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Cover: Orchids of Goa: front—*Aerides maculosa*; back—*Pinalia reticosa* © Jeewan Singh Jalal.



## OBSERVATIONS ON THE EX SITU MANAGEMENT OF THE SUMATRAN RHINOCEROS *DICERORHINUS SUMATRENSIS* (MAMMALIA: PERISSODACTYLA: RHINOCEROTIDAE): PRESENT STATUS AND DESIDERATA FOR CONSERVATION

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**Abstract:** The Sumatran Rhinoceros is approaching extinction. A few dozen animals remain, dispersed in dwindling Indonesian rainforest with only a few years of likely survival time. Eight rhinos belonging to two subspecies are in controlled breeding centres. The Sumatran Rhinoceros differs markedly from the other four species of Rhinocerotidae and requires management according to specific protocols. Several Sumatran Rhinoceros have died in zoos, owing to lack of knowledge concerning their particular dietary requirements and their high sensitivity to anthropogenic activities. Recently more positive results, including successful births, have been achieved with the aid of scientific research, which continues to examine factors required for successful conservation and accommodation efforts.

**Keywords:** Asiatic Two-horned Rhinoceros, behaviour, captivity, endangered species, ecology, forest protection, nutrition.

**Abstrak:** Badak Sumatera menuju kepunahan. Hanya beberapa lusin saja tersisa, tersebar di hutan hujan Indonesia yang semakin menipis dengan tinggal beberapa tahun waktu bertahan hidup. Delapan badak, satu milik subspecies, berada di pusat penangkaran terkendali. Badak Sumatera berbeda dari empat spesies lain dari Rhinocerotidae dan membutuhkan pengelolaan menurut protokol khusus. Beberapa Badak Sumatera mati di kebun binatang, karena kurangnya pengetahuan tentang persyaratan diet khusus dan sensitivitas mereka yang tinggi terhadap aktivitas antropogenik. Baru-baru ini hasil yang lebih positif, termasuk kelahiran yang berhasil, dicapai dengan bantuan penelitian ilmiah, yang terus memeriksa faktor-faktor yang diperlukan untuk upaya konservasi dan akomodasi yang berhasil.

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## INTRODUCTION

The emergence of the Asiatic Two-horned Rhinoceros, popularly known as the Sumatran Rhinoceros *Dicerorhinus sumatrensis* (Fischer 1814), has been dated to the lower Miocene between 23 and 16 million years ago (Tougaard et al. 2001). The species has shown little morphological change since then, leading some to refer to Sumatran Rhinos as “living fossils” (Groves 2017). Historically these rhinos have a large distribution area that once included northeastern India, Bangladesh, Myanmar, Thailand, southern China, Indochina, Malaysia, and Indonesia. Currently, only about 50 Sumatran Rhinos remain in small populations scattered in refuges in Sumatra and in Borneo.

Three subspecies have been described: the Sumatran or Southern Asiatic Two-horned Rhinoceros *Dicerorhinus sumatrensis sumatrensis* (Fischer, 1814) (Image 1). The range of this subspecies once extended from the southernmost parts of Myanmar and Thailand (Kra Isthmus) through peninsular Malaysia to the Indonesian island of Sumatra. Very small dispersed populations are still present in Sumatra. The Northern Asiatic Two-horned Rhinoceros *Dicerorhinus sumatrensis lasiotis* (Buckland, 1872) (Image 2), which is likely extinct, once ranged from the northeastern part of the Indian subcontinent to northern Myanmar and parts of Indochina; reports of occurrence from as far east as Sichuan are dated during the Song Dynasty (960–1279) (Rookmaaker 1980). The Bornean Two-horned Rhinoceros *Dicerorhinus sumatrensis harrissoni* (Groves, 1965) (Image 3) was historically present in much of Borneo; a few individuals survive in a small area in the heart of the island.

Sumatran Rhinos are by far the smallest of the five living species of rhino. On average, they weigh 600–950 kg, stand 1.0–1.5 m tall at the shoulder, and are about 2.0–3.0 m long (IRF 2019). The head is 70–80 cm long and the tail varies in length from 35 to 60 cm. This species has two horns, dark grey to black in colour, which in the wild are usually very smooth and form a slender cone that is curved backwards. A typical front horn of the Sumatran Rhinoceros is 15–25 cm long, although there is a horn 80cm long in the British Museum collection. The smaller second (posterior) horn is normally much smaller, seldom more than a few cm in length, and it is often not more than an irregular knob. *D. sumatrensis* has distinctive reddish-brown skin, which in the wild is variably covered with short bristly hair. In captivity the hair can grow out to a shaggy fur owing to less abrasion from vegetation. The ear edges have a prominent fringe of longer hairs, and the tail terminates with a tuft of

thicker hairs. Two prominent folds in the skin circle the body behind the front legs and before the hind legs, and lesser folds occur on the neck and at the base of the legs.

The Sumatran Rhino is a solitary folivore of the southeastern Asian lowland and mountain (i.e., moss) rainforests. It is an induced ovulator, with females ovulating in response to external stimuli during or before mating rather than ovulating cyclically or spontaneously. This is the first example reported within the Perissodactyla (Roth et al. 2001). The gestation period lasts 16 months and females produce a single calf every 3–4 years. The typical low density of rhino populations is likely attributable to their dietary specialization for eating specific leaves that tend to be highly localized. Consequently, Sumatran Rhinos require large, undivided and undisturbed areas, which have all but vanished (Cannon et al. 2009). *Dicerorhinus sumatrensis* is listed as Critically Endangered by the IUCN Red List (van Strien et al. 2008). The biggest threats to Sumatran Rhinos are poaching for their horn, inbreeding depression, and loss of habitat due to anthropogenic development. The horn is used in Asia as a medicine against fever and pain, and trade in rhino horn between Borneo and other source areas in southeastern Asia and China likely began more than 2,000 years ago with the origin of traditional Chinese medicine. Use of rhino horn has recently reached a plateau as a “status symbol” among the rich populations of China, Viet Nam (Milliken 2012) and Thailand (pers. info.).

Over the centuries, the Sumatran Rhinoceros has been exterminated over most of its range. In 2003 fewer than 300 Sumatran Rhinos were living in the wild. Most of these were in Bukit Barisan Selatan, Gunung Leuser and Way Kambas National Parks Sumatra, Indonesia, although a few were found in Borneo. By 2019 the situation had deteriorated considerably with no more than 80 rhinos left, for the most part in Gunung Leuser National Park (IRF 2019).

## BACKGROUND

Sumatran Rhinos are rarely seen in the wild, confounding efforts to study them by direct observation (van Strien 1985) and limiting knowledge concerning their numbers, ecological aspects and management in controlled environments. In 1985 van Strien (1985) estimated that as many as 800 Sumatran Rhinos remained, while less than 30 years later, Nardelli (2014) estimated about 75 were still alive. Recently, some experts have estimated that as few as 30 animals survive



Image 1. Sumatran or Southern Asiatic Two-horned Rhinoceros *Dicerorhinus sumatrensis sumatrensis*. © Sumatran Rhino Sanctuary.



Image 2. Northern Asiatic Two-horned Rhinoceros *Dicerorhinus sumatrensis lasiotis* at the London Zoo, ca. 1890. © Zoological Society of London.



Image 3. Bornean Two-horned Rhinoceros *Dicerorhinus sumatrensis harrissoni* at BRS in Sabah. © Jeremy Hance.

(Hance 2017). From this apparent rate of decline, it would appear that the last wild populations of Sumatran Rhinos will soon be gone. This may be viewed as a total conservation failure. While all rhino habitats are strictly protected by legislation, in reality many areas are subject to large-scale human encroachment that the national park management has neither the means nor political support to prevent. Thus establishment of Sumatran Rhino populations in well managed conservation areas will be a vital component of future conservation strategy.

One of the conclusions reached at the Sumatran Rhinoceros Crisis Summit in Singapore (31 March–04 April 2013) (Lees 2013) was that ex situ facilities holding Sumatran Rhinos ought to participate in the following essential tasks: 1) form “insurance” populations to re-establish or genetically invigorate wild populations, granted that strong protection measures are in force; 2) undertake research to improve knowledge of rhino biology; 3) promote the Sumatran Rhino as a “flagship species” to draw attention to the biodiversity spots they inhabit and educate the local communities on the importance of conservation. Nevertheless, and despite problems in captivity such as high mortality and poor gestation mostly resolved (Roth 2003), these resolutions are redundant topics for discussions pro and contra diverging conservation strategies (Hance 2018a,b).

The existing ex situ population of the Sumatran Rhinoceros is not viable (Lees 2013; Putnam 2013). Hazardous inertia has left the tiny group concentrated at the Sumatran Rhino Sanctuary (SRS) on Sumatra Island in Indonesia on its own to sustain the survival of the species, perhaps for no more than a few decades, unless more rhinos are captured without delay and moved into controlled areas. Scientific research has proved useful at solving technical “how to” problems but not at working out precise “whether to” efforts. We cannot expect science to do any more than feed data into ethical or political decisions, which are lacking.

