. 您食用、想食用穿山甲的原因是 (多选题)**

- 5.1 新鲜、猎奇
- 5.2 一种普通食物
- 5.3 显示特殊身份, 可带来优越感, 炫耀心理
- 5.4 有特殊的药用价值或保健价值
- 5.5 风味独特, 营养丰富
- 5.6 其他

**6. 您拒绝食用穿山甲的原因是 (多选题)**

- 6.1 违反国家相关法律
- 6.2 血腥、残忍
- 6.3 物种种群数量下降, 破坏生态平衡
- 6.4 味道差, 风味不佳
- 6.5 野生动物是传播人畜共患病的媒介
- 6.6 其他

**7. 您认为穿山甲的哪个价值对人类最重要?**

- 7.1 经济价值
- 7.2 药用价值
- 7.3 食用价值
- 7.4 生态价值
- 7.5 皮革价值
- 7.6 观赏价值
- 7.7 其他

**8. 您是否支持穿山甲做为药材药用**

- 8.1 支持
- 8.1 不支持
- 8.2 不知道

**9. 您日常通过哪些途径了解穿山甲保护的有关信息? (多选题)**

- 9.1 书籍
- 9.2 电脑
- 9.3 微信 (公众号新媒体)
- 9.4 学校知识
- 9.5 新闻
- 9.6 政府宣传
- 9.7 电视
- 9.8 其他

**10. 您愿意为乌岩岭保护区穿山甲做一些工作吗?**

- 10.1 非常愿意
- 10.2 愿意
- 10.3 不愿意
- 10.4 无所谓

**11. 您认为可以怎样去保护穿山甲? (多选题)**

- 11.1 增大执法力度
- 11.2 参与穿山甲保育的志愿工作
- 11.3 拒绝食用野生动物
- 11.4 保护动物的生存环境
- 11.5 劝告朋友, 教育别人不食用穿山甲
- 11.6 其他

**12. 您认为可以怎么样去调动人们保护穿山甲的积极性 (多选题)**

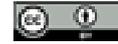
- 12.1 宣传普及
- 12.2 展开相关知识的讲解活动
- 12.3 推出相应的周边商品与软件应用
- 12.4 成为一名保护志愿者
- 12.5 制作相应穿山甲保护主体的影视作品
- 12.6 其他

**13. 发现有人偷猎穿山甲或食用穿山甲, 你会怎么做?**

- 13.1 事不关己高高挂起
- 13.2 自己也参与其中, 分享利益
- 13.3 马上报警举报
- 13.4 想要举报怕得罪人怕报复叫第三方报警

**14. 如果您举报, 您知道向谁举报? (多选题)**

- 14.1 森林公安局
- 14.2 110
- 14.3 120
- 14.4 119
- 14.5 其他



## Field friendly method for wild feline semen cryopreservation

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**Abstract:** The aim of this study was to develop a field-friendly method for free-living jaguar and cougar semen cryopreservation. Six captive Jaguars *Panthera onca* and three captive Cougars *Puma concolor* were chemically restrained with a combination of medetomidine (0.08–0.1 mg/kg) and ketamine (5 mg/kg). Semen was collected through a tomcat urinary catheter with an open end, diluted for a final concentration of  $50 \times 10^6$  sperm/mL in a TRIS-egg yolk extender and packaged into 0.25 mL straws. We compared two cooling methods: CoolA - in which straws were placed in a glass tube that was placed in a glass bottle containing water (600mL at 38°C) and transferred to a polystyrene container (12L) containing an 11cm column of ice and water at room temperature; CoolB - where the glass bottle - straws kit was transferred to a 4.26L cooler containing nine blocks (81cm<sup>3</sup>) of Ice Foam recyclable ice, previously frozen in liquid nitrogen. The sperm volume varied from 2 to 720  $\mu$ l for the jaguars and from 80 to 140  $\mu$ l for the cougars. Sperm concentration varied from 224 to  $5,115 \times 10^6$  sperm/mL for the jaguars and from 485.7 to  $562.5 \times 10^6$  sperm/mL for the cougars. Concerning the cooling treatments, there was no difference in frozen-thawed sperm quality between the methods, in both species. Thereby, the cooling method using recyclable ice frozen in liquid nitrogen can be used for semen cryopreservation in wild felines, eliminating the need for electric energy.

**Keywords:** Cryopreservation, free-living, *Panthera onca*, *Puma concolor*, spermatozoa.

**Resumo:** O objetivo deste estudo foi desenvolver um método de campo amigável para a criopreservação de sêmen de onça-pintada e onça-parda de vida livre. Seis onças-pintadas em cativeiro (*Panthera onca*) e três onças-pardas em cativeiro (*Puma concolor*) foram anestesiadas com uma combinação de medetomidina (0,08 a 0,1mg/kg) e cetamina (5mg/kg). O sêmen foi coletado através de um cateter urinário tomcat com extremidade aberta, diluído para uma concentração final de  $50 \times 10^6$  spz / mL em diluente de gema de ovo TRIS e embalado em palhetas de 0,25mL. Comparamos dois métodos de resfriamento: CoolA - em que as palhetas foram colocados em um tubo de vidro que foi colocado em uma garrafa de vidro contendo água (600mL a 38°C) e transferido para um recipiente de poliestireno (12L) contendo uma coluna de 11cm de gelo e água à temperatura ambiente; CoolB - onde as palhetas em garrafa de vidro foram transferidas para uma caixa térmica de 4,26 L contendo nove blocos (81cm<sup>3</sup>) de gelo reciclável, previamente congelado em nitrogênio líquido. O volume espermático variou de 2 a 720 $\mu$ l para as onças-pintadas e de 80 a 140 $\mu$ l para os pumas. A concentração espermática variou de 224 a  $5,115 \times 10^6$  spz/mL para as onças-pintadas e de 485,7 a  $562,5 \times 10^6$  spz/mL para as onças-pardas. Em relação aos tratamentos de resfriamento, não houve diferença na qualidade dos espermatozoides descongelados entre os métodos em ambas as espécies. Desse modo, o método de resfriamento usando gelo reciclável congelado em nitrogênio líquido pode ser usado para a criopreservação de sêmen em felinos selvagens, eliminando a necessidade de energia elétrica.

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For **Author details & Author contribution** see end of this article.



## INTRODUCTION

The Jaguar *Panthera onca* and the Cougar *Puma concolor* are apex predators and play a crucial role in the prey population control, thus both are considered keystone species for the ecosystems conservation (Crawshaw Jr. 1991). Threats such as deforestation and human activity are resulting in a reduced Jaguar and Cougar population in Brazil, and both species are classified as Vulnerable by the Brazilian Red Book of Threatened with Extinction Fauna (ICMBio 2018a). The conservation of such species depends on several actions that can reduce their vulnerability. These actions are defined in the National Action Plan for Big Cats Conservation (NPBigcat) (ICMBio 2018b) produced by the Brazilian Ministry of Environment.

One of the recommended actions by the NPBigcat is to develop assisted reproduction programs, which aim to help increase the genetic variability of the species. Sperm cryopreservation is an assisted reproduction technique that enables keeping viable sperms for an indeterminate period (Silva et al. 2004). In addition, semen cryopreservation allows translocation of genetic material among populations, dispensing the transport of individuals, which reduces the stress caused by the translocation and the risks of transmission of infectious diseases (Wildt 1990).

For cryopreservation, sperm must be cooled from body temperature to 5°C and only then frozen in nitrogen vapor (-70°C) and finally stored in liquid nitrogen at -196°C (Budhan Pukazhenthil et al. 1999; D. Zambelli et al. 2010). Several automatic cooling and freezing equipment are available in the market, however, they are large and require electricity. There are also portable containers for sperm cooling and transportation, which use recyclable ice. Nevertheless, they also need electricity to freeze the ice for 12h before being used. Thus, these devices are not feasible for use in free-living felines, since capture sites are often difficult to access and without electricity. This difficulty is clearly demonstrated when we evaluate the articles published in scientific journals, in which only two papers describe the characteristics of fresh sperm in free-living Jaguars, but they did not cryopreserve the samples (Morato et al. 2001; Araujo et al. 2018). Therefore, one of the challenges in developing assisted reproduction techniques in free-living cats is the lack of portable and electricity-free devices. Thus, this study was aimed to develop a field friendly method for Jaguar and Cougar semen cryopreservation.

## MATERIALS AND METHODS

### Animals

Captive Jaguars (n=6) and cougars (n=3) were used from three different institutions: two Jaguars and two Cougars at Mata Ciliar Association (Jundiá – SP; -23.178°S, -46.941°W), one Jaguar and one Cougar at Paulínia Zoo (Paulínea – SP; -22.764°S, -47.153°W) and three Jaguars at a non-governmental organization NEX - No Extinction (Corumbá de Goiás – GO; -15.859°, -48.476°W). The animals were housed in enclosures with natural lighting, with water ad libitum and fed a meat-based diet. Animal ages were estimated based on medical records of the respective maintainers.

The present study had authorization for scientific activities issued by SISBIO / ICMBio / MMA under no. 46031-4, approved by the Ethic Committee on Animal Use of the School of the Federal University of Viçosa (CEUA-UFV) under protocol no. 79/2015 and was registered in the SISGEN National System for the Management of Genetic Heritage and Associated Traditional Knowledge (Register A327AAC).

### Semen collection

Males were fasted for 12 hours without food and water before chemical restraint, that was performed using anesthetic darts fired with a blowpipe and containing medetomidine (0.08–0.1 mg/kg, Precision Pharmacy, CA, USA) and ketamine (5mg/kg, Dopalen, Vetbrands, SP, Brazil). After semen collection, anesthesia was reversed using Atipamezole (0.25mg/kg, Precision Pharmacy).

The semen was collected by urethral catheterization as described by Araujo et al. (2018). Briefly, 20–40 min after medetomidine administration a semi-rigid tomcat urinary catheter (w/ open end, 3FR, 130mm long) was introduced into the urethra and negative pressure was applied (by a 1mL syringe) to increase suction effect and semen collection. The semen was then placed in a pre-warmed (38°C) 2mL plastic tube and kept in a water bath at 38°C.

### Semen evaluation and processing

Immediately after collection, the semen was diluted (2:1) in maintenance medium (MM; TRIS 24g/L; citric acid 14g/L; glucose 8 g/L; amikacin 2g/L; egg yolk 200g/L; Nutricell, SP, Brazil). Then, subjectively evaluated for forward progressive motility (FPM) on a scale from 0 to 5, where 0 represented no forward movement and 5 represented steady, rapid forward progression; and progressive motility (PM) from 0% to 100%, in

increments of 5% under a 200x magnifying microscope (CBRA 2013). The sperm concentration was measured using a Neubauer chamber.

An aliquot of each diluted semen sample was fixed in Karnovsky fixative (Karnovsky 1965) and later evaluated for sperm morphology (200 cells/ejaculate) under phase-contrast microscopy (1000x magnification). Individual cells were classified as normal, major defects or minor defects in terms of their perceived adverse effects on male fertility (Blom 1973).

The sperm plasma membrane function was accessed by the hypo-osmotic swelling test (HOST), as described by Araujo et al. (2015). Semen was incubated in 100 mOsmol/kg sucrose solution (1:4) at 38 °C for 30 min, and fixed in Karnovisk fixative (Karnovsky 1965). One hundred sperms were evaluated under phase-contrast microscopy (1000x magnification) and those with bent or coiled tail were considered functional – this number was corrected by excluding the sperms with bent and coiled tail in the morphology test.

### Sperm cryopreservation

After evaluation, the semen concentration was standardized for  $100 \times 10^6$  sperm/mL in MM and then diluted (1:1) in cryopreservation media (12% glycerol, 1% de Equex STM Paste in MM). Thus, semen was

cryopreserved in TRIS-egg yolk extender with a final concentration of  $50 \times 10^6$  sperm/mL, 6% glycerol, 0.5% de Equex STM Paste. Samples were package into 0.25mL straws (IMV Technologies, NOR, France).

For each ejaculate, two cooling methods were evaluated: Cooling A (CoolA); we used the previously described method (Deco-Souza et al. 2013; Araujo et al. 2015) in which straws were placed in a glass tube that was placed in a glass bottle containing water (600 mL at 38 °C) and transferred to a polystyrene container (12L) containing an 11cm column of ice and water at room temperature, for 1.5h. The cooling rate was  $-0.53^\circ\text{C}/\text{min}$  (from 38 to 5°C). Cooling B (CoolB); where straws were cooled for 1.5h in a 4.26L cooler container containing nine blocks –  $81\text{cm}^3$  each – of Ice Foam recyclable ice, previously frozen in liquid nitrogen (Image 1). For this the straws were placed in a glass tube that was placed in a glass bottle containing water (600mL at 38°C) and transferred to the cooler. This glass bottle was surrounded by the ice foam blocks. The amount of Ice Foam was previously defined to reach a cooling rate similar to the CoolA group.

Cryopreservation was performed by placing the straws horizontally over a freezing rack inside a Styrofoam container filled with liquid nitrogen and exposed to nitrogen vapor at 10cm above liquid for



Image 1. A & B—Packing the ice foam blocks | C—Collings of semen | D—in the CoolB. © Gediendson Ribeiro de Araujo

15 minutes (Deco-Souza et al. 2013). Afterwards, the straws were immersed in liquid nitrogen ( $-196^{\circ}$ ).

The straws were thawed in a water bath at  $38^{\circ}\text{C}$  for 30s and transferred to a plastic tube where they were maintained during the evaluation. Each frozen-thawed sample was assessed as the fresh semen and for sperm motility, using a computer assisted sperm analysis (CASA) system and staining with fluorescent probes.

#### Frozen-thawed semen evaluation

The plasmatic and acrosomal membranes were assessed using a combination of three fluorescent probes: propidium iodide (PI; Sigma–Aldrich Co. LLC.– P4170), Hoechst 33342 (H342; Molecular Probes–H1399) and Peanut agglutinin conjugated with fluorescein isothiocyanate (FITC-PNA; Sigma–Aldrich Co. LLC. –L7381). The frozen-thawed semen ( $10\mu\text{l}$ ) was incubated with  $10\mu\text{l}$  of H342 ( $25\mu\text{g}/\text{mL}$  in DPBS) and  $60\mu\text{l}$  of FITC-PNA ( $10.3\mu\text{g}/\text{mL}$  in sodium citrate 3% in DPBS) at  $38^{\circ}\text{C}$ . After 8min,  $2\mu\text{l}$  of PI ( $0.5\text{mg}/\text{mL}$  in DPBS) were added and incubated for another 2min. The sperm were evaluated by epifluorescence microscopy (Nikon H550S, excitation:  $365\text{nm}$ ; emission:  $410\text{nm}$ ) and were classified based on the fluorescence emitted from each probe as: DI – damaged plasma membrane and intact acrosome (only the nucleus emitting red fluorescence); II – intact plasma membrane and intact acrosome (only the nucleus emitting blue fluorescence); DD – damaged plasma membrane and damaged acrosome (the nucleus emitting red fluorescence and the acrosomal region emitting green fluorescence); and ID – intact plasma membrane and damaged acrosome (the nucleus emitting blue fluorescence in acrosome region and emitting green fluorescence).

The sperm motility was accessed using the sperm class analyzer CASA system (Microptic S.L., Spain) with the following settings described by Lueders et al. (2012) in African lions: negative phase (Ph-) with green filter; particle size 5–85; connectivity 14 at of capture of 50fps and 40/50 images; drifting 10; static VCL  $25\mu\text{m}/\text{s}$ ; slow/medium VCL  $65\mu\text{m}/\text{s}$ ; rapid  $100\mu\text{m}/\text{s}$ ; STR 75%; and VAP setting  $7\mu\text{m}/\text{s}$ . Semen sample ( $4\mu\text{L}$ ) at  $25 \times 10^6$  sperm/mL was loaded onto a pre-warmed disposable Leja 4 Chamber Slides (Leja Products BV, The Netherlands) and accessed by total motility (%), progressive motility (%), velocity average pathway – VAP ( $\mu\text{m}/\text{s}$ ), velocity straight line – VSL ( $\mu\text{m}/\text{s}$ ), velocity curved line – VCL ( $\mu\text{m}/\text{s}$ ), amplitude lateral head – ALH (mm), beat cross-frequency – BCF (Hz), straightness – STR (%), and linearity – LIN (%).

#### Statistical analysis

Data on sperm quality from CoolA versus CoolB groups were analyzed using Bayesian t-test with unequal variances (Kery 2010). Data from fresh semen versus CoolA and CoolB groups were analyzed using simple variance (one-way ANOVA) with fixed effect with hierarchical Bayesian modeling. This method of analysis allows inferences about the population and is indicative of the probability that the parameters estimated for each group are derived from the same distribution. According to McCarthy (2007) and Kery (2010) the specification model was:

$$y_{ijk} = \alpha_j(i) + \epsilon_i$$

$$\epsilon_i \sim \text{Normal}(0, \sigma^2)$$

In this model,  $y_{ijk}$  corresponds to the data  $K$  observed from animal  $i$  in the population  $j$ ,  $\alpha_j(i)$  corresponds to the expected value for the data in the population  $j$ , and the residual  $\epsilon_i$  corresponds to the random deviation of the sperm parameter of the animal  $i$  of the mean of its population  $\alpha_j(i)$ .

Observations that did not meet the assumptions of normality were assessed using a Shapiro–Wilk test (Royston 1982) with a significance of  $p < 0.05$  and were log-transformed. Marginal posterior distributions of parameters were estimated using Markov Chain Monte Carlo (MCMC) methods. Analyses were implemented in program R (R Development Core Team 2011) using the rjags package, JAGS version 3.2.0. Each of the MCMC chains was run for 100,000 iterations; the first 20,000 iterations were discarded to allow for burn-in. Convergence was assessed by visually inspecting trace plots to ensure a reasonable exploration of the parameter space, and a potential scale reduction factor of  $< 1.02$  for each variable (Gelman & Rubin 1992). Results were back-transformed, if necessary. At each MCMC step, we calculated the Bayesian equivalent to a  $p$ -value by assessing whether the mean of one group was greater than the other.

#### RESULTS

Semen collection by urethral catheterization was effective in all animals, with good volume and concentration (Table 1), however, one Jaguar and one Cougar only ejaculated seminal fluid and thus were not considered for statistical analysis in frozen-thawed semen.

In Jaguars there were differences ( $p < 0.05$ ) in sperm FPM, sperm PM and HOST between fresh and frozen-thawed sperm, there was no difference ( $p < 0.05$ )

**Table 1. Quality of fresh semen collected by urethral catheterization after medetomidine administration in captive Jaguars (*Panthera onca*, N=6) and Cougars (*Puma concolor*, N=3).**

	Jaguar	Cougar
Volume (µl)	292.0 ± 326.6	106.7 ± 30.6
Concentration (x 10 <sup>6</sup> sperm/ mL)	2091.4 ± 1816.2	524.1 ± 54.3
Total number of spermatozoa (x10 <sup>6</sup> )	316.6 ± 399.0	56.5 ± 16.3

**Table 2. Fresh and frozen-thawed Jaguar (*Panthera onca*, N=5) sperm evaluation.**

	Fresh	Frozen-thawed	
		CoolA	CoolB
FPM	3.6 ± 0.4 <sup>a</sup>	2.3 ± 0.3 <sup>b</sup>	2.4 ± 0.3 <sup>b</sup>
PM (%)	73.0 ± 14 <sup>a</sup>	31.0 ± 19 <sup>b</sup>	38.6 ± 17.7 <sup>b</sup>
HOST (%)	55.0 ± 9.5 <sup>a</sup>	26.4 ± 5.8 <sup>b</sup>	24.3 ± 6.5 <sup>b</sup>
Normal sperm (%)	60.7 ± 6.8 <sup>a</sup>	46 ± 11.4 <sup>b</sup>	47.8 ± 5.3 <sup>b</sup>
Major defects (%)	21 ± 6.6 <sup>a</sup>	24.6 ± 12.6 <sup>a</sup>	24.8 ± 6.4 <sup>a</sup>
Minor defects (%)	18.3 ± 12.2 <sup>a</sup>	29.4 ± 7.6 <sup>a</sup>	27.4 ± 3.9 <sup>a</sup>
DI		55 ± 18.7 <sup>a</sup>	39 ± 10.0 <sup>a</sup>
II		21.2 ± 15.7 <sup>a</sup>	23.4 ± 13.8 <sup>a</sup>
DD		23.6 ± 15.3 <sup>a</sup>	37.6 ± 10.5 <sup>a</sup>
ID		0.4 ± 0.9 <sup>a</sup>	0.0 ± 0.0 <sup>a</sup>
Total Motility*		28.4 ± 14 <sup>a</sup>	28.8 ± 5.9 <sup>a</sup>
Progressive motility*		2.0 ± 1.9 <sup>a</sup>	1.8 ± 1.2 <sup>a</sup>
VAP*		10.5 ± 4.5 <sup>a</sup>	10.5 ± 3.4 <sup>a</sup>
VSL*		6.6 ± 4.3 <sup>a</sup>	6.8 ± 3.1 <sup>a</sup>
VCL*		23.5 ± 6.4 <sup>a</sup>	23.5 ± 3.8 <sup>a</sup>
ALH*		2.2 ± 1.8 <sup>a</sup>	2.1 ± 1.4 <sup>a</sup>
BCF*		7.2 ± 6.2 <sup>a</sup>	8.5 ± 6.2 <sup>a</sup>
STR*		58.6 ± 13.4 <sup>a</sup>	62.0 ± 12.5 <sup>a</sup>
LIN*		26.3 ± 11.4 <sup>a</sup>	27.8 ± 9.7 <sup>a</sup>

between CoolA and CoolB parameters (Table 2).

\*Data accessed by sperm class analyzer. Means ± S.D. Means within columns with different letters differ significantly (p<0.05). FPM – Sperm forward progressive motility; PM – sperm progressive motility; HOST – hypo-osmotic swelling test; velocity average pathway – VAP (µm/s); velocity straight line – VSL (µm/s); velocity curved line – VCL (µm/s); amplitude lateral head – ALH (mm); beat cross-frequency – BCF (Hz); straightness – STR (%) and linearity – LIN (%). DI: damaged plasma membrane and intact acrosome; II: intact plasma-membrane and intact acrosome; DD: damaged plasma membrane and damaged acrosome; ID: intact plasma membrane and damaged acrosome.

**Table 3. Fresh and frozen-thawed Cougar (*Puma concolor*, N=2) sperm evaluation.**

	Fresh	Frozen-thawed	
		CoolA	CoolB
FPM	3.0 ± 0 <sup>a</sup>	2.8 ± 0.3 <sup>a</sup>	2.8 ± 0.3 <sup>a</sup>
PM (%)	70.0 ± 0 <sup>a</sup>	50.0 ± 14.1 <sup>a</sup>	50.0 ± 14.1 <sup>a</sup>
HOST (%)	39.5 ± 6.4 <sup>a</sup>	13.5 ± 3.5 <sup>b</sup>	25.0 ± 1.4 <sup>b</sup>
Normal sperm (%)	40.5 ± 7.8 <sup>d</sup>	23.5 ± 2.1 <sup>d</sup>	31.5 ± 0.7 <sup>d</sup>
Major defects (%)	41.0 ± 12.7 <sup>d</sup>	26.5 ± 2.1 <sup>d</sup>	36 ± 5.6 <sup>d</sup>
Minor defects (%)	18.5 ± 4.9 <sup>d</sup>	44.5 ± 6.4 <sup>e</sup>	32.5 ± 6.4 <sup>de</sup>
DI		38.0 ± 1.4 <sup>a</sup>	50.5 ± 6.4 <sup>a</sup>
II		44.0 ± 9.9 <sup>a</sup>	39.0 ± 12.7 <sup>a</sup>
DD		17.0 ± 7.1 <sup>a</sup>	9.5 ± 4.9 <sup>a</sup>
ID		1.0 ± 1.4 <sup>a</sup>	1.0 ± 1.4 <sup>a</sup>
Total Motility*		40.0 ± 4.7 <sup>a</sup>	36.3 ± 3.2 <sup>a</sup>
Progressive motility*		6.3 ± 1.7 <sup>a</sup>	5.8 ± 4.3 <sup>a</sup>
VAP*		20.8 ± 4.6 <sup>a</sup>	21.3 ± 4.1 <sup>a</sup>
VSL*		13.7 ± 4.3 <sup>a</sup>	14.7 ± 3.9 <sup>a</sup>
VCL*		40.7 ± 6.7 <sup>a</sup>	39.6 ± 9.2 <sup>a</sup>
ALH*		3.6 ± 0.1 <sup>a</sup>	3.1 ± 0.3 <sup>a</sup>
BCF*		12.9 ± 2.8 <sup>a</sup>	14.3 ± 1.7 <sup>a</sup>
STR*		65.3 ± 6.3 <sup>a</sup>	68.4 ± 5.3 <sup>a</sup>
LIN*		33.3 ± 5.1 <sup>a</sup>	36.9 ± 1.3 <sup>a</sup>

As we saw in Jaguars, there were no differences (p>0.05) in sperm quality between the CoolA and CoolB for the cougars (Table 3), however, HOST and minor defects increased in frozen-thawed semen.

\*Data accessed by sperm class analyzer. Means ± S.D. Means within columns with different letters differ significantly (p<0.05). FPM – sperm forward progressive motility; PM – sperm progressive motility; HOST – hypo-osmotic swelling test; velocity average pathway – VAP (µm/s); velocity straight line – VSL (µm/s); velocity curved line – VCL (µm/s); amplitude lateral head – ALH (mm), beat cross-frequency – BCF (Hz); straightness – STR (%) and linearity – LIN (%). DI: damaged plasma membrane and intact acrosome; II: intact plasma-membrane and intact acrosome; DD: damaged plasma membrane and damaged acrosome; ID: intact plasma membrane and damaged acrosome.

## DISCUSSION

The results for fresh semen quality shows that urethral catheterization after medetomidine administration (CT) was effective for semen collection in Jaguars and

Cougars. Thus, this may be an alternative method for electroejaculation.

In Jaguars and in Cougars, the semen volume was lower than previously described (5.3 to 11 mL and 0.45 to 3.4 mL, respectively) (Wildt et al. 1988; Morato et al. 1998, 1999, 2001, 2004; Paz et al. 2000, 2003, 2006, 2007; Swanson et al. 2003; Deco et al. 2010). All those studies, however, used the electroejaculation (EE) for semen collection. It is well known that EE stimulates contractions of the smooth muscles and subsequently the accessory sex glands, which increases the seminal volume (Ball 1986), resulting in more diluted semen samples. On the other hand, with the CT we collected more concentrated semen samples than described in literature in both species. The total number of spermatozoa, however, was smaller than described for Cougars (Wildt et al. 1988; Deco et al. 2010). Because of the small number of Cougars used in this study, we cannot state if this result was related to the collection method or to the animals. The semen volume and concentration were good enough for cryopreservation and the CT was much more practical to be used than EE.

The SPM and PM (3.6 and 76%, respectively) in Jaguars were superior than previously described (2.2 to 3.3 and 50.6 to 64%, respectively) (Morato et al. 1998, 1999; Swanson et al. 2003; Silva et al. 2004; Paz et al. 2006). On the other hand, in Cougars the SPM and PM were superior to the 2.5–3 and 40–50 % described by Miller et al. (1990) and similar to the 3.5 and 75% described by Deco et al. (2010). Both parameters were considered good quality for cryopreservation.

In the present study, Jaguars had more normal sperm (60.7%) than Cougars (40.5%); as well as more normal sperm than described in literature (46.7% (Morato et al. 1998); 49% (Morato et al. 1999); 31.7% (Paz et al. 2000); 50% (Morato et al. 2001); 57.3% (Swanson et al. 2003); 48.7% (Paz et al. 2003)). Cougars had higher or even similar normal sperm than described for the species (26% (Wildt et al. 1988); 1–18 % (Miller et al. 1990); 8.6% (B. Pukazhenthil et al. 2001); 46.13% (Deco et al. 2010)). Felines usually have high proportion of pathologic sperm in the ejaculate, however, the etiology and impact of those in fertility is controversy (Howard et al. 1986). Several factors may affect sperm morphology; although, nutrition and stress are the main factors in captive animals.

After cryopreservation sperm quality reduced in both species. This is expected for any species as cryopreservation damages sperm, impairing their ability to fertilize oocyte. Despite the reduction in the quality of frozen-thawed sperm, SPM and PM values were similar

to those described for Jaguars (SPM: 2.7 and PM 30% (Paz et al. 2000); SPM 3.1 and PM 26.7% (Paz et al. 2007)) and for Cougars (SPM 2.5 and PM 42% (Deco-Souza et al. 2013)). To obtain semen samples from wild animals is always a challenge, because of the reduced number of captive animals (several of them are vasectomized) and the difficulty of accessing free-living animals. Therefore, frozen-thawed semen must be used, even if they are of poor-quality. For this, we can use artificial insemination via laparoscopy – depositing sperm closer to the site of fertilization – or even the intracytoplasmic sperm injection – ICSI. In addition, studies should be done to increase sperm quality after thawing, thus increasing the efficacy of its use for assisted reproduction programs.

For sperm cryopreservation sample must be cooled (from body temperature to 5°C), frozen (in liquid nitrogen vapor at -70°C) and stored (in liquid nitrogen at -196°C). Sperm cell also may be stored at the cooling temperature, however, it remains viable only for a few days. Several protocols and equipment are evaluable for carnivore semen cryopreservation (and cooling) (Zambelli et al. 2002; Luvoni et al. 2003; Tsuitsui et al. 2003; Macente et al. 2012). Some of those are also used for wild felids (Paz et al. 2007; Deco-Souza et al. 2013; Araujo et al. 2015; Jorge Neto et al. 2019). In these cases, cooling was performed using refrigerators, automatic cooling and / or freezing equipment, or even in portable containers using previously frozen recyclable ice. All these methods depend on electricity and cannot be used in the field, as in several places there is no electricity available.

The CoolA method was successfully used for cougar and ocelot semen cryopreservation (Deco-Souza et al. 2013; Araujo et al. 2015), however, it still needs electricity to store ice. Thus, we used nontoxic recyclable ice to reach the same cooling rate (CoolB), with the advantage of being frozen and kept in liquid nitrogen – which is necessary for the later stages of semen freezing. This enables this method to be used in fields where there is no energy available. There was no difference in sperm quality in both cooling methods, demonstrating that the CoolB may be used for semen cryopreservation from the felines. This makes it feasible for sperm banks to use semen from free-living animals, increasing the genetic resources of these species.

## CONCLUSION

The cooling method using recyclable ice frozen in liquid nitrogen offers good semen quality and may be

used for feline semen cryopreservation, eliminating the need of electricity. Thus, this is a more practical method to be used in the field.

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## Habitat structure determines the abundance of the Endangered Sharpe's Longclaw *Macronyx sharpei* (Aves: Passeriformes: Motacillidae) at Timau montane grasslands in central Kenya

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**Abstract:** Understanding the habitat selection and structure of a species is critical for developing evidence-centered conservation actions. Sharpe's Longclaw *Macronyx sharpei*, a passerine bird endemic to Kenya, is threatened by reductions in habitat size and quality that have left it inhabiting a small and highly fragmented range. From January to June 2016 we investigated the abundance and density of Sharpe's Longclaw in Marania farm located in Meru county in the northern sector of Mt. Kenya, where no previous study had been done. Population abundance and density were determined using the flush and count method. We observed that these birds were exclusively found in grasslands, being most abundant in habitats of short grass with tussocks, and less so in areas with tall grass. This habitat specificity indicates a key requirement for survival of Sharpe's Longclaw populations in this area. We recommend surveys in and around Marania farm to determine the distribution of suitable habitats for this species, and that the farm be designated an Important Bird Area. Further studies should also focus on determining the intensity of grazing that is compatible with conservation of Sharpe's Longclaw populations.

**Keywords:** Conservation, density, endemic, grasslands, passerine bird, population.

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## INTRODUCTION

Abundance of bird species is largely influenced by the spatial and temporal distribution of key resources (McCain 2009). Elevation and slope affect vegetation structure, site productivity, distribution, composition, and secondary biotic interactions (Girma et al. 2017). The conservation of Sharpe's Longclaw (Image 1) requires a detailed understanding of population sizes and distribution, and habitat quality and availability. This endemic and endangered species is restricted to grasslands at 1,800–3,500 m altitude (BirdLife International 2018). It has been reported at higher elevations, but this has not been confirmed (Borghesio et al. 2013). Much of past research has been concentrated in the Kinangop grasslands in the southern parts of Nyandarua County (Muchai 1998; Muchai et al. 2002; Ndang'ang'a et al. 2002; Mwangi et al. 2012; Borghesio et al. 2013). The species occurs at low densities throughout its range (BirdLife International 2015). In Kinangop grasslands, Ndang'ang'a et al. (2002) recorded a density of 1.2 individuals/ha while Muchai et al. (2002) and Mwangi et al. (2012) found an overall mean density of  $0.85 \pm 0.21$  individuals/ha and  $1.24 \pm 0.15$  individuals/ha, respectively. At Lake Ol' Bolossat, Wamiti et al. (2008) recorded a density of (0.004–0.06 individuals/ha). There are few precise breeding records (Keith et al. 1992). The highland grasslands that are strongholds for Sharpe's Longclaw (Muchai 1998; Muchai et al. 2002; Ndang'ang'a et al. 2002; Borghesio et al. 2013) also provide nesting, feeding, and breeding habitats for the eastern African endemic and near-threatened Jackson's Widowbird *Euplectes jacksoni*, the regionally threatened Long-tailed Widowbird *E. progne*, and the Afro-tropical highland biome-restricted species Hunter's Cisticola *Cisticola hunteri* (Bennun & Njoroge 1999).

The Timau high altitude grasslands in Kenya have recently undergone significant reduction, primarily due to habitat conversion to crop lands (Kimani et al. 2015). The alarming decline of local grassland habitat is linked to land sub-division within family units and sale of land parcels, resulting in native grassland loss and fragmentation. The local people living in the Kenyan highlands whose livelihood mainly revolves around small-scale farming play a large role in habitat fragmentation (Muchai 1998; Ndang'ang'a et al. 2002; Kimani et al. 2015). The main threat to native grassland habitat is conversion, especially through cultivation and establishment of woodlots of exotic species (Muchai 1998; Muchai et al. 2002; Borghesio et al. 2013). These factors have exacerbated the pressure on



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**Image 1. Sharpe's Longclaw *Macronyx sharpei***

highland grassland biodiversity, and the establishment of large-scale farming for commercial crops that are more profitable than livestock has also contributed to reduction of native grassland habitats.

Lack of appropriate information on the population status of Sharpe's Longclaw prevents efficient management of the habitats necessary to guide conservation efforts. Collection of such information on population size, abundance, and density are important when deciding where to allocate resources in conservation and research activities, and to provide empirical data to evaluate existing management strategies. These data are essential for the IUCN Red List of Threatened Species assessments. The overall objective of this study was to investigate population abundance and density of Sharpe's Longclaw in different habitat types in Marania farm, following reports that the species was present there. A detailed understanding of population size, spatial distribution and demographic trends will inform future management decisions and conservation interventions.

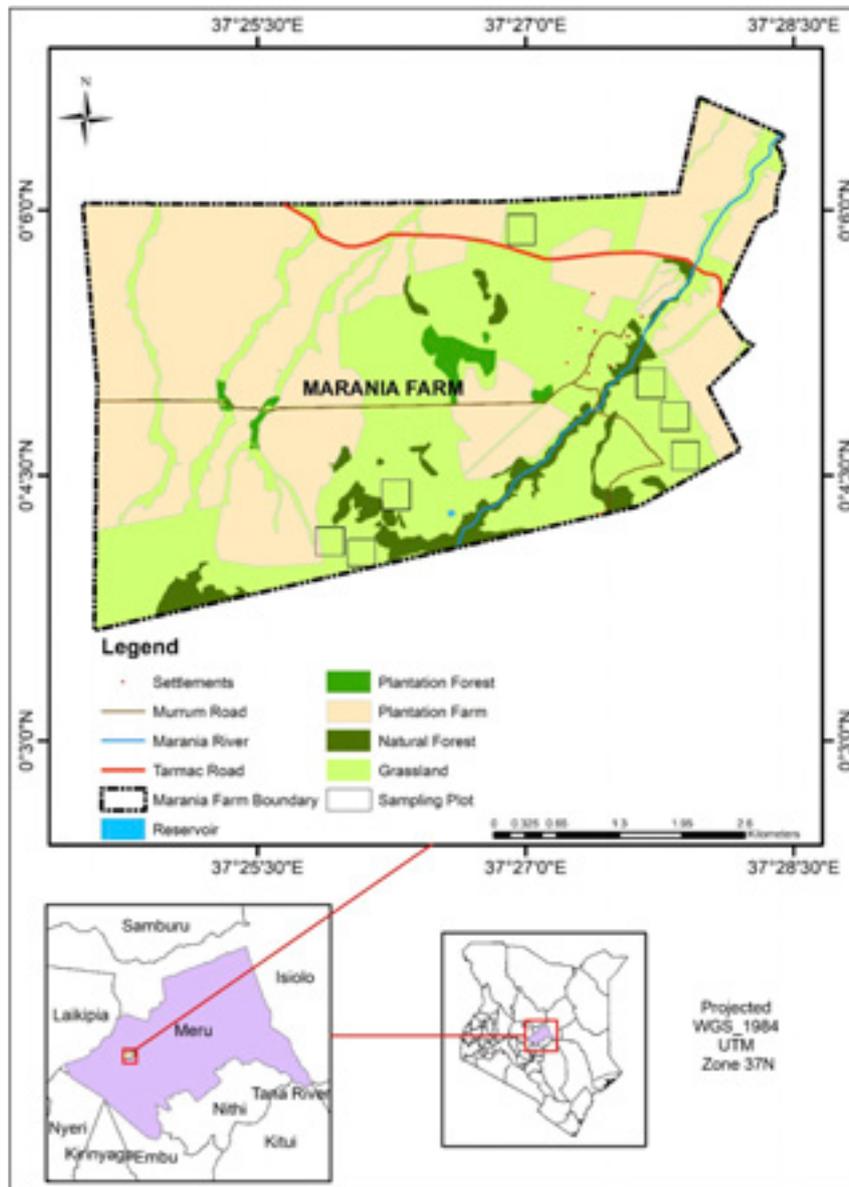


Figure 1. The location of Marania farm, Meru County, Kenya.

## MATERIALS AND METHODS

The population of Sharpe's Longclaw was studied in Marania farm, Meru County (0.080–0.070 °N and 37.458–37.367 °E), part of the northern section of Mt. Kenya that offers a previously unstudied fragmented population of the species. Marania farm is an approximately 2,580ha privately owned farm bordering Mt. Kenya National Park on the north-eastern side. The elevation of Marania farm grasslands where this study was carried out ranged from 2400 to 2800 m. In Marania farm, rearing of livestock (sheep and beef cattle) that forage in the native grasslands is controlled by paddocks, although the animals are supplemented with hay during

the dry seasons. There is also natural vegetation in the valleys and hill tops.

Grasslands in this farm cover an area of 865ha while the rest is under cultivation and interspersed with natural and planted forests. The farm has crops such as wheat, canola, peas, maize, and a small portion of mixed crops. They also practice animal husbandry. During the study, there were approximately 150 cattle grazing in the study area, over 400 sheep, and about 20 horses. The average annual rainfall in the area ranges 380–2,500 mm with a bimodal rainfall pattern in March–May and October–December (Gakuubi & Wanzala 2012).

Sharpe's Longclaw is a monogamous, sedentary

species restricted to high altitude, open, short grasslands. It is territorial and insectivorous, feeding particularly on grasshoppers and beetles. Birds live in permanent groups of two–seven individuals depending on the quality of their habitat (Muchai 1998; Muchai et al. 2002).

**Sampling Design and Census**

During the first month of the study (20 January–20 February 2016), a survey of Marania farm was done to determine appropriate study plots through purposive sampling. The study area was divided into six grassland plots (Figure 1) measuring an average of  $2.25 \pm 0.12$  (SD) ha (range 2.21–2.45 ha). Three of the plots were characterized as short grass with dense tussocks (SGWDT) and the other three as tall grass (TG) following Muchai et al. (1998). The plots were separated by different matrices with either natural forest, farm cultivation or plantation forests. Grass height at plots was classified as SGWDT and ranged between 10–20 cm while those of TG were 30cm and above, following design employed by Muchai (1998).

The plots were monitored for a period of five months (February–June 2016). Sharpe's Longclaw (SLC) is known to breed during the onset of rains or shortly after rains (Kimani et al. 2015). The study partly coincided with the breeding season but, only for a short period between April and May (Muchai 1998). This was done deliberately to ensure the breeding population was present. Due to the limitation of time, the study lasted only five months, in which February and March were dry while April–June were wet months. Censuses were conducted at each study plot at different times of the day (spread in three 4-hour long observation periods; 06.00–10.00 h, 10.00–14.00 h, 14.00–18.00 h) to give a spread of data on a spatial and temporal spread throughout the day. Each study plot was intensively searched once every week; 20 censuses were undertaken in each of the six study plots. Study plots were intensively searched using a flush-out and count method (Muchai 1998; Muchai et al. 2002)

where two people dragged a 50m rope on opposite ends to flush out the birds for easier sighting. Flushed out Sharpe's Longclaws were recorded, and the position they flew to was noted to avoid double counting. The original position of the bird was marked using a hand-held global positioning system unit (Garmin etrex 20).

**Statistical analysis**

Bird abundance in grasslands was examined in relation to plot size, grass height and presence/absence of tussocks using a generalized linear model via Poisson regression (Table 1). Abundance per plot was calculated as the total number of individuals counted divided by the number of sessions the birds were counted in that plot. Mean density was calculated as the mean abundance divided by the size in hectares of the plot. Generalized linear model via Poisson regression was used to determine which of the independent variables explained population abundance in the grassland habitat.

**RESULTS**

**Mean abundance**

The mean  $\pm$  SE abundance in short grass with dense tussocks (SGWDT) was  $4.53 \pm 0.30$  while in tall grass (TG) it was  $2.23 \pm 0.29$ . Figure 2 illustrates a significant difference in mean abundance between SGWDT and TG ( $P= 0.01$ ,  $df=40$ ,  $t = -6.95$ ).