Unfortunately, conservation is not only scientific, it is multi-faceted and, according to anthropomorphic standards, aesthetically biased (e.g., “beautiful” tiger vs. “ugly” rhinoceros) even, requiring social science aspects as well as biological sciences to lead towards the proper solutions.

In April 2016, an attempt to capture a female rhino in Kalimantan, the Indonesian region of Borneo, ended with its loss (Meijaard 2016). In 2018 a decision was finally taken to capture isolated Sumatran Rhinos and scrupulous conservationists started to reunite those “lost-in-the-woods” rhinos (IRF 2018a); on 25 November 2018 a female Sumatran Rhinoceros, Pahu, was safely

captured. On the same day and month in 1985, the male Torgamba was the first to be rescued by Save the Sumatran Rhino, a project of the Indonesian Directorate General of Forest Protection and Nature Conservation (PHPA) and the UK’s Howletts & Port Lympne Wildlife Parks (H&PL) (King 2013; King and Beer 2018). Between 1985 and 1994, 16 rhinos from Sumatra followed Torgamba’s safe arrival at H&PL, in the care of zoological institutions in Indonesia (Jakarta, Bogor, and Surabaya zoos), the UK (H&PL) and the USA (Cincinnati, Los Angeles, New York, and San Diego zoos, which had joined the project). Let us hope this coincidence of dates is a good sign that the ongoing capture and translocation will be as successful as the precedents.

## OVERVIEW OF THE CURRENT STATUS OF THE SUMATRAN RHINOCEROS IN A CONTROLLED ENVIRONMENT

### The situation at Borneo Rhino Sanctuary in Sabah

The Sumatran Rhinoceros is now officially extinct in Malaysia since Iman, a 25 year old female died in a sanctuary in Malaysia’s Sabah state on 23 November 2019. Forced by circumstances, Malaysian scientists, with the help of the Leibnitz Institute for Zoo and Wildlife in Germany, were pursuing artificial reproduction technology options. ART has so far shown some degree of success in the White Rhinoceros *Ceratotherium simum* and the Greater One-horned Rhinoceros *Rhinoceros unicornis* (Roth 2006; Hildebrandt et al. 2018). So much is unknown about the Sumatran Rhinoceros’ biology, fertility, and reproduction that these techniques seem less likely to succeed in the near future than natural conception, on time to propagate the rhinos. In any case and with the possible extinction of *D. sumatrensis*, it is important to preserve cryogenically as much genetic material as possible—starting with oocytes and gametes.

According to Agil et al. (2008), Sumatran Rhinos have a low sperm concentration (oligozoospermia) and a small volume of ejaculate. This may be one more *sumatrensis*’ peculiarity or a cause of the Allee effect—e.g., anthropogenic alteration of population size leading to lack of genetic diversity and demise. Recent scientific research attempting to resurrect extinct species from cells has not been considered here because it is still remote from guarantees and may be a possible diversion to the present efforts to save the Sumatran Rhinoceros via experimented methodologies.



Image 4. An early map of the Sumatran Rhino Sanctuary. Reproduced by Tomasz Cofta.

#### The situation at Sumatran Rhino Sanctuary in Indonesia

The few remaining Sumatran Rhinos (three males and four females), are presently at SRS in order to breed them under the best possible conditions with the potential for reintroduction of offspring to the wild. At SRS they are carefully monitored and kept under scientific protocols in a semi-wild condition. SRS is within Way Kambas National Park and covers an area of about 100ha between Way Kanan and Way Negarabatin, within an area of approximately 10,000ha.

Rhinos are kept in individual areas of 10–20 ha, connected at the center to permit mating (Image 4). Every 20–25 days, the male is introduced to the female (YABI 2019).

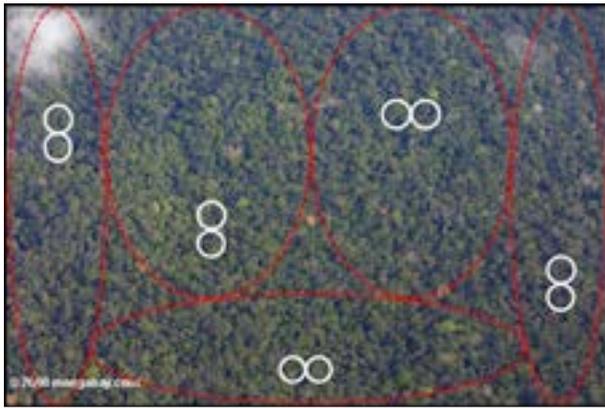
Harapan was born in Cincinnati Zoo on 29 April 2007 to female Emi and male Ipuh, and was their third and last calf. Harapan spent time in three US zoos during his first eight years of life: Cincinnati Zoo, White Oak Conservation Center in Florida, and Los Angeles Zoo. He was moved to the Sumatran Rhino Sanctuary (SRS) on 01 November 2015.

Bina, estimated to have been born around 1985, was one of the last Sumatran Rhinos to be captured and relocated within Indonesia. She, who was about

Breaking news: Two Northern White rhino (*Ceratotherium simum cottoni*) in-vitro embryos were successfully created at Avantea Laboratories in Cremona, Italy. “Researchers from Kenya, Italy, the Czech Republic, United States and Germany are still fine-tuning the implantation procedure before the embryos are transferred into a surrogate mother, but are hopeful a Northern White rhino calf can be born via surrogacy within the next three years” (Wingard 2019).

18 years old at capture, lived in an area of southern Sumatra called Bina Samakta, in Bengkulu province. The region was home to a significant population of Sumatran Rhinos, but the construction of several villages, large oil palm plantations and a logging concession and consequent rampant poaching, left the province with few rhinos.

Rosa, in late 2003, was rescued and brought to the sanctuary. Rhino Protection Units working in Bukit Barisan Selatan National Park received reports from local villagers that a young female Sumatran Rhino had



**Image 5. An example of possible layout of double breeding units (white) with potential protected forest areas (red). Aerial photo credit: Mongabay.com**

frequently been observed walking along one of the main roads, crisscrossing the park and browsing vegetation in villages around the park boundaries. She exhibits none of the shy, solitary behaviour associated with her species.

Ratu, was born around 2000 in Way Kambas NP, the protected area where the sanctuary is located. On 20 September 2005, rangers received reports that this female Sumatran Rhinoceros had been spotted in Braja Asri Village at about 04.00h. They rescued her and brought her to SRS.

Andalas, the Sumatran Rhino conceived and born at Cincinnati Zoo, the first one produced in captivity in 112 years, is the result of ground-breaking researches undertaken by American zoos, the Indonesian Government and the Sumatran Rhino Sanctuary. A worldwide news sensation, he was sent to Los Angeles Zoo when he was two years old and then brought to SRS four years later.

Andatu was born in the early morning of Saturday, 23 June 2012 at the Sumatran Rhino Sanctuary in Way Kambas National Park. His father is Andalas and his mother is Ratu.

Delilah was born in the early morning hours of Thursday, 12 May 2016 at the Sumatran Rhino Sanctuary. Her father was also Andalas and mother, Ratu (IRF 2018b).

Pahu, the female recently captured (25 November 2018) in Kalimantan, is presently kept in a new facility on the island. Husbandry experts and veterinarians are monitoring her health and assessing her breeding viability. They indicated she was in good health, fit for transport to a designated sanctuary located less than 160km from capture site, where she arrived safely.

### Sumatran Rhinos conceived and born in controlled environments

Only one Sumatran Rhinoceros had been conceived and born ex situ before 13 September 2001, a hybrid between *D. s. sumatrensis* and *D. s. lasiotis*, at the time considered full species. The event took place at the zoo of Alipore, Calcutta, on 30 January 1889 (Sanyal 1889 in Rookmaaker et al. 1998). Cincinnati Zoo & Botanical Gardens was the first facility to repeatedly breed *D. s. sumatrensis* using a planned and managed reproduction protocol. In the nineties, scientists using endocrinology analysis and ultrasonography set off research on the reproductive physiology of the species (Schaffer et al. 1994; Roth et al. 1997). The major scientific breakthrough in the discovery of induced ovulation in female Sumatran Rhinos, at the Center for Conservation and Research of Endangered Wildlife in Cincinnati (Roth et al. 1998), produced the male Sumatran Rhinoceros Andalas (Roth et al. 2001; Roth 2002). Cincinnati Zoo's breeding techniques subsequently led to the birth of a female, Suci, on 30 July 2004 and another male, Harapan, on 29 April 2007.

The Cincinnati Zoo's breeding pair was rescued from the wild during the Indonesian-American Save the Sumatran Rhino project: Ipuh, the male, was captured on 23 July 1990, in Ipuh, Bengkulu, in southwestern Sumatra. He was transferred to San Diego Zoo on 10 April 1991, then to Cincinnati Zoo on 24 October 1991. Emi, the female, was captured as a subadult on 6 March 1991 also in Ipuh. She was moved to Los Angeles Zoo on 23 November 1991, then to Cincinnati Zoo on 5 August 1995.

Success followed success and at the SRS two rhinos were born: on 23 June 2012 the female Ratu gave birth to the male Andatu, the first Sumatran Rhinoceros conceived and born ex situ in southeastern Asia. Ratu mated with Andalas in March 2011 and took a 16-month pregnancy to term. Andalas, born at Cincinnati Zoo, had been brought to Indonesia from Los Angeles Zoo when he was six years old.

Ratu mated again with Andalas on 22 January 2015 and took a 16-month pregnancy to term. On 12 May 2016, a female, Delilah (Image 6), was born to the same pair (Arsan 2016) at the Sumatran Rhino Sanctuary. She weighed approximately 20kg at birth, markedly less than her brother, Andatu, who weighed 27kg.

### Considerations

Due to the extreme urgency to mitigate extinction of the Sumatran Rhinoceros, ex situ management is a critical component in the conservation of this critically



**Image 6. Dr. Zulfi Arsan checking baby Delilah's health. Photo credit: Sumatran Rhino Sanctuary**

endangered species.

Natural reproduction in a controlled environment can be achieved through: a) optimum ex situ facilities, b) sorting out the reproduction conundrum, c) best operated feeding protocol, and d) a deep understanding of the species' behavioural ecology.

When compared with other endangered species in controlled environments, some aspects of the ecology and biology of the Sumatran Rhinoceros are still poorly known. Several essential elements of their ecology are based on scientific and methodical evidence: the most outstanding finding was that the female is an induced ovulator. The Sumatran Rhino's reproductive physiology is no longer a mystery. Know-how, skills and means have been difficult to acquire and marked with deep sorrows before this extraordinary mammal prospered and its complete reproduction cycles succeeded, resulting in five healthy calves growing to adults. These successes demonstrate the impact scientific research can have on breeding endangered species. Even so, no rhino species breeding has been consistent in controlled conditions so far, and their propagation continues to be further investigated to identify the reasons for below optimal reproduction (Roth et al. 2018).

Because they have poor eyesight, rhinos communicate primarily by vocal and olfactory signals. The Sumatran Rhinoceros is the most creative vocalizer among the extant rhino species, and its vocalization has a number of similarities with that of the Humpback Whale *Megaptera novaeangliae* (Muggenthaler et al. 1993, 2003). Several characteristics of whales were probably in place 25 million years ago at the latest and these traits have not changed over millions of years (Slater et al. 2010). The many conversation expressions combined with olfactory and auditory clues including

infrasounds—extreme frequencies that fall outside the normal response curve for the human ear—trigger a variety of mental states (Wiseman 2014), some of which may interfere with the rhino's breeding activity. For example, a male may subdue others sending "specific messages".

Psychosomatic weakness resulting from emotional stress can be a cause of severe disorders such as digestive and breeding complications. These conditions should also be investigated using the techniques available for the Black Rhinoceros *Diceros bicornis* and the White Rhinoceros *Ceratotherium simum* (Carlstead et al. 2005), and new research carried out.

In future, an animal's psycho-physical condition and consciousness (Griffin 2001; Andrews 2015) will undoubtedly have a much broader application in the management of several species for their relevant influence over the animals' welfare. The Sumatran Rhinoceros has proved to be an extremely sensitive species, one of the most difficult to adapt to controlled environment.

#### Nutrition

Nutritional aspects are of particular significance for health and, perhaps, for the reproductive difficulties of Sumatran Rhinos in captivity (Dierenfeld et al. 2000). Paul Reinhart, the Cincinnati Zoo's Sumatran Rhinos' keeper at the time of the breeding successes says: "We didn't know much about the Sumatran Rhino, not many people did. We assumed you could keep them like Indian rhino and like black [rhinos], feed [them] high-quality alfalfa grain, browse... and that was not the case, not even remotely the case... The animals didn't thrive in captivity until we logged on to feeding them large amounts of browse, which we got from San Diego and Florida." (Hance 2018b).

The Sumatran Rhinoceros belongs to the leaf-eating taxa, a relatively small number of species that depend strictly on the forest as selectors of specific foliage on which their diet is based. These unusual animals are better identified as folivores because a large number of species—the Black Rhinoceros included—among ungulates, primates and other orders are recognized as browsers: generalist vegetation eaters. Most folivores have specialized stomachs, with their own kind of bacterial flora, to digest leaves, which are abundant yet all-but-void of nutrition but very rich in leaf fibre (also known as insoluble or long fibre) content. These rhinos consume foliage from a wide range of rainforest tree species but at different intensities, indicating that the Sumatran Rhinoceros is a selective folivore.

Knowledge of general and seasonal food preferences of this megafolivorous mammal allows a better prediction of animal movements and therefore can assist in conservation efforts in situ.

If the folivore's extreme nutritional feeding pattern is ignored, or confused with the browser's habit, the risk of malnutrition in folivores within controlled facilities will persist (Nardelli 2013). Most tropical wild leaves are low in iron content, on the contrary of those from temperate arboreal species, mainly broad-leaved deciduous and usually sourced to feed browsers in temperate ex situ facilities; iron causes the deadly iron storage disease (ISD) or hemochromatosis, a disorder resulting from deposition of excess iron into insoluble iron clusters in soft tissue (Watanabe et al. 2016). Deciduous temperate forests also have a higher leaf concentration of sodium, potassium, and calcium, hence the consumption of saltlicks above all as source of sodium by several rainforest mammals. Some Sumatran Rhinos died of ISD in zoos, proving that presently this species is only safe feeding on its native foliage. These high adaptations lock folivores into their own world and make them vulnerable to changes.

The Sumatran Rhinoceros is an opportunistic feeder, taking a mouthful here and there rather than feeding intensively and systematically from one source. This species' cheek teeth are brachydont, adapted to retain a branch and pluck just the leaves, nodding-turning its head. The long-term supply of fresh leaves in large quantities and variety is a priority in managing this species. These rhinos prefer fast-growing, sun-loving plants found in forest openings created by fallen trees, although the rhinos are also found in higher density in primary forests.

From 1975 to 1980, Van Strien (1985) sampled 150 plants, mainly dicotyledonous species, and established that the Sumatran Rhinoceros does not eat fruit and monocotyledons (grasses and sedges) including the wild banana (*Musa* sp.), a very tall "grass" common in some areas. In 2016 Candra et al. (2016) listed 211 species of plants consumed by Sumatran Rhinos and research by Awaliah et al. (2018) found that the Sumatran Rhinos in the SRS area feed on 61 plant species; leaves constitute 75–85 percent of total food intake. The rhino keepers supply 51 types (Image 7). At SRS each rhino consumes daily 36–47 kg ( $x 7 = 252\text{--}329$  kg), a massive burden for the surrounding forest. Data on the type, amount and proportion of the Sumatran Rhino's favourite leaves are still lacking or are not known with certainty, thus specific research activities need to be persistent. It is however known that leaves in tropical forests are defended by



Image 7. Keeper at the Sumatran Rhino Sanctuary carrying a "mouthful" of leaves and saplings. © Sumatran Rhino Sanctuary.

having low nutritional quality, great toughness, and a wide variety of secondary metabolites (Coley & Barone 1996) and because of the poor nutritional quality of mature leaves, Sumatran Rhinos consume the more nutritious young leaves when possible.

#### Controlled environment

The quality of ex situ environments is fundamental for successful conservation breeding. As custodians of the last Sumatran Rhinos, we are responsible for ensuring their limited habitats are safe and healthy for them to prosper.

The Sumatran Rhino Sanctuary located in Way Kambas National Park is home to the only Sumatran Rhinos breeding in controlled environment in the world. This tiny population is pivotal in the managed breeding program for the species' recovery and for research. Built in 1996–1998 by the International Rhino Foundation (IRF) and the Indonesian Rhino Foundation (YABI), the original SRS facility was constructed within a vast, circular, single element split into a number of enclosures

to obtain triangular sections, bordering each other on two sides—according to the standards of that time. Each rhino resides in one subdivision of approximately 20ha of fenced forest. The seven Sumatran Rhinos at the SRS prosper in these large territories and receive state-of-the-art veterinary care and nutrition (IRF 2018b). In addition, SRS staff provide optimal care by physically checking the rhinos regularly (Image 6); however, they have to be moved around (evidently some paddocks are kept empty in turns) to allow the plants to re-grow (Bittel 2018).