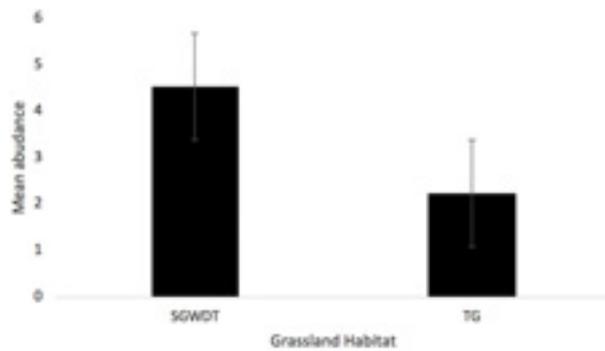
**Determinants of Sharpe's Longclaw abundance**

Three variables were the significant determinants: grass height ( $\beta =0.021$ ,  $P=0.050$ ), tussocks presence/absence ( $\beta=1.101$ ,  $P=0.001$ ) and interaction of grass height and tussocks presence /absence ( $\beta= -0.059$ ,  $P<0.001$ ) (Table 1). The equation of the fitted model was:

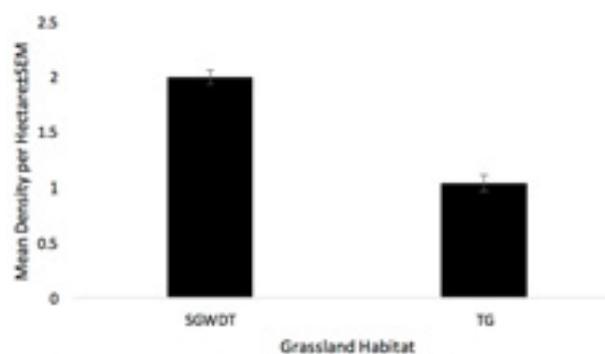
$$\text{Abundance} = 1.188 + 0.021 \text{ grass height} + 1.101 \text{ tussocks presence/absence} - 0.059 \text{ grass height} * \text{tussocks presence/absence}.$$

**Table 1. Generalized linear models via Poisson regression examining the relationship between grass height (GLHT), tussock presence/absence (TUPA) and their interaction (TUPA \* GLHT) on Sharpe's Longclaw abundance in Marania farm grasslands.**

Parameter	Estimate $\pm$ SE	95% confidence limits	Wald chi-square	Df	Sig.
Intercept	1.19 $\pm$ 0.18	0.83, 1.55	42.43	1	< 0.01
Grass height	0.02 $\pm$ 0.01	-0.00, 0.04	3.84	1	0.05
Tussock presence/absence	1.10 $\pm$ 0.34	0.427, 1.78	10.25	1	0.001
TUPA * GLHT	-0.059 $\pm$ 0.01	-0.086, -0.03	18.52	1	< 0.001



**Figure 2.** Comparison of mean abundance of Sharpe's Longclaw between short grass with dense tussocks (SGWDT) and tall grass (TG).



**Figure 3.** Mean population density of Sharpe's Longclaw in short grass with dense tussocks and tall grass in Marania farm.

### Mean density

As birds did not occur in non-grassland habitats, the six grassland plots had a mean density of  $0.78 \pm 0.37$ SD. The mean density was  $2.00 \pm 0.06$  and  $1.04 \pm 0.07$  Sharpe's Longclaw per ha for SGWDT and TG, respectively (Figure 3). There was a significant difference between mean densities in SGWDT and TG (Mann-Whitney W-test = 89.0, df = 42,  $P = 0.0001$ ).

### DISCUSSION

Sharpe's Longclaw population abundance and densities had a clear association with habitat variables. The birds revealed a strong preference for areas of short grass with dense tussocks. Areas of tall grass were less preferred, and birds occurred there at lower densities. This preference for a specific grassland habitat matches that reported in studies by Muchai et al. (1998, 2002) and Mwangi et al. (2012) in Kinangop grasslands.

It has been observed that many endemic bird species have high densities on grazed pastures due to co-

evolution with large grazing mammals, for instance the Chestnut-collared Longspur *Calcarius ornatus* (Kantrud 1981; Knopf & Rupert 1996). Low grazing intensity results in long grass and bush encroachment, while intense grazing destroys grass tussocks (Borghesio et al. 2013). Muchai et al. (2002) found that the persistence of Sharpe's Longclaw in the grasslands depends on intermediate levels of disturbance, resulting from grazing by mammalian herbivores. Our findings are consistent with those of Muchai et al. (2002), in that all the areas where we found Sharpe's Longclaw had grazing, especially by cattle. Although we did not have adequate data to verify a relationship with various grazers, we suggest that the stocking rate might be more important than the species, as shown in Sliwinski & Koper (2015). Besides domestic animals, wild antelopes (Bushbucks *Tragelaphus scriptus*, Duiker *Neotragus moschatus*) were regularly observed during the survey period, while Cape Buffaloes *Syncerus caffer* and African Elephant *Loxodonta africana* from the neighboring Mount Kenya National Park sometimes were reported to break fences and graze as well (unpublished data). These wild animals might also influence grassland height (Ogada et al. 2008) and ultimately Sharpe's Longclaw, but the data we had did not allow for testing of their effects on grassland height and structure. Field observations showed that Sharpe's Longclaw used tussocks mainly to rest during the hottest part of the day, which also agrees with observations by Muchai et al. (2002).

The height of grass plays an overriding role in determining habitat segregation and food specialization among bird species (Fisher & Davis 2011). Interspecific competitive exclusion is believed to be the main mechanism explaining occurrence or specialization of birds in grassland vegetation of different heights (McDonald 2017). Therefore, at least within grassland systems, mosaics of short and longer vegetation are likely to hold the maximum benefit for many farmland birds (Benton et al. 2003). Maphisa et al. (2017) argue that a combination of grass height and cover is more essential than just grass height alone or grass cover alone. It would be plausible to argue that Sharpe's Longclaw would probably spend more time being vigilant to detect predators other than carrying out other essential life process in tall grass due to tall grass obscuring their visibility (Muchai et al. 2002).

Although effect of patch size was not investigated in this study, results by Mwangi et al. (2012) showed large patches of grassland that are favoured by Sharpe's Longclaw compared to small ones. Consistent with this finding, Marania farm, being a large grassland under the

same management, is a potential Sharpe's Longclaw conservation site if properly managed. A year-long study is recommended to understand the breeding strategies that are exhibited by the Sharpe's Longclaw. In addition, our findings indicated that tall grass was equally good for Jackson's Widowbird *Euplectes jacksoni*, a Near Threatened species. This species had over 40 nests in the tall grasses. Large patches of grassland would be ideal for conservation of various species in different categories of threats.

### Conclusion and Recommendations

The findings of this study demonstrate that Timau grasslands still hold suitable and extensive habitat for the endemic and Endangered Sharpe's Longclaw. The study established that the mean population abundance was higher for short grass with dense tussocks compared with tall grass. Mean density was also higher in habitat of short grass with dense tussocks. In comparison to previous studies, it was acknowledged that Marania appeared better than other parts, like Kinangop grassland, previously thought to be the world stronghold of the species.

Sharpe's Longclaw is threatened by a very rapid and continuing reduction in the extent and quality of its habitat (Birdlife 2018). It is, therefore, imperative for conservationists to collaborate with farms such as Marania farm to adopt suitable management practices due to the role they play in conservation of this grassland-dependent bird species. Surveys in neighbouring farms should also be carried out to determine abundance and the extent of population distribution of Sharpe's Longclaw, and to assess the suitability of its habitat. This will be critical in guiding a discussion with the landowners on the merits of designating the farm/grasslands the status of BirdLife International Important Bird Area (IBA). This would be an important task that can be undertaken by the Sharpe's Longclaw Working Group. One way of recognizing the role Marania farm play in conserving Sharpe's Longclaw would be designating the area as an IBA to allow easy marketing of the area as a key tourist attraction site (avi-tourism) for Meru County. Being an endemic species that is becoming rare in its formerly known areas like Kinangop, good marketing would take keen bird watchers to Marania farm where it would be easy to find and perhaps photograph the Sharpe's Longclaw in its natural habitat.

Further research needs to be undertaken for both wet and dry seasons in order to understand if the species is affected by seasonal dynamics. Further research is also needed to shed light on the most appropriate

conservation measures. More information is required to determine the best grazing regimes optimal for Sharpe's Longclaw with economic benefits for easier adoption.

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## Avifaunal diversity of some selected water bodies of Khanapur Taluka, Belagavi District, Karnataka, India

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**Abstract:** An observation on avian species composition, density, and diversity was carried out at three selected water bodies of Khanapur Taluka, Belagavi District, for a period of 11 months in 2014. During the study, a total of 28 species belonging to 12 families from five orders were recorded. Of these, Ciconiiformes was represented by seven families followed by Coraciiformes by two families while Anseriformes, Charadriiformes and Gruiformes were represented by one family each. Among the three water bodies studied, maximum species composition (26 species) was recorded from Nandgad pond which also hosts Lesser Adjutant Stork and Woolly-necked Stork categorized as 'Vulnerable' and Painted Stork, River Tern and Oriental White Ibis as 'Near Threatened' by the IUCN Red List. Bidi Minor Irrigation Tank stands second with 17 species while the minimum was recorded at Hebbal Minor Irrigation Tank with 11 species. Based on our observation, a smaller habitat with habitat heterogeneity can attract more assemblages of avifauna and also result in increased species richness and diversity.

**Keywords:** Birds, richness, relative abundance, wetland birds.

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**Author contribution:** GK has played a crucial role in monitoring and guiding the work. HN has carried out field survey, analysis and documentation.

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## INTRODUCTION

Birds occupy a significant position in ecology and human society and play prominent and diverse roles as pollinators, seed dispersers, pest controlling agents, bio-indicators of an ecosystem, and also in religion & culture. Since time immemorial, they have been a source of food, medicine, feathers for ornaments, and amusements. Presently, they are confronting various threats from climate change and human interferences such as loss of habitat through inflow of domestic and industrial effluents, agricultural runoffs, degradation of wetlands, agricultural expansion, overgrazing of the grasslands, and urbanization leading to deforestation (Grimmett et al. 2011). Diversity of avifauna is one of the most important ecological indicators to evaluate the quality of habitats.

In Karnataka, most of the work is being concentrated on survey, distribution, diversity studies, and status of avifauna. Rajashekara & Venkatesha (2010) recorded diversity and abundance of water birds from Bangalore City lakes, Basavarajappa (2006) has studied avifauna of agro-ecosystems from maidan region of Davangere. Diversity studies in and around Shivamogga District has been carried out by workers like Nazneen et al. (2001) and Dinesh et al. (2007) at Kuvempu University campus, Shivamogga and checklist of avifauna from Gudavi Bird Sanctuary by Dayananda (2009). Distribution and diversity from Lakkavalli range forest of Bhadra Wildlife Sanctuary by Harisha & Hosetti (2009) have studied diversity of Shivamogga City. Assessment and status, diversity, and threats of loss of wetlands of Davangere District by Harisha & Hosetti (2018). Barve & Warriar (2013) have conducted a study on bird diversity from Sharavathy landscape. Bhat et al. (2009) have studied diversity of wetland bird species in Anekere wetland of Karkala. Manjunath & Joshi (2012) have observed species composition, relative abundance and avian classification based on feeding guilds around Chandrampalli Dam of Chincholi from Gulbarga region. Birasal (2015) has studied on occurrence of terrestrial birds in Haveri and neighboring districts. Diversity, richness and conservational threats of migratory wetland birds of Magadi Bird Sanctuary, Gadag District was studied by Kaulgud et al. (2016).

Publications on avifaunal status and diversity from Belagavi District can be traced from the surveys by Patil & Hiragond (2013) along Ghataprabha River near Shettihalli. The objective of the present work was to study species composition, diversity, and status of the wetland birds in some water bodies of Khanapur Taluk.

## MATERIALS AND METHODS

### Study Area

Khanapur Taluk is located towards the western part of Belagavi District of Karnataka. It is about 26km away from district headquarters. The total geographical area of the taluk is 1726.11km<sup>2</sup> with geographical co-ordinates of 15.63° N 74.52° E. It has an average area elevation of 649m. The study was carried out from February to December 2014 from three lentic water bodies, namely, Bidi Minor Irrigation Tank (MIT) (15.583°N, 74.640°E), Nandgad pond (15.580°N & 74.583°E), and Hebbal MIT (15.752°N & 74.563°E) located on the State Highway 93 of Khanapur Taluka (Figure 1). The two minor irrigation tanks were constructed by minor irrigation department of Government of Karnataka while Nandgad pond was constructed in 2010 under Jala Samvardhane Yojana Sangha, a registered society established by the Department of Water Resources, Government of Karnataka for community-based tank management.

### Methods

Survey of birds was conducted early morning 06.00–09.30 h during the first week of every month from February to December, 2014. Observations of the birds were made with the help of Olympus binocular (OLYMPUS 10X50 DPS I, Field 6.5°) and CANON EOS 600D lens kit was used for photography. Birds observed during the study period were classified into families and orders as per Grimmett et al. (2011). The density of the birds was counted using fixed-radius point count method with the expression; Density:  $D^{\wedge} = n/a = n/k\pi w^2$ , where, D = density, A = size of the survey region, k = number of points in the region, n = number of birds counted, and a =  $k\pi w^2$ , the total size of the survey plots. Six circular point count sites each with a 20-m radius were selected. The total area of each point count site was 1,257m<sup>2</sup>. The density of birds was expressed as total birds/ha. The point count sites were selected based on the roosting, feeding sites, and accessibility (Buckland et al. 2008) and during certain times counts were made with the help of photographs taken during the study (Whitworth et al. 2007).

Relative abundance of all bird species was calculated by the expression

$$\text{Relative abundance} = \frac{\text{No. of individuals of the species}}{\text{Total no. of individuals of all species}} \times 100$$

The diversity and evenness was calculated by using Shannon-Weiner diversity index and the formulae are expressed as

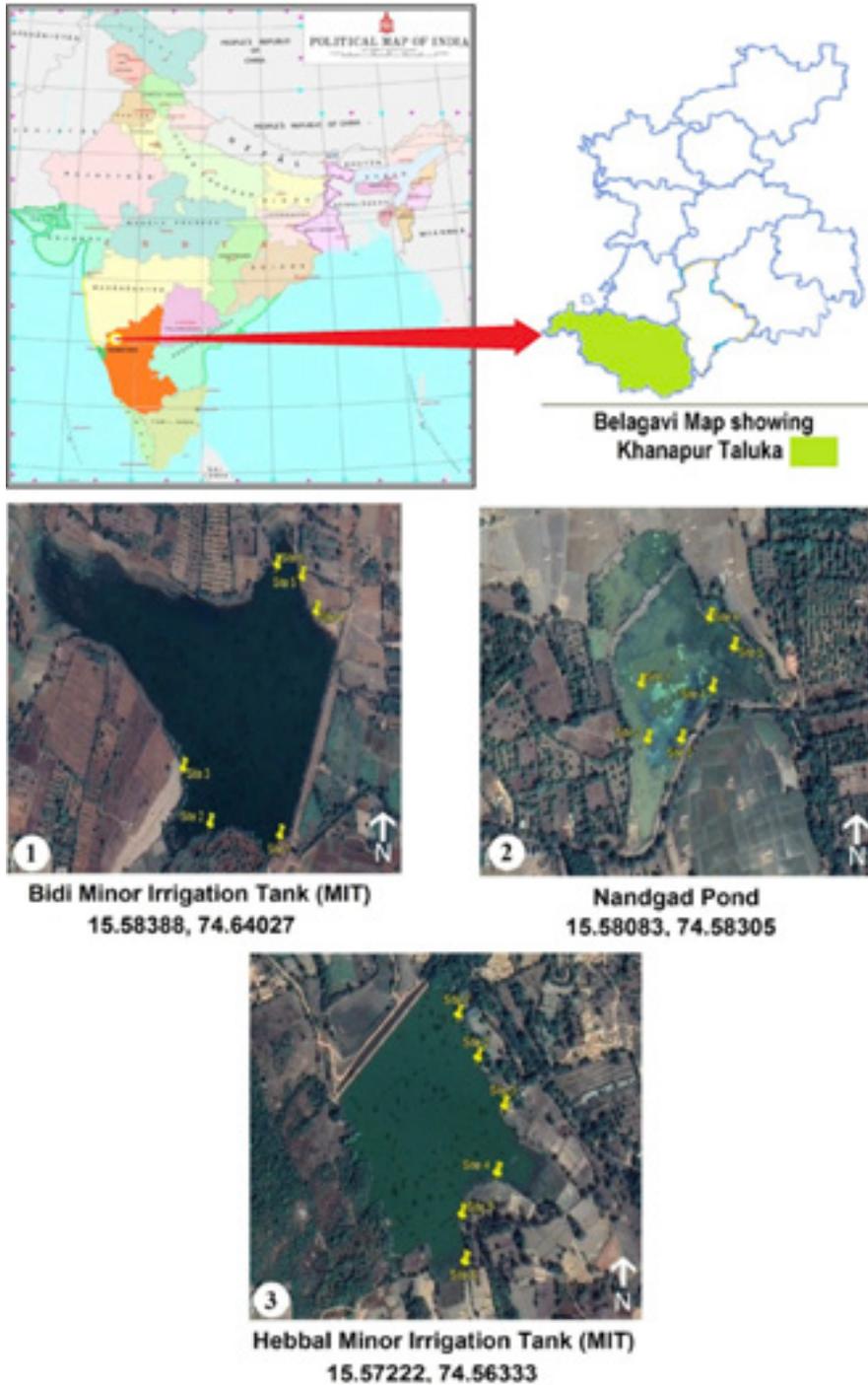


Figure 1. Map of study area showing the three selected water bodies of Khanapur Taluk, Belagavi District, Karnataka State, India.

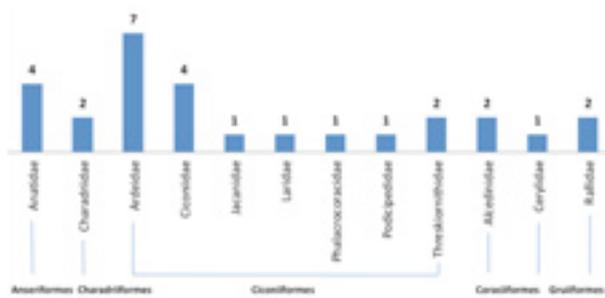
$$H' = - \sum_{i=1}^s (P_i * \ln p_i)$$

where,  $p_i$  = fraction of entire population made up of species 'i',  $\ln$  = natural log and  $\sum$  = sum from species 1 to species S. Shannon-Weiner evenness index:  $E_H = H'/H_{max}$  where,  $H'$  = Shannon-Weiner diversity index, and  $H_{max}$  is the  $\ln S$  where S is the number of species encountered in the group.

## RESULTS

### Species Composition, Abundance, Density and Relative Abundance

During the study, a total of 28 species belonging to 12 families from five orders were recorded. Of these, Ciconiiformes was represented with seven families followed by Coraciiformes with two families while Anseriformes, Charadriiformes, and Gruiformes were



**Figure 2: Number of avian species represented from each families and their respective orders at three selected water bodies of Khanapur taluka during 11 month study.**

represented with one family each. Among them, family Ardeidae dominated with seven species, followed by Anatidae and Ciconiidae with four species each. Families Charadriidae, Threskiornithidae, Alcedinidae, and Rallidae were represented with two species each while Laridae, Phalacrocoracidae, Podicipedidae, Cerylidae, and Jacaniidae were represented with one species each (Figure 2). Maximum species composition was recorded from Nandgad pond with 26 species followed by 17 species of birds from Bidi minor irrigation tank and 11 species from Hebbal minor irrigation tank.

#### Site 1: Bidi Minor Irrigation Tank (MIT)

The species composition at Bidi MIT reveals a total of 17 species representing eight families belonging to four orders from this pond (Table 1). Order Ciconiiformes dominated with 12 species belonging to five families namely, Ardeidae represented with five species, Ciconiidae with three species, Threskiornithidae with two species, Laridae and Phalacrocoracidae representing with one species each. Order Anseriformes represented with two species from family Anatidae and Order Charadriiformes also with two species belonging to family Charadriidae. Order Coraciiformes, however, was represented by only one species belonging to family Cerylidae. The periodicity of Indian Spot-billed Duck in this water body was found prominent as it was observed continuously for seven months (March–October 2014) followed by Little Egret *Egretta garzetta* and Cattle Egret *Bubulcus ibis* that were recorded in five months. Great Egret *Casmerodius albus*, Asian Openbill Stork *Anastomus oscitans*, Painted Stork *Mycteria leucocephala*, Eurasian Spoon Bill *Platalea leucorodia*, Pied Kingfisher *Cerylerudis travancoreensis* were spotted only once during the study period.

Asian Openbill Stork was the only migrant while Bronze-winged Jacana *Metopidius indicus* was recorded as a resident bird. The rest of the birds were either

residents or local migrants. As per the IUCN Red List, Lesser Adjutant Stork *Leptoptilos javanicus* is categorized as ‘Vulnerable’, Painted Stork *Mycteria leucocephala*, River Tern *Sterna aurantia* and Oriental White Ibis *Threskiornis melanocephalus* are Near Threatened (NT), and the remaining species are grouped as ‘Least Concern’. All the birds that were observed during the study period were of schedule IV of WPA, 1972 except Eurasian Spoonbill *Platalea leucorodia* which is included in Schedule I (Table 4).

At Bidi MIT, the abundance and density (birds/ha) of Indian Spot-billed Duck *Anas poecilorhyncha* of Anatidae family was the maximum (72 individuals and 95.45 birds/ha) throughout the study period. Statistics for other birds are provided in Table 1.

In this water body, Indian Spot-billed Duck showed the highest relative abundance (41%) followed by Lesser Adjutant Stork (16%), and Cattle Egret (12%). Minimum relative abundance was recorded by Oriental White Ibis (9%) and River Tern (7%). The rest of the species like Little Egret, Eurasian Spoonbill, Red-wattled Lapwing, and Little Ringed Plover, Black-crowned Night Heron, Great Egret, Median Egret, Little Cormorant recorded the least (1%) relative abundance (Figure 3).

The mean density of Lesser Adjutant Stork (*Leptoptilos javanicus*,  $33.14 \pm 19.50$ ) was the maximum followed by Indian Spot-billed Duck (*Anas poecilorhyncha*,  $23.8 \pm 28.94$ ), Oriental White Ibis (*Threskiornis melanocephalus*,  $18.56 \pm 8.57$ ), Comb Duck (*Sarkidiornis melanotos*,  $9.935 \pm 4.823$ ), Cattle Egret (*Bubulcus ibis*,  $9.542 \pm 10.998$ ), River Tern (*Sterna aurantia*,  $8.83 \pm 6.726$ ), Eurasian Spoonbill (*Platalea leucorodia*,  $6.62 \pm 1.996$ ), Great Egret (*Casmerodius albus*,  $2.65 \pm 0.799$ ), Median Egret (*Mesophoyx intermedia*,  $2.645 \pm 1.223$ ) and Little Egret (*Egretta garzetta*,  $1.852 \pm 1.071$ ) while the minimum was recorded by Red-wattled Lapwing (*Vanellius indicus*,  $1.32 \pm 0.637$ ), Little Ringed Plover (*Charadrius dubius*,  $1.32 \pm 0.556$ ), Black-crowned Night Heron (*Nycticorax nycticorax*), and Little Cormorant (*Phalacrocorax niger*) ( $1.32 \pm 0.533$ ), respectively. Asian Openbill Stork (*Anastomus oscitans*), Painted Stork (*Mycteria leucocephala*), and Pied Kingfisher (*Cerylerudis travancoreensis*) recorded mean density of  $1.32 \pm 0.397$ , respectively (Table 5).

#### Diversity (H) and Equitability or Evenness (E)

During the 11 month study, the highest diversity ( $H' = 2.2315$ ) was recorded during June, followed by July 2014 ( $H' = 1.56064$ ), while it was minimum ( $H' = 0.24491$ ) in October 2014. Highest evenness ( $E_H = 0.96968$ ) was observed in July while it was lowest ( $E_H = 0.32372$ ) in

**Table 1. Species composition, monthly abundance and density (birds/ha) of birds recorded at Bidi minor irrigation tank from February to December 2014. (N=305).**

Order	Family	Common name	F	M	A	M	J	J	A	S	O	N	D
Anseriformes	Anatidae	Comb Duck	-	<b>3</b> 3.97	<b>12</b> 15.90	-	-	-	-	-	-	-	-
		Indian Spot-billed Duck	-	<b>26</b> 34.46	<b>72</b> 95.45	<b>18</b> 23.86	<b>4</b> 5.30	<b>2</b> 2.65	<b>2</b> 2.65	-	<b>2</b> 2.65	-	-
Charadriiformes	Charadriidae	Red-wattled Lapwing	-	-	-	<b>1</b> 1.32	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	-
		Little ringed Plover	-	-	-	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	-	-
		Black Crowned Night Heron	-	-	-	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	<b>1</b> 1.32	-
		Cattle Egret	-	<b>2</b> 2.65	<b>4</b> 5.30	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	<b>28</b> 37.12	-
Ciconiiformes	Ardeidae	Great Egret	-	-	-	-	<b>2</b> 2.65	<b>2</b> 2.65	-	-	-	-	-
		Little Egret	-	<b>2</b> 2.65	<b>1</b> 1.32	<b>2</b> 2.65	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	-
		Median Egret	-	-	-	-	<b>3</b> 3.97	<b>1</b> 1.32	-	-	-	-	-
		Asian Openbill Stork	-	-	-	-	-	-	-	-	-	-	<b>1</b> 1.32
		Lesser Adjutant Stork	-	-	<b>49</b> 64.96	-	-	-	-	-	-	-	-
Ciconiiformes	Laridae	Painted Stork	-	<b>1</b> 1.32	-	-	-	-	-	-	-	-	-
		River Tern	-	-	<b>17</b> 22.53	<b>2</b> 2.65	<b>1</b> 1.32	-	-	-	-	-	-
		Little Cormorant	-	<b>1</b> 1.32	-	-	<b>1</b> 1.32	-	-	-	-	-	-
		Oriental white Ibis	-	<b>7</b> 9.28	-	-	-	-	-	-	-	-	<b>21</b> 27.84
		Eurasian Spoon bill	-	-	<b>5</b> 6.62	-	-	-	-	-	-	-	-
Ciconiiformes	Cerylidae	Pied Kingfisher	-	-	-	-	<b>1</b> 1.32	-	-	-	-	-	-
		17	0	42	160	24	17	6	2	0	30	23	1

Note: The numerical values given in **BOLD** are the **abundance** and those mentioned in decimal are the density values (birds/ha). -Birds not found.



Table 2. Species composition, monthly abundance, and density (birds/ha) of birds recorded at Nandagad Pond from February to December 2014. (N=357).

Order	Family	Common name	F	M	A	M	J	J	A	S	O	N	D	
Anseriformes	Anatidae	Cotton Teal	-	-	-	-	-	-	-	2 2.65	-	-	-	
		Indian Spot-billed Duck	-	-	-	5 6.62	1 1.32	-	-	-	1 1.32	12 15.90	-	
		Lesser Whistling Duck	-	-	-	4 5.30	-	-	3 3.97	4 5.30	-	2 2.65	-	
Charadriiformes	Charadriidae	Red wattled Lapwing	-	-	-	-	-	-	-	2 2.65	2 2.65	4 5.30	-	
		Black Crowned Night Heron	-	-	-	4 5.30	1 1.32	-	-	-	-	-	-	
		Cattle Egret	3 3.97	12 15.90	18 23.86	9 11.93	3 3.97	-	-	1 1.32	1 1.32	19 25.18	-	
Ciconiiformes	Ardeidae	Great Egret	-	-	3 3.97	-	-	-	-	-	-	-	-	
		Grey Heron	1 1.32	-	2 2.65	1 1.32	-	1 1.32	-	1 1.32	-	1 1.32	-	
		Little Egret	-	-	4 5.30	-	1 1.32	-	-	1 1.32	-	-	-	
		Median Egret	-	-	2 2.65	-	3 3.97	-	-	-	-	-	18 23.86	
		Indian Pond heron	-	-	1 1.32	2 2.65	1 1.32	-	-	1 1.32	-	-	-	
		Asian Openbill Stork	-	-	1 1.32	-	1 1.32	7 9.28	6 7.95	-	2 2.65	2 2.65	-	
		Lesser Adjutant Stork	-	-	-	-	-	-	-	-	-	-	1 1.32	
		Painted Stork	3 3.97	-	1 1.32	-	1 1.32	-	-	-	-	-	-	
		Woolly necked Stork	5 6.62	-	-	-	1 1.32	-	-	-	-	1 1.32	7 9.28	2 2.65

Order	Family	Common name	F	M	A	M	J	J	A	S	O	N	D
Ciconiiformes	Jacaniidae	Bronze winged Jacana	<b>1</b> 1.32	-	<b>5</b> 6.62	<b>2</b> 2.65	<b>2</b> 2.65	-	-	<b>2</b> 2.65	<b>1</b> 1.32	<b>9</b> 11.93	-
	Lariidae	River Tern	-	-	<b>2</b> 2.65	<b>1</b> 1.32	<b>1</b> 1.32	-	-	<b>1</b> 1.32	<b>1</b> 1.32	-	-
	Phalacrocoracidae	Little Cormorant	-	-	<b>1</b> 1.32	-	<b>1</b> 1.32	-	<b>1</b> 1.32	<b>6</b> 7.95	<b>1</b> 1.32	<b>4</b> 5.30	-
	Podicipedidae	Little Grebe	-	-	-	-	-	-	<b>1</b> 1.32	-	<b>2</b> 2.65	-	-
	Threskiornithidae	Oriental white Ibis	-	-	<b>2</b> 2.65	-	-	-	-	-	<b>3</b> 3.97	<b>38</b> 50.37	-
		Eurasian Spoon bill	-	<b>4</b> 5.30	<b>6</b> 7.95	<b>1</b> 1.32	<b>1</b> 1.32	<b>6</b> 7.95	-	<b>1</b> 1.32	-	-	-
		Common Kingfisher	<b>1</b> 1.32	-	-	-	<b>2</b> 2.65	-	-	-	-	-	-
		White throated Kingfisher	-	-	<b>1</b> 1.32	-	-	-	-	-	-	-	-
		Pied Kingfisher	<b>1</b> 1.32	<b>1</b> 1.32	-	-	<b>2</b> 2.65	<b>1</b> 1.32	-	-	-	<b>2</b> 2.65	-
		Eurasian Coot	-	-	-	-	-	<b>13</b> 17.23	<b>1</b> 1.32	<b>5</b> 6.62	<b>4</b> 5.30	<b>3</b> 3.97	-
Gruiformes	Rallidae	Purple Swamp hen	-	-	-	-	<b>3</b> 3.97	<b>2</b> 2.65	<b>1</b> 1.32	<b>1</b> 1.32	<b>7</b> 9.28	-	
5	12	26	15	17	49	20	30	33	14	28	22	127	2

Note: The numerical values given in **BOLD** are the abundance and those mentioned in decimal are the density values (birds/ha).  
- Birds not found

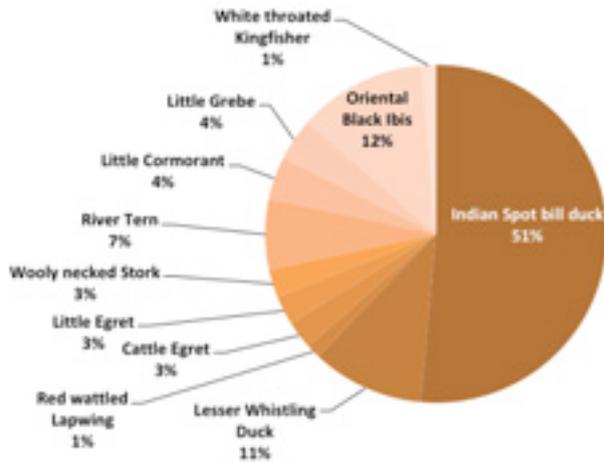


Figure 5. Relative abundance of all bird species observed at Hebbal MIT during 11 month study.

with three species. Coraciiformes was represented with three species from two families, Alcedinidae family with two species and one species from Cerylidae family, Gruiformes and Charadriiformes represented by Rallidae with two species and Charadriidae with one species.

Avifauna of Nandgad pond is similar to that of Bidi MIT except for the presence of the Woolly-necked Stork *Ciconia episcopus* categorized as 'Vulnerable' (Table 4). Most of the species recorded maximum density during the post monsoon season (November) (see Table 1).

The relative abundance of the birds at Nandgad pond shows that, Cattle Egret was highest with 18% followed by Oriental White Ibis 12%, Eurasian Coot recorded 7%, Bronze winged Jacana 6%, Indian Spot bill Duck, Asian Open bill Stork and Eurasian Spoon bill 5% each, Lesser Whistling Duck, Purple Swamp hen, Little Cormorant and Woolly necked Stork 4% each, Red wattled Lapwing, Little Egret, River tern, Pied Kingfisher recorded 2% each whereas, Cotton Teal, Black crowned Night Heron, Great Egret, Indian Pond Heron, Lesser Adjutant Stork, Painted Stork, Little Grebe, Common Kingfisher recorded 1% each (Figure 4).

The maximum mean density at Nandgad Pond was recorded by Oriental White Ibis (*Threskiornis melanocephalus*, 18.996 ± 15.048), followed by others (Table 5).

**Diversity (H) and Equitability or Evenness (E<sub>H</sub>)**

Shannon-Weiner diversity index (H) and Equitability (E<sub>H</sub>) of avifauna of Nandgad pond are given in Table 6. Maximum diversity (H'=2.58955) was recorded in June followed by October (H'=2.43694) and September (H'=2.31407) 2014. Minimum was recorded during



Source: Annual Rainfall Report of 2014, Directorate of Economics & Statistics, Bangalore, Govt. of Karnataka, May, 2015.

Figure 6. Monthly variation in rainfall recorded from three selected waterbodies of Khanapur taluk from February to December, 2014.

March (H'=0.75294) 2014. Highest evenness was recorded in October (E<sub>H</sub>=0.95009) followed by June (E<sub>H</sub>=0.93398) and September (E<sub>H</sub>=0.90219) 2014 and minimum (E<sub>H</sub>=0.68535) during March 2014. Species richness was observed to be a maximum of 16 species in June 2014 followed by April and November with 14 species each. Least species richness (1 species) was recorded in December 2014.

**Site 3: Hebbal Minor Irrigation Tank (MIT)**

A total of 11 species belonging to nine families was recorded from Hebbal MIT (Table 3). Maximum species (6) were recorded during April and June while minimum of one species during February, July and December 2014. Of which, Ciconiiformes were represented with a maximum of seven species distributed into six families i.e.: Ardeidae (2) and Ciconiidae, Laridae, Phalacrocoracidae, Podicipedidae, and Threskiornithidae were represented with one species each. Minimum numbers of species were represented from Charadriiformes and Coraciiformes (Table 4).

The abundance and density (birds/ha) at Hebbal MIT was comparatively less than other two water bodies (Table 3). Out of 11 species recorded, Indian Spot-billed Duck *Anas poecilorhyncha* of Anatidae was observed with the highest density (30 individuals and 39.77 birds/ha) in April and a minimum (8 and 10.60 birds/ha) in May 2014 (Table 3).

The relative abundance of all birds of Hebbal MIT reveals that, Indian Spot-billed Duck was maximum (51%) followed by Oriental White Ibis (12%) and Lesser Whistling Duck (11%). Little Grebe and Little Cormorant recorded 4% each and Woolly necked Stork, Little Egret and Cattle Egret (3% each) recorded minimum abundance. White throated kingfisher and Red wattled Lapwing recorded least relative abundance (Figure 5).

**Table 3. Species composition, monthly abundance, and density (birds/ha) of birds recorded at Hebbal minor irrigation tank from February to December 2014. (N=74).**

Order	Family	Common Name	F	M	A	M	J	J	A	S	O	N	D
Anseriformes	Anatidae	Indian Spot-billed Duck	-	-	<b>30</b> 39.77	<b>8</b> 10.60	-	-	-	-	-	-	-
		Lesser Whistling Duck	-	-	-	<b>8</b> 10.60	-	-	-	-	-	-	-
Charadriiformes	Charadriidae	Red wattled Lapwing	-	-	-	-	<b>1</b> 1.32	-	-	-	-	-	-
		Cattle Egret	-	-	-	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	-	-
		Little Egret	-	-	<b>2</b> 2.65	-	-	-	-	-	-	-	-
Ciconiiformes	Ciconiidae	Woolly necked Stork	-	-	<b>2</b> 2.65	-	-	-	-	-	-	-	-
		River Tern	-	-	-	-	<b>1</b> 1.32	<b>2</b> 2.65	-	-	-	-	<b>2</b> 2.65
	Phalacrocoracidae	Little Cormorant	-	-	<b>1</b> 1.32	<b>1</b> 1.32	<b>1</b> 1.32	-	-	-	-	-	-
		Little Grebe	-	-	<b>2</b> 2.65	<b>1</b> 1.32	-	-	-	-	-	-	-
Gruiformes	Threskiornithidae	Oriental white Ibis	<b>1</b> 1.32	-	<b>7</b> 9.28	-	<b>1</b> 1.32	-	-	-	-	-	-
		White throated Kingfisher	-	-	-	-	<b>1</b> 1.32	-	-	-	-	-	-
4	9	11	1	0	44	19	6	2	0	0	0	2	

Note: The numerical values given in BOLD are the abundance and those mentioned in decimal are the density values (birds/ha). - Birds not found.

**Table 4. Status and Occurrence of wetland birds of three water bodies of Khanapur Taluk.**

Common name & Scientific name	Residential status*	IUCN Status*	WPA, 1972 Schedule **	Bidi MIT	Nandgad Pond	Hebbal MIT
Comb Duck <i>Sarkidiornis melanotos</i>	R/LM	LC	IV	✓	-	-
Cotton Teal <i>Nettapus coromandelianus</i>	R/LM	LC	IV	-	✓	-
Indian Spot-billed Duck <i>Anas poecilorhyncha</i>	R/LM	LC	IV	✓	✓	✓
Lesser Whistling Duck <i>Dendrocygna javanica</i>	R/LM	LC	IV	-	✓	✓
Red wattled Lapwing <i>Vanellius indicus</i>	R/LM	LC	IV	✓	✓	-
Little Ringed Plover <i>Charadrius dubius</i>	R/WM	LC	IV	✓	-	-
Black-crowned Night Heron <i>Nycticorax nycticorax</i>	R/LM	LC	IV	✓	✓	-
Cattle Egret <i>Bubulcus ibis</i>	R/AM	LC	IV	✓	✓	-
Great Egret <i>Casmerodius albus</i>	R/LM	LC	IV	✓	✓	-
Grey Heron <i>Ardea cinerea</i>	R/WM	LC	IV	-	✓	-
Little Egret <i>Egretta garzetta</i>	R/LM	LC	IV	✓	✓	✓
Median Egret <i>Mesophoyx intermedia</i>	R/LM	NR	IV	✓	✓	-
Indian Pond Heron <i>Ardeola grayii</i>	R/LM	LC	IV	-	✓	-
Asian Openbill Stork <i>Anastomus oscitans</i>	M/LR/lc	LC	IV	✓	✓	-
Lesser Adjutant Stork <i>Leptoptilos javanicus</i>	R/LM	VU	IV	✓	✓	-
Painted stork <i>Mycteria leucocephala</i>	R/LM	NT	IV	✓	✓	-
Woolly-necked Stork <i>Ciconia episcopus</i>	R/LM	VU	IV	-	✓	✓
Bronze winged Jacana <i>Metopidius indicus</i>	R	LC	IV	-	✓	-
River Tern <i>Sterna aurantia</i>	R/LM	NT	IV	✓	✓	✓
Little Cormorant <i>Phalacrocorax niger</i>	R/LM	LC	IV	✓	✓	✓
Little Grebe <i>Tachybaptus ruficollis</i>	R/LM	LC	IV	-	✓	✓
Oriental White Ibis <i>Threskiornis melanocephalus</i>	R/LM	NT	IV	✓	✓	✓
Eurasian Spoon bill <i>Platalea leucorodia</i>	R/LM	LC	I	✓	✓	-
Common Kingfisher <i>Alcedo atthis taprobana</i>	R/WM/SM	LC	IV	-	✓	-
White-throated Kingfisher <i>Halcyon smyrnensis</i>	R/LM	LC	IV	-	✓	✓
Pied Kingfisher <i>Ceryle rudis travancoreensis</i>	R/LM	LC	IV	✓	✓	-
Eurasian Coot <i>Fulica atra</i>	R/WM	LC	IV	-	✓	-
Purple Swamphen <i>Porphyrio porphyrio</i>	R/LM	LC	IV	-	✓	-

Note: LC—Least Concern | VU—Vulnerable | NT—Near Threatened | NR—Not Recognized | Ra—Rare | ✓ Present; - Absent, R—Resident | M—Migrant | LM—Local Migrant | WM—Winter Migrant | Com—Common | LCom—Locally Common | VCom—Very Common | MIT—Minor Irrigation Tank | \*—Handbook of Indian Wetland Birds and their Conservation, ZSI (2005) | \*\* Wildlife Protection Act, (1972) – Schedule Species Birds Database.

Table 5. Mean density of birds recorded at three water bodies of Khanapur Taluk from February to December 2014.