Knowledge of the Sumatran Rhino's consciousness is lacking. Their conditioned responses to stimuli should be researched and analyzed, as these factors could assist in increasing survival and reproduction rates. From a series of photographs, videos and personal observations, the presence of and interactions with human contacts apparently are not causing visible stress on Sumatran Rhinos in the controlled environment. What may not be possible to recognize without specific studies could be the mutual stress induced by other rhino(s) in adjacent enclosure(s), or other reasons. If animals are calm or seem to be calm, it doesn't mean that underlying tensions are not present. Zulfi Arsan, SRS head veterinarian, reports: "*Sumatran Rhinos are solitary animals that become violent when housed together.*" (Bittel 2018). In a former controlled breeding centre, it was recommended to introduce only one female into a male enclosure because of their solitary habit, to avoid serious injuries being inflicted on the female (Zainuddin et al. 2005). Nevertheless, a short-term skirmish between male and female is usual at the time of introducing the two for mating; in fact such an event is widespread among a number of solitary species.

The SRS enclosures built in 1996 are adjacent to each other; the animals likely consider these environments "confined", considering the views in this article, and thus these conditions can be causes of undetected stress. With new and up-to-date knowledge of the ecology of this species, new structures should be located in separate areas, designed and created to meet the unique requirements of the Sumatran Rhinoceros. New controlled field centres for Sumatran Rhinos are likely better positioned when they are separate and at distant locations, and with newly developed fenced areas. At the same time, all known rhinos, whether in situ or in controlled breeding centres, must be managed as one population (Ellis 2013).

Keeping any animal species in a single location is an unsafe, if not hazardous, practice (Nardelli 2016). Where a species' population has been reduced to isolated

individuals or a segregated group, the need is critical to establish at least a new, viable population, either in situ or ex situ or, better, both, without procrastinating, to avoid the risk of spreading pathogens over whole areas, or to prevent catastrophic events that can decimate the remaining animals. The first concern when planning is the health and safety of the rhinos. Disasters—whether close to the SRS such as in 2003 at the Sungai Dusun Conservation Centre in Malaysia, where a bacterial infection wiped out all rhinos in two weeks (Vellayan et al. 2004), or far away in the Democratic Republic of Congo in central Africa, where in June 2012 armed rebels led by a poacher attacked the Okapi Wildlife Reserve Epulu Station headquarters and killed seven people and all 14 Okapi *Okapia johnstoni* (Hance 2013)—represent hard experiences that justify the construction of new facilities in distant areas, as suggested by the Indonesian delegates at the Sumatran Rhinoceros Crisis Summit in Singapore in 2013. The news that pathogenic bacteria have been detected in Borneo Rhino Sanctuary and Sumatran Rhino Sanctuary (Borneo Rhino Sanctuary Programme 2018; Wahyuni et al. 2018), is a sign that innovative SRS logistic solutions are necessary to increase safety standards.

## Desiderata Nutrition

The assessment of leaf nutritional status can bring important and essential information for direct actions in the conservation breeding of the Sumatran Rhinoceros. Thus, in view of the recent decision by the Indonesian government to count the remaining Sumatran Rhinos throughout the present distribution areas, a comprehensive quantitative and qualitative vegetation



Image 8. SRS senior staff (L–R): Inov, Sumadi Hasmaran (Facilities Manager), Yohadi, Rois and Dr. Andriansyah (Veterinarian) at the Sumatran Rhino Sanctuary, Indonesia. © Cathy Dean.

survey and analysis of the rhinos' feeding leaves is highly recommended.

Sumatran Rhinos are believed to experience little feeding competition but field studies so far lack sufficient examination of competition from other taxa, except humans (e.g., Asiatic Elephant *Elephas maximus* ssp., Asiatic Tapir *Tapirus indicus*, other large and medium-sized terrestrial rainforest mammals). Terrestrial existence, large body size, and folivory are correlated (Palo & Robbins 1991). Van Strien (1985) reported: "From the total amount of undergrowth (about ½ to 1½ kg per square meter) the leaves and stems suitable as rhino food weighed between 260 and 520 grammes (fresh weight) per square metre. Re-growth of leaves and stems varied from 0.7 (in the forest) to 3.8 (near the river) grams per day per square metre. It seems from these figures that the average production of browse suitable for the rhino is probably not more than 1 gram per day per square metre. There are a few hundreds of grams of browse standing on each square metre, but it takes a long time, up to a year or so, for replacement".

To better assess the consequences, future investigations should include other connections to feeding competition such as modification of ranging patterns, changes in activity, and decreased fecundity. Information gained from such studies may advance our current knowledge of Sumatran Rhinoceros ecology and better define their conservation plans. Best possible feeding in controlled environment, hopefully of an increasing number of Sumatran Rhinos, may possibly become a handicap for the optimum care of animals eating about 50kg daily of both specific and varied kinds of foliage (Candra et al. 2016). Suitable leaves may start to run out from the surroundings of a congregate, highly populated breeding centre, their re-growth could be too slow to fill the demand, or they may grow too high to be reached by the gatherers, not to mention the ever-present logging predation. Furthermore, because folivores depend on such an ephemeral food source and plant phenologies, this may select for more elaborate life history traits. Isolating new enclosures and allowing large distances between them will allow a larger quantity and variety of leaves to be harvested for a much longer time, with ease and with less damage to the vegetation that will re-grow in good health.

#### Controlled field conservation centres and units

From his experience as former curator of H&PL and director of the Save the Sumatran Rhino project in Indonesia, the author suggests that several vast forested areas measuring 20–50 ha apiece be fenced, in the

region of one percent of the natural home ranges of female–male Sumatran Rhinos.

Two enclosures should be adjacent to each other [shaped e.g., , to keep rhinos apart and to offer each animal sufficient and secluded space. Such double units should be sited several kilometres away (an expert veterinary team will assess the safest distance) from each other, to avoid any physical and perceptible interference between the rhinos.

The small portion where the two enclosures connect will be the pair's "meeting point", which can be opened when managers decide to allow male and female to mate, or closed to allow gestation, birth and the young's growth under natural physical and mental conditions. In a 2008 study, Terry Roth asserts: "... a scientific method for accurately predicting when the female would be receptive to the male was developed so that animals could be paired safely. Stimuli causing induced ovulation include the physical act of coitus or mechanical stimulation simulating this, sperm and pheromones. Sumatran females exhibit unusual progesterone patterns when not mated". SRS veterinarians monitor the female's ovarian follicular development via ultrasound examination before the animals mate. When follicles reach 20–22 mm in diameter, the time is right to put the two rhinos together (Terry Roth pers. comm. April 2013).

#### Habitat protection

This new concept of *controlled field conservation centres* should be considered because it contributes to preserving not only the forest areas occupied by the enclosures but also of much larger portions of habitat. The forest surrounding the 'controlled field units' will have to be preserved for: a) the rhino's safety and welfare, b) the food reservoirs and buffer zones, and c) the activity of the keepers, the food gatherers and the Rhino Protection Units' guards. As a result, a whole, much larger area can be saved once several controlled field units become operational, ultimately making use of the entire forest allocated to a new Sumatran Rhino sanctuary (Image 5). Such controlled and managed field conservation centres could become central to the future protection of this rhino as well as other species in need of human intervention.

#### Animal welfare

Hutchins & Kreger (2006) stated in 2006: "*Perceiving, retaining and replicating the species best conditions for their behaviour performances are essential for their welfare, and this is particularly important if conservation centres hope to reintroduce animals to the wild in the*

*future*". Animal welfare is a fundamental consideration in curatorial management, and although animal wellbeing can be measured systematically only to a certain degree (Hill & Broom 2009), behavioural habits can be lost if the specific taxon's natural ecology is not comprehensively studied and properly applied. It is noteworthy that much of their welfare depends on some people's inborn endowment to interpret their needs (Aspinall 1976). Accordingly, ex situ wildlife management and breeding is not a subject of university teaching.

*"The welfare of any sentient animal is determined by its individual perception of its own physical and emotional state"* (Webster 2016). How do Sumatran Rhinos obtain, process and exercise information if those states and processes are not directly assessable? Behavioural ecology can shed light on issues of cognition and on an ecological approach to cognition—environment information, then cognitive planning, leading to behaviour—should provide the evidence.

For the purpose of biological conservation, several aspects of management are important and poor quarters and environments are responsible for permanent changes in behaviour and physiology (Hofer & East 1998).

Housing Sumatran Rhinos in a species-appropriate area where they are able to perform normal activities and make independent choices should be considered fundamental for their well-being. To that end, managers could go to greater lengths to provide their animals items (e.g., mud wallows and saltlicks) that encourage exploration of a greater diversity of behaviours and that encourage maximal use of space. A further possibility is corridors that allow animals to move from one space to another. Not only does this provide the option for animals to choose one location over another, but it also increases the space available for them to roam, and it enlarges the diversity of stimuli that the animal can possibly experience in each of the different settings.

### **Behavioural ecology**

Several animal species communicate through all their senses including by means humans do not have. In recent years, the study of animal communication has expanded rapidly as has information on their consciousness (Bekoff et al. 2002) and has allowed the discovery of mesmerizing phenomena. For example, the Sumatran Rhinoceros emits an infrasound whistle followed by a sharp burst of air that can travel for kilometres (Muggenthaler 2003). Such complex communication, infrasounds included, in addition to the known capacity of the sensory organs to influence cognitive activities result in behaviour

remarkably similar to what humans define as social behaviour, although the Sumatran Rhinoceros is solitary and generally avoids contact with other rhinos in nature.

Reproductive competition occurs when an individual's capacity to conceive has diminished due to the presence of a conspecific. Most animal species resolve this problem by living solitarily (Emlen 1982): one more reason to manage the Sumatran Rhinos separately in several controlled field centres. Another aspect to consider is the behaviour of adult breeding males in the presence of sub-adult and/or adult non-breeding males; a psychological submission may develop, under controlled conditions, which could inhibit the non-breeding males. In fact, dominant male rhinos were present in situ during Borner (1979) and van Strien (1985) field studies, as reported by the authors. Furthermore, in spite of "social" designates to do with more than one individual (Waal & Tyack 2003)—the behaviour of a pair of animals may even be called social—socialising induces stress.

Stress in this context means the effects resulting from causes of various origins in rhinos, which interrupt homeostasis and cause harm because they diminish biological functions and ultimately result in reduced health conditions and a negative factor that favours the action of glucocorticoids, which cause infertility in mammals (Broom & Johnson 1993). Measuring faecal glucocorticoids, or their metabolites, may be useful for well-being studies in controlled environments—especially in assessing short-term responses to stressors e.g. capture, transportation and translocation are important stressors—and can contribute non-invasively to the work of biologists (Metrione & Harder 2011).

Carlstead & Brown (2005) presented evidence showing that social tension may cause chronic stress in the Black Rhinoceros and the White Rhinoceros, and established that non-cycling female rhinoceroses had more variable corticoid concentrations and higher rates of stereotypic pacing, an indicator of high stress levels. This factor gives the managed population a sustainability struggle that is observed in each species. Psychosomatic weaknesses, a probable cause of severe disorders, should also be investigated using the techniques available for the Black Rhinoceros and the White Rhinoceros (Carlstead & Brown 2005), and new research carried out.

Ex situ conservation centres with more than one female Black Rhinoceros have a lower reproductive rate and a later age of first birth. Probably, there is a density-dependent restraining effect on breeding function among females in confined environments

(Carlstead et al. 1999a,b). A physiological evaluation of welfare in managed animals can be obtained non-invasively through analysis of adrenal hormones in saliva. Adrenalin hormones measure activity in the sympathetic–adrenal medullary system and in the hypothalamic–pituitary–adrenocortical system (Palme 2012). Salivary corticosterone concentrations can determine stress in White rhinos (Schmidt & Sachser 2000).

A survey study conducted on Black rhinos *ex situ* surprisingly found that more aggression and assertiveness contributed positively to a female's chances of breeding (Carlstead et al. 1999a,b). The reproductive rates of Black Rhinoceros and White Rhinoceros in controlled environments are unsustainably low. Evidence shows that to a large extent social signals may cause chronic stress in rhinos, and this element contributes to the sustainability problems observed in each species of managed populations (Carlstead & Brown 2005; Metrione et al. 2007). The concentration of glucocorticoids (or their metabolites) can be measured in various body fluids or excreta. Above all, faecal samples offer the advantage that they can be easily collected and this procedure is feedback free. Thus, such methods are a valuable tool in a variety of research fields such as animal welfare in handling, housing and transport and also in ethological and environmental studies.

Scientific research on the behaviour of the Sumatran Rhinoceros should be expanded to include cognitive ethology—the comparative study of mental phenomena—including both conscious and unconscious mental states. A lot of effort is expended on the care of animals but only rarely is the inner world of those sentient beings well thought-out in strategic planning.

## CONCLUSIONS

There is reason to believe that the Sumatran Rhinoceros can continue to exist, providing that animals will still be around for a sufficient time to be rescued, or survivors that have lost contact with each other are not all genetically or reproductively ruined. Populations lose genetic diversity at a rate proportional to the inverse of their effective population size (Frankhman 1996), thus the surviving, small, *D. sumatrensis* populations are rapidly losing genetic diversity through drift (random loss of alleles across generations). To re-establish viable populations in numbers sufficient to maintain genetic diversity, it is imperative not only to capture the few remaining individuals, wherever they may be, but to

induce them to breed under the best conditions as a matter of urgency.

Food preferences of Sumatran Rhinoceros probably trigger short-term movements of individuals outside their home ranges, conservation actions should therefore aim at enlargements and connectivity of its habitats utilizing controlled field centres. The species' selective feeding habits may result in individuals moving into areas with highly preferred food resources, which can be areas of high mortality risks, once known to poachers. Habitat connectivity projects should pinpoint areas that allow these rhinos to access higher elevated areas, secluded and less accessible to humans.

With the rapid destruction of tropical forests and the threat of global climate change, a greater understanding of the importance of what has worked and what would work, is essential to the preservation of the megafolivorous Sumatran Rhinoceros.

In controlled environments, animal species which are difficult to observe in the wild can increase our knowledge of ecologic aspects that influence their habitat utilization within fragmented landscapes and can assist in animal husbandry and the planning of current and future conservation efforts. It is essential and urgent to match ongoing efforts for *in situ* protection with *ex situ* breeding, and to optimize this species' peculiar requirements inside strictly protected areas and in controlled field centres. The critically endangered Sumatran Rhino is a perfect example of the need for conservation measures that follow a One Plan Approach paradigm. The One Plan Approach, initially proposed by the IUCN SSC Conservation Planning Specialist Group (CPSG), considers all populations of the species, *in situ* and *ex situ*, under different conditions of management, engaging all responsible parties and all available resources from the very start of any species conservation planning initiative, as per Byers et al. (2013): “*The One Plan approach aims to establish new partnerships, ensure that intensively managed populations are as useful as possible to species conservation, increase the level of trust and understanding among conservationists across all conditions of management of a species and accelerate the evolution of species planning tools. Integrated species planning is not a new concept. Such holistic conservation efforts have led to several well-known conservation successes, from Golden lion tamarins in Brazil to Puerto Rican Crested toads in the Caribbean to Arabian oryx in the Middle East*”.

The Sumatran Rhinoceros is of special interest because, with the Javan Rhinoceros *Rhinoceros sondaicus*, it is one of the largest mammal species that

depends on undisturbed rainforest and for that reason can be regarded as an important indicator species. Despite the vigorous attempts by a handful of people to protect it, time is running out for the Sumatran Rhinoceros: a foremost phylum-genetic diversity loss (Davis et al. 2018). In the present status of wildlife, it is difficult to reconcile the actions of leaving a species to become extinct or allowing individuals to solely survive in ex situ breeding centres, albeit with unavoidable negative experiences. The Sumatran Rhinoceros represents the emblematic example of such a perplexing state of affairs.

Is saving the Sumatran Rhino mission possible? Yes! It will require a collaborative effort, following hard-and-fast rules, and optimal management conditions.

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## REVISITING GENETIC STRUCTURE OF WILD BUFFALOES *BUBALUS ARNEE* KERR, 1792 (MAMMALIA: ARTIODACTYLA: BOVIDAE) IN KOSHI TAPPU WILDLIFE RESERVE, NEPAL: AN ASSESSMENT FOR TRANSLOCATION PROGRAMS

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**Abstract:** Koshi Tappu Wildlife Reserve (KTWR) has the last remaining Nepalese population of the Endangered Asiatic Wild Buffalo (*Bubalus arnee* Kerr, 1792). Individual animals protected inside KTWR may be of purely wild, domestic or hybrid origin, and the wild population is under potential threat due to habitat loss and genetic introgression from feral backcrosses. Identification of genetically pure wild individuals is important for identifying animals for translocation to other areas within their former range. In this study we have sequenced a highly variable 422bp region of the Cytochrome b gene of 36 animals, and added 61 published sequences of both River and Swamp Buffalo from Italy and some southern Asian countries including India. The haplotype diversities ranged from 0.286–0.589 with slightly higher diversities in domesticated individuals. The AMOVA analysis revealed that 97.217% of the genetic variation was contained within groups and 2.782% occurred among groups. An overall fixation index ( $F_{ST}$ ) was found to be 0.02782 ( $p > 0.05$ ). Phylogenetic relationships derived through a reduced median network and maximum parsimony analyses reconfirmed the ancestral nature of the Wild Water Buffalo. Moreover, this study has reviewed recent achievements of molecular research in wild buffalo, assessed the technical capacities of research institutes in Nepal to conduct molecular research required for identifying pure wild individual in KTWR and more importantly initiated DNA bank and DNA sequence library of buffalos, which will enable an international collaboration for advanced molecular research in the future.