Common names	Scientific Names	BIDI MIT		NANDGAD POND		HEBBAL MIT	
		MEAN	SD	MEAN	SD	MEAN	SD
Comb Duck	<i>Sarkidiornis melanotos</i>	9.935	4.823	-	-	-	-
Cotton Teal	<i>Nettapus coromandelianus</i>	-	-	2.65	0.799	-	-
Indian Spot-bill Duck	<i>Anas poecilorhyncha</i>	23.86	28.949	6.29	4.925	25.185	12.096
Lesser Whistling Duck	<i>Dendrocygna javanica</i>	-	-	4.305	2.280	10.6	3.196
Red wattled Lapwing	<i>Vanellus indicus</i>	1.32	0.637	3.533	1.786	1.32	0.397
Little Ringed Plover	<i>Charadrius dubius</i>	1.32	0.556	-	-	-	-
Black Crowned Night Heron	<i>Nycticorax nycticorax</i>	1.32	0.533	3.31	1.607	-	-
Cattle Egret	<i>Bubulcus ibis</i>	9.542	10.998	10.93	9.686	1.32	0.533
Great Egret	<i>Casmerodius albus</i>	2.65	0.799	3.97	1.197	-	-
Grey Heron	<i>Ardea cinerea</i>	-	-	1.541	0.891	-	-
Little Egret	<i>Egretta garzetta</i>	1.852	1.071	2.646	1.675	2.65	0.799
Median Egret	<i>Mesophoyx intermedia</i>	2.645	1.223	10.16	7.124	-	-
Indian Pond heron	<i>Ardeola grayii</i>	-	-	1.652	0.935	-	-
Asian Openbill Stork	<i>Anastomus oscitans</i>	1.32	0.397	4.195	3.305	-	-
Lesser Adjutant Stork	<i>Leptoptilos javanicus</i>	33.14	19.550	1.32	0.556	-	-
Painted Stork	<i>Mycteria leucocephala</i>	1.32	0.397	2.203	1.235	-	-
Woolly-necked Stork	<i>Ciconia episcopus</i>	-	-	4.238	3.156	2.65	0.799
Bronze winged Jacana	<i>Metopidius indicus</i>	-	-	4.162	3.653	-	-
River Tern	<i>Sterna aurantia</i>	8.833	6.726	1.586	0.909	2.206	1.086
Little Cormorant	<i>Phalacrocorax niger</i>	1.32	0.533	3.088	2.741	1.32	0.616
Little Grebe	<i>Tachybaptus ruficollis</i>	-	-	1.985	0.856	1.985	0.856
Oriental White Ibis	<i>Threskiornis melanocephalus</i>	18.56	8.578	18.996	15.048	3.973	2.769
Eurasian Spoon bill	<i>Platalea leucorodia</i>	6.62	1.996	4.191	3.194	-	-
Common Kingfisher	<i>Alcedo atthis taprobana</i>	-	-	1.985	0.856	-	-
White throated Kingfisher	<i>Halcyon smyrnensis</i>	-	-	1.32	0.397	1.32	0.397
Pied Kingfisher	<i>Ceryle rudis travancoreensis</i>	1.32	0.397	1.852	1.071	-	-
Eurasian Coot	<i>Fulica atra</i>	-	-	6.888	5.276	-	-
Purple Swamp Hen	<i>Porphyrio porphyrio</i>	-	-	3.708	2.848	-	-

- indicates birds not found.

Table 6. Analysis of diversity (H), equitability (E<sub>H</sub>), and species richness (SR) from three water bodies of Khanapur Taluk from February to December 2014.

Sites	Diversity, Equitability & Sp. richness	F	M	A	M	J	J	A	S	O	N	D
Bidi Minor Irrigation Tank	H	-	1.25178	1.38641	0.8947	2.2315	1.56064	0	-	0.24491	0.35565	0
	E <sub>H</sub>	-	0.64328	0.71247	0.55591	0.93060	0.96968	0	-	0.35333	0.32372	0
	SR	-	7	7	5	11	5	1	-	2	3	1
Nandgad pond	H	1.73204	0.75294	2.15257	1.59104	2.58955	1.64755	1.53663	2.31407	2.43694	2.16523	0
	E <sub>H</sub>	0.89009	0.68535	0.81566	0.81763	0.93398	0.79230	0.85761	0.90219	0.95009	0.82045	0
	SR	7	3	14	7	16	8	6	13	13	14	1
Hebbal Minor Irrigation Tank	H	0	-	1.06103	1.19328	1.79166	0	-	-	-	-	0
	E <sub>H</sub>	0	-	0.59217	0.74143	0.99994	0	-	-	-	-	0
	SR	1	-	6	5	6	1	-	-	-	-	1

- indicates birds not found.

Maximum mean density was observed in Indian Spotbill Duck (25.185 ± 12.096) and followed by others (Table 5).

**Diversity (H) and Equitability or Evenness (E)**

Shannon-Weiner diversity index and Equitability of avifauna of Hebbal MIT is given in Table 6. Highest diversity (H'=1.79166) and equitability (E<sub>H</sub>=0.99994) was observed in June, while minimum diversity (H'=1.06103) and evenness (E<sub>H</sub>=0.59217) was recorded in April 2014. Diversity and evenness was zero during February and December 2014, as only one species was recorded.

Based on the monthly rainfall data, the rains had begun in the month of April 2014 (63mm) and slightly fluctuated till June. Maximum rainfall (510mm) was recorded in July 2014 while the minimum (10mm) was in December 2014 (Figure 6).

**DISCUSSION**

Habitat heterogeneity, climatic conditions, rainfall and vegetation cover are the factors that govern the composition, density, abundance and diversity of the avifauna (Gonzalez et al. 2009 & Lorenzon et al. 2016). Habitat selection plays a prominent role in bringing variations in the distribution of avifauna. The food resources, roosting and nesting grounds for local and migratory species might influence their diversity and distribution (Paracuellos 2006). In the present study the two minor irrigation tanks are quite identical with respect to the water spread area and depth but vary in vegetation cover. The eastern side of the bund at Hebbal minor irrigation tank has *Acacia auriculiformes* trees in majority while at the western side there is a hillock surrounded with shrubs and small tree species. The water body did not support any kind of vegetation nor topographic variation that could attract birds other than waterfowls and a few storks. Indian Spot-billed Ducks recorded maximum density (30 individuals) and relative abundance (51%) during mid-summer and Lesser Whistling Ducks (11%). Little cormorants, Little Grebe, Woolly-necked Stork, River Tern, and Oriental White Ibis made an occasional presence. The species composition and diversity at Hebbal MIT is considered to be fewer when compared with the two other water bodies. Hence the diversity and evenness was low during mid-summer while it gradually increased during May and reached its maximum diversity, evenness and species richness during the monsoon (June 2014). No birds were recorded for the rest of the period. The minor

irrigation tank just acted as feeding grounds as it lacked proper vegetation cover and roosting sites as well.

Bidi MIT is shallower than Hebbal MIT. It has a few elevated patches in the middle of the tank. During the dry season the water level recedes to expose land to form temporary islands. During mid-summer (April) Indian Spotbill Ducks and Lesser Adjutant Storks were recorded maximum with respect to their density and relative abundance of 41% and 16% respectively when compared with the two other water bodies. The diversity and evenness appeared quite fluctuating along with the variation in the rainfall. During the onset of summer (March) the diversity and evenness was quite low. A slight increase in diversity and evenness was observed with the beginning of the rains (65mm) in April but decreased again in May 2014 though there was an increase in rainfall. In June, however, with a slight decline in rainfall (63mm) the diversity and species richness rose to its highest value. In the month of July, the rainfall was 510mm which was maximum during which there was decline of avifaunal diversity and species richness.

Shallow water bodies with variations in depth might be the factor for aggregation of birds compared to deeper tank and support more species and greater densities (Helmers 1992; Colwell & Taft 2000). Nandgad pond is smaller as well as shallower than other two water bodies and with varied depths. It is not only surrounded by orchards and paddy fields but also harbors rooted and emergent plants. It provides suitable site for the wetland birds with respect to their roosting, feeding and nesting needs. Vegetation like *Nymphaea* sp., *Nymphoides indica* was more prevalent in most part of the pond that attracted waders like, Bronze-winged Jacanas, egrets, herons, storks and ibises. Submerged vegetation includes: *Charasp.*, *Vallisneria* sp., *Ceratophyllum* spp that are preferred by Eurasian Coot, Purple Swamp Hen. It also attracted swimming and diving birds like Cotton Teal, Indian Spot-billed Duck, Little Cormorants as well. High vegetation cover forms a suitable habitat for breeding birds and also for overall species richness (Porej 2004). Cattle Egret (*Bubulcus ibis*) reported with highest relative abundance of 18% and was more frequently sighted. This is due to its ability of exploring various kinds of feeding grounds and adaptability to different habitats as suggested by Seedikkoya et al. (2007) & Patankar et al. (2007). Based on the diversity values, maximum diversity was observed during monsoon (June) in all three water bodies. Nandgad Pond witnessed the highest diversity and species richness. The increase in avian diversity at Nandgad pond is attributed to the heterogeneity in the flora that provides vegetation

cover for feeding and nesting purposes. This factor has attracted high assemblages of wetland birds than in the two other minor irrigation tanks. Similar observations were also made by Kottawa-Arachchi & Gamage (2015). The conditions at Nandgad pond appeared similar to Bidi minor irrigation tank with respect to reduction of species richness and diversity status during monsoon season. The species richness reduced to half and a drastic decline was seen in the diversity and evenness value in July and continued till August, 2014. Increase in rains had flooded the floating vegetation and reduced the riparian area of the pond which acted as roosting as well as feeding sites of most of the birds. Similar observations have been made by Canepuccia et al. (2007). The scenario gradually changed during the end of the monsoon (September) with increase in diversity, evenness and species richness. The post monsoon season (October) recorded a slight increase in diversity and species richness while the birds were more evenly distributed than in any other month. With the decline of the rainfall the pond witnessed growth of *Eleocharis* sp. and *Cyperus* sp. at the riparian zone and submerged vegetation which resulted in the assemblage of birds like Indian Spot-billed Duck, Eurasian coots, Oriental White Ibis, Red-wattled Lapwing, Asian Openbill Stork, Woolly necked Stork, Purple Swampphen, and Bronze-winged Jacana which depend on floating and emergent vegetation.

According to Paracuellos (2006) & Gonzalez et al. (2009), in larger water bodies, with more habitat types, all species have access to their preferred feeding zones in long shores or wide inner areas of deep open water. In smaller water bodies, however, due to the proximity of the shore to the centre of the pond, the birds that usually feed close to the shore probably continue having available resources in shallow waters with abundant emergent vegetation, whereas those which also frequently select inner zones lose proportionally more feeding space and therefore, have less resource availability. In the present study, Nandgad pond with diverse emergent, submerged and floating vegetation attracted more aggregation, diversity as well as species richness of birds. Similar reports are made by Fairbairn & Dinsmore (2001); Lorenzon et al. (2016).

## CONCLUSION

The study on avifaunal diversity of three water bodies of Khanapur Taluk suggests that water bodies support a good number of residential, local migratory

and migratory birds. The two minor irrigation tanks witness less disturbances as they are away from the state highway whereas, Nandgad pond lies next to it and is prone to frequent anthropocentric activities like; release of clay idols and other related wastes during festivals, discharge of non-biodegradable wastes, washing clothes and heavy vehicles, pumping of waters during the dry seasons. It also suffers from siltation, which results in low water holding capacity. Unscientific excavations at the riparian area can become a stress factor on the pond ecosystem as well as the avifauna. Proper attention is needed from the public as well as the local governing bodies towards the protection and conservation of these habitats so that they can be promising sites for resident as well as migratory birds and other aquatic fauna. In addition there is a need for assessment of water quality and trophic status, and characteristic plankton population which influence the abundance and diversity of the wetland birds.

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## Herpetofauna of Shuklaphanta National Park, Nepal

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**Abstract:** We present 71 herpetofauna species in Shuklaphanta National Park (ShNP) and its buffer zone based on field surveys, rescue records, photo, and literature records. The list comprises 15 currently known species of amphibians and 56 species of reptiles. We recorded *Laudanka Vine Snake Ahaetulla laudankia* as a new species record for Nepal. Likewise, four frog species, namely, *Uperodon globulosus*, *Polypedates taeniatus*, *Hoplobatrachus crassus*, and *Minervarya peirrei*; and one skink, *Sphaenomorphus maculatus*; one agamid, *Laudakia tuberculata*; one turtle, *Pangshura tentoria circumdata*; and 10 snakes, *Eryx conicus*, *E. johnii*, *Coelognathus helena*, *C. radiatus*, *Chrysopelea ornata*, *Dendrelaphis tristis*, *Lycodon striatus*, *Oligodon arnensis*, *Psammophis cf condanarus*, and *Ophiophagus hannah* are new records for ShNP. Unregulated and illegal collection, road mortality, intentional killing are the observed threats to the herpetofauna. Our aim of this study is to compile species richness and advocate for more rigorous inventories in future providing updated information of herpetofauna of ShNP.

**Keywords:** *Ahaetulla laudankia*, amphibians, new records, reptiles, Terai-Arc Landscape.

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**Author contribution:** All authors have made equal contributions. YBR and SB collected and compiled field data; SB wrote the manuscript; SB, LPP, YBR and NS reviewed and approved the final draft.

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## INTRODUCTION

Protected area systems (PAs) are treated as conservation tools for the protection of habitat and species (Geldmann et al. 2013) and have been established for the conservation of ecosystems, constituent species, and services provided by them (Campos & Nepstad 2006; Dudley 2008). Several PAs in Nepal have been successful in achieving international conservation targets such as Aichi Biodiversity Targets. The success of the PAs in Nepal is primarily measured by increment to the charismatic species. Hence, management interventions inside the PAs have been prioritized only for species such as Tiger *Panthera tigris*, Greater One-horned Rhinoceros *Rhinoceros unicornis*, Asian Elephant *Elephas maximus*, and Snow Leopard *Panthera uncia*. Protected areas with such charismatic species are considered higher conservation value while taxa like amphibians and reptiles even within the same PAs are dubbed as low priority species (Bhattarai et al. 2017a). The Gharial, however, is the only reptile which has gained conservation focus in Nepal (Acharya et al. 2017; Bhattarai et al. 2018a) and none of the amphibians have been accorded with the highest degree of protection under National Parks and Wildlife Conservation Act, 1973 of Nepal.

Shuklaphanta National Park (ShNP) is located in the southwestern corner of lowland (known as Terai) Nepal. The Terai is the most productive fertile land with the highest succession rate of plant communities that govern the dispersal and dynamics of faunal species. The change in plant and animal communities due to succession, stochastic events, introduction of exotics, management interventions, and other factors (e.g., climate change) may locally extirpate some species before their formal documentation (Bhattarai et al. 2017a). Because amphibians and reptiles are overlooked species for conservation, their distribution pattern, conservation status, and ecological information from many PAs including ShNP are poorly documented. To understand changes in herpetofauna communities and to propose management strategies to reduce risks demands data on amphibians and reptiles of the park. The knowledge of site specific species richness is the first step to collate data, categorize status, trends of the species, and develop long-term population research and monitoring (Tuberville et al. 2005). Therefore, herein we provide a current update of amphibians and reptiles of the ShNP that will help in formulating their future conservation strategies and conservation management intervention.

## MATERIALS AND METHODS

### Study Area

The Shuklaphanta National Park (ShNP) (80.095–80.361 °N, 28.763–29.047 °E), was established in 1976 as a Royal Shuklaphanta Wildlife Reserve and accorded to a national park in 2017; it covers 305km<sup>2</sup> with open grasslands, river beds, and mixed forests (Figure 1). The buffer zone of the park was declared with an additional area of 243.5km<sup>2</sup> in 2004 (Poudyal & Chaudhary 2019). The climate of ShNP is subtropical with an average maximum temperature of 37°C and the average minimum of 7°C. Annual rainfall may range over 2,016mm (DNPWC 2003).

The park is connected to the Pilibhit Tiger Reserve in India, and Dudhwa Tiger Reserve towards the south-east via Laljhadi forest corridor and to Nandhaur Wildlife Sanctuary in India towards the north-west via Boom-Brahmadev forest corridor and Mahakali River. The aquatic and terrestrial habitats of ShNP contain more than 665 plant species belonging to 438 genera and 118 families (DNPWC 2003).

The ShNP comprises the Terai, Bhabar, and Chure/Sivaliks, and its vegetation can be broadly classified into forests, grassland, and aquatic habitat (wetlands). Although several variations in species association may lead to formation of many forest types, they are primarily grouped into Sal forest and deciduous riverine forest. The vegetation is dominated by Sal *Shorea robusta* and includes other associated plants such as *Terminalia tomentosa*, *T. bellirica*, and *Lagestromea parviflora*. The ShNP has the largest herd of Swamp Deer *Rucervus duvaucelii* in the world, provides prime habitat for Hog Deer *Axis porcinus*, Spotted Deer *Axis axis*, and many endangered species such as the Hispid Hare, Royal Bengal Tiger, Greater One-horned Rhinoceros, and Asian Elephants (DNPWC & DFSC 2018).

### Field Methods

We recorded all the amphibians and reptiles during regular anti-poaching operations and wildlife monitoring in ShNP. Regular day and night field patrolling is conducted to increase the deterrence against possible poaching of wildlife. We included all the opportunistic observations of herpetofauna during anti-poaching field operation and wildlife monitoring (such as camera traps for large carnivores, rhino monitoring, swamp deer translocation, and census) from January 2017 to September 2019 in and around the ShNP. We also incorporated literature records, reliable photographs, and rescue records from the buffer zone. The nocturnal

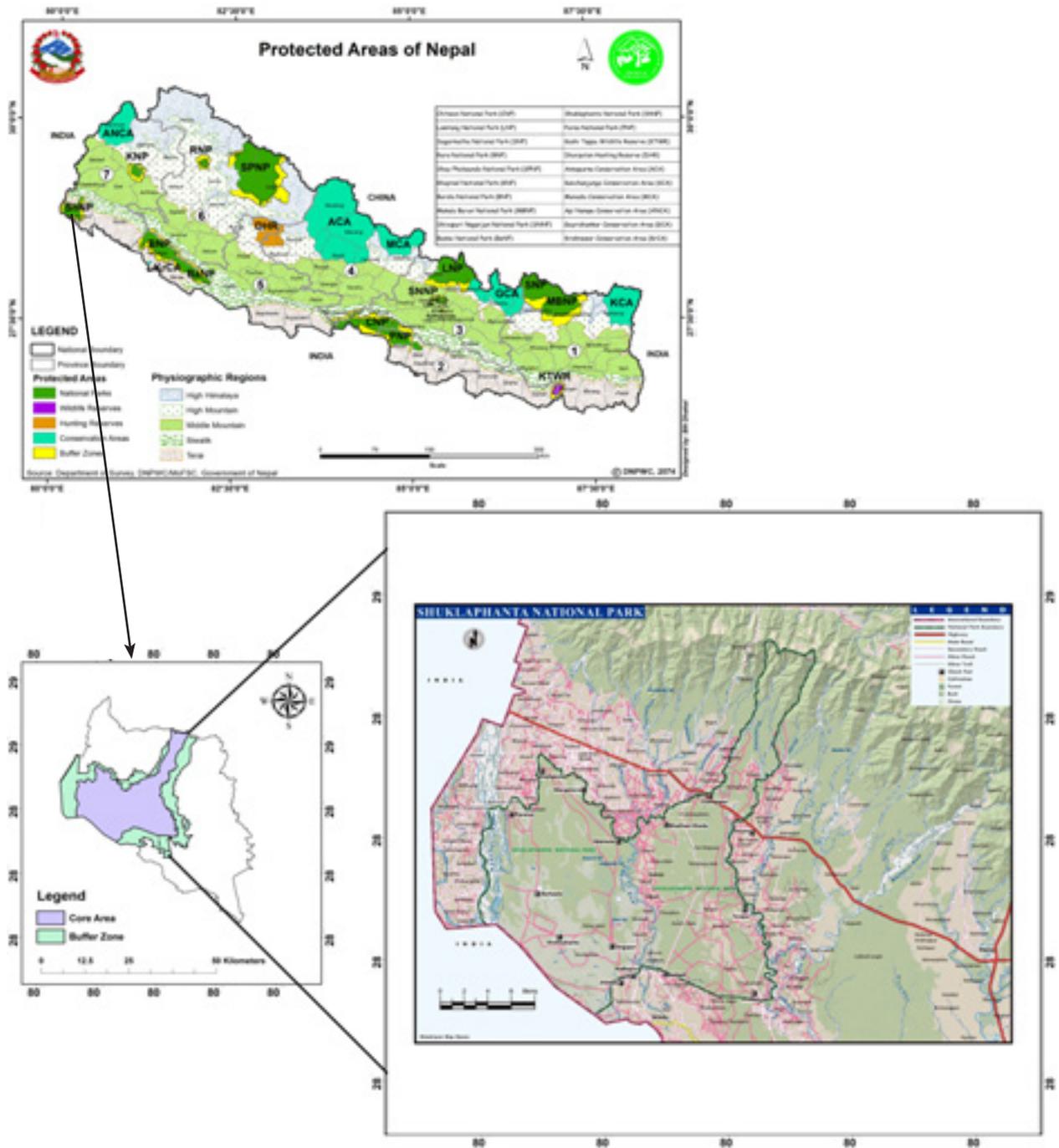


Figure 1. Shuklaphanta National Park.

anti-poaching patrolling activities aided with flashlights helped us in detecting calling frogs. A national east-west highway runs through the ShNP; we incorporated opportunistic road kill data of herpetofauna in our study. Upon detection, the individuals were either captured by hand or photographed using Canon1300D. Crocodiles were monitored along the river bank. We used published literature and field guides (Smith 1935, 1943; Schleich & Kästle 2002; Shah & Tiwari 2004; Ahmed et al. 2009;

Vasudevan & Sondhi 2010; Das & Das 2017) to identify the herpetofauna.

**RESULTS**

With a combination of field surveys, rescue records, photographic evidence, and literature records, the herpetofauna of the ShNP accounted for 71 species (15

**Table 1. Collated list of herpetofauna of Shuklaphanta National Park. The plus sign (+) refers to presence and minus sign (-) refers to absence of the species by the authors.**

	Species Name	Common Name	This study	Schleich & Kästle (2002)	Shah & Tiwari (2004)	Kästle et al. (2013)	IUCN Red List status	Remarks
<b>AMPHIBIANS</b>								
Family: Bufonidae								
1	<i>Duttaphrynus melanostictus</i>	Common Asian Toad	+	-	+	-	LC	
2	<i>Duttaphrynus stomaticus</i>	Marbled Toad	+	-	+	-	LC	
Family: Microhylidae								
3	<i>Microhyla</i> sp.	Narrow Mouth Frog	+	-	+	-	-	
4	<i>Uperodon globulosus</i>	Globular Balloon Frog	+	-	-	-	LC	New for ShNP
5	<i>Uperodon systoma</i>	Marbled Balloon Frog	+	+	+	+	LC	
6	<i>Uperodon taprobanicus</i>	Painted Frog	+	+	+	+	LC	
Family: Dicroglossidae								
7	<i>Euphlyctis cyanophlyctis</i>	Skittering Frog	+	+	+	-	LC	
8	<i>Minervarya pierrei</i>	Pierre's Cricket Frog	+	-	-	-	-	New for ShNP
9	<i>Minervarya syhadrensis</i>	Syhadra Cricket Frog	-	-	+	-	-	
10	<i>Minervarya teraiensis</i>	Terai Cricket Frog	+	-	+	-	-	
11	<i>Hoplobatrachus crassus</i>	Jerdon's Bull Frog	+	-	-	-	LC	New for ShNP
12	<i>Hoplobatrachus tigerinus</i>	Indian Bull Frog	+	+	+	-	LC	
13	<i>Sphaerotheca</i> sp.	Burrowing Frog	+	+	-	-	LC	
Family: Rhacophoridae								
14	<i>Polypedates maculatus</i>	Common Tree Frog	+	+	+	-	LC	
15	<i>Polypedates taeniatus</i>	Terai Bush Frog	+	-	-	-	LC	New for ShNP
<b>REPTILES</b>								
Family: Crocodylidae								
16	<i>Crocodylus palustris</i>	Mugger Crocodile	+	+	+	+	VU	
Family: Geoemydidae								
17	<i>Melanochelys tricarinata</i>	Sal Forest Turtle	+	-	-	+	VU	
18	<i>Melanochelys trijuga</i>	Black Pond Turtle	+	+	+	+	NT	
19	<i>Pangshura tecta</i>	Indian-roofed Turtle	+	-	-	+	LC	
20	<i>Pangshura tentoria</i>	Indian Tent Turtle	+	-	-	-	LC	New for ShNP
Family: Testudinidae								
21	<i>Indotestudo elongata</i>	Elongated Tortoise	+	+	+	-	CR	
Family: Trionychidae								
22	<i>Chitra indica</i>	Narrow-headed Softshell Turtle	-	-	-	+	EN	
23	<i>Lissemys punctata</i>	Indian Flapshell Turtle	+	-	+	+	LC	
24	<i>Nilssoniana gangetica</i>	Gangetic Softshell Turtle	+	-	+	+	VU	
25	<i>Nilssoniana hurum</i>	Peacock Softshell Turtle	+	-	-	+	VU	
Family: Agamidae								
26	<i>Calotes versicolor</i>	Common Garden Lizard	+	+	+	-	LC	
27	<i>Laudakia tuberculata</i>	Rock Lizard	+	-	-	-	LC	New for ShNP
28	<i>Sitana schleichi</i>	Shuklaphanta Sitana		+	+	+	NA	
29	<i>Sitana sivalensis</i>	Shivalik Sitana	+	-	+	-	NA	

	Species Name	Common Name	This study	Schleich & Kästle (2002)	Shah & Tiwari (2004)	Kästle et al. (2013)	IUCN Red List status	Remarks
Family: Gekkonidae								
30	<i>Hemidactylus cf. brookii</i>	Brook's Gecko	+	-	+	+	NA	
31	<i>Hemidactylus flaviviridis</i>	Yellow-bellied Gecko	+	+	+	+	NA	
32	<i>Hemidactylus frenatus</i>	Common House Gecko	+	-		-	LC	
33	<i>Hemidactylus garnotii</i>	Indo-pacific Gecko	-	+	+	-	NA	
Family: Scincidae								
34	<i>Asymbplepharus sikimensis</i>	Sikkim Ground Skink	-	-	+	-	NA	
35	<i>Eutropis carinata</i>	Common Grass Skink	+	-	+	-	LC	
36	<i>Eutropis dissimilis</i>	Striped Skink	+	-	+	-	NA	
37	<i>Eutropis macularia</i>	Bronze Grass Skink	+	+	+	+	NA	
38	<i>Lygosoma albopunctata</i>	Suppled Grass Skink	+	+	+	+	NA	
39	<i>Sphenomorphus maculatus</i>	Sal Forest Skink	+	-	-	-	NA	New for ShNP
Family: Varanidae								
40	<i>Varanus bengalensis</i>	Bengal Monitor Lizard	+	-	+	-	LC	
41	<i>Varanus flavescens</i>	Golden Monitor Lizard	+	-	+	-	LC	
Family: Erycidae								
42	<i>Eryx conicus</i>	Common Boa	+	-	-	-	NA	New for ShNP
43	<i>Eryx johnii</i>	Red Sand Boa	+	-	-	-	NA	New for ShNP
Family: Pythonidae								
44	<i>Python bivittatus</i>	Burmese Python	+	+	+	+	VU	
Family: Colubridae								
45	<i>Ahaetulla laudankia</i>	Laudanka Vine Snake	+	-	-	-	NA	New for Nepal
46	<i>Ahaetulla nasuta</i>	Common Vine Snake	+	-	+	-	NA	
47	<i>Boiga forsteri</i>	Forsten's Cat Snake	+	+	+	+	LC	
48	<i>Boiga trigonata</i>	Common Cat Snake	+	-	+	-	LC	
49	<i>Chrysopelea ornata</i>	Ornate Gliding Snake	+	-	-	-	NA	New for ShNP
50	<i>Coelognathus helena</i>	Common Trinket Snake	+	-	-	-	NA	New for ShNP
51	<i>Coelognathus radiatus</i>	Copper-headed Trinket Snake	+	-	-	-	LC	New for ShNP
52	<i>Dendrelaphis tristis</i>	Bronzeback Tree Snake	+	-	-	-	NA	New for ShNP
53	<i>Lycodon aulicus</i>	Common Wolf Snake	+	+	+	+	LC	
54	<i>Lycodon jara</i>	Twin-spotted Wolf Snake	+	+	+	-	LC	
55	<i>Lycodon striatus</i>	Barred Wolf Snake	+	-	-	-	NA	New for ShNP
56	<i>Oligodon arnensis</i>	Banded Kukri Snake	+	-	-	-	NA	New for ShNP
57	<i>Oligodon kheriensis</i>	Red kukri Snake	+	+	+	+	NA	
58	<i>Ptyas mucosa</i>	Rat Snake	+	+	+	+	NA	
59	<i>Sibynophis sagittarius</i>	Cantor's Black-headed Snake	+	-	+	-	NA	
Family: Homalopsidae								
60	<i>Enhydryis enhydryis</i>	Common Smooth Water Snake	-	-	+	-	LC	
61	<i>Ferania sieboldii</i>	Siebold's Water Snake	-	-	+	-	LC	
Family: Lamprophiidae								

	Species Name	Common Name	This study	Schleich & Kästle (2002)	Shah & Tiwari (2004)	Kästle et al. (2013)	IUCN Red List status	Remarks
62	<i>Psammophis condanarus</i>	Sand Snake	+	-	-	-	LC	New for ShNP
Family: Natricidae								
63	<i>Amphiesma stolatum</i>	Striped Keelback	+	+	+	+	NA	
64	<i>Fowlea piscator</i>	Checkered Keelback	+	+	+	-	NA	
Family: Elapidae								
65	<i>Bungarus caeruleus</i>	Common Krait	+	-	+	-	NA	
66	<i>Bungarus fasciatus</i>	Banded Krait	+	+	+	-	NA	
67	<i>Naja kaouthia</i>	Monocled Cobra	-	+	+	+	LC	
68	<i>Naja naja</i>	Common Cobra	+	-	+	-	NA	
69	<i>Ophiophagus hannah</i>	King Cobra	+	-	-	-	VU	New for ShNP
Family: Typhlopidae								
70	<i>Indotyphlops braminus</i>	Brahminy Blind Snake	+	-	+	-	NA	
Family: Viperidae								
71	<i>Daboia russelii</i>	Russell's Viper	+	+	+	+	LC	



Figure 2. Herpetofaunal species richness in the ShNP

species of amphibians represented by eight genera in four families and 56 species of reptiles represented by 37 genera in 17 families) (Table 1).

All the recorded amphibians comprised anurans only. Among reptiles, the family Colubridae was the most speciose with 15 species followed by Scincidae with six species, Elapidae with five species, families Agamidae, Gekkonidae, Geoemydidae, and Trionychidae each with four species, Varanidae, Erycidae, Homalopsidae, and Natricidae each with two species, Crocodylidae, Testudinidae, Pythonidae, Typhlopidae, and Viperidae each with single species (Figure 2).

Our study documented 18 species of herpetofauna that have not been reported before from ShNP. We report an arboreal snake—Laudanka Vine Snake *Ahaetulla laudankia*—as a new snake species record for Nepal based

on photo vouchers. Four frog species, namely, Jerdon's Bull Frog *Hoplobatrachus crassus*, Globular Balloon Frog *Uperodon globulosus*, Pierre's Cricket Frog *Minervarya pierrei*, and Six-lined Tree Frog *Polypedates taeniatus* are new records for ShNP. Similarly, we recorded the Indian Tent Turtle *Pangshura tentoria circumdata*, Sal Forest Skink *Sphaenomorphus maculatus*, Common Trinket Snake *Coelognathus helena*, Copper-headed Trinket Snake *C. radiatus*, Bronzeback Tree Snake *Dendrelaphis tristis*, Barred Wolf Snake *Lycodon striatus*, Banded Kukri Snake *Oligodon arnensis*, Sand Snake *Psammophis cf. condanarus*, and King Cobra *Ophiophagus hannah* as new records for ShNP. Likewise, our record of the Sand Snake *Psammophis cf. condanarus* in the ShNP is the third locality record for Nepal after 42 years of its first record by Kramer (1977) in Chitwan National Park. The new locality of the *Psammophis cf. condanarus* in the ShNP is ca. 550km west of Chitwan National Park.

### Species Accounts

#### AMPHIBIANS

##### Bufonidae Gray, 1825

##### Common Asian Toad *Duttaphrynus melanostictus* (Schneider, 1799)

This is the most common toad in the Terai and Churia region of Nepal (Bhattarai et al. 2018a). It has dorsal skin with two series of warts, tympanum distinct, two large parotid glands (Image 1).

We observed the individuals from Majhgaun,

Arjuni, Hirapur, Shuklaphanta, Malumela, Lalpani area, Badenikheda, Mahendranagar, Chandmari (NTNC-Shuklaphanta Conservation Program office complex), Majhgaun (ShNP headquarters). We frequently observed this species around park guard posts during the monsoon and road killed individuals on the national highway that passes through the ShNP and other roads in the buffer zone.

**Marbled Toad *Duttaphrynus stomaticus* (Lütken, 1864)**

This species is sympatric with *D. melanostictus* but it is comparatively smaller than *D. melanostictus*. We frequently observed the individuals around human habitation including park offices and posts, open grasslands, and river banks. The species lacks a cranial ridge and parotid glands and has irregular warts on the dorsal skins (Image 2). The juveniles were observed with red tipped warts.

**Dicroglossidae Anderson, 1871**

**Skittering Frog *Euphlyctis cyanophlyctis* (Schneider, 1799)**

It is the commonest dicroglossid frog in low land Terai of Nepal (Bhattarai et al. 2018a). We found it in all natural and constructed ponds inside ShNP and water bodies in the fringe area. Individuals from ponds and water logged areas had dark patch on their bodies which was absent on the individuals from the river (Image 3).

**Pierre's Cricket Frog *Minervarya pierrei* (Dubois, 1975)**

The type locality of this species is in Nepal. We observed this species at Beldandi, Hirapurphanta, and Arjuni (Image 4). The individuals of this species have longer dorso and dorso-lateral folds compared with *M. teraiensis* with dark patches on thighs and with or without mid-dorsal line. Nanhoe & Ouboter (1987) consider *M. pierrei* as a synonym of *M. limnocharis*. The Nepalese *Minervarya* spp. warrant molecular studies to resolve their taxonomy and genetic identity. This is the first record of the species from ShNP.

**Syhadra Cricket Frog *Minervarya syhadrensis* (Annandale, 1919)**

The record of this species in ShNP is based on Shah & Tiwari (2004).

**Terai Cricket Frog *Minervarya teraiensis* (Dubois, 1984)**

We recorded this species from Shuklaphanta, Majhgaun, Beldandi, Hirapurphanta, and Barkaula (Image 5). The individuals of this species had broad cream-coloured mid dorsal line and body with dorsal



Image 1. Common Asian Toad *Duttaphrynus melanostictus* from NTNC-SCP camp.



Image 2. Marbled Toad *Duttaphrynus stomaticus* from NTNC-SCP camp.



Image 3. Skittering Frog *Euphlyctis cyanophlyctis* from Chaudhar River.



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Image 4. Pierre's Cricket Frog *Minervarya pierrei*.

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Image 5. Terai Cricket Frog *Minervarya teraiensis* from Majhgaun.

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Image 6. Jerdon's Bull Frog *Hoplobatrachus crassus*.

longitudinal folds. This species is distributed across the whole Nepalese Terai below 400m (Schleich & Kästle 2002).

#### Jerdon's Bull Frog *Hoplobatrachus crassus* (Jerdon, 1854)

We observed this species from Chandmari, Beldandi, and Arjuniphanta. The individuals of this species are smaller than the Indian Bull Frog *H. tigerinus* and lack mid-dorsal and dorsolateral lines. The dorsal skin is warty but ventral is smooth (Image 6). This species has been well documented from eastern Nepal (Schleich & Kästle 2002). Shah & Tiwari (2004) reported its distribution up to Bardia National Park. This is the first record of the species from ShNP.

#### Indian Bull Frog *Hoplobatrachus tigerinus* (Daudin, 1803)

This species is the largest among all frogs in the Terai region of Nepal (Bhattarai et al. 2018a). This species (Image 7) has cream or yellow coloured mid and dorso-lateral lines from tip of the snout to posterior end which is lacking in *H. crassus* (Image 6). This Bull Frog is also well distributed in adjoining Indian states in Uttarakhand (Vasudevan & Sondhi 2010) and Uttar Pradesh (Das et al. 2012). During the breeding season, we observed yellow coloured breeding males in chorus.

#### Burrowing Frog (*Sphaerotheca* sp.)

We recorded this species from Majhgaun, Beldandi (Image 8). Nepal was reported to have four species of *Sphaerotheca*, namely, *S. breviceps*, *S. maskeyi*, *S. rolandae*, and *S. swani* based on morphological characters (Schleich & Kästle 2002). Among them, recent molecular studies have delimited the distribution range of *S. rolandae* in Sri Lanka and *S. breviceps* is now confined to southern India (Karnataka, Andhra Pradesh, and Tamil Nadu) (Prasad et al. 2019) and two species, namely, *S. maskeyi* and *S. swani* were described from Nepal. Dubois (1999, 2000) synonymized *S. maskeyi* and *S. swani* as *S. pluvialis*. Later, Dahanukar et al. (2017) resurrected them as valid species. The *Sphaerotheca* spp. from Nepal were described based on morpho-taxonomy. As recent studies have delimited the distribution range of *Sphaerotheca* sp. and due to variation in colour patterns, morphological characters among *Sphaerotheca* of ShNP, we could not ascertain the species found in ShNP and advocate for genetic studies of *Sphaerotheca* of Nepal as suggested by Prasad et al. (2019).



Image 7. Indian Bull Frog *Hoplobatrachus tigerinus* a breeding male.



Image 10. Globular Balloon Frog *Uperodon globulosus*.



Image 11. Marbled Balloon Frog *Uperodon systoma*.



Image 8. Burrowing Frog *Sphaerotheca* sp.



Image 12. Painted Frog *Uperodon taprobanicus*.



Image 9. *Microhyla* sp.



Image 13. Common Tree Frog *Polypedates maculatus*.



Image 14. Terai Bush Frog *Polypedates taeniatus*.

### Microhylidae (Günther, 1858)

#### Narrow-mouthed Frog *Microhyla* sp.

We observed *Microhyla* sp. from altered habitats at Shuklaphanta wildlife camp and home stay area (Image 9). Calls were frequently heard during July–August. The previous report of *Microhyla ornata* from the ShNP by Shah & Tiwari (2004); taxonomic revision of South Asian microhylid frogs by Garg et al. (2019) restricted the distribution range of *Microhyla ornata* in southern India. Similarly Khatiwada et al. (2017) described *Microhyla taraiensis* from Jhapa in far-east Nepal and reported occurrence of *Microhyla nilphamarensis* in Chitwan; this warrants genetic studies of *Microhyla* population from Shuklaphanta National Park to ascertain its taxonomic identity.

#### Globular Balloon Frog *Uperodon globulosus* (Günther, 1864)

We observed this species from Majhgaun (Image 10) being the first record from ShNP. We recorded this

species in the fringe areas of the park after a heavy shower during the monsoon season. It has a globular body with a pointed snout. The earlier records of this species is restricted to central and eastern Terai and the protected areas of Terai Nepal (Schleich & Kästle 2002; Shah & Tiwari 2004; Bhattarai et al. 2017a, 2018a). We also observed individuals in Bardia National Park which implies that this species has wide distribution in Terai Nepal.

#### Marbled Balloon Frog *Uperodon systoma* (Schneider, 1799)

We recorded individuals of this species from the headquarters of the ShNP at Majhgaun. The dorsum of this species is marbled with dark or dark brown and the ventral side is either white or yellow (Image 11). The calls of this species is frequently heard in paddy fields in the buffer zone area during the monsoon season. The occurrence of this species in Nepal was first confirmed by Schleich & Kästle (2002) from Kanchanpur District, 15km east of Mahendranagar.

#### Painted Frog *Uperodon taprobanicus* (Parker, 1934)

We recorded this species from Badenikheda and Arjuni (Image 12). This is a fossorial frog but also observed in tree cavities. The occurrence of this species was first reported by (Schleich & Kästle 2002) from Mahendranagar in the far-west and Jhapa in the far-east of Nepal. Shah & Tiwari (2004) and Kästle et al. (2013) also reported this species from ShNP. Shah & Tiwari (2004) added Chitwan and Parsa; Bhattarai et al. (2017a, 2018a) recorded from Beeshazar and associated lakes, and Parsa National Park, respectively.

### Rhacophoridae Hoffman, 1932

#### Common Tree Frog *Polypedates maculatus* (Gray, 1830)

We observed this species from Majhgaun and Badenikheda area (Image 13). It was Günther (1861) who first reported this species from Nepal. This species is widely distributed in Nepal from the entire Terai region to the mid hills (Schleich & Kästle 2002).

#### Terai Bush Frog *Polypedates taeniatus* (Boulenger, 1906)

We frequently observed individuals of this species from Majhgaun, 24 no. pillar of Shuklaphanta grassland, Shikari tal and Baba tal area (Image 14). In Nepal, this species was first recorded by Anders et al. (1998) from Chitwan National Park and Koshi Tappu Wildlife Reserve. Shah & Tiwari (2004) recorded it from Bardia National Park. Bhattarai et al. (2018b) recorded an amplexus between *P. maculatus* and *P. taeniatus*. Das et al.

(2012) recorded this species from Katarniaghat Wildlife Sanctuary, Uttar Pradesh India. This is the first record of *P. taeniatus* from ShNP.

## REPTILES

### Agamidae Gray, 1827

#### Oriental Garden Lizard *Calotes versicolor* (Daudin, 1802)

This is the most common agamid lizard in Nepal distributed from the Terai region to the mountain zone (Schleich & Kästle 2002). Likewise, Shah & Tiwari (2004) reported the distribution of this lizard from all protected areas of Nepal. In ShNP, we frequently observed this species from Majhgaun, Hirapurphanta, Shuklaphanta, Arjuni, Malumela and Chure/Sivalik areas of the park (Image 15).



Image 15. Oriental Garden Lizard *Calotes versicolor*.

#### Rock Lizard *Laudakia tuberculata* (Gray, 1827)

We recorded this species from Chure/Sivalik range along Syali River bank and other small rivulets of the park. This is the first record of *L. tuberculata* for ShNP (Image 16).



Image 16. Rock Lizard *Laudakia tuberculata*.

#### Shuklaphanta Fan-throated Lizard *Sitana schleichi* (Anders & Kästle, 2002)

This is an endemic lizard to Nepal described from Shuklaphanta National Park. Nepal has three species of lizards belonging to the genus *Sitana*, namely, *S. fusca*, *S. schleichi*, and *S. sivalensis*. The Nepalese *Sitana* are different from the Indian species in having small dewlaps that do not extend the forearm and no overlapping scales on dewlaps (Schleich & Kästle 2002; Deepak et al. 2016). Likewise, *S. sivalensis* has a wider distribution from central Nepal to Uttarakhand, India (Vasudevan & Sondhi 2010). Among three species of *Sitana* in Nepal, Shuklaphanta Fan-throated Lizard (*S. schleichi*) is the smallest species. Shah & Tiwari (2004) and Kästle et al. (2013) also recorded this species from ShNP.