**Keywords:** Asian Wild Buffalo, conservation, Cytochrome b, phylogenetics.

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For **Author details**, **Author contribution**, **Acknowledgements** and **Nepali abstract** see end of this article.



## INTRODUCTION

The domesticated Water Buffalo *Bubalus bubalis* Kerr, 1792 is one of the most important dairy and draft animals in southern Asia. Buffaloes are broadly categorized into two general 'breeds' sometimes characterized as subspecies: river (*Bubalus bubalis bubalis*) and swamp buffalo (*Bubalis bubalis carabanesis*). Despite having distinct morphological and behavioural traits and different karyotypes between these two categories of buffalo, they interbreed easily and produce progeny with intermediate chromosomes (Mishra et al. 2015). Swamp and river buffalo have different purposes and are found in different geographical areas. The swamp type, with wide-spreading horns and some white markings, is more similar to Wild Buffalo *Bubalus arnee* (Kumar et al. 2007; Mishra et al. 2015).

The Government of Nepal (GoN) established Koshi Tappu Wildlife Reserve (KTWR), an IUCN Category IV protected area (Heinen 1995), in 1976 primarily to conserve the last Nepalese population of Wild Buffalo (Heinen 1993). Wild Buffalo cohabit the reserve with highly backcrossed feral buffalo thought to have been released in the area in the 1950s (Dahmer 1978). Wild Water Buffalo *Bubalus arnee* (Kerr, 1792) is considered Endangered globally (Kaul et al. 2019), with isolated populations in KTWR and selected areas of Bhutan, India, Thailand and possibly Myanmar and Cambodia (Groves 1996; Heinen & Srikosamatara 2003; Choudhury & Barker 2014). In 2016, 433 Wild Buffaloes were counted in KTWR (2016).

Despite the possibility of interbreeding between wild and domesticated buffalo, it is important to assign extant individuals to wild and other types where possible to broaden our understanding of the genetic structure of different types and to maintain genetic fingerprinting of wild breeds for their conservation. The introgression from domestic to wild population is female-mediated, therefore mitochondrial DNA (mtDNA) sequencing is likely to be helpful in the identification of group-specific mitotypes. The presence of wild-specific or domestic-specific haplotypes in either group would allow us to identify hybrids (Lau et al. 1998; Flamand et al. 2003). Furthermore, mtDNA sequence variations have been widely applied in mammals to study inter- and intra-species phylogenetic relationships (Kikkawa et al. 1997; Lau et al. 1998; Conroy & Cook 1999; Kuwayama & Ozawa 2000; Kumar et al. 2007).

Conservation decisions on translocation should be based on putative wild, feral and domestic genetic assignments reliably performed through standard

and widely accepted techniques. Therefore, selecting individuals for translocation programs, identification of wild individuals through detailed molecular study of the buffalo population protected in the reserve is a high priority for the Nepal government. In addition, understanding the genetic makeup of Wild Buffalo could be used as the basis for genetic improvement of domestic stock. National capacity building to conduct advanced molecular studies should be initiated from collecting blood and faecal samples, creating a DNA reference library and carrying out genetic research on various aspects such as population genetics, breeding behaviours among different buffalo types, disease dynamics, and food habits of buffalo population in the reserve. We present results of DNA sequence variation in the partial but variable cytochrome b gene among purely wild, feral and domesticated individuals and future prospects for advancing genetic research on Wild Buffaloes inhabiting KTWR in eastern Nepal.

## MATERIAL AND METHODS

### Identification of organizations

Nepal Government, Ministry of Forest and Environment is working together with local communities and national and international conservation partners to protect wild animals and restore their natural habitats. With the aim of establishing viable populations of endangered species in different areas and safeguarding them from poaching and natural calamities such as flood, fire and epidemics, the Ministry of Forests and Environment, Department of National Parks and Wildlife Reserve (DNPWC) has been regularly translocating species to their original natural habitats, viz.: One-horned Rhinoceros and Wild Water Buffalo. In 2016, 18 individuals of Wild Water Buffaloes were translocated from Koshi Tappu Wildlife Reserve (KTWR) to Chitwan National Park. Translocation was carried out by a team of 60 people including three veterinarians and 12 wildlife technicians led by DNPWC with support from the World Wildlife Fund Nepal, the USAID-supported Hariyo Ban Program, National Trust for Nature Conservation, Biodiversity Conservation Centre (NTNC-BCC), and the Zoological Society of London (Nepal). A series of consistent identification criteria that are based on phenotypic and behavioural characteristics were used to choose Wild Buffaloes from different herds for translocation. Given the availability of highly polymorphic genetic markers, the expert team strongly recommended to adopt genetic translocation as an

effective and reliable management strategy. For this management strategy to be implemented effectively the team further emphasized the need for institutional strengthening and capacity building of national laboratories to conduct genetic research on buffalo before translocating them from the reserve in future. Wildlife veterinarians, biologists and research scholars from DNPWC and NTNC-BCC collected blood and faecal samples of buffaloes and initiated a genetic study in the wildlife laboratory of NTNC-BCC and molecular biotechnology laboratory of the Nepal Academy of Science and Technology.

### Sampling and blood collection

A total of 42 blood and faecal samples (Table 1) were collected mainly from individuals residing in and around KTWR located in the Terai of southeastern Nepal (Figure 1). Animals were provisionally divided into three classes: domestic (D, n=11), hybrid (H, n=11), and wild (W, n=20) based on the consistent phenotypic and behavioural criteria (Dahmer 1978; Heinen & Paudel 2015) and location of herds sampling (domestic buffalo were sampled from the villages nearby KTWR while wild and feral were sampled using a location map of natal herds prepared by the reserve) and behavioural and

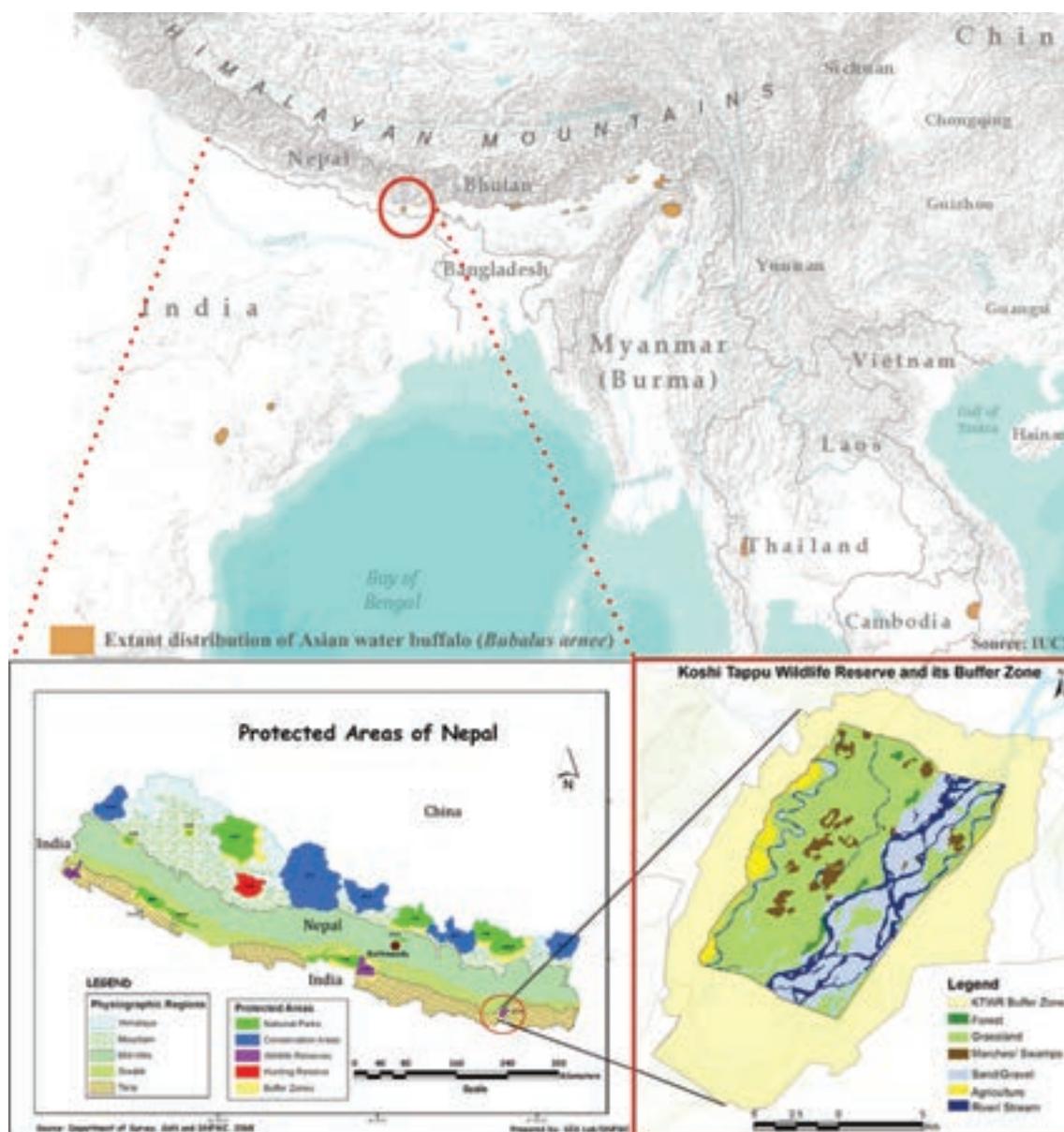


Figure 1. Map of an extant distribution of Asian Water Buffalo *Bubalus arnee* and location of the study area in Koshi Tappu Wildlife Reserve, Nepal.