#### Sivalik Fan-throated Lizard *Sitana sivalensis* (Schleich, Kästle and Shah, 1998)

This species was described from Shivpur, Kapilbastu, Nepal. It is found in open dry grass patches in Sivalik/Churia hill range or foot hills of the Sivalik range. We recorded this species from Kuwadanda (Image 17). Shah & Tiwari (2004) also recorded the occurrence of *S. sivalensis* in ShNP. Studies on habitat use by these two related species in ShNP and genetic studies are suggested to ascertain the taxonomic ambiguity of Nepalese *Sitana* spp.



Image 17. Sivalik Fan-throated Lizard *Sitana sivalensis*.

## Gekkonidae Gray, 1825

### Brook's Gecko *Hemidactylus cf. brookii* (Gray, 1845)

We observed the individuals of this species from Kuwadanda, Majhgaun, and Barkaula areas (Image 18). The individuals of this species have strongly keeled tubercles and tails with spines. This species is regarded as species complex (Rösler & Glaw 2010; Kathriner et al. 2014; Lajmi et al. 2016) with one of the most diverse clades within *Hemidactylus* (Agarwal et al. 2019). Considering this taxonomic uncertainty, we suggest detailed molecular studies on Nepalese *H. brookii* complex.



Image 18. Brook's Gecko *Hemidactylus cf. brookii*.

### Yellow-bellied Gecko *Hemidactylus flaviviridis* (Rüppell, 1835)

This is a common gecko in ShNP, found in park posts, army posts, and in houses in the buffer zone area (Image 19). This species is also found in cattle sheds in Chure/Sivalik areas of the park.



Image 19. Yellow-bellied Gecko *Hemidactylus flaviviridis*.

### Common House Gecko *Hemidactylus frenatus* (Dumeril & Bibron, 1836)

We recorded this species from Majhgaun, Beldandi, Badenikheda, Radhapur, Pipariya, Hirapurphanta, Dhakka, and Champapur. It is easily identified from other *Hemidactylus* spp. having reduced inner digit and smooth skin with round scattered tubercles.

### Indo-Pacific Gecko *Hemidactylus garnotii* (Dumeril & Bibron, 1836)

This species in Nepal was first recorded from Pokhara in 1954 (Schleich & Kästle 2002). Later, colonized in other parts of the country especially in the lowlands. The record of this species in ShNP is based on Shah & Tiwari (2004).

## Scincidae Gray, 1825

### Sikkim Ground Skink *Aymblepharus sikimensis* (Blyth, 1854)

The record of this species in ShNP is based on Shah & Tiwari (2004).

### Common Grass Skink *Eutropis carinata* (Schneider, 1801)

This is one of the commonly sighted species in the Terai and Chure/Sivalik region of Nepal (Bhattarai et al. 2018a). We observed this species in Majhgaun, Malumela, Shuklaphanta, and Paliya areas basking on open grasslands (Image 20).



Image 20. Common Grass Skink *Eutropis carinata*.

### Striped Grass Skink *Eutropis dissimilis* (Hallowell, 1857)

We observed this species from Hirapurphanta and



Image 21. Striped Grass Skink *Eutropis dissimilis*.



Image 22. Bronze Grass Skink *Eutropis macularia*.



Image 23. Sal Forest Skink *Sphenomorphus maculatus*.

Garjamani. This species is easily identified from other *Eutropis* spp. with a white stripe below the eyes (Image 21).

#### Bronze Grass Skink *Eutropis macularia* (Blyth, 1853)

Observed from Hirapurphanta, Arjuni basking on the open grassland. Individuals were also observed at Chandmari, Arjuni, Beldandi, and Shuklaphanta grassland (Image 22). This species is also frequently observed in agricultural fields during April–May.

#### Sal Forest Skink *Sphenomorphus maculatus* (Blyth, 1853)

We observed this species from Chure area of ShNP basking on open river beds in Sal *Shorea robusta* mixed forest (Image 23). This species is frequently observed in ShNP especially under low canopy Sal forest area. This species is a new record for ShNP.

#### Varanidae Merrem, 1820

##### Bengal Monitor Lizard *Varanus bengalensis* (Daudin, 1802)

Individuals were frequently observed at Malumela, Chandani-Dodhara, Hirapurphanta, Arjuni, Shuklaphanta grassland, Majhgaun, Chandmari, and around human habitations in the buffer zone area (Image 24). One sub-adult individual was rescued from Tilkeni Village and released inside the park. We also observed a road kill on the national highway near Arjuni post.

##### Golden Monitor Lizard *Varanus flavescens* (Hardwicke & Gray, 1827)

This species was frequently observed at fringe areas of the park from Pipariya, Beldandi, and Majhgaun areas (Image 25). Three individuals were rescued each from Mahendranagar, Pipariya Village and Majhgaun and released in ShNP. In Nepal, this species is facing multiple threats like habitat destruction, poaching (Bhattarai et al. 2018a), however, Ghimire & Shah (2014) mentioned that the species tolerates habitat modification in Kanchanpur.

#### Typhlopidae Merrem, 1820

##### Brahminy Blind Snake *Indotyphlops braminus* (Daudin, 1803)

We recorded the individuals during wetland management activities from Sundariphanta and one individual was also observed at Majhgaun (Image 26). This species is the smallest snake species of Nepal, is a fossorial species, and is known to have parthenogenetic reproduction.



Image 24. Bengal Monitor Lizard *Varanus bengalensis*.



Image 27. Common Sand Boa *Eryx conicus*.



Image 25. Golden Monitor Lizard *Varanus flavescens* rescued from Mahendranagar.



Image 28. Laudanka Vine Snake *Ahaetulla laudankia*. Top: fullbody, Bottom: close-up of head.



Image 26. Brahminy Blind Snake *Indotyphlos braminus*.

### Erycidae Bonaparte, 1840

#### Common Sand Boa *Eryx conicus* (Schneider, 1801)

We recorded this species from Hirapurphanta and Parkhedi school compound, Majhgaun (Image 27). The earlier records of this species were from eastern Nepal to Bardia National Park only (Schleich & Kästle 2002; Shah & Tiwari 2004; Bhattarai et al. 2017a, 2018a; Pandey et al. 2018). Recently, Devkota et al. (2019) recorded this species from Sainamaina, Rupandehi District also. This is a new record of the species from ShNP.

#### Red Sand Boa *Eryx johnii* (Russell, 1801)

We recorded this individual from Pipariya. One road kill was also observed near Arjuni post during translocation of Swamp Deer from ShNP to Chitwan National Park. Shah & Tiwari (2004) reported this species from Bardia National Park. This is the first record of *E. johnii* from ShNP and the locality where the species was recorded is ca. 200km west of Bardia National Park.

### Colubridae Opperl, 1811

#### Laudanka Vine Snake *Ahaetulla laudankia* Deepak, Narayanan, Sarkar, Dutta & Mohapatra, 2019

This is a newly described species from India by Deepak et al. (2019) based on the specimens from Odisha and Rajasthan. This is a Brown Vine Snake that looks like a dried stem of bottle gourd for which the species name has been latinized. We recorded this species from Larighat area of the ShNP (Image 28). The morphological characters of our specimen corresponded to the original description collected from Odisha and Rajasthan (for details see Deepak et al. 2019). Recently, Patel et al. (2019) also reported its occurrence from another Indian state from Gujarat. We report it as a new snake species record for Nepal. We first recorded it from ShNP. Later, we also received photographic evidence of this species from the buffer zone village Banu Gaun (gaun=village) of Bardia National Park and also observed at Patna tal and Lami tal of Chitwan National Park. This indicates that *A. laudankia* has a wider distribution in Nepal. We suggest detailed inventory of this newly described species.

#### Green Vine Snake *Ahaetulla nasuta* (Lacépède, 1789)

We recorded this species from Shuklaphanta and Darakphanta (Image 29). This species was also recorded by Shah & Tiwari (2004).

#### Forsten's Cat Snake *Boiga forsteni* (Duméril, Bibron & Duméril, 1854)

We recorded this species from Malumela and Shuklaphanta area (Image 30). Previous studies by



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Image 29. Green Vine Snake *Ahaetulla nasuta*.



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Image 30. Forsten's Cat Snake *Boiga forsteni*.



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Image 31. Common Cat Snake *Boiga trigonata*.

Schleich and Kästle (2002); Shah & Tiwari (2004) and Kästle et al. (2013) also reported the occurrence of this species in ShNP.

**Common Cat Snake *Boiga trigonata* (Schneider, 1802)**

We rescued this species, one each from the buffer zone villages (Beldandi and Majhgaun) and released them in the park (Image 31). We also observed one individual at Shuklaphanta grassland.

**Ornate Gliding Snake *Chrysopelea ornata* (Shaw, 1802)**

A juvenile individual was observed near the bridge of Bahuni River on the way to Shuklaphanta grassland during swamp deer translocation from the ShNP to Chitwan National Park in May 2017 (Image 32). This is a new record of the species from ShNP. The nearest locality record of this snake is Bardia National Park (Shah & Tiwari 2004).

**Common Trinket Snake *Coelognathus helena* (Daudin, 1803)**

We observed the individuals from Garjamani, Hirapurphanta, and Beldandi. We also found one road kill near Majhgaun during swamp deer translocation from ShNP to Chitwan National Park. This is a new record from ShNP.

**Copper-headed Trinket Snake *Coelognathus radiatus* (Boie, 1827)**

One dead specimen probably killed by elephant staff because of its aggressive nature was found near Shuklaphanta post during swamp deer translocation in 2017. This is a new snake species record from ShNP.

**Bronzeback Tree Snake *Dendrelaphis tristis* (Daudin, 1803)**

This species was frequently observed at Majhgaun, Pipariya, Shuklaphanta, Singhpur, and Hirapurphanta (Image 33). This is a new snake species record from ShNP.

**Common Wolf Snake *Lycodon aulicus* (Linnaeus, 1758)**

We recorded this species from Majhgaun, Beldandi, and Homestay areas (Image 34). It is also frequently seen at Pipariya and Mahendranagar.

**Twin-spotted Wolf Snake *Lycodon jara* (Shaw, 1802):** We recorded this species from Shuklaphanta wildlife camp, Majhgaun, and Hirapurphanta. An individual was also rescued from a Homestay area (Image 35).



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Image 32. Ornate Gliding Snake *Chrysopelea ornata* (juvenile).



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Image 33. Bronzeback Tree Snake *Dendrelaphis tristis*.



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Image 34. Common Wolf Snake *Lycodon aulicus*.



Image 35. Twin-spotted Wolf Snake *Lycodon jara*.



Image 36. Barred Wolf Snake *Lycodon striatus*.



Image 37. Banded Kukri Snake *Oligodon arnensis*.

#### **Barred Wolf Snake *Lycodon striatus* (Shaw, 1802)**

We recorded this species from Beldandi and Majhgaun area. This is a new snake species record from ShNP (Image 36).

#### **Banded Kukri Snake *Oligodon arnensis* (Shaw, 1802)**

We frequently observed this species from Majhgaun, Arjuni, Pipariya, and Beldandi areas (Image 37). This is a new snake species record from ShNP.

#### **Coral Red Kukri Snake *Oligodon kheriensis* (Acharji & Ray, 1936)**

We observed this species from Majhgaun, Beldandi, Jhilmila, and Arjuni post (Image 38). Individuals were also rescued from buffer zone villages. In Nepal, this species was first recorded by Schleich & Kästle (2002) from Mahendranagar, Kanchanpur District. Later, Pandey et al. (2016) provided locality records of this species from Chitwan and Jhapa showing its wider distribution in Nepal. We also received photographic evidence of this species from Gobraila Village, Bardia National Park and Dhangadhi, Kailali District.

#### **Common Rat Snake *Ptyas mucosa* (Linnaeus, 1758)**

Individuals were frequently observed within and outside of the park (Image 39). We recorded this species from Majhgaun, Beldandi, Gobraiya, Pipariya, Arjuni, and Parkhedi area. We also frequently rescued them from the buffer zone villages.

#### **Cantor's Black-headed Snake *Sibynophis sagittarius* (Cantor, 1839)**

This species was observed at Majhgaun, Dhakka and Barkaula areas (Image 40).

#### **Homalopsidae (Jan, 1863)**

#### **Common Smooth Water Snake *Enhydris enhydris* (Schneider, 1799)**

The record of this species is based on Shah & Tiwari (2004).

#### **Siebold's Water Snake *Ferania sieboldii* (Schlegel, 1837)**

The record of this species is also based on Shah & Tiwari (2004).

#### **Lamprophiidae Fitzinger, 1843**

#### **Sand Snake *Psammodon cf. condanarus* (Merrem, 1820)**

We recorded a dead specimen from Shuklaphanta grassland during a grassland management activity (Image 41). The first record of this snake in Nepal was from Chitwan National Park by Kramer (1977). Later,



Image 38. Coral Red Kukri Snake *Oligodon kheriensis*.



Image 41. Sand Snake *Psammophis cf. condanarus*.



Image 39. Common Rat Snake *Ptyas mucosa*.



Image 42. Striped Keelback *Amphiesma stolatum*.



Image 40. Cantor's Black-headed Snake *Sibynophis sagittarius*.



Image 43. Checkered Keelback *Fowlea piscator*.

Schleich & Kästle (2002) reported it from Koshi Barrage, Sunsari District ca. 340km east of Chitwan National Park. Our record in ShNP is 550km west from Chitwan National Park. This is the first record for ShNP and third locality record for Nepal; however, we suggest detailed molecular studies of this species for further validation of taxonomic identity.

#### Natricidae Bonaparte, 1838

##### Striped Keelback *Amphiesma stolatum* (Linnaeus, 1758)

This is a commonly sighted snake species in and around ShNP. We frequently observed this species at Majhgaun, Lallare, Beldandi, Pipariya, Dhakka, Arjuni, and Hirapurphanta (Image 42). Road kill individuals on national highway through the park were also observed.

##### Checked Keelback *Fowlea piscator* (Schneider, 1799)

This species is frequently observed in water bodies (lakes, rivers) within the park and agricultural fields in the buffer zone (Image 43). Purkayastha et al. (2018) reallocated this species to the genus *Fowlea*.

#### Elapidae Boi, 1827

##### Common Krait *Bungarus caeruleus* (Schneider, 1801)

This species is most commonly sighted among all other kraits in ShNP. It was also frequently rescued from buffer zone villages, namely, Majhgaun, Pipariya, and Parkhedhi (Image 44).

##### Banded Krait *Bungarus fasciatus* (Schneider, 1801)

One individual was photographed at Majhgaun (Image 45).

##### Monocled Cobra *Naja kaouthia* (Lesson, 1831)

We recorded an individual of this species from Shuklaphanta post. The individual was spotted entering a toilet of the Shuklaphanta post. Records of this species in ShNP are also reported by Schleich & Kästle (2002), Shah & Tiwari (2004), and Kästle et al. (2013).

##### Common Cobra *Naja naja* (Linnaeus, 1758)

This species was recorded from Majhgaun, Hirapurphanta, Beldandi, Shuklaphanta and also rescued from Homestay area, Pipariya and Suksaal areas (Image 46). One dead individual was also observed in Garjamani Village and it was killed when it entered the kitchen.

##### King Cobra *Ophiophagus hannah* (Cantor, 1836)

We observed an adult individual at Bichuwa. We could only photograph posterior part of the snake as it was moving into a dense bush. We also observed a dead



Image 44. Common Krait *Bungarus caeruleus*.



Image 45. Banded Krait *Bungarus fasciatus*.



Image 46. Common Cobra *Naja naja*.



Image 47. Burmese Python *Python bivittatus* swallowing a Spotted Deer.



Image 48. Sal Forest Turtle *Melanochelys tricarinata*.



Image 49. Black Pond Turtle *Melanochelys trijuga*.

individual north of Kalapani area of ShNP. Thapa et al. (2019) mentioned the presence of the King Cobra in 37 districts of Nepal and a single locality record from Far-west/Sudoorpaschim Province. Therefore, our record is the first from the ShNP and second locality for the Far-west Province. The previous nearest King Cobra record from Nigali, Kailali by Thapa et al. (2019) is north-east, ca. 60km away by aerial distance. We also received photographs of a dead King Cobra from Godawari, Kailali District. The forests of Godawari, Kailali is contiguous with Chure/Sivalik area of ShNP with similar habitat. Therefore, it is highly likely that the King Cobra also occurs in the Chure/Sivalik range of ShNP.

### Pythonidae Fitzinger 1826

#### Burmese Python *Python bivittatus* Kuhl, 1820

We recorded this species from Beldandi, Malumela, Majhgaun, Mangalsera, Pipariya, Arjuni, Radhapur, and Hirapurphanta (Image 47). We observed a python swallowing a Spotted Deer *Axis axis* at Shuklaphanta grassland. We also rescued more than 50 individuals from Majhgaun (n=10), Tilkeni (n=12), Khairbhatti (n=15), Gobraiya (n=4), Pipariya (n=1), Katan (n=3), and Baibaha (n=7). This is the largest snake in Nepal. This is the only snake species in Nepal which has been accorded the highest degree of protection under the National Parks and Wildlife Conservation Act, 1973. The occurrence of the Burmese Python in Nepal was first confirmed by O'Shea (1998) from Bardia National Park. Barker & Barker (2008) mentioned three disjunct populations of Burmese Python, viz., Chitwan, Bardia, and Corbett populations; however, according to Shah & Tiwari (2004) and Bhattarai et al. (2017b) the Burmese Pythons are widely distributed in Nepal from <100m to 2800m and even rescued from fringe villages of community forests outside protected areas in Nepal (Bhattarai 2012).

### Viperidae Opperl 1811

#### Russell's Viper *Daboia russelii* (Shaw & Nodder, 1797)

We recorded this species from Barkaula area and Dakhnaghat area. The earlier studies by Schleich & Kästle (2002), Shah & Tiwari (2004), and Kästle et al. (2013) also mentioned the occurrence of Russell's Viper in ShNP.

### Geoemydidae Theobald 1868

#### Sal Forest Turtle *Melanochelys tricarinata* (Blyth 1856)

We recorded this turtle from Malumela, Pipariya, and Shuklaphanta grassland areas. An individual was encountered crossing the patrolling route during an anti-poaching operation between Malumela and Solgaudi Lake (Image 48).

**Black Pond Turtle *Melanochelys trijuga* (Schweigger, 1812)**

The individuals of this species were observed basking on the banks of the lakes inside ShNP. We frequently observed them at Sikari tal (tal=lake), Rani tal, Lami tal, Tara tal, Solgaudi, and Malumela (Image 49).

**Indian Roofed Turtle *Pangshura tecta* (Gray, 1831)**

Frequently observed at Rani tal, Baghmara and Malumela area (Image 50). The species is also frequently seized from local communities when they collect either for food or for sale. The hard shelled turtles and elongated tortoise have higher demands in local market as the businessmen believe them to be a sign of good luck (Bhattarai et al. 2018a).



Image 50. Indian Roofed Turtle *Pangshura tecta*.

**Indian Tent Turtle *Pangshura tentoria* (Gray, 1834)**

We recorded this species basking on the banks of Rani tal and Bahuni River. One dead specimen was also recorded at Chaudhar Khola (Image 51). Based on Schleich & Kästle (2002), we ascertained this species as *Pangshura tentoria circumdata* having a pink ring on its carapace and yellow coloured plastron with irregular black patch. This species is a new record from ShNP.



Image 51. Indian Tent Turtle *Pangshura tentoria*.

**Testudinidae Batsch 1788**

**Elongated Tortoise *Indotestudo elongata* (Blyth, 1854)**

We recorded this species from the foot hills of Chure/ Sivalik in ShNP (Image 52). Earlier records of this species in ShNP was by Shah & Tiwari (2004). This is one of the most sought after species for the illegal pet trade in Nepal.



Image 52. Elongated Tortoise *Indotestudo elongata*.

**Trionychidae Fitzinger 1826**

**Narrow-headed Softshell Turtle *Chitra indica* (Gray, 1831)**

The record of this species in ShNP is based on Kästle et al. (2013).

**Indian Flapshell Turtle *Lissemys punctata* (Bonnaterre, 1789)**

We recorded this species from Malumela, Hirapurphanta, Pipariya, Badenikheda, Rani tal, Shikari tal, and Beldandi (Image 53). Based on the description provided by Aryal et al. (2010), we ascertain this turtle as *L. punctata andersoni*.

**Gangetic Softshell Turtle *Nilssonina gangetica* (Cuvier, 1825)**

We recorded this species from Bahuni River and Chaudhar River. Local people are frequently arrested by



Image 53. Indian Flapshell Turtle *Lissemys punctata*.



Image 56. Mugger Crocodile *Crocodylus palustris*.



Image 54. Gangetic Softshell Turtle *Nilssonina gangetica*.

park authorities during illegal collection of this species (Image 54).

**Peacock Softshell Turtle *Nilssonina hurum* (Gray, 1831)**

We recorded this species from Rani tal, Bahuni River, and Chaudhar River (Image 55). The juveniles of this species have four to six eye rings on the carapace. This species is also heavily poached in the area.

**Crocodylidae Cuvier, 1807**

**Mugger Crocodile *Crocodylus palustris* (Lesson, 1831)**

We recorded this species from Chaudhar River, Bahuni River, Rani tal, Baba tal, Solgaudi, Mahakali River, Shikari taal, Sundariphanta khalla, and Gobriaya nullah (Image 56). We also rescued five mugger crocodiles from human habitation and private fish ponds from Bhasi (n=1), Khairbhatti (n=2), Chandani-Dodhara (n=1), and Gobraiya (n=1).



Image 55. Peacock Softshell Turtle *Nilssonina hurum* (juvenile).

**DISCUSSION**

Our study provided crucial information on the species richness and distribution of herpetofauna in Shuklaphanta National Park and its buffer zone. Out of 71 recorded species, 18 species are new to ShNP. Among the newly recorded 18 species, one snake species *Ahaetulla laudankia* is new to Nepal. The herpetofauna of ShNP (n=71 species) is comparable with other protected areas of the Terai region of Nepal. For example, Zug & Mitchell (1995) and Lamsal (2014) recorded 55 species of herpetofauna from Chitwan National Park. Recently, Pandey et al. (2018) updated the list of snakes of Chitwan National Park with records of 32 species. Bhattarai et al. (2018a) recorded 51 species of herpetofauna from Parsa National Park and GoN (2015) mentioned the occurrence

of 42 species in Bardia National Park. This indicates that the species richness (n=71) in ShNP is relatively higher.

The earlier studies (such as Schleich & Kästle 2002; Shah & Tiwari 2004; Aryal et al. 2010; Kästle et al. 2013) did not provide exact locality information of species present in ShNP. Some other studies (e.g., Shrestha & Sheshtha 2008; Subedi 2011) were confined to gray literature such as dissertations and technical reports. Despite their scientific importance these have not been published for readers widely.

### Conservation concerns

Among the species we compiled, 39 species have been listed in IUCN Red List threat category (Table 1). Among them, one species has been listed as Critically Endangered (CR), one as Endangered (EN), six as Vulnerable (VU), one species as Near Threatened (NT), and 30 species as Least Concern (LC) (IUCN 2019). Two reptiles, namely, Golden Monitor Lizard *Varanus flavescens* and Python *Python* sp. have been accorded the highest degree of protection under the National Parks and Wildlife Conservation Act, 1973 of Nepal. ShNP is the type locality for the agamid lizard *Sitana schleichi* which is endemic to Nepal.

ShNP supports the largest herd of Swamp Deer. Active habitat management has been practiced to hold the population size of Swamp Deer and provide sufficient prey for tigers and leopards. The active habitat management interventions only for select species like tigers, rhinos, swamp deer, however, has caused to cost for survival of herpetofauna. We observed a *Python bivittatus* at Kuwadanda between Barkaula and Syauli posts and a nesting female of *Nilssonina hurum* badly injured due to intentional fire for grassland management. We also recorded several road kills of amphibians and reptiles on the national highway that passes through ShNP. Among the snakes we documented, only six species were venomous, however, all snake species have suffered vindictive killing. Reptiles (especially turtles and monitors) are poached for food. The ShNP has also the problem of feral animals inside the park, the intensity of loss due to feral animals (such as dogs, domestic cats, and cattle) are to be studied on the population dynamics of local herpetofauna. Herpetofauna face severe anthropogenic pressure due to habitat alteration and pesticide use in the buffer zone. Such pressure has extirpated the Gharial *Gavialis gangeticus* from Mahakali River.

We failed to document the endemic lizard *Sitana schleichi* in ShNP and suggest collection based detailed inventory for this species to ascertain its genetic identity with its congeners. We believe the record of False

Cobra *Pseudoxenodon macrops* in ShNP by Schleich & Kästle (2002) was mistakenly included and others (such as Shah & Tiwari 2004; Subedi 2011; Kästle et al. 2013) followed Schleich & Kästle (2002). The locality records of *Pseudoxenodon macrops* in Nepal (except in ShNP) is limited to mid-mountains from >1,000m to almost 3,000m (Santosh Bhattarai pers. obs. 15.viii.2019). Therefore, we delist the occurrence of *Pseudoxenodon macrops* from ShNP. Subedi (2011) reported the occurrence of *Python molurus* in ShNP. We are confident that observations by Subedi (2011) were taxonomic misidentification and we treat all the observations as *Python bivittatus*.

### Conservation implications

The ShNP supports an impressive herpetofauna species richness; however, the observed threats such as intentional killing and poaching of herpetofauna for illegal trade and consumption are of grave concern. Such illegal activities and accelerated killings have depleted some herpetofauna (e.g., the last individual of Gharial from Chaudhar River in ShNP was seized in 1993 when one of the authors of this paper (NS) filed a case against the poacher). Similarly, forest fires during April–May are also common in the area. The impacts of fire on the herpetofauna has not yet been studied in ShNP. The ShNP frequently conducts conservation awareness sessions for local communities focusing on large charismatic species only. We strongly suggest such conservation initiatives must advocate for herpetofauna as well. The east-west highway bisects ShNP and we frequently observed road kills of wildlife. Regular road survey will provide us with quantitative data on species loss due to vehicular movement. Our study provides an updated information on species richness of herpetofauna in ShNP and opens avenue for species-based detailed inventories such as population dynamics, effects of anthropogenic pressures, and forest fires on herpetofauna. The results of the study are also very useful for conservation planning of the park.

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## Varying colour pattern, yet genetically similar: Pebble Crab *Seulocia vittata* (Stimpson, 1858) (Brachyura: Leucosiidae) from the southeastern coast of India

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**Abstract:** Five adult specimens of leucosiid crab *Seulocia vittata* (Stimpson, 1858) were recently collected off the coast of Palk Bay, southern India. Typical morphological examination revealed the presence of two colour patterns: grey and red. Interestingly, molecular analysis based on the barcoding gene cytochrome oxidase sub unit I (COI) revealed that both grey and red colour patterns in *S. vittata* showed 0% sequence divergence between the specimens. This indicates a situation of reverse cryptic behavior in this crab. Surprisingly, the evolutionary and ecological processes leading to the absence of genetic divergence and variation in morphology (colour pattern) in *S. vittata* complex remain to be addressed.

**Keywords:** Colouration in crab, DNA barcoding, leucosiid, Mandapam, molecular phylogeny, Tamil Nadu.

**Tamil abstract:** லுக்ோசிட் நண்டு சிலோசியா விட்டாட்டா (ஸ்டிம்ப்ஸன், 1858), பாக் விரிகுடாவின் கடற்கரையில் (5 மாதிரிகள்) சமீபத்தில் சேகரிக்கப்பட்டன. வழக்கமான உருவ பரிசோதனையின் மூலம் அதன் இரண்டு வண்ண வடிவங்கள்: சாம்பல் மற்றும் சிவப்பு நிறம் இருப்பது கண்டறியப்பட்டது. மேலும், மூலக்கூறு டிஎன்ஏ பார்ஓடிங் சைட்டோக்ரோம் ஆக்சிடேஸ் சப்்யூனிட் 1 (COI) ஐ அடிப்படையாகக் கொண்ட பகுப்பாய்வு மேற்கொண்டபோது சாம்பல் மற்றும் சிவப்பு நிறங்கள் கொண்ட நண்டு வகையின் வேறுபாடு 0% ஆக இருந்தது. இந்த நிலைமை ரிவேர்ஸ் கிரிப்டிக் பிஓஹெவியர் ஐ குறிக்கின்றது. ஆச்சரியப்படும் விதமாக, சி. விட்டாட்டா வகை நண்டுகளில் மரபணு மாறுபாடு மற்றும் உருவவியல் (வண்ண முறை) மாறுபாடு இல்லாததற்கு, அதன் பரிணாம வளர்ச்சி மற்றும் சுற்றுச்சூழல் செயல்முறைகளே காரணமாக இருக்கக்கூடும் என்பது இன்னும் விவாதத்திற்குரிய ஒன்றாகும்.

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**Author details:** SANJEEVI PRAKASH is a marine biologist, specialized with the taxonomy of coral-reef dwelling decapod crustaceans. Currently, he is focusing on the integrative approach such as morphology and molecular taxonomy to understand the cryptic species diversity in the coral reef associated caridean shrimps of the world. AMIT KUMAR is a marine biologist. He has been using molecular markers to understand marine biodiversity. He is also interested in studying how marine organisms respond to predicted climate change stress conditions.

**Author contribution:** SP and AK designed and performed the study. SP identified the species using morphology. AK did the phylogeny. SP and AK interpreted the results and wrote the manuscript.

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## INTRODUCTION

Pebble crab or leucosiid crab belonging to the family Leucosiidae (Samouelle, 1819) is rich in diversity (Ng et al. 2008; Galil & Ng 2015). It mostly inhabits the sandy and silty areas adjacent to seagrass beds, coral reef flats, as well as intertidal areas usually buried in the sand (Naderloo & Apel 2012; Ng & Komatsu 2016). Leucosiid crabs of India have a long history where the key to the Indian *Leucosia* was first provided by Alcock (1896) during an “Investigator” expedition. At present, 97 species belonging to 35 genera of the family Leucosiidae have been reported from India (Trivedi et al. 2018). While revisiting the Leucosiidae classification, Galil (2005) proposed a new genus *Seulocia* which differs from other genera in the shared characters such as third to sixth abdominal somites fused in males and the straight shaft in the first pleopod of males twisted once on its axis. So far, 11 species have been described in this genus (Ng et al. 2008; WoRMS 2019), of which six species *S. cristata* (Galil, 2005), *S. pubescens* (Miers, 1884), *S. pulchra* (Galil, 2005), *S. rhomboidalis* (De Haan, 1841), *S. truncata* (Alcock, 1896), and *S. vittata* (Stimpson, 1858) have been recorded in Indian waters (Trivedi & Vachhrajani 2017; Trivedi et al. 2018). All of these above records were mainly based on the morphological characteristics and lack of information on genetic relatedness among them.

DNA barcoding along with morphological examination has been considered as a useful tool for the validation of species (Madhavan et al. 2020). This method can effectively identify cryptic species due to differences in their genetic character (Bucklin et al. 2007). No such study has been reported for the genus *Seulocia*. Hence, in the present study along with the detailed morphological examination, we used mitochondrial cytochrome oxidase subunit I (COI) gene to validate the taxonomy of *S. vittata* from the southeastern coast of India.

## MATERIALS AND METHODS

The fish landing centre at Mandapam in Ramnad District of Tamil Nadu, India is one of the major landing sites in the southeastern coast of India (9.286°N & 79.153°E). A total of five crab specimens were hand-picked from the freshly discarded by-catch of commercial trawlers at the fish landing during June–July 2019. Specimens were quickly cleaned to remove sediments and photographed (Cannon Powershot G16) in the

field to record fresh colouration. The specimens were preserved in 95% ethanol and brought to Sathyabama Marine Research Station, Rameswaram for further detailed examination.

### Morphological examination

The specimens were examined by comparing key morphological features and photographs described by Galil (2005). Four specimens (3 male, 1 female) were in red colouration (LR) and 1 male was in typical bluish-grey colour (LG) as described by Galil (2005). The carapace length (cl in mm) was measured from the tip of the rostrum in the anterior region to the posterior border of the carapace. The carapace width (cw in mm) was measured from the lateral margins of the carapace. The specimens were then deposited in the national zoological collection of the Marine Biological Regional Centre (MBRC), Zoological Survey of India (ZSI), Chennai, Tamil Nadu, India.

### Molecular identification

One representative each of red and grey coloured specimens was subjected to molecular identification. Total genomic DNA from the propodus/meri region of the major cheliped of the crab was extracted using OMEGA BIO-TEK E.Z.N.A. Blood & Tissue DNA Kit, USA following the manufacturer’s protocol. PCR amplification was done for the mitochondrial cytochrome oxidase subunit I (COI) gene using LCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) primers (Folmer et al. 1994). Each PCR contained 12.5μL 2X PCR master mix (Ampliqon, Denmark), 2.5μL each of the two primers (10nM), and 2.5μL of template DNA (10–20 ng) and water to make a final volume of 25μL. PCR conditions were as follows: initial denaturation at 95°C for 10 min, 35 cycles of 95°C for 45 sec, 50°C for 45 sec, and 72°C for 45 min and final extension at 72°C for 10 min. PCR products were then visualized on 1% agarose and products with the high intensity band were sequenced with ABI Prism 3730 Genetic Analyzer based on BigDye Terminator Chemistry.

Chromatograms were visualized, edited, and contigs were prepared using consensus sequences from both the strands in the BioEdit (Hall 1999). Sequences obtained in the present study were deposited in NCBI GenBank. Sequences of COI from the present study were then compared with published COI sequences of related taxa from NCBI GenBank using BLASTn tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). COI sequences of species of the genus *Seulocia* and other

related genus belonging to the family Leucosiidae were downloaded and aligned in the web version of Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). The alignment consisted of two sequences obtained in the present study (MN786514, MN786515), two published sequences of the genus *Seulocia*: *S. vittata* (MH675982), *S. latirostrata* (MH675981) as ingroup terminals, as well as four sequences from other genera as outgroup terminal: *Leucosia rubripalma* (MH675986), *L. craniolaris* (MH675985), *Euclosiana scitula* (MH675980), and *E. crosnieri* (MH675978). The pairwise genetic distance between the species was determined by the Kimura 2-parameter method (Kimura 1980) using MEGA 7 (Kumar et al. 2016). The phylogenetic tree was constructed using maximum likelihood method based on the Tamura-Nei model in MEGA 7 (Kumar et al. 2016). Bootstrap test was conducted using 1,000 replications to get the best topology from a 75% majority rule consensus tree (Felsenstein 1985).

## RESULTS

### Systematic accounts

Order Decapoda (Latreille, 1802)  
 Infraorder Brachyura (Latreille, 1802)  
 Family Leucosiidae (Samouelle, 1819)  
 Genus *Seulocia* (Galil, 2005)  
*Seulocia vittata* (Stimpson, 1858), Image 1 & 2.

### Restricted Synonymy

*Cancer craniolaris*; Herbst, 1783: 90, pl. 2, fig. 17.  
*Leucosia craniolaris*; Fabricius, 1798: 350 (part); K. Sakai, 1999: 19, pl. 7E.  
*Leucosia vittata* Stimpson, 1858: 159; Shen & Dai, 1964: 28, fig.; Chhappgar, 1968: 609; Chen & Sun, 2002: 436, fig. 197, pl. 16.8.  
*Leucosides craniolaris*; Rathbun, 1910: 310 (part).  
*Leucosia sinica* Shen & Chen, 1978: 80, pl. 2, figs 12, 13, text-fig. 5; Huang, 1994: 580; Chen & Sun, 2002: 440, fig. 199.

**Materials examined:** Grey colouration: MBRC/ZSI D1-609, 11.vii.2019, 1 male, (cl 22mm; cw 20mm), India, Tamil Nadu, Mandapam fish landing site (Palk Bay), 9.286°N & 79.153°E, depth 10–15 m, col. Prakash & Amit Kumar.

Red Colouration: MBRC/ZSI D1-610, 11.vii.2019, 1 male and 1 female, (cl 22 each; cw 19 and 20), same collection data as above.

MBRC/ZSI D1-610, 11.vii.2019, 2 males, (cl 19 and 22; cw 18 and 20), same collection data as above.

### Short description

Carapace sparsely punctate anteriorly, anterior margin tridenticulate with median denticle slightly larger than the adjacent ones. Anterolateral margin with minute beaded lines. Margin of epibranchial angle of carapace finely milled, epimeral margin evenly milled throughout. Posterior margin of carapace slightly rounded in male specimens (Both Grey and Red) and rounded in female specimens (Red). Thoracic sinus deep, pterygostomian region anteriorly defined by scalloped, overhanging and oblique margin. Fused abdominal segment bearing granule medially in male, smooth without granules in females. Merus of the major cheliped periform, tubercles on the lateral margins, few tubercles (3–5) on the dorsal as well as ventral region. The upper margin of carpus and palm smooth and lower margins periform, movable finger with upper margin carinate (both grey and red). Meri of the remaining pereopods bearing beaded lines on the dorsal and ventral margin, carpi prominently carinate dorsally. Propodi of the pereopods carinate in dorsal and ventral margins, dactyli flat and non-carinate on the lateral margins.

### Colouration – Grey (Male)

Carapace bluish-grey, with median dorsal reddish-brown band becoming broader posteriorly (Image 1A–C). Presence of two oblique bands of reddish-brown on each side diverging from the front. Major cheliped meri, carpi and pal with a combination of reddish-brown and bluish-grey band, distal region reddish, fixed and movable finger whitish anteriorly and reddish posteriorly. Meri, carpi, propodi, and dactyli of the remaining pereopods with a combination of reddish and white bands, tip of the dactyli brown to black. Abdominal region almost whitish (Image 1B). Terminal end of the maxillipeds dark bluish-grey (Image 1C).

### Colouration – Red (Male and Female)

Anterior region of carapace bluish-grey, latter half of the carapace brick red to reddish-brown in colour in females (Image 2A–C). In males, carapace with bluish-grey extended to latter half, posterior region of carapace brick-red (Image 2D–F). Oblique bands are not visible on carapace (Image 2D). Dorsal and ventral region of the meri, carpi, and propodi of the major chelipeds brick-red to reddish colour, dactyli of the movable dark red with a whitish tip (Image 2A,B). Meri of the remaining pereopods brick-red in colour, carpi, propodi, and dactyli dark brown to black in colour. Abdomen brick red with big black patch at the centre in both females and males

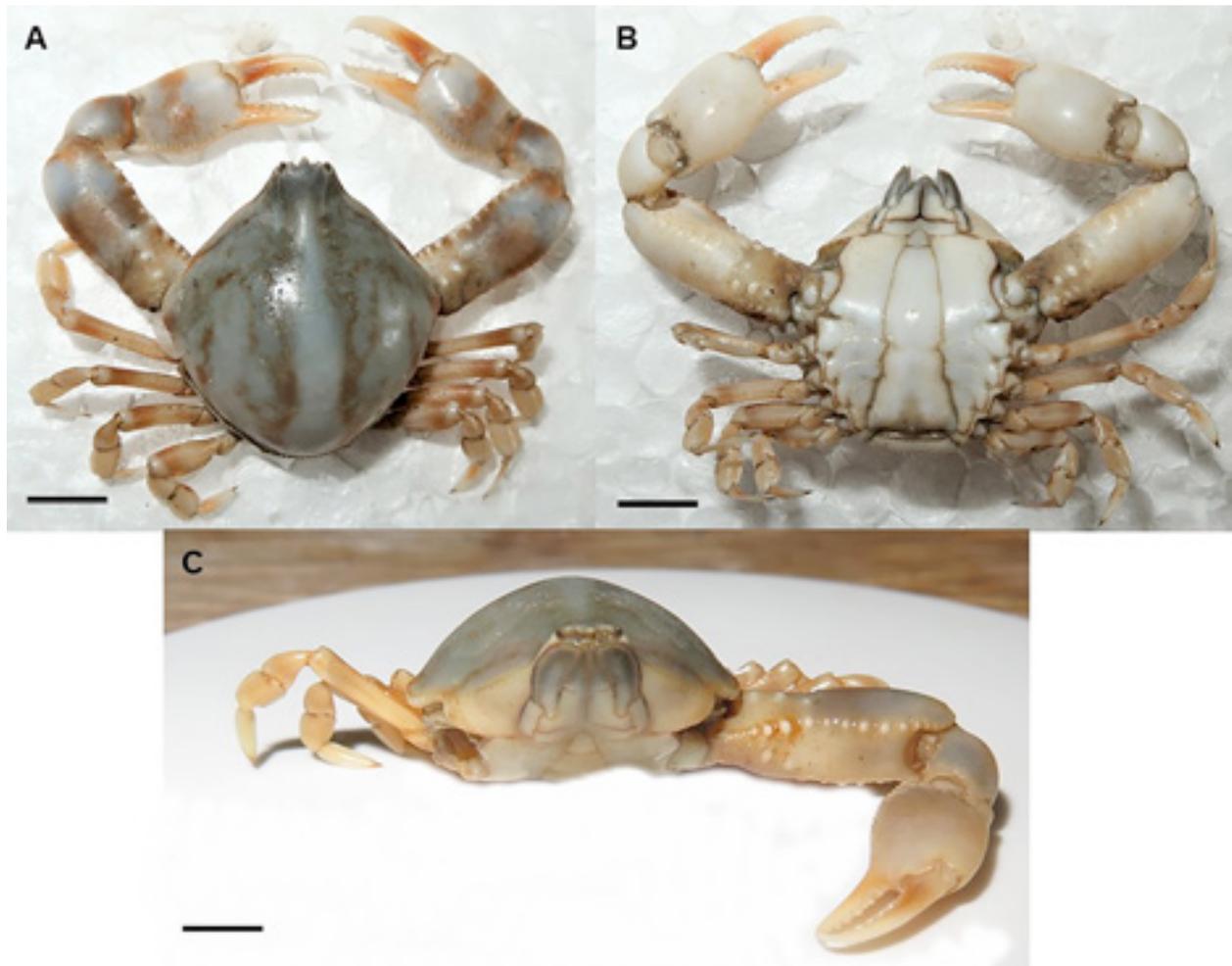


Image 1. Pebble crab *Seulocia vittata* (Stimpson, 1858) (Leucosiidae) male from Palk Bay with grey colour pattern. MBRC/ZSI D1-609. A—dorsal view | B—ventral view | C—frontal view of mouthparts. Scale bar: A–C = 6 mm. © Sanjeevi Prakash.

(Image 2B, 2E). Propodi and carpi of the major cheliped were black ventrally (Image 2B, 2E). Terminal end of the maxillipeds dark bluish-grey in both females and males (Image 2C, 2F).

#### DNA barcode and phylogenetic relationship

BLAST analysis revealed that the sequences for LG and LR exhibited 99.67% and 99.83% similarity with existing COI sequence of *Seulocia vittata* sequence in the NCBI GenBank. The phylogenetic analysis based on ML tree constructed using single mitochondrial gene fragment (COI – 653bp) resulted in tree topology that *S. vittata* (grey and red colour patterns) are closely related to *S. vittata* available in the GenBank (Figure 1). Out of 653 sites, 148 were parsimony informative sites. In the ML tree, all the *S. vittata* clustered together to form a monophyletic clade. In addition, *S. vittata* is sister to its congener comprising *Seulocia latirostrata* and other

related genera *Leucosia* and *Euclosiana* (Figure 1).

Furthermore, the calculated pairwise genetic distance of COI gene fragment using Kimura-2 parameter revealed that LR, LG, and published *S. vittata* in the GenBank have no genetic divergence. However, the genetic distance of 0.177 was calculated between *S. vittata* and *Seulocia latirostrata*, which is comparable to the genetic distance of more than 0.200 with other genera such as *Leucosia* and *Euclosiana* in the family Leucosiidae (Table 1).

#### DISCUSSION

A nomenclatural and taxonomical validation of the brachyuran crabs of the world includes over 6793 species belonging to 1,271 genera and 93 families (Ng et al. 2008). Interestingly, recent advances in the molecular techniques have received greater attention in



Image 2. Pebble crab *Seulocia vittata* (Stimpson, 1858) (Leucosiidae) females and males from Palk Bay – red colour pattern. MBRC/ZSI D1-610. A—Female, dorsal view | B—ventral view | C—frontal view of mouth parts | D—male, dorsal view | E—ventral view | F—frontal view of mouth parts. Scale bar: A–B = 6mm, C = 4mm; D–E = 5mm; F = 4mm. © Sanjeevi Prakash.

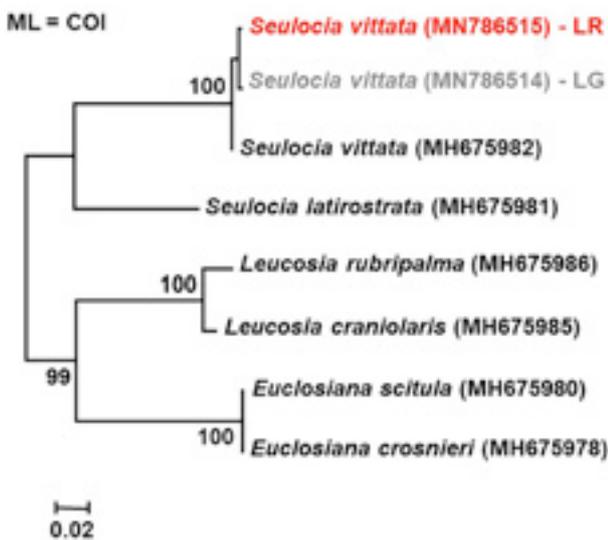


Figure 1. Maximum Likelihood phylogenetic tree of leucosiid crab *Seulocia vittata* (grey and red colouration) based on COI gene sequence data (653 bp, out of which 148 are parsimony informative sites). Numbers above or below the branches indicate bootstrap support based on ML. GenBank accession numbers are mentioned next to the species name in parentheses.

understanding the evolutionary perspectives of marine brachyuran crabs (Hultgren & Stachowicz 2008; Lai et al. 2013; Fratini et al. 2018; Mantelatto et al. 2018;

Chen et al. 2019). The integrative approach of both morphological and molecular analyses offers robust information not only on the taxonomic ambiguity of the species, including cryptic species (Baeza & Prakash 2019) but also in the monitoring of commercial crabs for seafood safety (Rath et al. 2018). In India, the molecular based study in brachyuran crabs was very limited (Vartak et al. 2015; Apreshgi et al. 2016; Ravichandran et al. 2017; Rath et al. 2018; Madhavan et al. 2020). In the present study, we performed the molecular phylogeny as well as identified the pairwise sequence divergence study of *Seulocia vittata* and its congeners based on the COI gene fragment. The ML tree suggests that *S. vittata* was sister to the *S. latirostrata* and other related species that are supported with high bootstrap values.

*Seulocia vittata* has a wide geographic distribution in the Indo-Pacific from Mauritius, India, Singapore, Malaysia, Thailand, Indonesia, China, and Philippines (Galil 2005). The present study represents a rediscovery of *S. vittata* in the south east coast of India after the original report of Alcock (1896) during the “investigator” expedition. Moreover, no information exists on its varying colour pattern. We observed that though the individuals of *S. vittata* differed in their colour pattern (grey and red variants), their genetic distance showed no variation between these two-colour forms. This

**Table 1.** Pairwise genetic distance calculated using Kimura 2-parameter based on COI gene fragment of *S. vittata* and other closely related taxa.

Species name	1	2	3	4	5	6	7
<i>Seulocia vittata</i> (MH675982)							
<i>Seulocia latirostrata</i> (MH675981)	0.177						
<i>Seulocia vittata</i> - LG (Present study)	0	0.177					
<i>Seulocia vittata</i> - LR (Present study)	0	0.177	0				
<i>Leucosia rubripalma</i> (MH675986)	0.214	0.212	0.214	0.214			
<i>Leucosia craniolaris</i> (MH675985)	0.202	0.196	0.202	0.202	0.026		
<i>Euclosiana scitula</i> (MH675980)	0.215	0.204	0.215	0.215	0.188	0.182	
<i>Euclosiana crosnieri</i> (MH675978)	0.215	0.204	0.215	0.215	0.188	0.182	0

indicates a situation of reverse cryptic behaviour in this crab. Surprisingly, the evolutionary and ecological processes leading to the absence of genetic divergence and variation in morphology (colour pattern) in *S. vittata* complex remain to be addressed. There could be several possible explanations: a) *S. vittata* possess the capacity to change colour and camouflage in nature as anti-predatory mechanisms (Stevens et al. 2014); b) the colour variation could be due to ecological adaptation to different depths and habitats such as reefs and open sand flats (Darnell 2012); c) morphological colour variation with low genetic structuring may indicate high dispersal capacities of *S. vittata* throughout the evolutionary history of this species. However, to validate the above hypotheses, extensive sampling efforts and detailed examinations at larger geographical scales are required.

Based on the outcome of this study, we recommend integrative taxonomic and phylogeographic approaches to demonstrate the extent and magnitude of species complexity in the leucosiid crabs. This goal needs to be prioritized as there is a recent increase in the trawl net operations in the south eastern coast of India that could lead to decline in the benthic biodiversity (Purohit 2017). This could cause profound implications in the conservation planning, stock assessment, biogeography, evolutionary as well as the natural history of leucosiids. Lastly, the species complexity in *S. vittata* will provide the opportunity to understand the important mechanisms of speciation among the leucosiid crabs.

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## Grasses of Kundadri Hills in the Western Ghats of Karnataka, India

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**Abstract:** The present communication provides a checklist of grass flora in Kundadri Hill of central Western Ghats, Karnataka. During the exploration, a total of 78 species belonging to two sub-families, 12 tribes, and 43 genera of Poaceae have been documented.

**Keywords:** Checklist, diversity, plant taxonomy, Poaceae.

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**Author details:** HANCHALI UDAYASHANKAR ABHIJIT is DST Inspire awardee and he has very much Interest in Plant taxonomy and diversity assessment. Presently working on diversity and distribution of grasses in the central Western Ghats of Karnataka, India and he was document the three additional grasses to the flora of Karnataka. YELUGERE LINGANAİK KRISHNAMURTHY is Professor in Applied Botany and he has expert in Plant Biodiversity - survey, documentation and conservation and fungal endophytes - Enhancement of plant growth by incorporation of beneficial fungi and fungal metabolite profiling, Exploration and characterization of lichens. Presently four of his students working on plant systematics.

**Author contribution:** HUA - carried out the field work, data collection, identification, photography, herbarium preparation, data interpretation, manuscript writing. YLK - carried out the field work, guided for data interpretation and manuscript writing.

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**INTRODUCTION**

Grasses are morphologically complex and diverse angiosperms and cover one-fifth of the earth’s land surface (Shantz 1954). Twenty-four percent of the earth’s vegetation is comprised of Grass; they grow in both tropical and temperate regions (Jain 1986). Globally, grasses are represented by 10,550 species belonging to 715 genera (Pathak 2013) whereas, in India 1,200 species belonging to 268 genera are documented (Karthikeyan et al. 1989; Moulik 1997). About 430 grass species are endemic to India, among them Indian peninsular region shows 55% endemism (Jain 1986).

India is one among the 17 mega biodiversity nations with 5,000 endemic flora (Nayar 1996). In India, the Western Ghats is one of the hot spots and the second most speciose center for endemism comprising 1,500 endemic flora (Nayar 1980). The Western Ghats is a long mountain range running parallel to the western coast of India. It consists of dense evergreen forests, grasslands, streams, and other wetlands. The region receives heavy precipitation from southwest monsoon and has good edaphic factors giving it a dense plant life (Gadgil 1996). Grass flora has been extensively studied in the northern and southern Western Ghats (Sreekumar & Nair 1991; Kabeer & Nair 2009; Potdar et al. 2012). The central Western Ghats, however, remains largely understudied. Therefore, in the present work, we explored the grass

diversity in Kundadri Hill, a hillock in central Western Ghats of Karnataka that records the highest rainfall in peninsular India (Manjunatha 2015).

**MATERIALS AND METHODS**

**Study area**

The Kundadri Hill (13.553–13.564 °N and 75.156–75.177 °E) is a monolith (Figure 1). It consists of tropical evergreen forest and patches of shola grasslands. Hill top having 17<sup>th</sup> century Jain temple of 23<sup>rd</sup> thirthankara Parshwanath and two ponds that hold water throughout the year. The Hill is located amidst the rain forests of central Western Ghats. It receives 7,620mm average annual rainfall and the average annual temperature is 23.5°C (Manjunatha et al. 2015). This area provides both rocky as well as soil substrates. The rock and its crevices act as a micro habitat for many annual specialized endemic grass communities (Porembski 2000).

**Data collection**

Opportunistic sampling method was used for the collection of grasses. The survey was conducted during August 2017–August 2019. The habitats for sampling was classified according to Bhat & Nagendran (2001) and Kabeer & Nair (2009). Our study site consisted of open areas, grasslands, road cuttings, rock crevices,



Figure 1. Study area -- Kundadri Hill.

forest area, moist places, shady places, and stream sides. Specimens were collected and identified using floras and research papers (Bor 1960; Bhat & Nagendran 2001; Potdar et al. 2012). Herbaria was prepared as described by Rao & Sharma (1990). The documented grasses are classified on the basis of Bor classification (1960) Voucher specimens are deposited in the herbarium, department of applied botany, Kuvempu University, Karnataka, India.

## RESULT AND DISCUSSION

The floristic assessment of the family Poaceae in different regions of the study area revealed a total of 78 species of 43 genera belonging to 12 tribes and two subfamilies. A detailed checklist of grass species, their subfamilies, tribe and habitat of each species (Table 1) and photographs of the selected species (Images 1–4) are provided. The subfamily Panicoideae shows 47 species of 27 genera and two tribes, of which 26 species belong to tribe Andropogoneae including four varieties and 21 species belong to tribe Paniceae. Genus *Ischaemum* shows maximum number of species (seven). The subfamily Pooideae shows 31 Species of 16 genera and 10 tribes, in that six species belong to Arundinellae, 10 Species belong to Eragrostaeae, and three species belong to both Chloridae and Isachneae. Two species in Garnotieae, Babuseae, and Sporoboleae and the tribe Aristidae, Centothecaeae, Oryzaeae have one species each. Genus *Arundinella* shows the maximum number of species (five) (Figure 2 & 3). *Apluda mutica* L. and *Indopoa paupercula* (Stapf) Bor., are the two monotypic genera recorded during the study. This indicates the small geographical area of Kundadri Hill having rich grass diversity. Open area, moist area, grasslands and, rock crevices are the common habitats in the study area. We documented 26% from the open area, 19% from grasslands, 18% from rock crevices, 13% from moist wet area, 9% from road cuttings, 8% from moist shady area, and 5% and 2% from forest area and stream side, respectively. According to IUCN Red List status all the documented species come under Not Evaluated (NE) category but regionally 27 species are rare and remaining 51 species are common to the study site (Figure 4). Open area, grasslands and rock crevices are the most suitable habitats for grasses in the study area.

Grass flora of northern Western Ghats and southern Western Ghats are well studied (Sreekumar & Nair 1991; Kabeer & Nair 2009; Potdar et al. 2012). Thomas et al. (2012) worked on Chasmophytic grasses of Vellinagiri Hills located in southern Western Ghats.

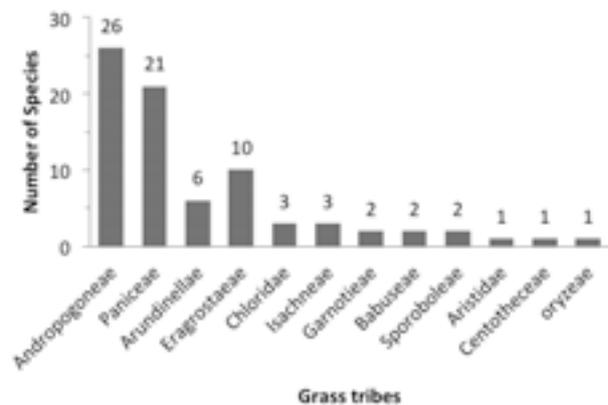


Figure 2. Tribes showing number of species.

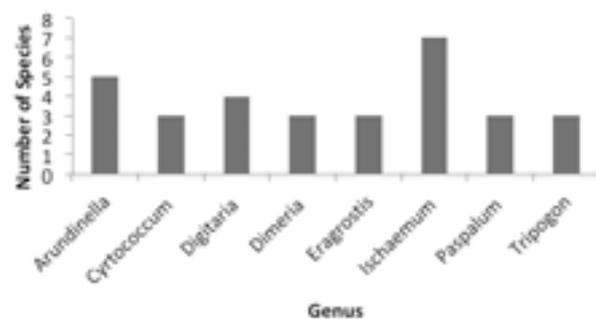


Figure 3. Top eight genera showing maximum number of species.

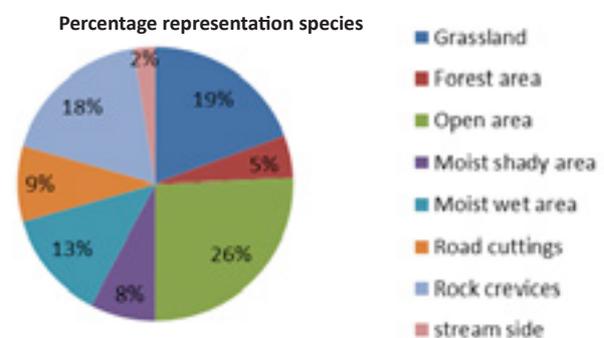


Figure 4. Habitat distribution of documented species.

They documented 30 species of wild chasmophytic grasses belong to 23 genera and dominated by four species of genera *Eragrostis*. Barbhuiya et al. (2013) recorded grasses in Barak Valley of southern Assam around 6,922km<sup>2</sup> area and provide the checklist of 98 grass species belonging to 49 genera. Region exhibit 32% of grass flora of Assam State. Various authors have explored the flora of central Western Ghats (Saldanha & Nicholson 1976; Yoganarasimhan et al. 1982; Saldanha 1984; Murthy 1990; Ramaswami et al. 2001). Poaceae, however, is underrepresented in these reports except

Table 1. Checklist of documented grasses in Kundadri Hill .

	Botanical name	Subfamily	Tribe	Habitat
1	<i>Acroceras munroanum</i> (Balansa) Henr.	Panicoideae	Paniceae	Moist shades
2	<i>Alloterospis cimicina</i> (L.) Stapf	Panicoideae	Paniceae	Open area
3	<i>Apluda mutica</i> L.	Panicoideae	Andropogoneae	Road cuttings
4	<i>Aristida setaceae</i> Retz.	Pooideae	Aristideae	Open area
6	<i>Arthraxon hispidus</i> (Thunb.) Makino	Panicoideae	Andropogoneae	Rock crevices
5	<i>Arthraxon hispidus</i> var. <i>santapau</i> (Bor) Welzen	Panicoideae	Andropogoneae	Rock crevices
7	<i>Arthraxon lanceolatus</i> (Roxb.) Hochst	Panicoideae	Andropogoneae	Moist shades
8	<i>Arundinella ciliata</i> (Roxb.) Nees ex Miq.	Pooideae	Arundinelleae	Road cuttings
9	<i>Arundinella nepalensis</i> Trin.	Pooideae	Arundinelleae	Stream side
10	<i>Arundinella pumila</i> (Hochst. ex A.Rich.) Steud.	Pooideae	Arundinelleae	Road cuttings
11	<i>Arundinella purpurea</i> Hochst. ex Steud.	Pooideae	Arundinelleae	Grasslands
12	<i>Arundinella tuberculata</i> Munro ex Lisboa	Pooideae	Arundinelleae	Grasslands
13	<i>Bambusa arundinacea</i> (Retz.) Willd.	Pooideae	Bambuseae	Forest area
14	<i>Capillipedium huegelii</i> (Hack.)Stapf	Panicoideae	Andropogoneae	Moist shades
15	<i>Centotheca lappacea</i> (L.) Desv.	Pooideae	Centothecae	Open area
16	<i>Chloris barbata</i> Sw.	Pooideae	Chlorideae	Open area
17	<i>Chrysopogon aciculatus</i> (Retz.) Trin.	Panicoideae	Andropogoneae	Open area
18	<i>Chrysopogon hackeli</i> (Hook.f.) C.E.C.Fisch.	Panicoideae	Andropogoneae	Grasslands
19	<i>Coelachne simpliciuscula</i> (Wight & Arn. Ex Steud.) Munro ex Benth	Pooideae	Isachneae	Moisty area
20	<i>Cymbopogon martinii</i> (Roxb.)	Panicoideae	Andropogoneae	Grasslands
21	<i>Cynodon dactylon</i> L.	Pooideae	Chlorideae	Open area
22	<i>Cynodon radiatus</i> Roth ex Roem. & Schult	Pooideae	Chlorideae	Open area
23	<i>Cyrtococcum deccanense</i> Bor.	Panicoideae	Paniceae	Moist shades
24	<i>Cyrtococcum longipes</i> (Wight & Arn. ex Hook.f.) A.Camus	Panicoideae	Paniceae	Moist shades
25	<i>Cyrtococcum oxyphyllum</i> (Steud.) Stapf	Panicoideae	Paniceae	Moist shades
26	<i>Dactyloctenium aegyptium</i> (L.) Willd.	Pooideae	Eragrosteae	Open area
27	<i>Dichanthium annulatum</i> (Forssk.) Stapf	Panicoideae	Andropogoneae	Open area
28	<i>Digitaria bicornis</i> (Lam.) Roem. & Schult.	Panicoideae	Paniceae	Open area
29	<i>Digitaria ciliaris</i> (Retz.) Koeler	Panicoideae	Paniceae	Open area
30	<i>Digitaria longiflora</i> (Retz.) Pers.	Panicoideae	Paniceae	Grasslands
31	<i>Digitaria Radicosa</i> (J.Prisl.) Miq.	Panicoideae	Paniceae	Open area
32	<i>Dimeria lawsonii</i> (Hook.f.) C.E.C.Fisch.	Panicoideae	Andropogoneae	Grasslands
35	<i>Dimeria ornithopoda</i> Trin.	Panicoideae	Andropogoneae	Road cuttings
33	<i>Dimeria stapfiana</i> C.E.Hubb. ex Pilger	Panicoideae	Andropogoneae	Rock crevices
34	<i>Eleusine indica</i> (L.) Gaertn.	Pooideae	Eragrosteae	Open area
36	<i>Elytrophorus spicatus</i> (Willd.) A. Camus	Pooideae	Eragrosteae	Open area
37	<i>Eragrostis atrovirens</i> (Desf.) Trin. ex Steud.	Pooideae	Eragrosteae	Moisty area
38	<i>Eragrostis japonica</i> (Thunb.) Trin.	Pooideae	Eragrosteae	Open area
39	<i>Eragrostis uniolooides</i> (Retz.) Nees ex Steud.	Pooideae	Eragrosteae	Open area
40	<i>Eulalia trispicata</i> (Schult.) Henrard	Panicoideae	Andropogoneae	Grasslands
41	<i>Garnotia arundinacea</i> Hook.f.	Pooideae	Garnotieae	Road cuttings
42	<i>Garnotia tenella</i> (Arn. ex Miq.) Janowski	Pooideae	Garnotieae	Rock crevices
43	<i>Glyphochloa forficulata</i> (C.E.C.Fisch.) Clayton	Panicoideae	Andropogoneae	Rock crevices
44	<i>Glyphochloa mysorensis</i> (S.K.Jain & Hemadri) Clayton	Panicoideae	Andropogoneae	Rock crevices

	Botanical name	Subfamily	Tribe	Habitat
45	<i>Heteropogon contortus</i> (L.) P.Beauv. ex Roem. & Schult.	Panicoideae	Andropogoneae	Grasslands
46	<i>Imperata cylindrica</i> (L.) Raeusch. *	Panicoideae	Andropogoneae	Open area
47	<i>Indopoa paupercula</i> (Stapf) Bor	Pooideae	Eragrosteae	Rock crevices
48	<i>Isachne globosa</i> (Thunb.) Kuntze	Pooideae	Isachneae	Moisty area
49	<i>Isachne gracilis</i> C.E.Hubb.	Pooideae	Isachneae	Moisty area
50	<i>Ischaemum commutatum</i> Hack.	Panicoideae	Andropogoneae	Grasslands
51	<i>Ischaemum indicum</i> (Houtt.) Merr.	Panicoideae	Andropogoneae	Grasslands
52	<i>Ischaemum rugosum</i> Salisb.	Panicoideae	Andropogoneae	Moisty area
53	<i>Ischaemum semisagittatum</i> Roxb.	Panicoideae	Andropogoneae	Road cuttings
54	<i>Ischaemum timorensis</i> Kunth	Panicoideae	Andropogoneae	Grasslands
55	<i>Ischaemum tumidum</i> Stapf ex Bor	Panicoideae	Andropogoneae	Grasslands
56	<i>Ischaemum zeylanicum</i> Bor.	Panicoideae	Andropogoneae	Rock crevices
57	<i>Jansenella griffithiana</i> (Mull. Stuttg.) Bor	Pooideae	Arundinelleae	Road cuttings
58	<i>Leersia hexandra</i> Sw.	Pooideae	Oryzae	Moisty area
59	<i>Ochlandra scriptoria</i> (Dennst.) C.E.C.Fisch	Pooideae	Bambuseae	Forest area
60	<i>Oplismenus compositus</i> (L.) P.Beauv.	Panicoideae	Paniceae	Forest area
62	<i>Panicum curviflorum</i> Hornem.	Panicoideae	Paniceae	Moisty area
63	<i>Panicum repens</i> L.	Panicoideae	Paniceae	Moisty area
64	<i>Paspalum canarae</i> var. <i>canarae</i>	Panicoideae	Paniceae	Rock crevices
65	<i>Paspalum canarae</i> var. <i>fimbriatum</i> (Bor) Veldkamp	Panicoideae	Paniceae	Rock crevices
66	<i>Paspalum conjugatum</i> P.J.Bergius	Panicoideae	Paniceae	Open area
67	<i>Paspalum scrobiculatum</i> L.	Panicoideae	Paniceae	Moisty area
68	<i>Pennisetum hohenackeri</i> Hochst. ex Steud	Panicoideae	Paniceae	Moisty area
69	<i>Pennisetum purpureum</i> Schumach. *	Panicoideae	Paniceae	Open area
61	<i>Pseudechinolaena polystachya</i> (Humb., Bonpl. & Kunth) Stapf	Panicoideae	Paniceae	Forest area
70	<i>Sacciolepis indica</i> (L.) Chase	Panicoideae	Paniceae	Moisty area
71	<i>Setaria pumila</i> (Poir.) Roem & Schult.	Panicoideae	Paniceae	Grasslands
72	<i>Sporobolus fertilis</i> (Steud.) Clayton	Pooideae	Sporoboleae	Stream side
73	<i>Sporobolus piliferus</i> (Trin.) Kunth	Pooideae	Sporoboleae	Open area
74	<i>Themida tremula</i> (Nees ex Steud.) Hack	Panicoideae	Andropogoneae	Grasslands
75	<i>Themida triandra</i> Forssk.	Panicoideae	Andropogoneae	Grasslands
77	<i>Tripogon bromoides</i> Roem. & Schult.	Pooideae	Eragrosteae	Rock crevices
76	<i>Tripogon capillatus</i> Jaub. & Spach	Pooideae	Eragrosteae	Rock crevices
78	<i>Tripogon lisboae</i> Stapf	Pooideae	Eragrosteae	Rock crevices

\* non-native species.

for Bhat & Nagendran (2001), who have explored grasses and sedges in Dakshina Kannada and Udupi districts which act as a first grass flora of central Western Ghats. We recorded 78 species of grasses in our study site which is just around 155ha. This represents 6.5% of grass species recorded in India. This indicates the richness of grass species in the region. Much of the documented species are recorded in habitats like rock crevices and grasslands which are prone to change in land (Figure 4). Habitat disturbance is known to alter grassland species

composition (Joy 1992). Various disturbance factors like livestock grazing, ecotourism, and development affect Kundadri Hills. Similarly, threats like urbanization, encroachment, agricultural intensification, resource exploitation are plaguing the Western Ghats region (Gunawardene et al. 2007). Therefore, we stress the importance of conservation of these species and the habitat.



Image 1. A—*Arundinella ciliata* | B—*Arundinella pumila* | C—*Arundinella purpurea* | D—*Dactyloctenium aegyptium* | E—*Digitaria Radicosa* | F—*Dimeria stapfiana*.



Image 2. A—*Chrysopogon aciculatus* | B—*Garnotia tenella* | C—*Eulalia trispicata* | D—*Themida tremula* | E—*Themida triandra* | F—*Leersia hexandra*.



Image 3. A—*Setaria pumila* | B—*Tripogon lisboae* | C—*Paspalum canarae* var. *canarae* | D—*Paspalum scrobiculatum* | E—*Jansenella griffithiana* | F—*Sacciolepis indica*.



Image 4. A—*Ischaemum indicum* | B—*Eleusine indica*. | C—*Indopoa paupercula* | D—*Glyphochloa mysorensis* | E—*Heteropogon contortus* | F—*Pennisetum hohenackeri*.



Image 5. Spikelets diversity: A—*Apluda mutica* | B—*Arthraxon hispidus* var. *santapau* | C—*Dichanthium annulatum* | D—*Dimeria lawsonii* | E—*Glyphochloa forficulata* & *G. mysorensis* | F—*Ischaemum rugosum*.



Image 6. A—Kundadri Hill | B—adjacent hill of Kundadri | C—Jain temple at hill top | D & E—different habitats of grasses | F—collection of grasses.

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## Comparative phytosociological assessment of three terrestrial ecosystems of Wayanad Wildlife Sanctuary, Kerala, India

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**Abstract:** Phytosociological studies were conducted in three vegetation types in the WS II area of Wayanad Wildlife Sanctuary. In each vegetation type, 85 quadrats (10 x 10 m) were laid to quantify the vegetation. Natural forest showed comparatively higher species richness than plantation and vayal (swamps/low lying grassland). In natural forest 96 plant species were present while it was 70 and 66 respectively in plantation and vayal. Fabaceae was the dominant family in all the three vegetation types. The natural forest was dominated by *Chromolaena odorata*, followed by *Lantana camara*, *Mimosa pudica*, *Terminalia elliptica*, *Glycosmis pentaphylla*. In the plantations, *Chromolaena odorata*, *Tectona grandis*, *Mimosa pudica* and *Glycosmis pentaphylla* showed dominance. The vayal was dominated by *Arundinella leptochloa*. The second most dominant species in the vayal was *Chromolaena odorata*. Other dominant species were *Kyllinga nemoralis* and *Sporobolus tenuissimus*. Among the three, vayal recorded the highest Simpson Diversity Index. The highest Berger-Parker Dominance Index value in plantation indicates the presence of dominant species. Natural forests recorded highest Margalef Richness Index and the least was in vayal. The highest Pielou's Wiener Equitability Index in vayal indicated all species are evenly distributed.

**Keywords:** Invasive alien species, phytosociology, Simpson Diversity Index, Wayanad Wildlife Sanctuary, Western Ghats.

**Abbreviations:** C—Climber | H—Herb | IVI—Important Value Index | NF—Natural Forest | S—Shrub | T—Tree | WS—Wildlife Sanctuary | WS II—Wildlife Sanctuary II.

**സംഗ്രഹം:** വയനാട് വന്യജീവി സങ്കേതത്തിലെ WS II പ്രദേശത്തെ മൂന്ന് ആവാസ വ്യവസ്ഥകളിൽ ഫൈറ്റോസോളജിക്കൽ പഠനങ്ങൾ നടത്തി. ഈ ഓരോ ആവാസ വ്യവസ്ഥകളിലും, സസ്യങ്ങളെ കണക്കാക്കാൻ വേണ്ടി 85 ക്വാഡ്രാറ്റുകൾ (10x10 m) സ്ഥാപിച്ചു. തോട്ടം, വയൽ (ചതുപ്പുകൾ / താഴ്ന്ന പുൽമേടുകൾ) എന്നിവയേക്കാൾ താരതമ്യേന ഉയർന്ന ഇനം സസ്യ സമ്പന്നത പ്രകൃതിദത്ത വനങ്ങളിൽ കാണപ്പെട്ടു. പ്രകൃതിദത്ത വനങ്ങളിൽ 96 ഇനം സസ്യങ്ങളും തോട്ടത്തിലും വയലിലും യഥാക്രമം 70 ഉം 66 ഉം സസ്യ ഇനങ്ങളും കണ്ടെത്തി. മൂന്ന് ആവാസ വ്യവസ്ഥകളിലും ഫാബാസിയായിരുന്നു പ്രധാനപ്പെട്ട സസ്യ കുടുംബം. പ്രകൃതിദത്ത വനത്തിൽ ക്രോമോളീന ഒഡോറാറ്റയും, ലന്താന കമാറ, മിമോസ പുഡിക്ക, ടെർമിനാലിയ എലിപ്റ്റിക്ക, ഗ്ലൈക്കോസിസ് പെന്റാഫില്ല എന്നിവയാണ് മുഖ്യമായും കാണപ്പെട്ടത്. തോട്ടങ്ങളിൽ, ക്രോമോളീന ഒഡോറാറ്റ, ടെക്ടോണ ഗ്രാൻഡിസ്, മിമോസ പുഡിക്ക, ഗ്ലൈക്കോസിസ് പെന്റാഫില്ല എന്നിവ ആധിപത്യം പ്രകടമാക്കി. വയലിൽ ആധിപത്യം കാണിച്ചത് അരുണ്ടിനെല്ല ലെപ്റ്റോക്ലോവയാണ്. വയലിലെ ഏറ്റവും പ്രബലമായ രണ്ടാമത്തെ സസ്യ ഇനം ക്രോമോളീന ഒഡോറാറ്റയാണ്. കില്ലിംഗ നെമോലിസ്, സ്പോറോബോളസ് സെനുസിസിമസ് എന്നിവയായിരുന്നു മറ്റ് പ്രധാന സസ്യ ഇനങ്ങൾ. മൂന്ന് ആവാസ വ്യവസ്ഥകളിൽ വച്ച്, ഏറ്റവും ഉയർന്ന സിംസൺ വൈവിധ്യ സൂചിക രേഖപ്പെടുത്തിയത് വയലിലാണ്. പ്രബലമായ ജീവിവർഗങ്ങളുടെ സാന്നിധ്യം കാരണം തോട്ടത്തിലാണ് ഏറ്റവും ഉയർന്ന ബർഗർ-പാർക്കർ ആധിപത്യ സൂചിക രേഖപ്പെടുത്തിയത്. ഏറ്റവും ഉയർന്ന മാർഗ്ഗലേഫ് സമ്പന്ന സൂചിക രേഖപ്പെടുത്തിയത് പ്രകൃതിദത്ത വനങ്ങളിലാണ്, ഏറ്റവും കുറവ് വയലിലും. വയലിലെ ഉയർന്ന പിയാലോയുടെ വീനർ ഇക്വിറ്റബിലിറ്റി സൂചിക സൂചിപ്പിക്കുന്നത് എല്ലാ ഇനങ്ങളും തുല്യമായി വിതരണം ചെയ്യപ്പെടുന്നു എന്നാണ്.

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## INTRODUCTION

Forests are the principal bio-resources and repositories of natural wealth that support human well-being and ecological sustainability (Sarkar 2016). Phytosociological studies are necessary for protecting the biodiversity and natural plant communities (Rao et al. 2015). These are very essential components for understanding the changes accomplished in the past and future (Hamzaoglu 2006). The environmental safety of a country depends on the health of its forest area (Lloyd & Ghelard 1964) as it is the forest ecosystems which allocate disparate share to the world's biodiversity (Battles et al. 2001). For the conservation of biodiversity, it is crucial to attain forest sustainability (Chaubey et al. 1988). It is proven that long-term sustainability of forest ecosystems is greatly related to plant diversity and their phytosociological attributes. Most of the forests in the world today are under extensive anthropogenic disturbances and require careful management intervention to maintain overall biodiversity and sustainability (Kumar et al. 2006). As plants provide both food and habitat for other organisms (Das et al. 2015), the total forest diversity is a dependent factor of plant diversity. The overall strength of the forest rests on its plant composition, and hence the information on its composition, diversity and ecological aspects is of primary importance in conservation planning and implementation.

Tree species control the growth of other vascular plants as they regulate sunlight availability of the forest floor. Analysis and estimation of tree diversity, through which a combination of physical habitat, vegetation, physiognomy, species composition and community relationship are unlocked, are useful datasets in forest management interventions (Battles et al. 2004). The inherent variation within communities and ecosystems must be documented and used as base-line data to effectively predict the outcome of disturbances, such as regeneration and harvest methods on floristic diversity and richness (Sarkar 2015).

## MATERIALS AND METHODS

### Study area

The study was carried out in Wayanad Wildlife Sanctuary (WWS), Kerala State located in southern India, between October 2016 and February 2017. WWS is spread over to 344km<sup>2</sup> and comprises two discontinuous land areas of 77.67km<sup>2</sup> (WS-I) and 266.77 km<sup>2</sup> (WS-II)

(Figure 1). The larger of these two, WS-II lies within the geographical extremes of 11°35'–11°49'N and 76°13'–76°27'E. The other area WS-I lies within 11°50'–11°59'N and 76°02'–76°7'E. The phytosociological study was done in WS-II which has been divided into three forest ranges, namely, Muthanga, Kurichiat, and Sulthan Bathery. The dominant natural vegetation here is characterized by moist and dry deciduous forest (Image 1), teak and eucalyptus plantations (Image 2), and bamboo brakes (Management Plan 2012–2022). Swamps, which are low lying grasslands are spread over 715.79ha. The land area locally known as vayals (Image 3), represent an edaphic climax with its deep clayey soils and are waterlogged during the monsoon, but sustain grasses throughout the year.

The quadrat method was employed for phytosociological analysis of all vegetation. Three ecosystems, viz., natural forest (NF), plantation, and swamps/vayal (low lying grasslands) were compared. In each vegetation type, 85 quadrats (10 × 10 m) were randomly laid to quantify the tree vegetation. Tree species found within each quadrat were photographed. Those plants which could not be immediately identified were recorded by their vernacular names (information from range officer, beat officer, forest guards, and local people). These species were later identified and their scientific names recorded by consulting dendrologists, books, articles, and internet. The other vegetation inside the 10 × 10 m quadrat was further surveyed using 2 × 2 m nested quadrats. In the nested quadrats, for all the species identity, origin (native or alien), growth form (herb, shrub, and climber), and abundance of other vascular plant species were recorded. In order to analyse the diversity of tree vegetation, frequency, relative frequency, density, and relative density were calculated using the following formulae.

$$\text{Density (D)} = \frac{\text{Number of individuals}}{\text{Hectare}}$$

$$\text{Relative Density (RD)} = \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all species}} \times 100$$

$$\text{Abundance (A)} = \frac{\text{Total number of individuals of a species in all quadrats}}{\text{Number of quadrats of occurrence of the species}}$$

$$\text{Frequency (F)} = \frac{\text{Number of quadrats of occurrence of the species}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Relative frequency (RF)} = \frac{\text{Frequency of individual species}}{\text{Sum of frequency of all species}} \times 100$$

Importance value index (IVI) was calculated by adding relative frequency, relative density and relative

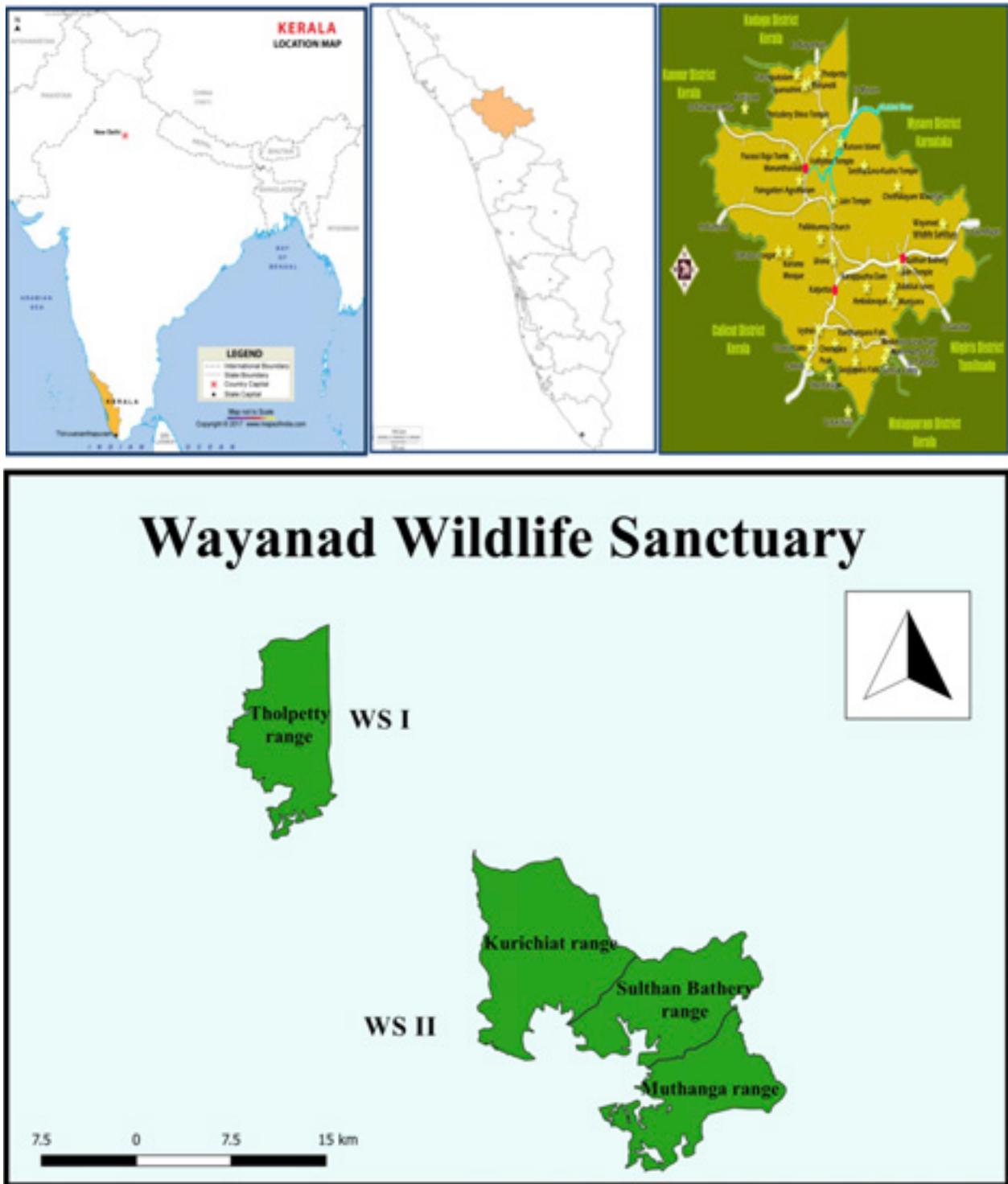


Figure 1. Location map of the study area.

basal area.

Species richness was calculated according to Margalef (1958). Diversity was calculated using Simpson’s diversity index (Simpson 1949). The evenness

was calculated in terms of Pielou’s equitability index (Pielou 1969). Dominance was calculated using Berger-Parker dominance index (Berger & Parker 1970).



Image 1. Natural forest in WS II.



Image 2. Plantation in WS II.



Image 3. Vayal in WS II.

## RESULTS AND DISCUSSION

Overall 129 plant species representing 111 genera were recorded from the three ecosystems (Table 2). Of these, 55 were trees, 24 were shrubs, 35 herbs, and 12 climbers (Table 1). Natural forest showed comparatively higher species richness than plantation and vayal. In natural forest there were 96 plant species. Plantation and vayal had 70 and 66 plant species, respectively. The species recorded in natural forest represented 84 genera in 46 families (Table 1). Fabaceae was the dominant family across the three ecosystems (Figure 2). In the natural forest alone, Fabaceae was represented by 12 species. The other dominant families were Poaceae, Asteraceae, Caesalpinaceae, Combretaceae, Verbenaceae and Euphorbiaceae.

Among the tree species *Anogeissus latifolia*, *Butea monosperma*, *Cassia fistula*, *Lagerstroemia microcarpa*, *Lannea coromandelica*, *Naringi crenulata*, *Olea dioica*, *Pterocarpus marsupium*, *Shorea roxburghii*, *Syzygium cumini*, *Tabernamontana alternifolia*, *Tectona grandis*, *Terminalia bellirica*, and *T. elliptica* were seen in all the three vegetation types. *Aporosa cardiosperma*, *Carallia brachiata*, *Dalbergia lanceolaria*, *Diospyros melanoxylon*, *Elaeocarpus variabilis*, *Gmelina arborea*, *Hydnocarpus pentandra*, *Miliusa tomentosa*, *Pongamia pinnata*, *Streblus asper*, and *Terminalia paniculata* were observed only in NF. In vayals, the trees, namely, *Careya arborea* and *Trewia nudiflora* were seen. In plantations, only *Ailanthus triphysa*, *Elaeocarpus tuberculatus*, *Mallotus tetracoccus*, and *Ziziphus mauritiana* were present.