**Table 1. Sample code, classification and individual details of Wild Buffalo *Bubalus arnee* sampled from Koshi Tappu Wildlife Reserve, Nepal. Blood samples were taken from individuals if not mentioned in sample type.**

Samples code	Group	Sex, age, date of collection	Sample type
BuffH1	Hybrid	Female, 10 month, 02.vii.2017	
BuffH2	Hybrid	Female, 6 years, 29.vi.2017	
BuffH3	Hybrid	Female, 2 years, 29.vi.2017	
BuffH4	Hybrid	Female, 2 years, 02.vii.2017	
BuffH5	Hybrid	Male, 2 years, 02.vii.2017	
BuffH6	Hybrid	Male, 2 years, 02.vii.2017	
BuffH7	Hybrid	Male, 2 years, 29.vi.2017	
BuffH8	Hybrid	Female, 2 years, 29.vi.2017	
BuffH9	Hybrid	Male, 10 month, 02.vii.2017	
BuffH10	Hybrid	Male, 1.5 years, 02.vii.2017	
BuffH11	Hybrid	Female, 8 month, 02.vii.2017	
BuffD12	Domestic	Female, 1.5 years, 02.vii.2017	
BuffD13	Domestic	Female, Adult, 27.vi.2017	
BuffD14	Domestic	Female, 10 years, 27.vi.2017	
BuffD15	Domestic	Male, 2 years, 01.vii.2017	
BuffD16	Domestic	Male, 2 years, 01.vii.2017	
BuffD17	Domestic	Male, 1 years, 01.vii.2017	
BuffD18	Domestic	Female, 12 years, 01.vii.2017	
BuffD19	Domestic	Male, 1 year, 29.vi.2017	
BuffD20	Domestic	Male, 1 year, 29.vi.2017	
BuffD21	Domestic	Male, 2.5 years, 27.vi.2017	
BuffD22	Domestic	Female, 1 year, 01.vii.2017	
BuffW23	Wild*	Male, Adult, 04.ii.2017	
BuffW24	Wild*	Male, Adult, 26.i.2017	
BuffW25	Wild*	Female, Sub-adult, 06.ii.2017	
BuffW26	Wild*	Female, Adult, 01.ii.2017	
BuffW27	Wild	Male, Adult	Fecal Sample
BuffW28	Wild**	Male, Adult, 05.ii.2017	
BuffW29	Wild**	Female, Adult, 01.ii.2017	
BuffW30	Wild**	Female, Adult, 01.ii.2017	
BuffW31	Wild**	Female, Adult, 06.ii.2017	
BuffW32	Wild**	Female, Adult, 05.i.2017	
BuffW33	Wild**	Female/2.5 years, 06.i.2017	
BuffW34	Wild	Female, Adult	Fecal sample
BuffW35	Wild	Male, Adult	Fecal Sample
BuffW36	Wild	Male, Adult	Fecal sample
BuffW37	Wild	Female, Adult	Fecal Sample
BuffW38	Wild*	Female, Adult, 31.i.2017	
BuffW39	Wild*	Female, Adult, 29.i.2017	
BuffW40	Wild*	Female, Adult, 27.i.2017	
BuffW41	Wild	Male, Adult	Fecal sample
BuffW42	Wild	Male, Adult	Fecal sample

\*—Translocated from KTWR | \*\*—Collected from Central Zoo, Lalitpur | Wild—20 | Hybrid—11 | Domestic—11.

anatomical phenotypic traits. All animals classified as domestic were river type buffalo with black bodies and curled horns (as in the Murrah breed of river buffalo), while those classified as wild had white chevrons, socks and tail tips, and larger, relatively straight, pale-coloured horns (Image 1) similar to swamp buffalos (Heinen 2002). Hybrid animals had intermediate phenotypes and may be first generation crosses or the result of various levels of backcrossing to either wild or domestic.

#### DNA Extraction, Polymerase Chain Reaction and Sequencing

Genomic DNA was extracted from blood using a Qiagen DNEasy Blood kit, according to the manufacturer's

protocol. For the faecal samples, a Qiagen QIAMP DNA Stool Mini Kit was used following the manufacturer's instructions. Extracted DNA samples were stored at 4°C until they were used for molecular analyses. Aliquots of extracted DNA were used for PCR and sequencing. The mitochondrial partial Cytochrome b gene of 422bp was amplified using primer pairs (L14724: 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' and H15149: 5'-AAACTGCAGCCCCTCAGAATGATATTGTCTCA -3') (Kocher et al. 1989). PCR was carried out with 3µl template DNA, 15µl of Hot Start Taq 2X Mastermix (New England Biolab, UK), 1µl of each primer and 7µl of nuclease free water in a total reaction volume of 30µl using an ABI Veriti™ Thermal Cycler (Model no. 9902). Of



**Image 1.** Translocation of Wild Buffalo and collection of blood samples from both domestic and Wild Buffalo: a–b—blood sample collection from domestic buffalo | c—Wild Buffalo *Bubalus arnee* | d–e—blood collection and translocation of Wild Buffalo from Koshi Tappu Wildlife Reserve to Chitwan National Park, Nepal.

the 42 samples, only 36 were amplified successfully and rest of the six didn't amplify even in multiple attempts due to poor DNA quality. The PCR conditions were an initial denaturation at 94°C for 10 minutes, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 55°C for 30s, elongation at 72°C for 45s and a final extension at 72°C for 10 minutes. The PCR products were electrophoresed at 100 volts for 30 minutes in 1.5% agarose gels, viewed in Gel Doc (Syngene InGenius) after staining with Sybr safe and photographed.

Amplified DNA fragments were purified using ExoSap-IT Express PCR Product Clean up (Affymetrix Inc., Santa Clara, CA, USA) following a cycle of 37°C for 15 minutes and 80°C for 1 minute in a thermo-cycler. High quality purified PCR amplicons were subjected to Dideoxy sequencing in a total volume of 10µl containing 1µl purified PCR product, 1µl Big Dye terminator sequencing mixture (V3.1) (BIGDYE Terminator Cycle Sequencing Kit, Applied Biosystems, Foster, CA, USA), 1.5µl sequencing buffer and 1.5µl primer (10µm). Sequencing was done at Nepal Academy of Science and Technology, Molecular Biotechnology Laboratory, in an ABI 3500XL automated DNA sequencer (Applied Bio-systems, Forster City, CA, USA). Sequencing for the majority samples was performed for one time but in the case sequence quality was low and/or polymorphism was observed then re-sequencing was done for confirmation. Successful sequences of 36 samples were deposited in GenBank (Accession no. MH718851–85)

#### Sequence alignment, haplotype identification and phylogeny

Raw sequence fragments were assembled, checked and edited with Sequencer 5.0 (Gene Codes Corp., Ann Arbor, MI, USA) and contigs of both reverse and forward primers were created. Sequences were aligned with ClustalX (Thompson et al. 1997) in BioEdit. In addition to our 36 sequences (W=15, H=10 and D=11), we included the 42 Nepalese samples (W=7, H=15 and D=20) of Flamand et al. (2003), sequenced and analysed by Zhang et al. (2016, KR009944–85), four Cytochrome b haplotypes from Indian river buffalo identified by Kumar et al. (2007, EF409939 H1-4) and 15 Cytochrome b haplotypes defined by Kikkawa et al. (1997), eight river buffalo from Bangladesh, Sri Lanka, Italy and Pakistan and seven swamp buffalo from Japan, Taiwan, Thailand, Philippines, Indonesia and Bangladesh (D34637–38, D88627–38, D88983).

All sequences were aligned and mitochondrial haplotypes were defined in DnaSP v5, and haplotype (h) and nucleotide diversity ( $\pi$ ) were estimated using DnaSP v5 (Librado & Rozas 2009). Genetic differentiation within and between buffalo groups (wild, hybrid and domestic) was estimated by an analysis of molecular variance (AMOVA) using 10,000 permutations in Arlequin v3.5 (Excoffier & Lischer 2010). Similarly, pairwise genetic divergences between groups ( $F_{ST}$ ) was also calculated and significances tested using 10,000 permutations in Arlequin v3.5.