*Biophytum reinwardtii* var. *reinwardtii*, *Crassocephalum crepidioides*, *Curculigo orchioides*,

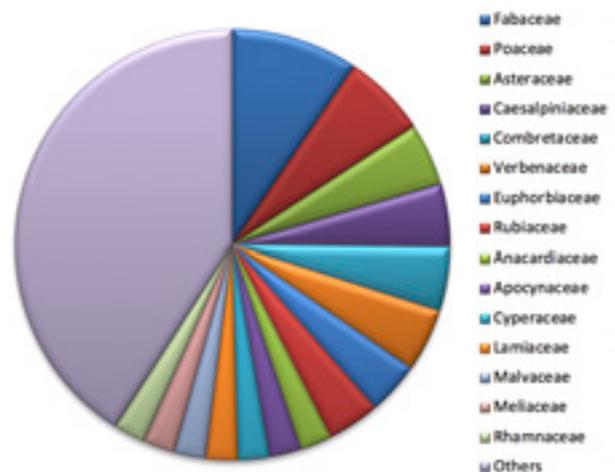


Figure 2. Family wise plant species in Wayanad WS.

**Table 1. Vegetation-type-wise distribution of species, genera, and families.**

	Herb	Shrub	Tree	Climber	Total no. of plant species	Family	Genus
Natural forest	17	21	46	9	96	46	84
Plantation	15	16	30	6	70	36	59
Vayal	26	10	24	3	66	31	60

**Table 2. List of all plant species in the WS II of sanctuary.**

	Binomial	Category	Family	NF	Plantation	Vayal
1	<i>Ageratum conyzoides</i> L.*	Herb	Asteraceae	+	-	+
2	<i>Ailanthus triphysa</i> (Dennst.) Alston	Tree	Simaboubaceae	-	+	-
3	<i>Annona squamosa</i> L.*	Tree	Annonaceae	+	-	+
4	<i>Anogeissus latifolia</i> Wall	Tree	Combretaceae	+	+	+
5	<i>Aporosa cardiosperma</i> (Gaertn.) Merr.	Tree	Euphorbiaceae	+	-	-
6	<i>Arundinella leptochloa</i> Hook.f.	Herb	Poaceae	-	-	+
7	<i>Axonopus compressus</i> P. Beauv.	Herb	Poaceae	-	-	+
8	<i>Barleria mysorensis</i> Heyne	Shrub	Acanthaceae	+	+	-
9	<i>Bauhinia malabarica</i> Roxb.	Tree	Caesalpiniaceae	-	-	+
10	<i>Bauhinia racemosa</i> Lam.	Tree	Caesalpiniaceae	+	+	-
11	<i>Biophytum reinwardtii</i> (Zucc.) Klotzsch	Herb	Oxalidaceae	+	+	+
12	<i>Butea monosperma</i> (Lam.) Taub.	Tree	Fabaceae	+	+	+
13	<i>Caesalpinia mimosoides</i> Lam.	Climber	Caesalpiniaceae	+	-	-
14	<i>Calotropis gigantea</i> (L.) W.T. Aiton	Shrub	Asclepiadaceae	-	-	+
15	<i>Calycopteris floribunda</i> (Roxb.) Lam.	Climber	Combretaceae	+	+	-
16	<i>Canthium coromandelicum</i> (Burm.f.) Alston	Shrub	Rubiaceae	+	-	-
17	<i>Carallia brachiata</i> Lour. Merr.	Tree	Rhizophoraceae	+	-	-
18	<i>Cardiospermum halicacabum</i> L.	Climber	Sapindaceae	+	+	-
19	<i>Careya arborea</i> Roxb.	Tree	Lecythidaceae	-	-	+
20	<i>Carmona retusa</i> (Vahl) Masamune*	Shrub	Boraginaceae	+	+	-
21	<i>Caryota urens</i> L.	Tree	Palmae	+	+	-
22	<i>Cassia fistula</i> L.	Tree	Caesalpiniaceae	+	+	+
23	<i>Catunaregam spinosa</i> (Thunb.) Tirveng.	Shrub	Rubiaceae	+	+	+
24	<i>Centella asiatica</i> (L.) Urb.	Herb	Umbelliferae	+	-	-
25	<i>Chamaecrista absus</i> (L.) H.S.Irwin & Barneby	Herb	Caesalpiniaceae	+	-	-
26	<i>Chonemorpha fragrans</i> (Moon) Alst.	Climber	Apocynaceae	-	-	+
27	<i>Chromolaena odora</i> (L.) King & Rob.*	Shrub	Asteraceae	+	+	+
28	<i>Cinnamomum verum</i> J.Presl	Tree	Lauraceae	+	+	-
29	<i>Cipadessa baccifera</i> Miq.	Shrub	Meliaceae	+	+	-
30	<i>Clerodendrum infortunatum</i> L.	Shrub	Verbenaceae	+	-	-
31	<i>Cosmostigma racemosum</i> Wight	Climber	Asclepiadaceae	+	-	-
32	<i>Crassocephalum crepidioides</i> S.Moore*	Herb	Asteraceae	+	+	+
33	<i>Curculigo orchioides</i> Gaertn.	Herb	Hypoxidaceae	+	+	+
34	<i>Curcuma neilgherrensis</i> Wight	Herb	Zingiberaceae	+	+	+
35	<i>Cyclea peltata</i> (Lam.) Hook.f. & Thoms.	Climber	Menispermaceae	+	+	-
36	<i>Cyperus pilosus</i> Vahl	Herb	Cyperaceae	-	-	+
37	<i>Dalbergia lanceolaria</i> L.f.	Tree	Fabaceae	+	-	-
38	<i>Dalbergia latifolia</i> Roxb.	Tree	Fabaceae	-	+	+

	Binomial	Category	Family	NF	Plantation	Vayal
39	<i>Dendrocalamus strictus</i> Nees	Shrub	Graminae	+	+	+
40	<i>Desmodium gangeticum</i> Blanco	Herb	Fabaceae	-	+	-
41	<i>Desmodium heterocarpon</i> (L.) DC.	Shrub	Fabaceae	+	+	-
42	<i>Desmodium laxiflorum</i> DC.	Herb	Fabaceae	+	+	-
43	<i>Desmodium pulchellum</i> (L.) Benth.	Shrub	Fabaceae	+	+	-
44	<i>Desmodium triflorum</i> (L.) DC.	Herb	Fabaceae	-	-	+
45	<i>Digitaria ciliaris</i> (Retz.) Koeler	Herb	Graminae	-	-	+
46	<i>Diospyros melanoxylon</i> Roxb.	Tree	Ebenaceae	+	-	-
47	<i>Elaeagnus kologa</i> Schltldl.	Climber	Elaeagnaceae	+	-	-
48	<i>Elaeocarpus tuberculatus</i> Roxb.	Tree	Elaeocarpaceae	-	+	-
49	<i>Elaeocarpus variabilis</i> Zmarzty	Tree	Elaeocarpaceae	+	-	-
50	<i>Elephantopus scaber</i> L.	Herb	Asteraceae	+	+	+
51	<i>Eleutheranthera ruderalis</i> (Sw.) Sch.Bip.*	Herb	Asteraceae	+	+	+
52	<i>Eragrostis tenella</i> (L.) P.Beauv. ex Roem. & Schult.	Herb	Poaceae	-	-	+
53	<i>Eucalyptus globulus</i> Labill.*	Tree	Myrtaceae	-	+	+
54	<i>Flacourtia indica</i> (Burm.f.) Merr.	Shrub	Flacourtiaceae	+	-	-
55	<i>Flemingia strobilifera</i> (L.) W.T.Aiton	Shrub	Fabaceae	-	-	+
56	<i>Glycosmis pentaphylla</i> (Retz.) DC.	Shrub	Rutaceae	+	+	+
57	<i>Gmelina arborea</i> Roxb.	Tree	Verbenaceae	+	-	-
58	<i>Gomphrena celosioides</i> Mart.*	Herb	Amaranthaceae	-	+	-
59	<i>Grangea maderaspatana</i> (L.) Poir.	Herb	Asteraceae	-	-	+
60	<i>Grewia tiliifolia</i> Vahl.	Tree	Tiliaceae	+	+	-
61	<i>Haldina cordifolia</i> (Roxb.) Ridsdale.	Tree	Rubiaceae	+	-	+
62	<i>Helicteres isora</i> L.	Shrub	Sterculiaceae	+	+	-
63	<i>Hemidesmus indicus</i> (L.) R.Br.	Climber	Periplocaceae	+	+	+
64	<i>Hydnocarpus pentandra</i> (Buch.-Ham.) Oken	Tree	Flacourtiaceae	+	-	-
65	<i>Hyptis suaveolens</i> (L.) Poit.*	Herb	Lamiaceae	+	+	-
66	<i>Jansenella griffithiana</i> (Müll.Hal.) Bor	Herb	Poaceae	-	-	+
67	<i>Kyllinga nemoralis</i> (J.R.Forst. & G.Forst.) Dandy	Herb	Cyperaceae	-	-	+
68	<i>Lagerstroemia microcarpa</i> Wight.	Tree	Lythraceae	+	+	+
69	<i>Lagerstroemia speciosa</i> Pers.	Tree	Lythraceae	+	-	-
70	<i>Lannea coromandelica</i> (Houtt.) Merr.	Tree	Anacardiaceae	+	+	+
71	<i>Lantana camara</i> L.*	Shrub	Verbenaceae	+	+	+
72	<i>Lepidagathis incurva</i> Buch.-Ham. ex D.Don	Herb	Acanthaceae	+	+	+
73	<i>Leucaena leucocephala</i> (Lam.) de Wit*	Herb	Mimosaceae	+	-	-
74	<i>Leucas aspera</i> Link	Herb	Lamiaceae	+	-	+
75	<i>Lindernia crustacea</i> (L.) F.Muell.*	Herb	Scrophulariaceae	+	-	-
76	<i>Ludwigia peruviana</i> (L.) H.Hara*	Shrub	Onagraceae	-	-	+
77	<i>Mallotus tetraococcus</i> Kurz	Tree	Euphorbiaceae	-	+	-
78	<i>Mangifera indica</i> Wall.	Tree	Anacardiaceae	+	-	-
79	<i>Melastoma malabathricum</i> L.	Shrub	Melastomataceae	+	-	+
80	<i>Melia azedarach</i> L.*	Tree	Meliaceae	+	+	-
81	<i>Melia dubia</i> Cav.	Tree	Meliaceae	+	+	-
82	<i>Mikania micrantha</i> Kunth*	Climber	Asteraceae	-	+	-
83	<i>Milium tomentosum</i> (Roxb.) Finet & Gagnep.	Tree	Annonaceae	+	-	-
84	<i>Mimosa pudica</i> L.*	Herb	Fabaceae	+	+	+

	Binomial	Category	Family	NF	Plantation	Vayal
85	<i>Mimusops elengi</i> Wight	Tree	Sapotaceae	+	+	-
86	<i>Mitracarpus hirtus</i> DC.*	Herb	Rubiaceae	+	+	+
87	<i>Naringi crenulata</i> (Roxb.) Nicolson	Tree	Rutaceae	+	+	+
88	<i>Olea dioica</i> Roxb.	Tree	Oleaceae	+	+	+
89	<i>Osbeckia aspera</i> Blume	Shrub	Melastomataceae	+	-	-
90	<i>Panicum trypheron</i> Schult.	Herb	Poaceae	-	-	+
91	<i>Persea macrantha</i> (Nees) Kosterm.	Tree	Lauraceae	+	+	-
92	<i>Phyllanthus emblica</i> L.	Tree	Euphorbiaceae	+	-	+
93	<i>Piper nigrum</i> L.	Climber	Piperaceae	+	-	-
94	<i>Pogostemon purpurascens</i> Dalzell	Herb	Lamiaceae	-	+	-
95	<i>Pongamia pinnata</i> (L.) Merr.	Tree	Fabaceae	+	-	-
96	<i>Premna tomentosa</i> Wild.	Tree	Verbenaceae	+	-	-
97	<i>Pterocarpus marsupium</i> Roxb.	Tree	Fabaceae	+	+	+
98	<i>Rauvolfia serpentina</i> Jacq.	Shrub	Apocynaceae	+	-	-
99	<i>Rhynchospora corymbosa</i> (L.) Britton	Herb	Cyperaceae	-	-	+
100	<i>Sacciolepis indica</i> (L.) Chase*	Herb	Poaceae	-	-	+
101	<i>Schleichera oleosa</i> (Lour.) Oken	Tree	Sapindaceae	+	+	-
102	<i>Schrebera swietenoides</i> Roxb.	Tree	Oleaceae	+	-	-
103	<i>Semecarpus anacardium</i> Roxb.	Tree	Anacardiaceae	-	+	-
104	<i>Senna spectabilis</i> (DC.) H. S. Irwin & Barneby*	Tree	Fabaceae	+	+	+
105	<i>Senna tora</i> Roxb.*	Herb	Caesalpiniaceae	+	+	+
106	<i>Shorea roxburghii</i> G. Don.	Tree	Dipterocarpaceae	+	+	+
107	<i>Sida acuta</i> burm. F.	Shrub	Malvaceae	+	+	+
108	<i>Sida alnifolia</i> L.	Shrub	Malvaceae	+	+	+
109	<i>Sida rhombifolia</i> L.	Shrub	Malvaceae	-	+	-
110	<i>Solanum aculeatissimum</i> Jacq.*	Shrub	Solanaceae	+	+	+
111	<i>Spathodea campanulata</i> Buch.-Ham. ex DC.*	Tree	Bignoniaceae	+	-	-
112	<i>Sporobolus tenuissimus</i> Kuntze	Herb	Poaceae	-	-	+
113	<i>Stachytarpheta jamaicensis</i> (L.) Vahl*	Shrub	Verbenaceae	+	+	-
114	<i>Streblus asper</i> Lour.	Tree	Moraceae	+	-	-
115	<i>Syzygium cumini</i> (L.) Skeels	Tree	Myrtaceae	+	+	+
116	<i>Tabernamontana alternifolia</i> Roxb.	Tree	Apocynaceae	+	+	+
117	<i>Tamilnadia uliginosa</i> (Retz.) Tirveng. & Sastre	Tree	Rubiaceae	+	-	+
118	<i>Tectona grandis</i> L.f.	Tree	Verbenaceae	+	+	+
119	<i>Terminalia bellirica</i> (Gaertn.) Roxb.	Tree	Combretaceae	+	+	+
120	<i>Terminalia cuneata</i> Roth	Tree	Combretaceae	+	-	+
121	<i>Terminalia elliptica</i> Willd.	Tree	Combretaceae	+	+	+
122	<i>Terminalia paniculata</i> Roth	Tree	Combretaceae	+	-	-
123	<i>Themeda triandra</i> Forssk.	Herb	Poaceae	-	-	+
124	<i>Trewia nudiflora</i> Wight	Tree	Euphorbiaceae	-	-	+
125	<i>Triumfetta rhomboidea</i> Jacq.	Shrub	Tiliaceae	+	+	-
126	<i>Vitex altissima</i> L.f.	Tree	Verbenaceae	+	-	-
127	<i>Ziziphus glabrata</i> B. Heyne ex Roth	Tree	Rhamnaceae	+	-	-
128	<i>Ziziphus mauritiana</i> Lam.	Tree	Rhamnaceae	-	+	-
129	<i>Ziziphus oenopia</i> (L.) Mill.	Climber	Rhamnaceae	+	+	+

\*indicates non-native species

*Curcuma neilgherrensis*, *Elephantopus scaber*, *Eleutheranthera ruderalis*, *Lepidagathis incurva*, *Mimosa pudica*, *Mitracarpus hirtus*, and *Senna tora* were the herbs seen in all the three vegetation types. *Centella asiatica*, *Chamaecrista absus*, and *Lindernia crustacea* were the herbs observed only in NF. In plantations, the herbs seen were *Acalypha paniculata*, *Desmodium gangeticum*, *Gomphrena celosioides*. *Arundinella leptochloa*, *Axonopus compressus*, *Cyperus pilosus*, *Desmodium trifolium*, *Digitaria ciliaris*, *Grangea maderaspatana*, *Jansenella griffithiana*, and *Kyllinga nemoralis* were observed only in vayal.

*Catunaregam spinosa*, *Dendrocalamus strictus*, *Glycosmis pentaphylla*, *Sida acuta*, *S. alnifolia*, and *Solanum aculeatissimum* are the shrubs that could be recorded in all three vegetation types. *Canthium coromandelicum*, *Carmona retusa*, *Clerodendrum infortunatum*, *Desmodium heterocarpon*, *D. pulchellum*, *Flacourtia indica*, *Glycosmis pentaphylla*, *Helicteres isora*, *Melastoma malabathricum*, *Osbeckia aspera*, *Rauvolfia serpentina*, *Sida acuta*, *S. alnifolia*, *Solanum aculeatissimum*, *Stachyphrynium jamaicensis*, and *Triumfetta rhomboidei* were the shrubs observed in NF. *Canthium coromandelicum*, *Clerodendrum infortunatum*, *Flacourtia indica*, *Osbeckia aspera*, and *Rauvolfia serpentina* were seen only in NF. *Carmona retusa*, *Catunaregam spinosa*, *Cipadessa baccifera*, *Dendrocalamus strictus*, *Desmodium heterocarpon*, *D. pulchellum*, *Glycosmis pentaphylla*, *Helicteres isora*, *Sida acuta*, *S. alnifolia*, *S. rhombifolia*, *Solanum aculeatissimum*, *Stachyphrynium jamaicensis*, and *Triumfetta rhomboidei* were the shrubs seen in plantation. *Calotropis gigantea*, *Catunaregam spinosa*, *Dendrocalamus strictus*, *Flemingia strobilifera*, *Glycosmis pentaphylla*, *Ludwigia peruviana*, *Melastoma malabathricum*, *Sida acuta*, *S. alnifolia*, and *Solanum aculeatissimum* were the shrubs commonly seen in vayal. Among these, *Calotropis gigantea* and *Flemingia strobilifera* were only seen in vayal.

Among the 11 climbers, *Hemidesmus indicus* and *Ziziphus oenoplia* were seen in all the vegetation types. *Caesalpinia mimosoides*, *Cosmostigma racemosum*, *Elaeagnus kologa*, and *Piper nigrum* were seen in NF. In vayal, *Chonemorpha fragrans* was only climber which was seen. No climber could be recorded in the plantation.

The vegetation analysis in NF showed that *Chromolaena odorata* has maximum abundance (81.6) and frequency (61.1) (Table 3). Next to *Chromolaena odorata*, *Stachytarpheta jamaicensis* (31.3) has maximum abundance. The abundance of *Senna spectabilis* and

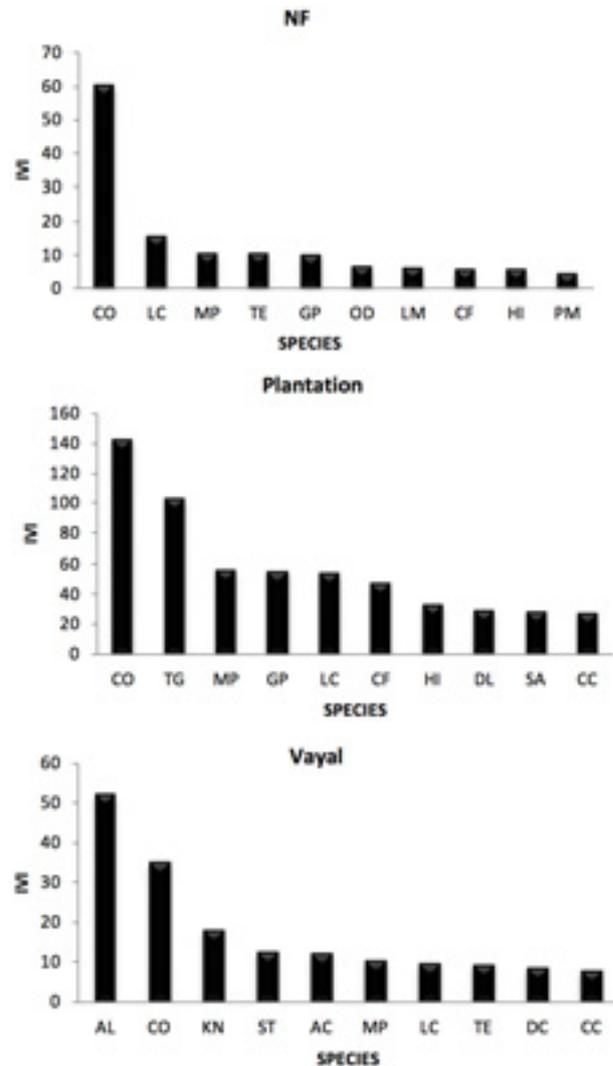


Figure 3. Ten dominant species encountered in each vegetation type: AC—*Axonopus compressus* | AL—*Arundinella leptochloa* | CC—*Crassocephalum crepidioides*\* | CO—*Chromolaena odorata*\* | CF—*Cassia fistula* | DC—*Digitaria ciliaris* | DL—*Dalbergia latifolia* | TG—*Tectona grandis* | GP—*Glycosmis pentaphylla* | HI—*Helicteres isora* | KN—*Kyllinga nemoralis* | LM—*Lagerstroemia microcarpa* | LC—*Lantana camara*\* | MP—*Mimosa pudica*\* | OD—*Olea dioica* | SA—*Sida alnifolia* | ST—*Sporobolus tenuissimus* | TE—*Terminalia elliptica* | PM—*Persea macrantha*.

\*indicates non-native species

*Lantana camara* were 17.7 and 9.8, respectively. The density of *Lantana camara* was 532.9 stems ha<sup>-1</sup>. After *Lantana camara*, *Glycosmis pentaphylla* (338.8 stems ha<sup>-1</sup>) and *Mitracarpus hirtus* (195.2 stems ha<sup>-1</sup>) were the densely seen plant species in NF. The most densely seen tree species in NF is *S. spectabilis* (188.2 stems ha<sup>-1</sup>). Among the first ten highly dense plant species in NF, five were IAPS. Maximum frequency in NF was shown by *Chromolaena odorata* (61.1) and *Lantana camara*

Table 3. Phytosociological analysis of vegetation in natural forest.

	Binomial	F	RF	D	RD	A	RBA	IVI
1	<i>Ageratum conyzoides*</i>	11.76	1.14	61.18	0.60	5.20	1.14	2.88
2	<i>Annona squamosa*</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
3	<i>Anogeissus latifolia</i>	9.41	0.91	12.94	0.13	1.38	0.91	1.95
4	<i>Aporosa cardiosperma</i>	5.88	0.57	8.24	0.08	1.40	0.57	1.22
5	<i>Barleria mysorensis</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
6	<i>Bauhinia racemosa</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
7	<i>Biophytum reinwardtii</i>	2.35	0.23	10.59	0.10	4.50	0.23	0.56
8	<i>Butea monosperma</i>	9.41	0.91	10.59	0.10	1.13	0.91	1.93
9	<i>Caesalpinia mimosoides</i>	1.18	0.11	17.65	0.17	15.0	0.11	0.40
10	<i>Calycopteris floribunda</i>	18.82	1.83	37.65	0.37	2.00	1.83	4.02
11	<i>Canthium coromandelicum</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
12	<i>Carallia brachiata</i>	15.29	1.48	37.65	0.37	2.46	1.48	3.33
13	<i>Cardiospermum halicacabum</i>	8.24	0.80	10.59	0.10	1.29	0.80	1.70
14	<i>Carmona retusa*</i>	15.29	1.48	18.82	0.18	1.23	1.48	3.15
15	<i>Caryota urens</i>	3.53	0.34	7.06	0.07	2.00	0.34	0.75
16	<i>Cassia fistula</i>	28.24	2.74	57.65	0.56	2.04	2.74	6.04
17	<i>Catunaregam spinosa</i>	10.59	1.03	11.76	0.11	1.11	1.03	2.17
18	<i>Centella asiatica</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
19	<i>Chamaecrista absus</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
20	<i>Chromolaena odorata*</i>	61.18	5.94	4996.47	48.69	81.6	5.94	60.5
21	<i>Cinnamomum verum</i>	11.76	1.14	52.94	0.52	4.50	1.14	2.80
22	<i>Cipadessa baccifera</i>	4.71	0.46	8.24	0.08	1.75	0.46	0.99
23	<i>Clerodendrum infortunatum</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
24	<i>Cosmostigma racemosum</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
25	<i>Crassocephalum crepidioides*</i>	8.24	0.80	11.76	0.11	1.43	0.80	1.71
26	<i>Curculigo orchoides</i>	3.53	0.34	44.71	0.44	12.6	0.34	1.12
27	<i>Curcuma neilgherrensis</i>	17.65	1.71	70.59	0.69	4.00	1.71	4.11
28	<i>Cyclea peltata</i>	17.65	1.71	27.06	0.26	1.53	1.71	3.69
29	<i>Dalbergia lanceolaria</i>	10.59	1.03	15.29	0.15	1.44	1.03	2.20
30	<i>Dendrocalamus strictus</i>	18.82	1.83	58.82	0.57	3.13	1.83	4.23
31	<i>Desmodium heterocarpon</i>	2.35	0.23	8.24	0.08	3.50	0.23	0.54
32	<i>Desmodium laxiflorum</i>	7.06	0.68	11.76	0.11	1.67	0.68	1.48
33	<i>Desmodium pulchellum</i>	5.88	0.57	7.06	0.07	1.20	0.57	1.21
34	<i>Diospyros melanoxylon</i>	7.06	0.68	9.41	0.09	1.33	0.68	1.46
35	<i>Elaeagnus kologa</i>	2.35	0.23	4.71	0.05	2.00	0.23	0.50
36	<i>Elaeocarpus variabilis</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
37	<i>Elephantopus scaber</i>	14.12	1.37	142.35	1.39	10.1	1.37	4.13
38	<i>Eleutheranthera ruderalis*</i>	7.06	0.68	31.76	0.31	4.50	0.68	1.68
39	<i>Flacourtia indica</i>	11.76	1.14	14.12	0.14	1.20	1.14	2.42
40	<i>Glycosmis pentaphylla</i>	36.47	3.54	338.82	3.30	9.29	3.54	10.3
41	<i>Gmelina arborea</i>	2.35	0.23	2.35	0.02	1.00	0.23	0.48
42	<i>Grewia tiliifolia</i>	14.12	1.37	20.00	0.19	1.42	1.37	2.93
43	<i>Haldina cordifolia</i>	5.88	0.57	9.41	0.09	1.60	0.57	1.23

F—Frequency | RF—Relative Frequency | D—Density, RD—Relative density | A—Abundance | RBA—Relative basal area | IVI—Importance Value Index.

	Binomial	F	RF	D	RD	A	RBA	IVI
44	<i>Helicteres isora</i>	27.06	2.63	50.59	0.49	1.87	2.63	5.74
45	<i>Hemidesmus indicus</i>	1.18	0.11	3.53	0.03	3.00	0.11	0.26
46	<i>Hydnocarpus pentandra</i>	5.88	0.57	5.88	0.06	1.00	0.57	1.20
47	<i>Hyptis suaveolens*</i>	3.53	0.34	24.71	0.24	7.00	0.34	0.93
48	<i>Lagerstroemia microcarpa</i>	31.76	3.08	38.82	0.38	1.22	3.08	6.54
49	<i>Lagerstroemia speciosa</i>	3.53	0.34	3.53	0.03	1.00	0.34	0.72
50	<i>Lannea coromandelica</i>	2.35	0.23	2.35	0.02	1.00	0.23	0.48
51	<i>Lantana camara*</i>	54.12	5.25	532.94	5.19	9.85	5.25	15.7
52	<i>Lepidagathis incurva</i>	15.29	1.48	29.41	0.29	1.92	1.48	3.25
53	<i>Leucaena leucocephala*</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
54	<i>Leucas aspera</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
55	<i>Lindernia crustacea*</i>	12.94	1.26	35.29	0.34	2.73	1.26	2.86
56	<i>Mangifera indica</i>	4.71	0.46	11.76	0.11	2.50	0.46	1.03
57	<i>Melastoma malabathricum</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
58	<i>Melia azedarach*</i>	4.71	0.46	5.88	0.06	1.25	0.46	0.97
59	<i>Melia dubia</i>	4.71	0.46	4.71	0.05	1.00	0.46	0.96
60	<i>Milium tomentosum</i>	1.18	0.11	2.35	0.02	2.00	0.11	0.25
61	<i>Mimosa pudica*</i>	48.24	4.68	149.41	1.46	3.10	4.68	10.8
62	<i>Mimusops elengi</i>	2.35	0.23	2.35	0.02	1.00	0.23	0.48
63	<i>Mitracarpus hirtus</i>	9.41	0.91	195.29	1.90	20.7	0.91	3.73
64	<i>Naringi crenulata</i>	20.00	1.94	40.00	0.39	2.00	1.94	4.27
65	<i>Olea dioica</i>	30.59	2.97	80.00	0.78	2.62	2.97	6.72
66	<i>Osbeckia aspera</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
67	<i>Persea macrantha</i>	22.35	2.17	40.00	0.39	1.79	2.17	4.73
68	<i>Phyllanthus emblica</i>	2.35	0.23	2.35	0.02	1.00	0.23	0.48
69	<i>Piper nigrum</i>	7.06	0.68	11.76	0.11	1.67	0.68	1.48
70	<i>Pongamia pinnata</i>	5.88	0.57	8.24	0.08	1.40	0.57	1.22
71	<i>Premna mollissima</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
72	<i>Pterocarpus marsupium</i>	8.24	0.80	8.24	0.08	1.00	0.80	1.68
73	<i>Rauvolfia serpentina</i>	3.53	0.34	5.88	0.06	1.67	0.34	0.74
74	<i>Schleichera oleosa</i>	16.47	1.60	22.35	0.22	1.36	1.60	3.41
75	<i>Schrebera swietenoides</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
76	<i>Senna spectabilis*</i>	10.59	1.03	188.24	1.83	17.7	1.03	3.89
77	<i>Senna tora*</i>	3.53	0.34	10.59	0.10	3.00	0.34	0.79
78	<i>Shorea roxburghii</i>	15.29	1.48	36.47	0.36	2.38	1.48	3.32
79	<i>Sida acuta</i>	3.53	0.34	3.53	0.03	1.00	0.34	0.72
80	<i>Sida alnifolia</i>	11.76	1.14	22.35	0.22	1.90	1.14	2.50
81	<i>Solanum aculeatissimum*</i>	18.82	1.83	29.41	0.29	1.56	1.83	3.94
82	<i>Spathodea campanulata*</i>	11.76	1.14	18.82	0.18	1.60	1.14	2.47
83	<i>Stachytarpheta jamaicensis*</i>	3.53	0.34	110.59	1.08	31.3	0.34	1.76
84	<i>Streblus asper</i>	7.06	0.68	7.06	0.07	1.00	0.68	1.44
85	<i>Syzygium cumini</i>	18.82	1.83	60.00	0.58	3.19	1.83	4.24
86	<i>Tabernaemontana alternifolia</i>	16.47	1.60	32.94	0.32	2.00	1.60	3.52
87	<i>Tamilnadia ulginosa</i>	1.18	0.11	1.18	0.01	1.00	0.11	0.24
88	<i>Tectona grandis</i>	20.00	1.94	42.35	0.41	2.12	1.94	4.29

	Binomial	F	RF	D	RD	A	RBA	IVI
89	<i>Terminalia bellirica</i>	5.88	0.57	5.88	0.06	1.00	0.57	1.20
90	<i>Terminalia cuneata</i>	3.53	0.34	3.53	0.03	1.00	0.34	0.72
91	<i>Terminalia elliptica</i>	50.59	4.91	72.94	0.71	1.44	4.91	10.5
92	<i>Terminalia paniculata</i>	14.12	1.37	14.12	0.14	1.00	1.37	2.88
93	<i>Triumfetta rhomboidea</i>	4.71	0.46	4.71	0.05	1.00	0.46	0.96
94	<i>Vitex altissima</i>	4.71	0.46	5.88	0.06	1.25	0.46	0.97
95	<i>Ziziphus glabrata</i>	20.00	1.94	20.00	0.19	1.00	1.94	4.08
96	<i>Ziziphus oenoplia</i>	5.88	0.57	7.06	0.07	1.20	0.57	1.21
	<b>Total</b>	<b>1029.41</b>	<b>99.89</b>	<b>8228.24</b>	<b>80.18</b>	<b>368.08</b>	<b>99.89</b>	<b>300.00</b>

\*indicates non-native species

**Table 4. Phytosociological analysis of vegetation in plantation.**

	Binomial	F	RF	D	RD	A	RBA	IVI
1	<i>Acalypha paniculata</i>	1.18	0.13	2.35	0.03	2.00	1.18	1.33
2	<i>Ailanthus triphysa</i>	5.88	0.64	7.06	0.09	1.20	5.88	6.61
3	<i>Anogeissus latifolia</i>	14.12	1.54	14.1	0.17	1.00	14.12	15.8
4	<i>Barleria mysorensis</i>	1.18	0.13	1.18	0.01	1.00	1.18	1.32
5	<i>Bauhinia racemosa</i>	1.18	0.13	1.18	0.01	1.00	1.18	1.32
6	<i>Biophytum reinwardtii</i>	2.35	0.26	5.88	0.07	2.50	2.35	2.68
7	<i>Butea monosperma</i>	2.35	0.26	2.35	0.03	1.00	2.35	2.64
8	<i>Calcyopteris floribunda</i>	2.35	0.26	4.71	0.06	2.00	2.35	2.67
9	<i>Cardiospermum halicacabum</i>	3.53	0.38	3.53	0.04	1.00	3.53	3.96
10	<i>Carmona retusa*</i>	3.53	0.38	3.53	0.04	1.00	3.53	3.96
11	<i>Caryota urens</i>	2.35	0.26	2.35	0.03	1.00	2.35	2.64
12	<i>Cassia fistula</i>	42.35	4.62	83.5	1.01	1.97	42.35	47.9
13	<i>Catunaregam spinosa</i>	21.18	2.31	31.7	0.38	1.50	21.18	23.8
14	<i>Chromolaena odorata*</i>	75.29	8.21	4943.53	59.56	65.6	75.29	143.1
15	<i>Cinnamomum verum</i>	4.71	0.51	11.76	0.14	2.50	4.71	5.36
16	<i>Cipadessa baccifera</i>	11.76	1.28	14.12	0.17	1.20	11.76	13.22
17	<i>Crassocephalum crepidioides*</i>	24.71	2.69	35.29	0.43	1.43	24.71	27.82
18	<i>Curculigo orchioides</i>	10.59	1.15	84.71	1.02	8.00	10.59	12.76
19	<i>Curcuma neilgherrensis</i>	15.29	1.67	72.94	0.88	4.77	15.29	17.84
20	<i>Cyclea peltata</i>	10.59	1.15	17.65	0.21	1.67	10.59	11.95
21	<i>Dalbergia latifolia</i>	27.06	2.95	34.12	0.41	1.26	27.06	30.42
22	<i>Dendrocalamus strictus</i>	14.12	1.54	37.65	0.45	2.67	14.12	16.11
23	<i>Desmodium gangeticum</i>	1.18	0.13	2.35	0.03	2.00	1.18	1.33
24	<i>Desmodium heterocarpon</i>	2.35	0.26	2.35	0.03	1.00	2.35	2.64
25	<i>Desmodium laxiflorum</i>	7.06	0.77	7.06	0.09	1.00	7.06	7.91
26	<i>Desmodium pulchellum</i>	9.41	1.03	14.12	0.17	1.50	9.41	10.61
27	<i>Elaeocarpus tuberculatus</i>	2.35	0.26	16.47	0.20	7.00	2.35	2.81
28	<i>Elephantopus scaber</i>	23.53	2.56	101.18	1.22	4.30	23.53	27.31
29	<i>Eleutheranthera ruderalis*</i>	1.18	0.13	4.71	0.06	4.00	1.18	1.36
30	<i>Eucalyptus globulus*</i>	12.94	1.41	75.29	0.91	5.82	12.94	15.26
31	<i>Glycosmis pentaphylla</i>	44.71	4.87	484.71	5.84	10.8	44.71	55.42

F—Frequency | RF—Relative Frequency | D—Density, RD—Relative density | A—Abundance | RBA—Relative basal area | IVI—Importance Value Index.

	Binomial	F	RF	D	RD	A	RBA	IVI
32	<i>Gomphrena celosioides*</i>	3.53	0.38	9.41	0.11	2.67	3.53	4.03
33	<i>Grewia tilifolia</i>	10.59	1.15	16.47	0.20	1.56	10.59	11.94
34	<i>Helicteres isora</i>	29.41	3.21	67.06	0.81	2.28	29.41	33.42
35	<i>Hemidesmus indicus</i>	8.24	0.90	68.24	0.82	8.29	8.24	9.95
36	<i>Hyptis suaveolens*</i>	1.18	0.13	4.71	0.06	4.00	1.18	1.36
37	<i>Lagerstroemia microcarpa</i>	17.65	1.92	20.00	0.24	1.13	17.65	19.81
38	<i>Lannea coromandelica</i>	1.18	0.13	1.18	0.01	1.00	1.18	1.32
39	<i>Lantana camara*</i>	45.88	5.00	322.35	3.88	7.03	45.88	54.77
40	<i>Lepidagathis incurve</i>	4.71	0.51	28.24	0.34	6.00	4.71	5.56
41	<i>Mallotus tetracoccus</i>	4.71	0.51	9.41	0.11	2.00	4.71	5.33
42	<i>Melia azedarach*</i>	1.18	0.13	1.18	0.01	1.00	1.18	1.32
43	<i>Melia dubia</i>	11.76	1.28	57.65	0.69	4.90	11.76	13.74
44	<i>Mikania micrantha*</i>	2.35	0.26	11.76	0.14	5.00	2.35	2.75
45	<i>Mimosa pudica*</i>	49.41	5.38	183.53	2.21	3.71	49.41	57.01
46	<i>Mimusops elengi</i>	1.18	0.13	1.18	0.01	1.00	1.18	1.32
47	<i>Mitracarpus hirtus</i>	11.76	1.28	147.06	1.77	12.5	11.76	14.82
48	<i>Naringi crenulata</i>	2.35	0.26	2.35	0.03	1.00	2.35	2.64
49	<i>Olea dioica</i>	16.47	1.79	42.35	0.51	2.57	16.47	18.78
50	<i>Persea macrantha</i>	1.18	0.13	4.71	0.06	4.00	1.18	1.36
51	<i>Pogostemon purpurascens</i>	3.53	0.38	50.59	0.61	14.3	3.53	4.52
52	<i>Pterocarpus marsupium</i>	2.35	0.26	2.35	0.03	1.00	2.35	2.64
53	<i>Schleichera oleosa</i>	18.82	2.05	92.94	1.12	4.94	18.82	21.99
54	<i>Semecarpus anacardium</i>	18.82	2.05	25.88	0.31	1.38	18.82	21.19
55	<i>Senna spectabilis*</i>	8.24	0.90	63.53	0.77	7.71	8.24	9.90
56	<i>Senna tora*</i>	3.53	0.38	38.82	0.47	11.0	3.53	4.38
57	<i>Shorea roxburghii</i>	12.94	1.41	17.65	0.21	1.36	12.94	14.56
58	<i>Sida acuta</i>	12.94	1.41	14.12	0.17	1.09	12.94	14.52
59	<i>Sida alnifolia</i>	25.88	2.82	54.12	0.65	2.09	25.88	29.35
60	<i>Sida rhombifolia</i>	8.24	0.90	8.24	0.10	1.00	8.24	9.23
61	<i>Solanum aculeatissimum*</i>	11.76	1.28	21.18	0.26	1.80	11.76	13.30
62	<i>Stachytarpheta jamaicensis*</i>	2.35	0.26	90.59	1.09	38.5	2.35	3.70
63	<i>Syzygium cumini</i>	8.24	0.90	20.00	0.24	2.43	8.24	9.37
64	<i>Tabernamontana alternifolia</i>	23.53	2.56	41.18	0.50	1.75	23.53	26.59
65	<i>Tectona grandis</i>	87.06	9.49	564.71	6.80	6.49	87.06	103.3
66	<i>Terminalia bellirica</i>	2.35	0.26	2.35	0.03	1.00	2.35	2.64
67	<i>Terminalia elliptica</i>	4.71	0.51	14.12	0.17	3.00	4.71	5.39
68	<i>Triumfetta rhomboidea</i>	15.29	1.67	23.53	0.28	1.54	15.29	17.24
69	<i>Ziziphus mauritiana</i>	5.88	0.64	5.88	0.07	1.00	5.88	6.59
70	<i>Ziziphus oenoplia</i>	14.12	1.54	25.88	0.31	1.83	14.12	15.97
	<b>Total</b>	<b>917.65</b>	<b>100.00</b>	<b>8300.00</b>	<b>100.00</b>	<b>321.66</b>	<b>100.0</b>	<b>300</b>

\*indicates non-native species

Table 5. Phytosociological analysis of vegetation in Vayal.

	Binomial	F	RF	D	RD	A	RBA	IVI
1	<i>Ageratum conyzoides*</i>	10.59	1.03	768.24	2.39	72.56	1.03	4.46
2	<i>Annona squamosa*</i>	1.18	0.11	22.35	0.07	19.00	0.11	0.30
3	<i>Anogeissus latifolia</i>	11.76	1.15	11.76	0.04	1.00	1.15	2.34
4	<i>Arundinella leptochloa</i>	83.53	8.16	11662.3	36.27	139.6	8.16	52.59
5	<i>Axonopus compressus</i>	17.65	1.72	2917.65	9.07	165.3	1.72	12.52
6	<i>Bauhinia malabarica</i>	1.18	0.11	1.18	0.00	1.00	0.11	0.23
7	<i>Biophytum reinwardtii</i>	4.71	0.46	4.71	0.01	1.00	0.46	0.93
8	<i>Butea monosperma</i>	2.35	0.23	2.35	0.01	1.00	0.23	0.47
9	<i>Calotropis gigantea</i>	3.53	0.34	3.53	0.01	1.00	0.34	0.70
10	<i>Careya arborea</i>	4.71	0.46	4.71	0.01	1.00	0.46	0.93
11	<i>Cassia fistula</i>	12.94	1.26	20.00	0.06	1.55	1.26	2.59
12	<i>Catunaregam spinosa</i>	4.71	0.46	4.71	0.01	1.00	0.46	0.93
13	<i>Chonemorpha fragrans</i>	1.18	0.11	1.18	0.00	1.00	0.11	0.23
14	<i>Crassocephalum crepidioides*</i>	40.00	3.91	80.00	0.25	2.00	3.91	8.06
15	<i>Curculigo orchioides</i>	7.06	0.69	11.76	0.04	1.67	0.69	1.42
16	<i>Curcuma neilgherrensis</i>	23.53	2.30	49.41	0.15	2.10	2.30	4.75
17	<i>Cyperus pilosus</i>	8.24	0.80	195.29	0.61	23.71	0.80	2.22
18	<i>Dalbergia latifolia</i>	4.71	0.46	4.71	0.01	1.00	0.46	0.93
19	<i>Dendrocalamus strictus</i>	7.06	0.69	11.76	0.04	1.67	0.69	1.42
20	<i>Desmodium triflorum</i>	15.29	1.49	712.94	2.22	46.62	1.49	5.21
21	<i>Digitaria ciliaris</i>	29.41	2.87	992.94	3.09	33.76	2.87	8.83
22	<i>Elephantopus scaber</i>	8.24	0.80	37.65	0.12	4.57	0.80	1.73
23	<i>Eleutheranthera ruderalis*</i>	4.71	0.46	37.65	0.12	8.00	0.46	1.04
24	<i>Eragrostis tenella</i>	21.18	2.07	1052.94	3.27	49.72	2.07	7.41
25	<i>Eucalyptus globulus*</i>	1.18	0.11	3.53	0.01	3.00	0.11	0.24
26	<i>Chromolaena odorata*</i>	89.41	8.74	5810.59	18.07	64.99	8.74	35.54
27	<i>Flemingia strobilifera</i>	2.35	0.23	7.06	0.02	3.00	0.23	0.48
28	<i>Glycosmis pentaphylla</i>	14.12	1.38	148.24	0.46	10.50	1.38	3.22
29	<i>Grangea maderaspatana</i>	7.06	0.69	11.76	0.04	1.67	0.69	1.42
30	<i>Haldina cordifolia</i>	35.29	3.45	38.82	0.12	1.10	3.45	7.02
31	<i>Hemidesmus indicus</i>	3.53	0.34	8.24	0.03	2.33	0.34	0.72
32	<i>Jansenella griffithiana</i>	18.82	1.84	203.53	0.63	10.81	1.84	4.31
33	<i>Kyllinga nemoralis</i>	24.71	2.41	4289.41	13.34	173.6	2.41	18.17
34	<i>Lagerstroemia microcarpa</i>	8.24	0.80	8.24	0.03	1.00	0.80	1.63
35	<i>Lansea coromandelica</i>	1.18	0.11	1.18	0.00	1.00	0.11	0.23
36	<i>Lantana camara*</i>	43.53	4.25	423.53	1.32	9.73	4.25	9.82
37	<i>Lepidagathis incurve</i>	1.18	0.11	3.53	0.01	3.00	0.11	0.24
38	<i>Leucas asper</i>	8.24	0.80	10.59	0.03	1.29	0.80	1.64
39	<i>Ludwigia peruviana</i>	1.18	0.11	7.06	0.02	6.00	0.11	0.25
40	<i>Melastoma malabathricum</i>	2.35	0.23	12.94	0.04	5.50	0.23	0.50
41	<i>Mimosa pudica*</i>	52.94	5.17	172.94	0.54	3.27	5.17	10.88
42	<i>Mitracarpus hirtus*</i>	18.82	1.84	137.65	0.43	7.31	1.84	4.11
43	<i>Naringi crenulata</i>	2.35	0.23	2.35	0.01	1.00	0.23	0.47

F—Frequency | RF—Relative Frequency | D—Density, RD—Relative density | A—Abundance | RBA—Relative basal area | IVI—Importance Value Index.

	Binomial	F	RF	D	RD	A	RBA	IVI
44	<i>Olea dioica</i>	7.06	0.69	8.24	0.03	1.17	0.69	1.40
45	<i>Panicum trypheron</i>	29.41	2.87	736.47	2.29	25.04	2.87	8.04
46	<i>Phyllanthus emblica</i>	4.71	0.46	4.71	0.01	1.00	0.46	0.93
47	<i>Pterocarpus marsupium</i>	5.88	0.57	9.41	0.03	1.60	0.57	1.18
48	<i>Rhynchospora corymbosa</i>	7.06	0.69	81.18	0.25	11.50	0.69	1.63
49	<i>Sacciolepis indica*</i>	30.59	2.99	250.59	0.78	8.19	2.99	6.76
50	<i>Senna spectabilis*</i>	7.06	0.69	84.71	0.26	12.00	0.69	1.64
51	<i>Senna tora*</i>	21.18	2.07	45.88	0.14	2.17	2.07	4.28
52	<i>Shorea roxburghii</i>	3.53	0.34	8.24	0.03	2.33	0.34	0.72
53	<i>Sida acuta</i>	10.59	1.03	18.82	0.06	1.78	1.03	2.13
54	<i>Sida alnifolia</i>	16.47	1.61	25.88	0.08	1.57	1.61	3.30
55	<i>Solanum aculeatissimum</i>	29.41	2.87	54.12	0.17	1.84	2.87	5.92
56	<i>Sporobolus tenuissimus</i>	56.47	5.52	632.94	1.97	11.21	5.52	13.00
57	<i>Syzygium cumini</i>	18.82	1.84	21.18	0.07	1.13	1.84	3.74
58	<i>Tabernamontana alternifolia</i>	2.35	0.23	2.35	0.01	1.00	0.23	0.47
59	<i>Tamilnadia uliginosa</i>	18.82	1.84	20.00	0.06	1.06	1.84	3.74
60	<i>Tectona grandis</i>	16.47	1.61	41.18	0.13	2.50	1.61	3.35
61	<i>Terminalia bellirica</i>	4.71	0.46	4.71	0.01	1.00	0.46	0.93
62	<i>Terminalia cuneata</i>	7.06	0.69	7.06	0.02	1.00	0.69	1.40
63	<i>Terminalia elliptica</i>	48.24	4.71	74.12	0.23	1.54	4.71	9.66
64	<i>Themeda triandra</i>	8.24	0.80	105.88	0.33	12.86	0.80	1.94
65	<i>Trewia nudiflora</i>	1.18	0.11	2.35	0.01	2.00	0.11	0.24
66	<i>Ziziphus oenoplia</i>	2.35	0.23	3.53	0.01	1.50	0.23	0.47
	<b>Total</b>	<b>1023.53</b>	<b>100.0</b>	<b>32156.4</b>	<b>100.0</b>	<b>997.96</b>	<b>100.0</b>	<b>300.0</b>

\*indicates non-native species.

**Table 6. Diversity attributes of three ecosystems.**

Ecosystem	Simpson's diversity index (1-D)	Berger-Parker dominance index	Margalef richness index	Pielou's equitability index
Natural Forest	0.61	0.62	10.76	1.002
Plantation	0.58	0.64	7.85	0.999
Vayal	0.80	0.36	6.46	1.19

(54.1). *Terminalia elliptica* (50.5) was the tree species having the highest frequency, followed by *Lagerstroemia microcarpa* (31.7) and *Olea dioica* (35.8). It is *Annona squamosa* which has the lowest frequency, abundance and density in NF.

In plantation, *Chromolaena odorata* (75.29) was recorded in maximum frequency, followed by *Glycosmis pentaphylla* (44.7), *Lantana camara* (44.5) and *Mimosa pudica* (44.9) (Table 4). After *Chromolaena odorata* (65.6), *Stachytarpheta jamaicensis* (38.5) recorded the second highest abundance. The highest frequency in plantation was for *Tectona grandis* (87.05). It was followed by *Chromolaena odorata* (75.29) and *Mimosa*

*pudica* (49.4). The least frequency was shown by *Barleria mysorensis*, *Bauhinia racemosa*, *Lannea coromandelica*, *Melia azedarach* and *Mimusops elengi*. *Chromolaena odorata* recorded the highest IVI, followed by *Tectona grandis*.

The most densely seen plant species in vayals was *Arundinella leptochloa* (11,662 stems ha<sup>-1</sup>) (Table 5). Density of *Chromolaena odorata* in vayal was (58,10.6 stems ha<sup>-1</sup>). The lowest density in vayal was recorded for *Bauhinia malabarica*, *Chonemorpha fragrans*, and *Lannea coromandelica*. The most abundantly seen plant species in vayals was *Kyllinga nemoralis* (173.6). It was followed by *Arundinella leptochloa* (165.3) and



*Axonopus compressus* (139.6). In vayals, *Ageratum conyzoides* (72.56) was more abundantly seen than *Chromolaena odorata*. The highest frequency in vayals was recorded for *Chromolaena odorata* (89.4) and *Arundinella leptochloa* (83.5).

The NF in WS II was dominated by *Chromolaena odorata* (60.56) (Figure 3). The second most dominant species in NF was *Lantana camara* (15.7). Other dominating species were *Mimosa pudica* (10.82), *Terminalia elliptica* (10.53), and *Glycosmis pentaphylla* (10.38). In the WS II plantation also, the dominance of *Chromolaena odorata* (143.06) was evident. The second most dominant species here was *Tectona grandis* (103.35). Other dominating species were *Mimosa pudica* (57.01), and *Glycosmis pentaphylla* (55.42). In vayal, *Arundinella leptochloa* (143.06) had the highest dominance. This was followed by *Chromolaena odorata* (35.54), *K. nemoralis* (18.17) and *Sporobolus tenuissimus* (13.0) in that order.

Among the three ecosystems (Table 6), vayals recorded the highest Simpson's diversity index, with plantations recording the least index value. In the vayal ecosystem, the predominance of many grass species has contributed to the higher index value. Moreover, vayals also recorded the highest Pielou's Wiener equitability index, which means that, in vayals, the plant species present are also more evenly distributed. The highest Berger-Parker dominance index for the plantations indicates the domination by selected species in this ecosystem which is also a reason for its reduced diversity index. The highest Margalef richness index was in natural forest followed by plantation and vayal.

## CONCLUSION

The paper assessed the phytosociological characters of the vegetation in three different ecosystems (Natural forest, plantation and vayal) of WS II area of Wayanad WS in Kerala State. The plant species diversity and the structural composition of flora found in these ecosystems were distinctly different. As expected, the highest species richness was found in NF and the least was in vayal. All the three ecosystems had their unique set of representative plant species. *Chromolaena odorata*, which is an invasive alien plant species (IAPS), however, was one of the dominant species in all three ecosystems. Besides the tree species, *Terminalia elliptica* and *Tectona grandis*, WS II of Wayanad WS was also observed to be largely invaded by *Chromolaena odorata*, *Lantana camara*, and *Mimosa pudica*, which are also invasive in nature.

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**Author contribution:** MVC—conducted the field study, data analyses, drafting the manuscript, provided literatures, photos of specimens, habitats. SG—conceptualized the research idea and peer reviewed the manuscript. AM—identification of the observed plant species.





## Piroplasmosis in a captive Grant's Zebra *Equus quagga boehmi* (Mammalia: Perissodactyla: Equidae) - a case study

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**Abstract:** An apparently healthy 2½-year-old male Grant's Zebra weighing approximately 200kg located at Nandankanan Zoological Park, Odisha, India, procured from Zoological Centre, Tel Aviv- Ramat Gan, Israel during September 2015 was noticed in a sitting position making frequent attempts to get up. The zebra was immobilised the same day with a combination of 1.96mg etorphine hydrochloride, 8.0mg of acepromazine and 40.0mg of xylazine hydrochloride to facilitate diagnosis and treatment. Clinical examinations did not reveal any signs suggestive of disease or disorder of the musculoskeletal system. Microscopic examination of blood smears stained in Giemsa's stain revealed the presence of intra-erythrocytic inclusions, either single or pairs, suggestive of haemoprotozoans, i.e., *B. caballi* and/or *T. equi*. The zebra was administered with two divided doses of imidocarb injection @4.0mg/kg b.wt. deep intramuscularly in the neck region with supportive therapy. Progressive improvement in posture, gait, and appetite were noticed following 24h of medication. Three more doses of imidocarb were administered at 72h intervals, each time after immobilisation.

**Keywords:** *Babesia caballi*, imidocarb, immobilisation, sub-clinical carriers, *Theileria equi*.

'Equine piroplasmosis', a tick borne haemoparasitic disease of Equidae (horses, mules, donkeys, and zebras), is widely distributed across the globe including tropical

and subtropical areas, and some temperate zones (Alhassan et al. 2005; Acici et al. 2008). Piroplasmosis is prevalent amongst Burchell's Zebra *Equus quagga burchellii* and Cape Mountain Zebra *Equus zebra zebra* in southern Africa (Lampen et al. 2009; Bhoora et al. 2010). It is caused by two morphologically distinct intra erythrocytic protozoans, viz., *Theileria equi* (formerly known as *Babesia equi*) and/or *Babesia caballi*. *T. equi* infection having shorter incubation period is more pathogenic than *B. caballi* (de Waal & van Heerden 2004). The disease appears in acute, sub-acute, and chronic forms with signs of fever, anaemia, icterus, hepatomegaly, edema, intravascular haemolysis, and haemoglobinuria. Mortality may reach up to 50% (de Waal 1992). Laminitis is one of the clinical signs of secondary complications (de Waal 1992). Antiprotozoan drugs are quite effective in bringing clinical recovery but fail to make the infected animal sterile. Hence, infected animals may remain life-long carriers of *T. equi* infections while *B. caballi* for up to four years (de Waal

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& van Heerden 2004). Thirty species of ixodid ticks of the genera *Dermacentor*, *Hyalomma*, and *Rhipicephalus* have been identified as vectors (de Waal 1992). It is also not possible to distinguish between *T. equi* and *B. caballi* infections based on clinical signs alone and mixed infections do occur (de Waal 1992). Available literature is silent about documentation of these infections amongst Grant's Zebras in Indian zoos. The present case describes a case report of Piroplasmosis in a Grant's Zebra *Equus quagga boehmi* at Nandankanan Zoological Park.

### Case history

On 28 December 2016 (15.30h Indian time), a 2½-year old apparently healthy male Grant's Zebra (approx. body weight 200kg) of Nandankanan Zoological Park (NKZP) was noticed in a sitting position making repeated attempts to get up. But the zebra failed to bear its weight on the hind limbs. When approaching close to the animal, it moved with difficulty and dragged its right hind limb fetlock on the ground. Initial attempt with an intramuscular injection of NSAID (non-steroid anti-inflammatory drug) of 10ml Melonex Power (M/S Intas pharmaceuticals Ltd, Ahmedabad, Gujarat, India) through a blow pipe did not result in any remission even after three hours post-administration.

Four zebras (two males + two females) including the present ailing one were procured from Zoological Center, Tel Aviv- Ramat Gan, Israel. As per health records, the zebras were apparently healthy during the time of procurement as well as on arrival at Nandankanan Zoological Park on 13 September 2015. The zebras were kept in 'pre-export' quarantine at Ramat Gan, Israel for a period of 30 days. During the quarantine period at Israel, they were screened against *Theileria equi* and *Babesia caballi* by complement fixation test (CFT) and test reports were negative for both. At NKZP, these zebras were housed in an open air exhibit enclosure of 3,510m<sup>2</sup> area.

Of these four zebras, two females died on 20 August 2016 and 29 October 2016 with the predominant signs of limping in one or more limbs that continued for a period of 20–60 days in spite of supportive treatment consisting of nervine stimulants, NSAID, and broad spectrum antibiotics (BSA), all given with the help of a blow pipe. Further investigations could not be initiated due to non-availability of supporting facilities during that concerned period.

Earlier painful experience of casualties in two valuable animals triggered efforts to immobilise the sick zebra in the late evening (20.00h) to extend all possible therapeutic measures.

### Clinical investigation

The zebra was darted using a drug mixture of 0.8ml of large animal imobilon (Novartis Animal Health, UK Limited, Frimley, South Africa) containing 1.96mg etorphine hydrochloride & 8.0mg of acepromazine and 0.4ml of Xylazil 100 (Troy Lab Pty Ltd, 35 Glendenning Road, Australia) containing 40.0mg of xylazine hydrochloride. This drug mixture was administered intramuscularly to the thigh muscle through 'Dist-Inject Syringe Projector Mod30N' from a distance of about 10m using a blue cartridge.

Detailed clinical examination was carried out including examination of hooves, joints, and other vulnerable body regions. Blood samples were collected with anticoagulants (EDTA & fluoride) and clot activator in three different sterile vials for further investigation.

Laboratory investigation was performed the same night with respect to haemato-biochemical and parasitological examinations to initiate a specific line of treatment. Blood smears were stained with Giemsa's stain and examined under oil immersion with the objective to detect haemoparasite. It revealed the presence of pear/oval shaped intra-erythrocytic inclusions, either single or pairs, suggestive of *Babesia* organism. Haemato-biochemical parameters with respect to Hb, TLC, DLC, sodium, potassium, ALP, AST, total protein, urea, creatinine, cholesterol, bilirubin, glucose, triglyceride, calcium, magnesium, and phosphorus were carried out following standard procedures (Table 1).

### Treatment

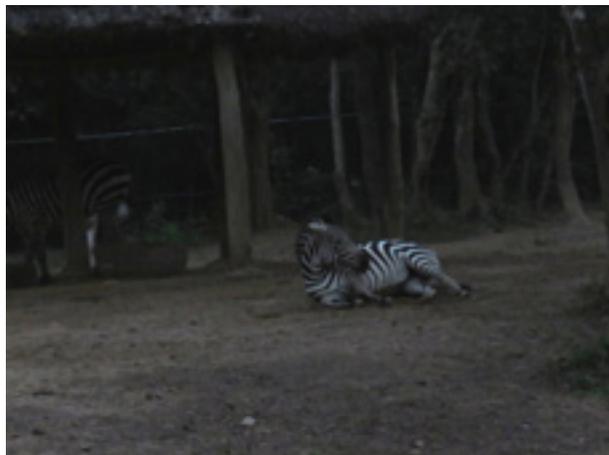
The sick zebra was administered Imicarb 8.0ml (M/S Sava Health Care Ltd. Sava House, Pune, India) deep i/m in two equally divided doses at the neck muscle. The dose was calculated @4.0mg imidocarb per kg body weight. Additional treatment included 1L Lactated Ringer's and 1L 5% dextrose, 2.0g Tazar (Piperacillin and Tazobactam from M/S Lupin Limited, Mumbai, India) and 15ml Optineurone (M/S Lupin Ltd, Gujarat, India).

The zebra was reversed after 40min of induction by intravenous injection of 0.8ml of large animal revivon (Novartis Animal Health) that contained 2.6mg of diprenorphine hydrochloride and 0.5ml Reverzine (Bomac Pty Limited, Hornsby, NSW 2077) that contained 5.0mg of yohimbine hydrochloride. All the activities were accomplished under artificial electric flood light (Image 1).

As a supportive therapy, the zebra was provided with mineral mixture (Bestomin Gold, Provimi Animal Nutrition India Pvt Ltd.) @ 30g/day, calcium granules (Orcal-P, TTK Healthcare Limited, Chennai, India) @ 50g/

**Table 1. Haemato-biochemical values of a 2½-year-old ailing male Grant's Zebra on different days of illness.**

Parameters	Haemato-biochemical values on different days of treatment		Reference values of horse (Radostits et al. 2006)
	28.xii.2016	01.i.2017	
Haemoglobin (g%)	15.5	15.4	11–19
TLC (cu mm)	14,350	11,600	5400–14300
DLC(%)			
Neutrophil	78	70	52–70
Eosinophil	02	01	0–7
Lymphocyte	18	26	21–42
Monocyte	02	03	0–6
Sodium (mEq/L)	134.5	131.6	132–146
Potassium (mEq/L)	4.1	4.0	3.0–5.0
ALP (IU/L)	225.6	210.9	140–4003
ALT (IU/L)	6.0	5.8	3.0–23
Total Protein (g/dl)	6.5	6.4	6.0–7.7
Urea (mg/dl)	59.7	70.2	10–24
Creatinine (mg/dl)	1.81	1.25	0.9–1.9
Cholesterol (mg/dl)	132.9	107.4	46–180
Bilirubin (mg/dl)	0.95	1.01	1.0–2.0
Glucose (mg/dl)	163.0	102.1	75–115
Triglyceride (mg/dl)	67.5	67.5	4.0–44.0
Calcium (mg/dl)	12.1	11.1	11.2–13.6
Magnesium (mg/dl)	2.2	2.2	2.2–2.8
Phosphorous (mg/dl)	3.7	4.1	3.1–5.6



**Image 1. Ailing Grant's Zebra after recovery from anaesthesia on 28.xii.2016 night. © Nandankanan Zoological Park.**

day and a commercially available herbal antirheumatic preparation (R-Compound from M/S Alarsin Pharmaceuticals, Mumbai, India) @ 10 tablets per day in its concentrate feed for a period of three months.

The sick zebra was segregated from the other male zebra to facilitate treatment and monitoring. Based on the literature (Radostits et al. 2006), three more doses of imidocarb injections were administered with the same dose and route at 72h intervals. Blood samples

were also collected during the post-treatment period to record haemato-biochemical alterations.

**RESULTS AND DISCUSSION**

The severity of clinical signs shown by the ailing zebra coupled with the earlier tragic end of two other zebras in the same enclosure warranted immediate intervention. Etorphine used in this case is the most recommended drug to immobilise the zebra. The drug combination, i.e., Etorphine, Acepromazine, and Xylazine were also used previously by different workers to immobilise zebras (Walzer 2003; Senthilkumar et al. 2005; Nath et al. 2012). Following tranquilisation the zebra started showing signs of anaesthesia four minutes post-injection period and complete immobilisation was achieved in seven minutes.

Clinical signs of equine piroplasmosis are often nonspecific. It may be confused with a variety of other viral diseases like equine influenza, encephalosis virus infection and equine infectious anaemia. The haemato-biochemical parameters analysed in the present case were found to be within reference range (Table 1). This showed the absence of any of the viral infections described above. Clinical examinations did not reveal any appreciable musculo-skeletal disorders or deformity correlating clinical signs exhibited by the zebra. Body



Image 2. Ailing Grant's Zebra in standing posture on 01.i.2017. © Nandankanan Zoological Park

vitals like rectal temperature, respiration, and heart rate were recorded as 99.3°F, 12 breaths/minute, and 70 beats/minute, respectively. Both rectal temperature and respiration rates were within the normal range. Heart beats were on a higher side as against the reference value of 28–40 bpm. This transient increase could be correlated with the excitement during pre and post tranquillisation procedure.

Anaemia and haemoglobinuria which is marked in case of *T. equi* infection (Soulsby 1982) was not seen here and posterior paralysis found in this case is common to *B. caballi* infection and not found in *T. equi* infection (Soulsby 1982).

Blood smear examination revealed the presence of pear/oval shaped intra-erythrocytic inclusions, either single or pairs (Image 3), suggestive of haemoprotozoans, i.e., *B. caballi* and/or *T. equi*. No tetrads/ maltese cross, specific for *T. equi*, (Soulsby 1982) were noticed here. Clinical signs coupled with parasitological examination confirmed the case to be equine piroplasmosis and more likely to be *B. caballi* infection. This corroborated the earlier report of Zwegarth et al. (2002) who detected both *B. caballi* and *T. equi* in zebras from two national parks in South Africa.

The present report is substantiated by the fact that *Theileria equi* and *Babesia caballi* infections are endemic in Israel (Levi et al. 2018). Most of the infected animals (equids) may remain as sub-clinical carriers of these parasites with no clinical signs and act as a source of infection (Friedhoff & Soulè 1996). Though these zebras were tested negative against *B. caballi* & *T. equi* through complement fixation test during pre-export quarantine period at Israel, the possibility of carrier state can't be ruled out as documented by Radostits et al. (2006) and

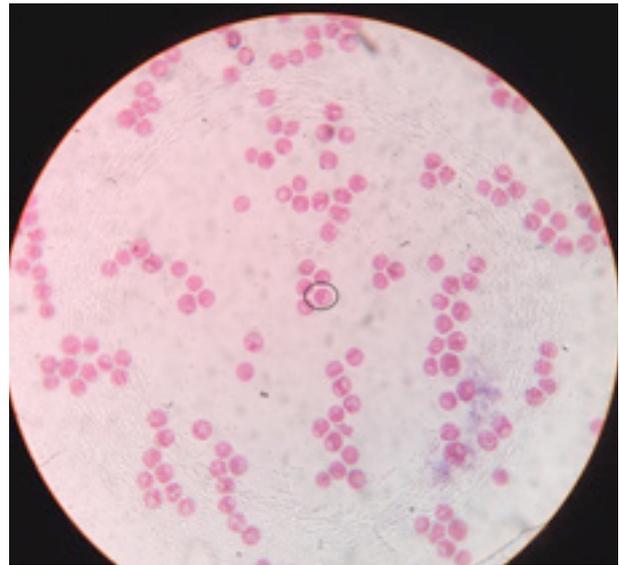


Image 3. Blood smear (Giemsa's stain) of an 2½-year-old male Grant's Zebra showing pyriform intra-erythrocytic *Babesia* sp. in pair or single under oil immersion objective. © Department of Veterinary Parasitology.

the zebra is suspected to have carried *B. caballi* and/or *T. equi* from Israel in sub-clinical stage.

Several techniques/tests are employed for the diagnosis of equine piroplasmosis that include clinical signs, direct demonstration of parasites in blood smears, serological assays, cell-culture, and PCR assays, however, the present diagnosis is based on the clinical signs, blood smear examination, and response to treatment. Advanced molecular techniques couldn't be carried out due to lack of facilities at that time. The clinical signs noticed here, i.e., sudden onset of impaired mobility with posterior paralysis were also akin to observations by other authors (Radostits et al. 2006; Kaandorp 2010).

Drugs available for the treatment of equine piroplasmosis are Diminazene for *B. caballi* and Parvaquone for *T. equi* infections (de Waal 1992). Imidocarb, which is considered to be the safest of all drugs available, is effective in treating clinical cases of both the protozoans (Radostits et al. 2006).

Within 24 hours after administration of the first dose of imidocarb injection, significant improvement was observed with respect to gait, movement, and appetite. The zebra could stand and walk with moderate speed (Image 2). The signs of limping subsided completely and appetite was regained within 72h after the first dose of imidocarb. In order to ensure proper administration of the required drug, the zebra was immobilised every time using the same drug and dose schedule.

This favourable response to imidocarb confirmed

our diagnosis of piroplasmosis. Imidocarb is the most trusted drug for the treatment of equine babesiosis (Radostits et al. 2006; Donnellan & Marais 2009). To the best of our knowledge, this seems to be the first report of the piroplasmosis in Grant's Zebra in Indian zoos.

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## *Eurylophella karelica* Tiensuu, 1935 (Insecta: Ephemeroptera: Ephemerellidae) – an additional species to the mayfly fauna of Ukraine and notes on distribution of the family in the country

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**Abstract:** A rare European species, *Eurylophella karelica* Tiensuu, 1935 (Ephemeroptera: Ephemerellidae) is reported for the first time from Ukraine. The larvae of the species were found within Ukrainian Polissya region in the Pripjat' River basin. Species list of family Ephemerellidae of Ukraine with notes on species distribution within the country is given in the present contribution.

**Keywords:** Checklist, distribution, mayfly, Pannota, species, Ukrainian Polissya region.

*Eurylophella* Tiensuu, 1935 is a Holarctic genus encompassing 19 recent species. Most of these species inhabit the Neartic realm, and only three of them occur within the western Palearctic region—*Eurylophella karelica* Tiensuu, 1935, *E. iberica* Keffermüller & Da Terra, 1978, and *E. korneyevi* Martynov, Palatov & Godunko, 2015 (Martynov et al. 2015). *Eurylophella karelica* is the most common and widely distributed, the other two are rare with restricted distribution. Distribution of *E. karelica* extends from north to south, and falls within the north-west of the European part of Russia, Finland, Lithuania, Latvia, Estonia, Poland, Hungary, and Slovenia (Martynov et al. 2015; Ozoliņš et al. 2017). This species has not yet been recorded in Belarus (Moroz & Lipinskaya 2014), but it is likely to be found in the future.

### MATERIALS AND METHODS

The material was collected with square hydrobiological hand net 25cm long on each side. The sample is stored in 90–95 % ethanol in the author's collection in the National Museum of Natural History, National Academy of Sciences of Ukraine (Kyiv, Ukraine); its inventory number (IN) is Riv19Eurkar. Toponyms and hydronyms are duplicated in Ukrainian in square brackets.

Habitus of *E. karelica* larva was photographed using a Leica Z16 APO stereomicroscope equipped with Leica DFC450 Digital Camera in the I.I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, and was subsequently processed with LAS Core 3.8 and Adobe® Photoshop™ CS5 software.

**Material:** Five larvae of *Eurylophella karelica*, Ukraine, Rivne region [Рівненська область], vicinity of Osnyts'k [Осницьк] Village, L'va [Льва] River, 51.284E, 27.145N and 51.284°E, 27.144°N (Image 1), about 145m, 22.xii.2018, leg. Martynov A.V. – IN Riv19Eurkar.

### RESULTS AND DISCUSSION

In Ukraine, *E. karelica* was recorded at Polissya [Полісся] region in potamal zone of L'va [Льва] River

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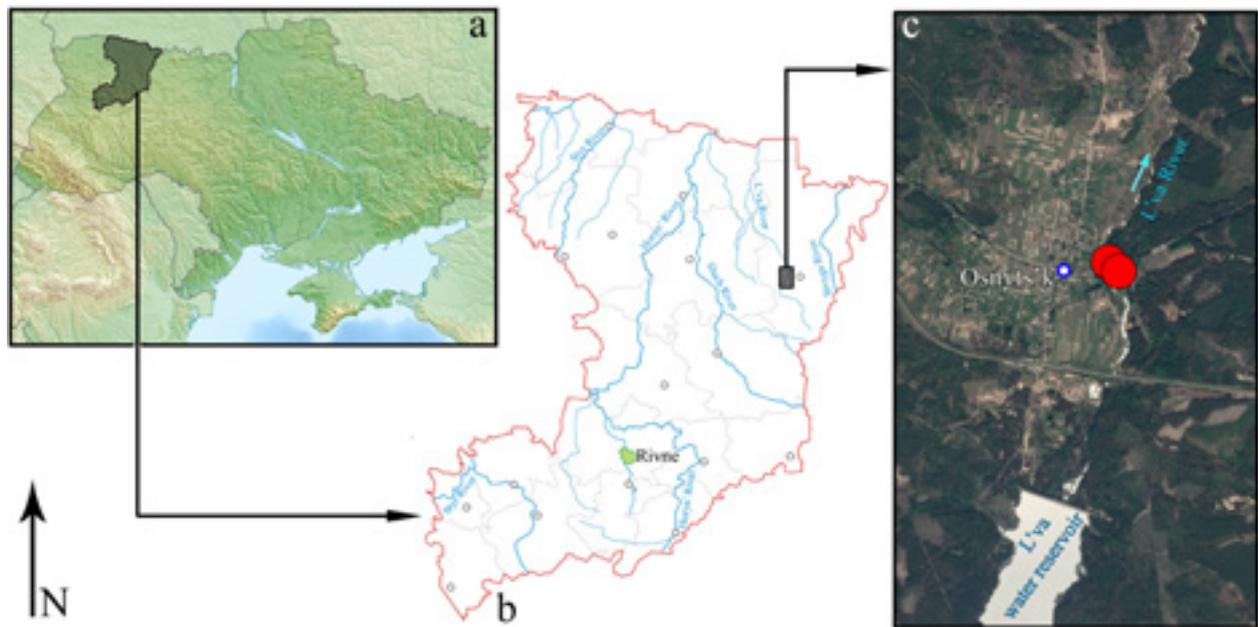


Image 1. Geographical location of *Eurylophella karelica* Tiensuu, 1935 collecting place: a—Rivne Region | b—vicinity of Osnyts'k Village on map of Rivne Region | c—total view of L'va River watercourse in vicinity of Osnyts'k Village.

(Image 2) that belongs to Pripjat' [Прип'ять] River basin. This finding is the first record of *E. karelica* from Ukraine. The Pripjat' River basin, where the species was found, differs in the presence of a relatively large number of waterbodies, with slightly impaired species compositions of aquatic insects, in comparison with vast parts of lowland Ukraine. Thus, during investigation of Ephemeroptera at the Pripjat' River basin in 2017–2018 several stenobiotic and rare species were registered (Martynov 2018); some of these species had not yet been recorded elsewhere in Ukraine.

#### Habitat characteristics

The section of the L'va River, where *E. karelica* larvae were collected (Image 2, 3), is situated downstream of a dam. The river has moderate current velocity (up to 0.3m/s), but under the bridge and above the dam rapids with strong current are present (up to 1m/s). The bottom of the river is silty, sandy (in some places silted), and stony (under the bridge). All larvae of the species were collected from dead aquatic vegetation (leaves, stems etc.) in small bays with silted bottom and almost lacking flow (Image 2). The additional parameters of waterbody in collecting sites measured during the sampling (22.xii.2018) were: water temperature 6°C, water hardness 84ppm, pH 8.9.

The following mayflies were also collected within the investigated section of the L'va River in winter (22.xii.2018) and spring (22.iii.2019)—*Nigrobaetis digitatus*

(Bengtsson 1912), *Cloeon dipterum* (Linnaeus, 1761), *Heptagenia sulphurea* (O.F. Müller, 1776), *H. flava* Rostock, 1878, *Kageronia fuscogrisea* (Retzius, 1783), *Leptophlebia marginata* (Linnaeus, 1767), *L. vespertina* (Linnaeus, 1758), *Paraleptophlebia submarginata* (Stephens, 1835), *Ephemera vulgata* Linnaeus, 1758, and *Caenis* sp. The registration of 11 mayfly species in this section of the L'va River, makes me consider it as one of the hotspots of mayfly species diversity in Ukrainian Polissya region.

#### Ephemerellidae of Ukraine and their distribution within country

In the first checklist of mayflies of Ukraine, five species of subfamily Ephemerellinae were mentioned (Godunko & Kłonowska-Olejnik 2003). *Eurylophella karelica*, recorded for Ukraine for the first time herein, belongs to another subfamily – Timpanoginae. Thereby, now species list of Ephemerellidae of Ukraine consists of six species from five genera and two subfamilies, and *E. karelica* is the most rare species among them.

#### Ephemerellinae

- *Serratella ignita* (Poda, 1761)
- *Ephemerella notata* Eaton, 1887
- *Ephemerella mucronata* (Bengtsson, 1909)
- *Teloganopsis mesoleuca* (Brauer, 1857)
- *Torleya major* (Klapálek, 1905)



Image 2–3. Larva of *Eurylophella karelica* Tiensuu, 1935 and its habitat: 2—total view of the L'va River in collecting cite (area outlined with white color - microhabitat mostly preferred by *E. karelica*) | 3—larva of *E. karelica*, dorsal view. Scale bar 2mm. © Alexander V. Martynov.

### Timpanoginae

· *Eurylophella karelica* Tiensuu, 1935 – first record for Ukraine

The Carpathian Mountains [Карпати], Transcarpathian [Закарпатська] Lowland, and eastern Carpathian Foothills [Передкарпаття] are the Ukrainian regions inhabited with the highest number of Ephemeroptera taxa; a total of five species are recorded here—*S. ignita*, *E. notata*, *E. mucronata*, *T. mesoleuca*, and *T. major* (Mikulski 1935; Godunko 2000; Godunko & Kłonowska-Olejnik 2003; Afanasyev 2006; Kovács & Godunko 2008). Such diversity is reasoned by abundance of waterbodies with unpolluted or moderately polluted rhithral and epipotamal zones preferred by a vast

number of mayfly species.

Except for *E. karelica*, also *S. ignita* inhabits some waterbodies of Ukrainian Polissya. Larvae of this last species were registered in Dnipro [Дніпро] River (Zimbalevskaya et al. 1989), Pripjat' River (Trylis et al. 2013), and Irsha [Ірша] River (50.759E & 29.411N) (original data). Such small numbers of species in the region may be due to the small number of flowing waterbodies preferred by Ephemeroptera, pollution and habitat modifications in main part of them.

Four species of Ephemeroptera, *Serratella ignita*, *Teloganopsis mesoleuca*, *Ephemeroptera notata*, and *E. mucronata* were recorded for Dniester [Дністер] River, natural border of Podolia [Подільська] Upland, and

eastern Carpathian Foothills (Telyuk 1982, 1992; Mikulski 1933).

Mayflies of Podolia and Volyn' [Волинська] Uplands, lowland, central, and southern regions of Ukraine are still poorly investigated. Most of the data are old and need confirmation due to significant changes and pollution levels in waterbodies, especially in central and southern regions.

The Crimean [Кримські] Mountains was investigated and summarized in detail (see overview in Prokopov & Godunko 2007). Only *Serratella ignita* was listed by Kiseleva & Yezernitskiy (1985) for this region, and there are no other mentions of the species for the Crimea [Крим]. According to Prokopov & Godunko (2007) the presence of this species in the fauna of the region needs confirmation.

Within physiographic regions of eastern Ukraine only one representative of Ephemerellidae—*Serratella ignita*—was registered. This species inhabits only rhithral and eupotamal zones of streams and headwaters of rivers within most elevated parts of Donetsk [Донецька] elevated areas, and the species is rare within it (Martynov 2014, 2016). The recorded population of *S. ignita* is geographically isolated and should be considered as glacial relicts in this territory.

It should be noted, that the diversity of Ephemerellidae in neighboring countries (Romania, Hungary, Slovakia, Poland, and European part of Russian Federation) does not exceed diversity of the family within Ukraine. The territory of Moldova is poorly investigated; two species of the family were recorded for Belarus only. Therefore, the Ephemerellidae species list given above should be considered as complete, and no other species of the family are expected to be registered in Ukraine in the near future.

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## Some new records of katydids (Orthoptera: Tettigoniidae) from Uttar Pradesh, India

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**Abstract:** In the present investigation intensive field surveys were undertaken to explore katydids of Uttar Pradesh. Five species of katydids from four genera, namely, *Letana* cf. *megastridula* Ingrisch, 1990, *Parasanaa donovani* (Donovan, 1834), *Sathrophyllia femorata* (Fabricius, 1787), *S. rugosa* (Linnaeus, 1758), and *Acanthoprius suspectum* (Brunner 1895) were recorded for the first time from Uttar Pradesh, India.

**Keywords:** *Acanthoprius*, Chandauli, Ghazipur, grasshoppers, *Letana*, Mirzapur, *Parasanaa*, *Sathrophyllia*.

Grasshoppers and katydids are an important group belonging to the order Orthoptera. Globally, more than 28,530 valid species of orthoptera have been catalogued in which more than 7,500 species of tettigoniids are known worldwide (Cigliano et al. 2018). Exploration of tettigoniids from Uttar Pradesh, India is not much more explored as compare to Maharashtra (Chandra & Gupta 2012). However, due to the scattered reporting on this group, exact estimation is unavailable also at the national level as well as from Uttar Pradesh. However, Shishodia et al. (2010) mentioned some 160 species from 72 genera of tettigoniids in his checklist of Orthoptera from India. Due to the scattered reporting on this group, exact estimation is unavailable at the national level, however, Shishodia et al. (2010) mentioned some 160

species from 72 genera of tettigoniids in his checklist of Orthoptera from India. They are dominant fauna of tropical and subtropical ecosystems. In India, most of the taxonomic studies of katydids are carried out from the northeastern Indian states as compare to other states (Shishodia et al. 2010).

Katydid are also called long-horned grasshoppers. They belong to the suborder Ensifera and the superfamily Tettigoniioidea. They are very easily identified by their very long antennae, (hence the common name) and sword- or sickle-shaped long ovipositor with four segmented tarsi. They are more similar to crickets than other grasshoppers. The Indian katydids are grouped in nine subfamilies of which Phaneropterinae is the most diverse and Phyllophorinae is the least diverse subfamily. *Letana* is a highly diverse genus of katydids (Tettigoniidae) in India (Shishodia et al. 2010). Some important notable works on the taxonomy and distribution of Tettigoniidae from India are done by Barman & Srivastava (1976), Barman (1993, 2003), Ingrisch & Shishodia (2000), Shishodia (2000), Shishodia & Tandon (2000), Kulkarni & Shishodia (2004, 2005), Shishodia & Barman (2004), Senthilkumar et al. (2006), Chandra et al. (2007), Senthilkumar (2010), Shishodia

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Figure 1 . Survey sites in Uttar Pradesh

et al. (2010), Srinivasan & Prabakar (2012), and Yadav (2016).

Uttar Pradesh is one of the largest states in India and considered biogeographically diverse ranging from plains to dry and plateau areas. Here, floral and faunal diversity is very varied but katydids are under-studied probably because of their nocturnal habit. Farooqi & Usmani (2016) recorded 13 species of katydids from this

Table 1. Coordinates of survey sites.

	Site	Coordinate
1	Barkachchha, Mirzapur	25.133°N & 82.564°E
2	Chahaniya, Chandauli	25.418°N & 83.211°E
3	Gai Ghat , Ghazipur	25.415°N & 83.559°E
4	Jangipur, Ghazipur	25.654°N & 83.557°E
5	Maujhi, Chandauli	25.260°N & 83.266°E
6	Nagra, Ballia	25.968°N & 83.871°E
7	Sahadatpur, Mau	25.924°N & 83.452°E

state. Hence, the authors have tried to explore the long-horned grasshoppers from Uttar Pradesh.

**Materials and Methods**

The present exploration was part of a series of surveys at several locations in eastern Uttar Pradesh during 2017 (Image 1). The katydids were collected using sweep nets, light traps, and by hand picking. Most collections were made during night and morning hours, i.e., 19.30–23.00 h and 07.00–10.00 h, respectively. The collected katydids were killed in a container containing ethyle acetate. Subsequently, the materials were cleaned with hair brush, pinned and relaxed (as per need) on the handmade stretching board of thermacol. It was kept for more than 60–72 hours for drying to avoid decomposition of the specimens. The identification is based on the morphological and genital features and classification was done according to Cigliano et al. (2018). The specimens collected were deposited in the Department of Entomology and Agricultural Zoology (RGSC), Faculty of Agriculture, Rajiv Gandhi South Campus, Banaras Hindu University, Mirzapur, Uttar Pradesh, India. The coordinates of the survey sites are presented in Table 1.

**Results**

Order: Orthoptera

Suborder: Ensifera

Superfamily: Tettigonioidea

Family: Tettigoniidae

**1. Subfamily: Phaneropterinae**

**Tribe: Letanini**

***Letana cf megastridula* Ingrisch, 1990** (Image 1)

1990. *Letana megastridula* Ingrisch, *Entomologica Scandinavica* 21(3): 258.

2007. *Letana megastridula* Chandra et al. *Zoos' Print Journal* 22(5): 2684.

2016. *Letana megastridula* Gaikwad et al. *Journal of Threatened Taxa* 8(2): 8534.



Image 1. *Letana cf. megastridula* Ingrisch, 1990.

Materials examined: RGSCE 006–007, 17.ix.2017, 1 male, 1 female, Uttar Pradesh, Chahaniya (Chandauli), grasses, coll. R.S. Yadav; RGSCE 008, 10.ix.2017, 1 female, Nagra (Ballia), grasses, coll. R.S. Yadav; RGSCE 009, 24.ix.2017, 1 male, Jangipur (Ghazipur), bushes, coll. R.S. Yadav; RGSCE 0010, 24.ix.2017, 1 female, Sahadatpura (Mau), bushes, coll. R.S. Yadav.

Diagnosis characters: Bluish-green, medium-sized insect. An antenna is about a double the length of the body. The tegmina is narrow, dorsal portion of tegmina is brown in colour, surpassing the hind knees and with forked radial areas (Ra); the left tegmina with broad stridulatory field. Body along with legs is reddish or blackish dots. Cerci expanded internally.

Distribution: Bihar, Chhattisgarh, Himachal Pradesh, Maharashtra, and Tamil Nadu (Shishodia et al. 2010), and Uttar Pradesh (present study).

Habitat: The species is more common in grasses.

Remark: The genus *Letana* is the most diverse taxon of katydids in India.

## 2. Subfamily: Pseudophyllinae

### i. Tribe: Cymatomerini

#### *Parasanna donovani* (Donovan, 1834) (Image 2)

2000. *Parasanna donovani*, Ingrisch & Shishodia, *Mitt. Munch. Ent. Ges.* 90 : 21.

2003. *Parasanna donovani*, Barman, *State Fauna Series, 9: Fauna of Sikkim, Part 2*, Zool. Surv. India: 199.

Materials examined: RGSCE 001, Uttar Pradesh, 5.ix.2017, 1 female, Maujhi (Chandauli), forest tree, coll. RS Yadav; RGSCE 002–003, 10.ix.2017, 2 females, Barkachchha (Mirzapur), shrubs, coll. D Kumar.

Diagnosis characters: Medium to large insect. Beautifully decorated. Antennae are brownish in colour. The head and body are brownish in colour whereas the pronotum is light yellow in colour with two brown spots. The tegmina are with three reddish-brown irregular spots between the radius and cubitus region. The ovipositors are reddish-brown in colour.



Image 2. *Parasanna donovani* (Donovan, 1834).

Distribution: Rajasthan, Sikkim (Shishodia et al. 2010), and Uttar Pradesh (present study).

Habitat: Small bushes are the preferred habitat of the species.

#### *Sathrophyllia femorata* (Fabricius, 1787) (Image 3)

1869. *Sathrophyllia femorata*, Walker, *Cat. Derm. Salt. Brit. Mus.*: 401.

1954. *Sathrophyllia femorata*, Beier, *Trab. Inst. Esp. ent. Madrid*: 236.

1993. *Sathrophyllia femorata*, Barman, *State Fauna Series, 3: Fauna of West Bengal, Part 4*, Zool. Surv. India: 359.

2003. *Sathrophyllia femorata*, Barman, *State Fauna Series, 9: Fauna of Sikldm, Part 2*, Zool. Surv. India: 197.

Materials examined: RGSCE 0011–12, 5.viii.2017, 2 females, Uttar Pradesh, Barkachchha (Mirzapur), shrubs, coll. D. Kumar.

Diagnosis characters: It is the large insect with brownish colour. The vertex of head excavated at the apex portion with two horny projections. The pronotum is strongly crested over. The tegmina is rounded at apex. Wing is close to the tegmina. The all femora are waved below. Subgenital plate is broad, styli conical and epiproct rounded with curved cerci. Sword shaped ovipositors with black at tip.

Distribution: Karnataka, Maharashtra, Sikkim, Tamil Nadu and West Bengal. (Shishodia et al. 2010), and Uttar Pradesh (present study).

Habitat: It is prevalent in bushes and forest tree.

Remark: Only female specimens were found.

#### *Sathrophyllia rugosa* (Linnaeus, 1758) (Image 4)

1758. *Gryllus (Tettiginia) rugosa* Linnaeus, *Systema*



© D. Kumar

Image 3. *Sathrophyllia femorata* (Fabricius, 1787)

*Naturae per Regna Tria Naturae*, (10<sup>th</sup> ed.): 430.

1815. *Conocephalus cornotus* Thunberg, *Mem. Acad. Imp. Sci. St. Peterburg*. 5: 277.

1906. *Sathrophyllia rugosa* Kirby, *Syn. Cat. Orth.*: 306.

1993. *Sathrophyllia rugosa* Barman, *State Fauna Series 3: Fauna of West Bengal, Part 4, Zool. Surv. India*: 284, 285.

2004. *Sathrophyllia rugosa* Shishodia & Barman, *State Fauna Series 10: Fauna of Manipur, Zool. Surv. India*: 144.

2016. *Sathrophyllia rugosa* Gaikwad et al., *Journal of Threatened Taxa* 8(2): 8536.

Materials examined: RGSCE 021–022, 5.viii.2017, 2 females, Uttar Pradesh, Barkachchha (Mirzapur), shrubs, coll. D Kumar.

Diagnosis characters: It is an ash coloured large insect. The pronotum has one anterior and several posterior teeth. The tegmina is slightly shorter than wings. The fore femur is stout with ventral margin and is strongly lamellate and with distinct lobe. The dorsal edge of mid femur is only faintly lamellar and mostly straight. Inner dorsal edge of hind tibia is with distinct teeth. The supra anal plate oval and little cut at apex. Ovipositor is toothed at apex.

Distribution: Karnataka, Madhya Pradesh, Manipur,

**Keys to some recorded katydids from Uttar Pradesh**

**Key to subfamilies**

- 1. Prosternal spines present; forewing oval and coastal areas with many transverse veins; ovipositor long and straight ..... Pseudophyllinae
- Prosternal spines absent; forewing not like above, without transverse costal veins; ovipositor short and bent upward ..... Phaneropterinae

**Key to tribes of Pseudophyllinae**

- 1. Mostly brownish in colour; bark like; pronotum with or without tubercles; tegmina rugose or not rugose; all femora compressed at exteroventral margin, more or less expanded ..... Cymatomerini
- Mostly greenish in colour, boat likes; pronotum without tubercles; tegmina not rugose always, covering the some part of pronotum; femora not like above ..... Phyllomimini (only one species)

**Key to tribes of Phaneropterinae**

- 1. Male having much more bifurcation into subgenital plate ..... Letanaeini (only one species)
- Not much more deep bifurcation into subgenital plate ..... Ducetini (not discussed here)

**Key to genera of Cymatomerini**

- 1. Pronotum mostly smooth; tegmina not rugose, wing infumated; radius of tegmina parallel, coarse with subcosta; front femora simple, only hind femora compressed ..... *Parasanaa*
- Pronotum mostly not smooth; tegmina rugose; anal area of wing well developed; frontal femora lamellate ..... *Sathrophyllia*



Image 4. *Sathrophyllia rugosa* (Linnaeus, 1758).

Meghalaya, Sikkim, Tamil Nadu and West Bengal (Shishodia et al. 2010), and Uttar Pradesh (present study).

Habitat: The species prefers shrubs, bushes and tall grasses

Remark: The fauna was recognized by its ash colour, rugose body and crested pronotum.

#### ii. Tribe: Phyllomimini

##### *Acanthoprion suspectum* (Brunner, 1895) (Image 5)

1895. *Aprion suspectum* Brunner von Wattenwyl, *Verhand. K. K. Zool. Bot. Ges. Wier*: 77.

1962. *Acanthoprion suspectum*, Beier, *Das Tierreich*, 73 : 155.

1993. *Acanthoprion suspectum*, Barman, *State Fauna Series 3: Fauna of West Bengal, Part 4, Zool. Surv. India*: 361.

2010. *Acanthoprion suspectum*, Shishodia et al., *Record Zoological Survey of India, Occ. Paper No. 314*: 314.

Materials examined: RGSC 0023–24, 10.xii.2017, 2 females, Uttar Pradesh, Gaihat (Ghazipur), sorghum and flower plant, coll. RS Yadav.

Diagnosis characters: Medium to large insect. The insect body is light green in colour. Fastidium conically



Image 5. *Acanthoprion suspectum* (Brunner, 1895)

produced with blunt apex. The head is conical in appearance. The antennal base is distinguishably elevated. The posterior portion of the pronotum is conical in shape. The tegmina are wrapping the whole body and uniformly in green colour. Ovipositor is sword-shaped, reddish-brown at apex.

Distribution: India: Kerala, Tamil Nadu and West Bengal (Shishodia et al. 2010), Meghalaya (Barman 1995), and Uttar Pradesh (present study).

Habitat: The species prefers shrubs and are more common during the month of August in this area from where the material was collected.

Remark: The fauna was much more mimics to leaves.

#### Conclusion and Summary

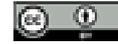
The five species of katydids discussed above are firstly recorded from Uttar Pradesh. Further more intensive field work may enrich katydids fauna of the Uttar Pradesh, India.

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## On the occurrence of Honey Badger *Mellivora capensis* (Mammalia: Carnivora: Mustelidae) in the northern Eastern Ghats of Andhra Pradesh, India

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The Honey Badger *Mellivora capensis* Schreber, 1776 popularly known as the Ratel, is a monotypic species of the small carnivore family Mustelidae, and is the sole member of its subfamily Mellivorinae. Although currently classified as Least Concern (LC) by the World Conservation Union (IUCN), its population is decreasing and sightings are extremely rare (Do Linh San et al. 2016).

The Honey Badger is widely distributed throughout Africa and western & southern Asia (Begg et al. 2005; Do Linh San et al. 2016), including most of India (Prater 1980; Menon & Daniel 2003). Although their status and distribution have been well documented in parts of Africa and western Asia (Kruuk & Mills 1983; Begg et al. 2003, 2005), there have been few studies on them from across different parts of India (Kumara & Singh 2007; Gupta et al. 2012; Gubbi et al. 2014; Krishnan et al. 2016), mainly from central India and the Western Ghats. There have been no published records of Honey Badgers from the Eastern Ghats of Andhra Pradesh. The current note presents the first record of the occurrence of Honey Badger from northern Eastern Ghats (NEG) of Andhra Pradesh State through camera trap images.

This record was obtained as part of a camera trapping study that is being undertaken currently by the authors to inventorise the mammal community and its diversity patterns across the NEG of Andhra Pradesh (Aditya & Ganesh 2017). The NEG is spread between 18.491–19.181 °N & 79.541–83.233 °E. The region is generally understood as the section of the Ghats stretching northwards between the Godavari River in Andhra Pradesh and the Mahanadi River in central Odisha. The NEG has an altitudinal range from 20m at the Godavari River to 1,690m at the Jindhagada Peak. The dominant forest type is moist deciduous, with some patches of semi-evergreen and dry deciduous forest (Champion & Seth 1968). There are no published studies on Honey Badgers in the NEG. Most of the forests in the NEG are administered as a cluster of reserved forests (RFs), and there is also one large protected area, the Papikonda National Park (Figure 1).

Passive digital infra red camera traps (Trail Cam and Bushnell 8mp, Scout Guard 20mp) were used in a stratified sampling framework across different elevation zones and habitat types across the NEG. Four cameras are being installed in selected grids, each measuring 5x5

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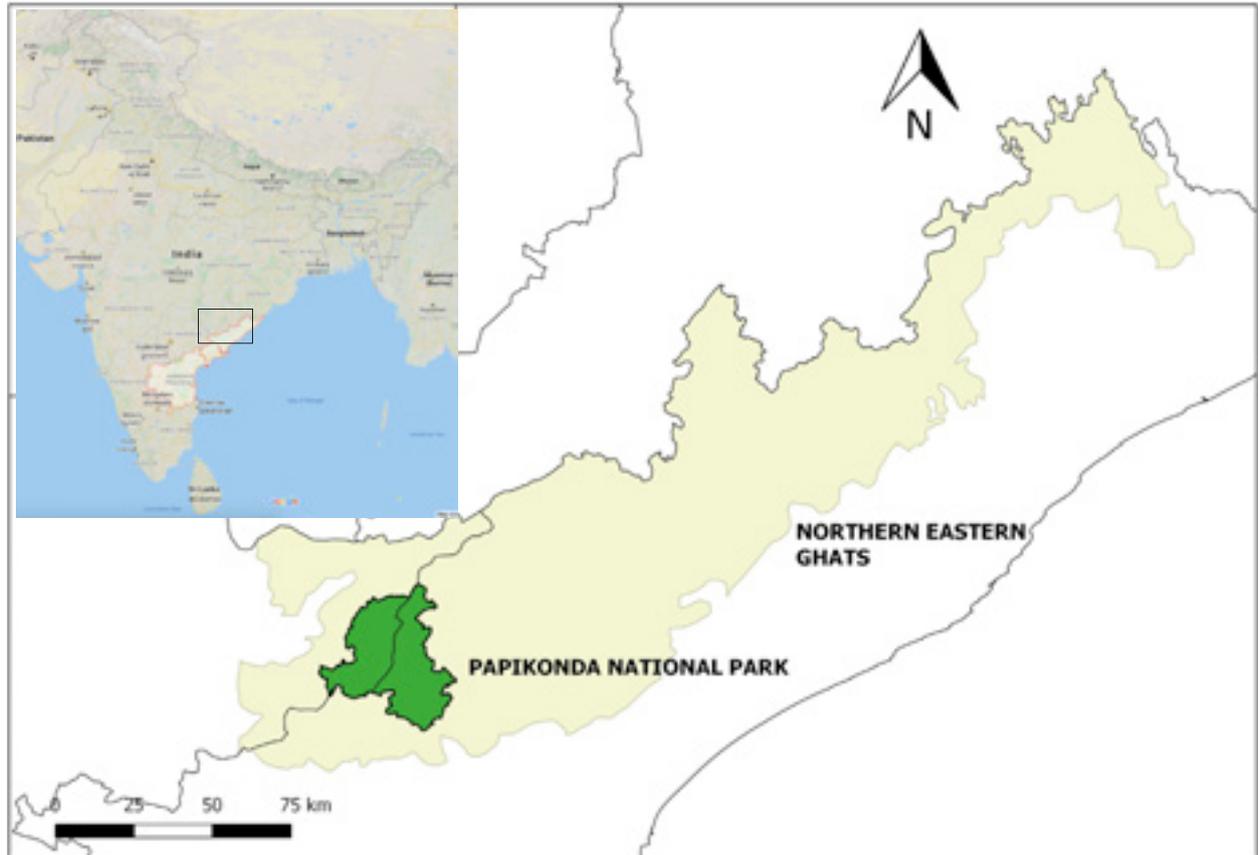


Figure 1. The northern Eastern Ghats of Andhra Pradesh, India.

km<sup>2</sup> for a period of seven trapping nights per camera trap (CT). A total of 30 grids representing various habitat types and elevation zones across the NEG were surveyed through CTs between October 2018 and February 2019. Therefore, the total trapping effort was 840 trap nights (=20,160 trap hours) which recorded one pic of Honey Badger.

The Honey Badger was previously recorded from sign surveys and community observations from the NEG (Aditya & Ganesh 2018), however, there were no photographic captures before this report. One CT recorded images of Honey Badger in the current study. The species was identified using a standard field identification guide for mammals (Menon & Daniel 2003). The species was recorded from a TrailCam IR camera trap located at 17.690 °N and 81.582 °E in the fairly dense moist deciduous forest at an elevation of 520m (Image 1). The image was captured at 00:18:00 on 18 October 2019. The location was in the East Godavari District of Andhra Pradesh, in the buffer of the Papikonda National Park. The location was about 15km south of the Sileru River separating Andhra Pradesh and Odisha states.



Image 1. Camera trap image of Honey Badger.

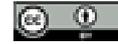
The CT captures of the Honey Badger confirms its presence in the NEG landscape (Aditya & Ganesh 2018). This record adds to the biological diversity and the conservation importance of the NEG in particular and the larger Eastern Ghats landscape in general (Goswami et al. 2018; Agarwal et al. 2012; Balaji & Satyanarayana 2016). Their presence in dense moist deciduous forest

indicates their suitability and adaptability to a wide variety of habitats, and could highlight their preference for this habitat in the NEG. Given that the Eastern Ghats is home to several rare and threatened species but is also among the least protected forest landscapes globally with only 3.53% of its area protected (Cardillo et al. 2006). This report underscores the need to implement stronger conservation measures, particularly in the face of rapid land-cover changes from development activities in the region such as the upcoming Polavaram Dam (Mohan 2006; Aditya & Ganesh 2018).

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## Assamese Cat Snake *Boiga quincunciata* (Wall, 1908) (Reptilia: Squamata: Colubridae) - new country record for Bhutan

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Opisthoglyphous snakes of the genus *Boiga* are currently represented by 34 species globally (Uetz et al. 2019). Snakes of this genus are distributed in China, India, southeastern Asia, Sundaland, Nepal, and Bhutan. In Bhutan, the genus is so far represented by *Boiga gokool* (Gray, 1835), *B. cyanea* (Duméril, Bibron & Dumeril, 1854), *B. multifasciata* (Blyth, 1861), *B. ochracea ochracea* (Theobald, 1868), *B. ochracea stoliczkae* (Wall, 1909), and *B. siamensis* (Nutaphand, 1971). *Boiga quincunciata* (Wall, 1908) was first described in India from upper Assam, and is subsequently reported from Arunachal Pradesh, Mizoram, and West Bengal (Wall 1908; Ghosh & Mukherjee 2019).

In Bhutan 69 species of snakes represented by eight families and 35 genera are so far reported (Smith 1943; Biswas 1976; Bauer & Gunther 1992; Das & Palden 2000; Mitra 2009; Wangyal & Gurung 2010; Wangyal 2011, 2012, 2013; Das et al. 2016; Koirala et al. 2016; Wangyal & Gurung 2017; Wangdi 2018). All these studies exclude record of *Boiga quincunciata* from Bhutan. Seven *Boiga* spp. are known to occur in adjoining areas of northeastern India (Ahmed et al. 2009) and out of these

six species occur in Bhutan.

The record is based on a dead specimen. The specimen was partially damaged in the neck region; it was collected for preservation. Before fixing in 10% formalin a small piece of flesh from the damaged region was cut and preserved in 100% ethanol for DNA extraction in the future. For long term preservation it is now stored in 75% ethanol. We studied the fresh specimen before preserving and detailed morphological examination was done after preservation. Identification is based on Wall (1908) and Das (2018). We measured body length using a standard measuring tape (L.C. 1mm). Dorsal scale count was made by using magnifying hand lens (5x optic zoom). Images were taken using Canon EOS 7D Mark II digital camera. GPS Garmin GPSMAP 62sc and datum WGS84 was used for recording data. Coordinates were represented in decimal degrees, corrected to three decimal digits. Habitat classification was followed (as by Grierson & Long 1983).

**Specimen examined:** Since there is no standard system in the country to allocate e-voucher numbers, specimen and digital copies of images are deposited by

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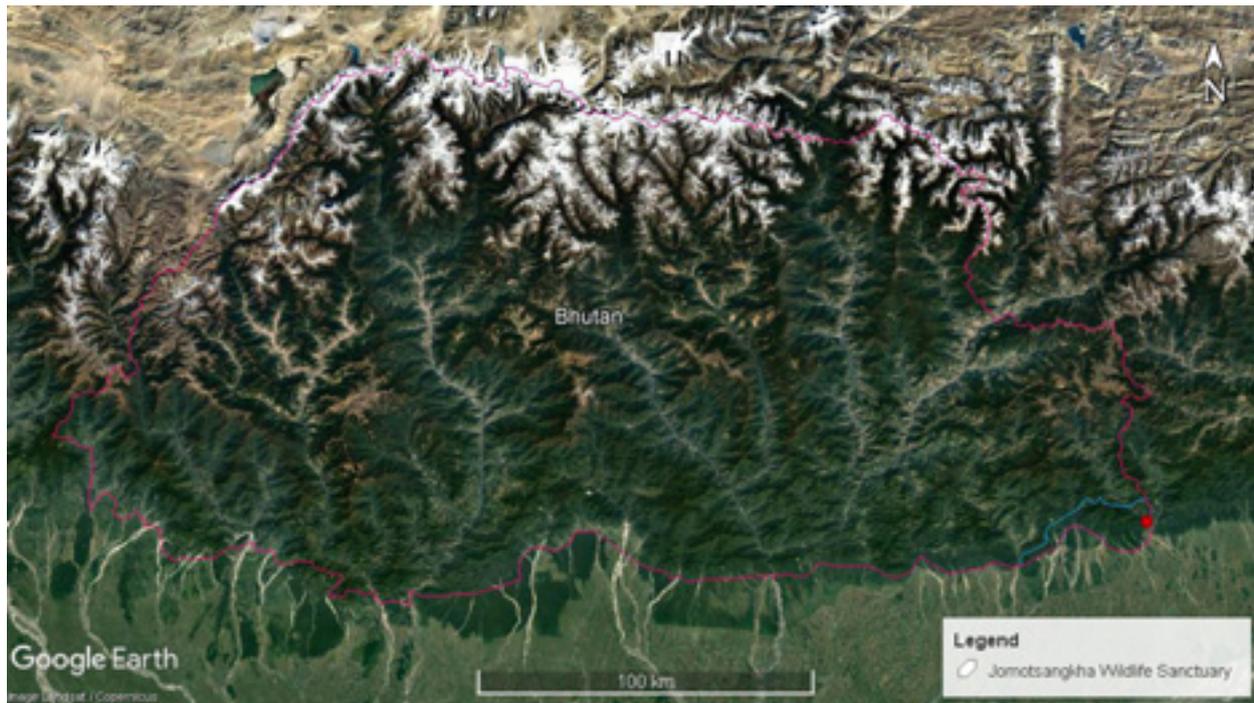


Figure 1. Bhutan map showing record place of *Boiga quincunciata*.

the author in Jomotsangkha Wildlife Sanctuary (JWS), Samdrupjongkhar, Bhutan for reference. Wall (1908) was referred to identify the species where it is described as *Dipsadomorphus quincunciata* from Assam.

**Morphology:** Rostral touches six shields; internasal two; nasals two subequal shields touching the 1<sup>st</sup> and 2<sup>nd</sup> superlabials; loreal one; preocular one, barely reaching crown. Postoculars two; temporals small scale-like, superposed anteriorly. Supralabials eight on the right side with the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> touching the eye (Image 1). Dorsal Scales 19:19:15; mid body or vertebral scales 310 (Image 2); ventrals 242; anal divided; subcaudals 119 (Image 3). Total body length 149cm. Tail length 33cm. Vertebrae enlarged with smooth dorsal part. Colour yellowish or greyish-brown above speckled with dark brown. A series of dark brown spots with white edge present on vertebrae. Venter yellowish-white with white or brown spots present on outer margins. Three longitudinal stripes present on nape. Head is distinct from neck; body is slender and elongate, eyes large with vertical pupil.

**Field Observation:** On 26 March 2019 at 07.30h, during the drive to Menjiwoong Village from Jomotsangkha Town (29.939°N & 92.101°E, 345m) (Figure 1; Image 4) the first author encountered a road kill of *Boiga quincunciata* near Jampani located at the left bank of Jomochu (a tributary of Dansari River in India).

The surrounding forest type falls under tropical forest with *Tetrameles nudiflora*, *Pterospermum acerifolium*, *Bombax ceiba*, *Duabanga grandiflora*, and *Ficus* species as major crown cover with thick undergrowth of climbers and large bamboos. Das (2018) had also made similar observation where this species inhabits wet evergreen forest, arboreal on undergrowth especially bamboo.

The most recent report on country's biodiversity by Wangyal & Gurung (2017) had listed only six species of cat snakes from Bhutan. Das et al. (2016) listed three species of *Boiga* (*siamensis*, *gokool*, and *ochracea*) from Royal Manas National Park. *Boiga ochracea* is known from Mongar 1,541m, Langthel and Taksha (Wangyal & Tenzin 2009; Tshewang & Letro 2018). *Boiga cyanea* had been recorded from Mongar at 1,929m and JWS at 340m (Wangyal & Tenzin 2009; Wangdi 2018). *Boiga multifasciata* is listed from Trashiyangtse, Lhunntse 1,170m, below 1,500m at Jigme Dorji National Park, Langthel and Taksha (Wangyal & Tenzin 2009; Koirala et al. 2016; Tshewang & Letro 2018). *Boiga siamensis* was recorded from JWS at 234m (Lekey Chaida pers. obs 22 May 2019).

Currently in India and Bhutan, *Boiga quincunciata* is known from Arunachal Pradesh: Bandardewa, Chimpur, Papum Pare, Pashighat (Borang et al. 2005; Sanyal & Gayen 2006); Assam: Tinsukia, Jeypore Reserve Forest, Garbhanga Reserve Forest, Manas Tiger Reserve, Borail



Image 1. Head of Assamese Cat Snake *Boiga quincunciata*.



Image 2. Unique arrangement of large mid dorsal scales.



Image 3. Ventral subcaudal scales.



Image 4. Habitat of *Boiga quincunciata* in Jomotsangkha Wildlife Sanctuary.

Wildlife Sanctuary (Wall 1908; Agarwal & Ghosh 1995; Sengupta et al. 2000; Abhijit Das pers. obs. 28 August 2012); West Bengal: Buxa Tiger Reserve (Ghosh & Mukherjee 2019); Sawleng, Mizoram (H.T. Lalremsanga pers. comm. August 2009); upper Myanmar (CAS 224439), and in JWS from Bhutan (this study).

In Assam, individuals were obtained within 40–200m elevation. In Mizoram the female individual was obtained from 950m elevation. The specimen collected from upper Myanmar was obtained from Kachin State at an elevation of 650m. The northern Bengal record is from 744m (Ghosh & Mukherjee 2019).

*Boiga quincunciata* is nocturnal and arboreal. Found both in mixed deciduous and evergreen forests., one was collected from a rocky crevice (17.45h) at ca. 2m above ground and other was from a tree ca. 5m above ground at around 21.00h (Sengupta et al. 2000). In

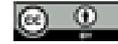
Barail Wildlife Sanctuary, one male individual was found active among bamboo along streams at 21.00h (Abhijit Das pers. obs. 28 August 2012). In Upper Burma, Smith (1940) obtained specimens from inside bamboo during winter month. He reports this snake as inoffensive. The female specimen from Garbhanga Reserve Forest laid two elongated eggs in July and another clutch of two eggs were laid two days after the first. Eggs measure  $45.35 \times 13.45$  mm (Das 2010).

With the confirmed occurrence of Assamese Cat Snake *Boiga quincunciata* in Jomotsangkha Wildlife Sanctuary, Bhutan now have seven *Boiga* species and 70 snakes in the herpetofauna list and this region should be prioritized for future survey efforts. Follow-up efforts should target in tropical and subtropical forest near riverine ecosystems, bamboo forest and even near human settlement to get additional distribution range of this snake in the country and region.

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## Loss of Critically Endangered Hawksbill Turtle nesting beach at EGA facility, Abu Dhabi, UAE

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The nesting beaches are critical resources for sea turtles, most beaches around the world are disturbed by natural pressures and direct or indirect human influence. EGA (Emirates Global Aluminium) beach, a stretch of ~0.64km length and an average breadth of 165m, located between 24.800°N, 54.702°E and 24.804°N, 54.705°E has historically been visited by Hawksbill Turtles *Eretmochelys imbricata* and used extensively for nesting (EAD 2016; Sharma 2018). A rapid survey was carried out at the EGA beach during low tide on 5 February 2019 to document the possibilities of sea turtle nesting, as a part of the environmental and ecological monitoring survey of Abu Dhabi ports, 2019. There were no signs of sea turtle nesting observed during the survey, however, the nesting and crawl-marks were well documented in our previous surveys from 2012 to 2017. According to the discussion with the local environmental surveyors, EGA points to the fact that Hawksbill Turtles *Eretmochelys imbricata* were the species visiting the EGA beach, which was corroborated by the dimensions of the observed crawl-marks during 2012 to 2017. During the survey, three old sea turtle nests (Image 1a–c) of 2016–2017 were recorded and it was marked and protected

with fishing cages by the EGA environmental team. Further, a reconnaissance survey was carried out in the entire EGA beach area (~0.64km) to record the trail of sea turtle nesting. This survey revealed there were no recent traces of sea turtle in the beach area. It was found that the beach is not any more conducive for sea turtle nesting as the shoreline was completely covered with mounds of dead seagrasses (Image 1d,e) which would restrict the access of sea turtle to the beach. In addition, the hatchlings must be able to return to the sea and the nest must not have any visual obstructions (Godfrey & Barreto 1995). Debris on the beach would also prevent successful nesting as the beach was completely covered with fragments including plastic and oyster shells (Image 1f–h). This sometimes causes a phenomenon called 'false crawl' where the females emerge from the water but do not lay eggs (Fujisaki & Lamont 2016) due to unfavorable conditions. There was one 'false crawl' (Image 1i) and one nest recorded during 2017, and this was the last evidence of sea turtles on EGA beach. Since then, there has been no nesting or crawl signs recorded. Additionally, artificial lighting from the industries might also have a negative impact on the nesting processes

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Image 1. a–c—old sea turtle nesting spots | d–e—dead seagrass mounds | f–h—debris including plastic and oyster shells | i—false crawl. © D. Adhavan.



Figure 1. Graphical representation of sea turtle nesting trend from 2012 to 2018.

of nesting females (Weishampel et al. 2016). With reference to that, a set of scientists worked closely with the community to minimize disturbance to nesting

females by avoiding bright lights and loud noise in the nesting area (Gulf News 2017). The turtle nesting status was comparatively reduced from 2016 onwards (Marine

Ecological Monitoring Project Survey (2012–2018). The trend of nesting status from 2012 to 2018 is graphically shown in Figure 1. It is also predicted, if there is no aid to protect the sea turtle nesting beaches, this magnitude of beach loss could literally be the point of no return for the nesting populations of the sea turtles to EGA beach. This would eventually affect seagrass meadows and coral reefs, and in turn, there would be an impact on the marine ecosystem.

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## *Meliola elaeocarpicola* sp. nov. (Ascomycetes, Meliolales) from Malabar Wildlife Sanctuary in Kerala State, India

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A huge number of meliolaceous fungi were reported from India and there was a requirement for the consolidation of this group and Hosagoudar (1996) published a monograph for India by including six genera and 378 species. The enthusiastic work on this group continued in Kerala. Hosagoudar & Abraham (1996a, b), Hosagoudar et al. (1997), Hosagoudar & Abraham (1998 a,b,c,d,e), Hosagoudar et al. (1998 a,b,c,d,e,f; 1999a,b), Goos & Hosagoudar (1998), Hosagoudar & Jacob Thomas (2013) contributed to Meliolales of Kerala and other southern parts of India; Hosagoudar (2008, 2013) for Meliolales of India and Hosagoudar & Agarwal (2008), for the world monograph are the subsequent works. This study describes a new species that belongs to the genus *Meliola* from Kerala State.

### MATERIALS AND METHODS

Infected leaves of *Elaeocarpus* sp. (*Elaeocarpaceae*) were collected and field notes were prepared regarding their nature of colonies, infection and the collection locality. For each collection, a separate field number was given. In the field, each infected plant part was collected separately in polythene bags along with the host twig (preferably with the reproductive parts, to facilitate the

identity of the corresponding host). These infected plant parts were pressed neatly and dried between blotting papers. After ensuring their dryness, they were used for microscopic study. Scrapes were taken directly from the infected host and mounted in 10% KOH solution. After 30 mins, KOH was replaced by Lactophenol. Both the mountants performed well as clearing agents and made the septa visible for taking measurements. To study the entire colony in its natural condition, a drop of high quality natural colored or transparent nail polish was applied to the selected colonies and carefully thinned with the help of a fine brush without disturbing the colonies. Colonies with hyper parasites showing a woolly nature were avoided. The treated colonies along with their host plants were kept in a dust free chamber for half an hour.

When the nail polish on the colonies dried fully, a thin, colorless or slightly apple rose colored (depending upon the colour tint in the nail polish) film or flip was formed with the colonies firmly embedded in it. In case of soft host parts, the flip was lifted off with a slight pressure on the opposite side of the leaves and just below the colonies. In case of hard host parts, the flip was eased off with the help of a razor or scalpel. A drop of dibutyl

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phthalate polystyrene xylene (DPX) was spread on a clean slide and the flip was spread properly on it. One or two more drops of DPX were added additionally on the flip and a clean cover glass was placed over it. By gently pressing on the cover glass, the excessive amount of DPX was removed after drying. Care was taken to avoid air bubbles.

These slides were labeled and placed in a dust free chamber for one to two days for drying. These permanent slides were then used for further studies. For innate fungi, sections were made and stained in cotton blue. After the study of each collection, part of the material was retained in the regional herbarium, Mar Thoma College Herbarium, Thiruvalla (MTCHT).

***Meliola elaeocarpicola* sp. nov. Lini K. Mathew  
(Figure 1, Image 1)**

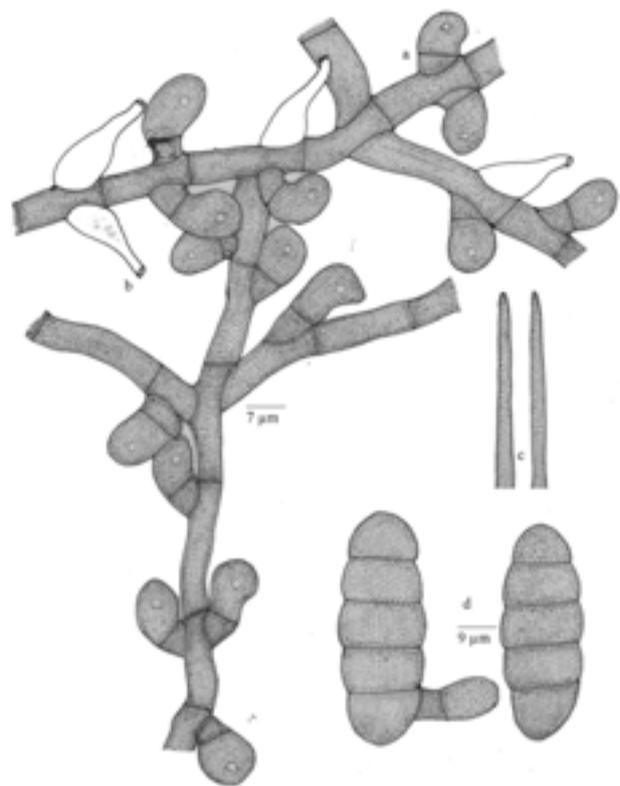
MycoBank # 835348

Colonies epiphyllous, dense, up to 5mm in diameter, rarely confluent. Hyphae straight to flexuous, branching alternate to opposite at acute to wide angles, loosely to closely reticulate, cells 13–20 x 3–6.6 µm. Appressoria alternate to opposite, antrorse to subantrorse to retrorse, spreading, straight to curved, 10–20 µm long; stalk cells cylindrical to cuneate, 3–5 µm long; head cells ovate, rarely globose, entire, 9–15 x 9–12 µm. Phialides mixed with appressoria, alternate to opposite, ampulliform, 13–20 x 6.6–10 µm. Mycelial setae scattered to grouped around the perithecia, simple, straight, acute, up to 650µm long. Perithecia scattered, up to 180µm in diameter; ascospores oblong to cylindrical, 4-septate, constricted at the septa, 33–40 x 13–17 µm.

On leaves of *Elaeocarpus* sp. (*Elaeocarpaceae*), Peruvannamuzhy, Malabar Wildlife Sanctuary, Calicut, Kerala, December, 26, 2014, MTCHT 106 (Type), TBGT 6999 (Isotype), collected by Lini K. Mathew.

*Appendiculella elaeocarpicola* Hosag. & Robin, J., *Asteridiella elaeocarpi-tuberculati* Hosag., *A. elaeocarpicola* Hansf. and *Meliola elaeocarpi* Yates are known on this host genus (Yates 1917; Hansford 1961;

Hosagoudar 1996, 2008, 2013; Hosagoudar et al. 1997; Hosagoudar & Agarwal 2008). *Meliola elaeocarpicola* differs from *Appendiculella elaeocarpicola* in the absence of perithecial appendages and flattened-globose perithecia. It differs from *Asteridiella elaeocarpicola* Hansf., *A. elaeocarpi-tuberculati* Hosag. in absence of perithecial wall cells and presence of mycelial setae (Hansford 1961; Hosagoudar 1996, 2008, 2013; Hosagoudar & Goos 1989; Hosagoudar & Agarwal 2008; Hosagoudar & Thomas 2013). *Meliola elaeocarpi* Yates was the only *Meliola* species on the host genus which was reported in 1917 from Philippines. The current species differs from *Meliola elaeocarpi* Yates



**Figure 1. *Meliola elaeocarpicola* sp. nov. Lini K. Mathew**  
A—appressorium | B—phialides | C—mycelial setae | D—ascospore.

**Comparative account**

Name	Beeli formula	Distinguishing characters				
		Colonies	Mycelial	Mycelial setae	Appressoria	Spore
<i>M. elaeocarpicola</i> sp. nov.	3113.3223	Epiphyllous	Hyphae straight to flexuous,	simple, straight, acute, up to 650µm long	alternate to opposite; head cells ovate, rarely globose, entire, 9–15 x 9–12 µm	oblong to cylindrical, 33–40 x 13–17 µm.
<i>M. elaeocarpi</i>	3112.4221 Philippines	amphigenous	Straight	simple, acute, obtuse up to 300µm long	Opposite, subglobose to ovoid	Subellipsoid, obtuse, 44– 50 x 18 µm

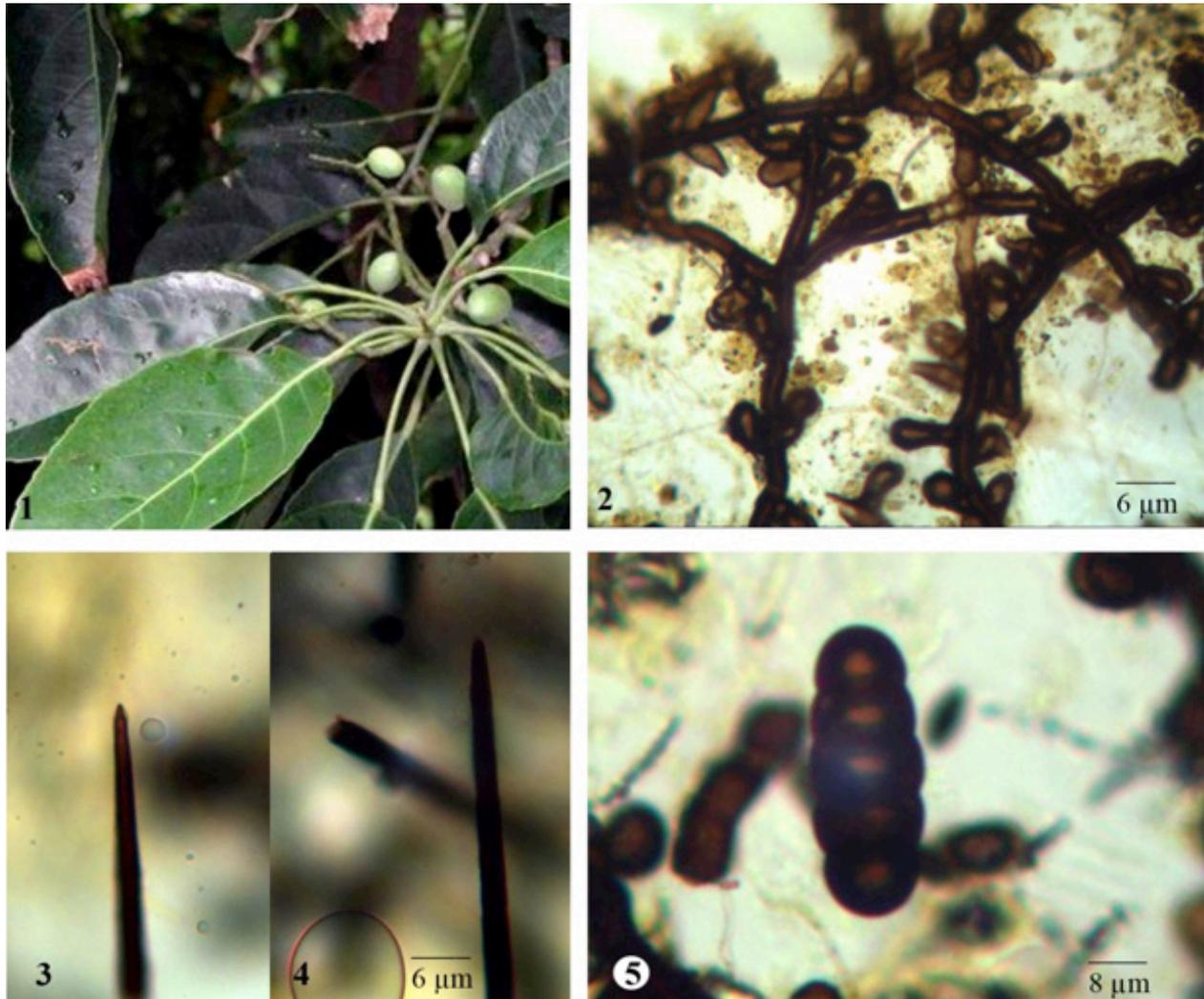


Image 1. *Meliola elaeocarpicola* sp. nov. Lini K. Mathew: 1—infected leaf of *Elaeocarpus serrates* (Elaeocarpaceae) | 2—appressorium mycelium with phialides | 3 & 4—apical portion of the mycelia setae | 5—germinating ascospore. © Jacob Thomas.

in the presence of alternate and opposite appressoria and comparatively smaller ascospores, whereas *Meliola elaeocarpicola* Yates has only opposite appressoria and larger ascospores.

**Etymology:** The specific epithet is based on the host genus.

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