Phylogenetic relationships among haplotypes were

derived through a reduced median network v4.6 (Bandelt et al. 1999). To identify phylogenetic lineages, maximum parsimony (MP) analysis was performed in PAUP 4.0b10 (Swofford 2002) using the heuristic search option with 1,000 random additions and the tree bisection-reconnection (TBR) swapping and the MULTrees option on. Branch support was provided by a bootstrap analysis of 10,000 replicates of heuristic searches, with the MULTrees option on and TBR swapping off. Consistency indices (CI) and retention indices (RI) were obtained in PAUP. In addition, a neighbor-joining (NJ) tree (Saitou & Nei 1987) was produced using MEGA7 (Kumar et al. 2016).

## RESULTS

### Sequence Variation and Divergence

The lengths of the 36 partial cytochrome b sequences including primers at both ends for all the three types of buffalo were 486bp. When primers on either end were removed the sequence lengths were 422bp. The aligned matrix without primers contained two variable sites (Table 2), however, when our data set was compared with the accession data of Zhang et al. (2016), Kumar et al. (2007), and Kikkawa et al. (1997), base substitutions at 13 nucleotide positions (variable sites) were obtained and, among them, three nucleotide positions were specific for river buffalo and 10 positions were specific for swamp buffalo. The sequence divergence within buffalo of Nepal and India was 0.24 to 0.49 %; however,

when compared with river buffalo sequence of Kikkawa et al. (1997), the divergence was slightly higher: 0.24 to 0.74 %. Sequence divergence within swamp buffalo was 0.24 to 0.98%. The sequence divergence between swamp and river buffalo was calculated to be 1.49 to 2.49%.

### Haplotype Identification, Differentiations, and Phylogenetic relations

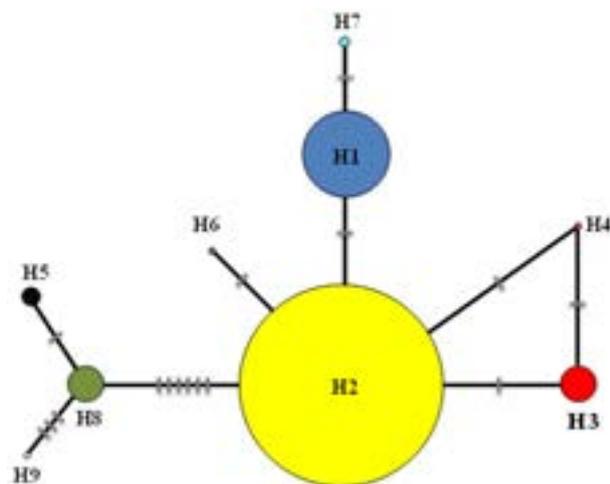
The 97 partial cytochrome b sequences, including 36 from this study, showed a total of 13 variable sites (Table 2, see Figure 2), which defined nine haplotypes. Nepalese buffalo had either haplotype H1, H2 or H3, but the three classes domestic (D), hybrid (H) and wild (W) were represented among both H1 and H2 (H1: D=23,W=18, H=17; H2: H=8, D=7, W=2 and H3: W=2). The most common haplotype (H2) was widely distributed among groups and represented by 66% of sequences (Nepal: D=23, W=18 and H=17; one of Kumar et al. (2007); and five of Kikkawa et al. (1997)). The second most common haplotype (H1) was represented by 20% of the samples (H=8, D=7, and W=2) and one each from Kumar et al. (2007) and Kikkawa et al. (1997). Three sequences of Nepalese samples, two from this study and one each from Zhang et al. (2016) and Kumar et al. (2007) were restricted to the third haplotype (H3). Of the remaining six haplotypes, one was reported by Kumar et al. (2007; H4) while the remaining five (H5 to H9) were defined by Kikkawa et al. (1997). Haplotypes 4, 6 and 7 were found in river animals from other countries (i.e., not Nepal) and haplotypes 5, 8 and 9 were specific

**Table 2.** Variable nucleotide positions for the partial Cytochrome b gene of the 36 accessions of the present study, 42 accessions of Zhang et al. (2016), four accessions of Kumar et al. (2007), and 15 accessions of Kikkawa et al. (1997).

Haplotypes	Nucleotide positions													Frequency	Remarks
	30	61	79	81	87	99	147	234	240	375	379	411	417		
H1	T	C	G	T	T	C	T	A	G	T	T	A	A	64	Nepal sample, Kikkawa et al. (1997), Kumar et al. (2007), Zhang et al. (2016)
H2	T	C	G	T	T	T	T	A	G	T	T	A	A	19	As above
H3	T	C	G	T	T	C	T	A	C	T	T	A	A	4	As above
H4	T	C	G	T	T	C	T	A	A	T	T	A	A	1	Kumar et al. (2007)
H5	C	C	G	C	T	C	C	G	G	C	C	A	G	2	Kikkawa et al. (1997)
H6	T	C	G	T	T	C	T	A	G	T	T	A	T	1	As above
H7	T	C	A	T	T	T	T	A	G	T	T	A	A	1	As above
H8	C	C	G	C	T	C	C	G	G	C	C	A	A	4	As above
H9	C	G	G	C	A	C	C	G	G	C	C	G	A	1	As above
<b>Total</b>														<b>97</b>	

**Table 3.** Haplotype diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) estimated from partial mitochondrial Cytochrome b sequences of 78 Nepalese buffalo of three different groups (wild, domesticated, and hybrid).

Buffalo		No. of haplotypes (H)	Sample size	$h$ (Haplotype diversity)	SD	$\pi$ (nucleotide diversity)	SD
Wild	This study	3	15	0.362	0.145	0.00095	0.00041
	Zhang et al. 2016	2	7	0.286	0.196	0.00071	0.00049
	Total	3	22	0.329	0.121	0.00086	0.00034
Hybrid	This study	2	10	0.467	0.132	0.000116	0.00033
	Zhang et al. 2016	2	15	0.476	0.092	0.00119	0.00023
	Total	2	25	0.453	0.072	0.00113	0.00018
Domesticated	This study	2	11	0.509	0.101	0.00127	0.00025
	Zhang et al. 2016	3	20	0.353	0.123	0.00092	0.00034
	Total	3	31	0.411	0.087	0.00106	0.00024
All this study		3	36	0.438	0.082	0.00116	0.00024
All Zhang et al. (2016)		3	42	0.382	0.076	0.00098	0.00021
Total		3	78	0.403	0.055	0.00105	0.00016

**Figure 2.** Reduced median network constructed with 422bp sequences of 9 cytochrome b haplotypes of the buffalo from Nepal, India, Pakistan, Bangladesh, Sri Lanka, Japan, Indonesia, Philippines, and Italy. Circle sizes in the network are proportional to the sequence frequencies over the data sets | colours of circles indicate different haplotype | largest circles are the most abundant haplotypes | slashes across the branches represent mutational steps.

to swamp buffalo.

Within groups, haplotype diversity was highest in hybrids followed by domesticated buffalo, and slightly lower in Wild Buffalo (Table 3). Overall haplotype and nucleotide diversities were 0.403 and 0.00105, respectively. Haplotypes were divided into two branches corresponding to river and swamp buffalo by six nucleotide mutations. River buffalo were found to be less diverse genetically than swamp buffalo. The AMOVA

conducted for 78 Nepalese buffaloes under three groups showed highest variation (97.21%) partitioned within groups and very little variation (2.782%) partitioned among them with an overall fixation index  $F_{ST}$  of 0.0278 ( $p > 0.05$ ) (Table 4). The pairwise genetic variations between groups also revealed non-significant low  $F_{ST}$  scores (wild vs hybrid,  $F_{ST} = 0.055$ ,  $p = 0.126$ ; wild vs domestic,  $F_{ST} = 0.002$ ,  $p = 0.416$  and hybrid vs domestic,  $F_{ST} = 0.020$ ,  $p = 0.546$ ). The maximum parsimony analysis of the 97 sequences revealed four distinct clades (clades A through D) with moderate to high bootstrap values (Figure 3). The three most parsimonious trees ( $CI = 1.00$ ,  $RI = 1.00$ , length = 15 steps) were recovered. Clade A contained all H1 sequences plus one H4 and one H6, Clade B contained all 19 H2 sequences plus one H7, Clade C contained all four sequences of Haplotype H3 and Clade D, with a high bootstrap value (99%), included all swamp animals (Haplotypes H5, H8, H9). The strong separation of swamp and river buffalo is also shown by the median joining network, with six mutations separating H8 and H2. Neighbour-joining (NJ) phylogenetic analysis showed the same topology (Supporting information, Appendix 1).

## DISCUSSION

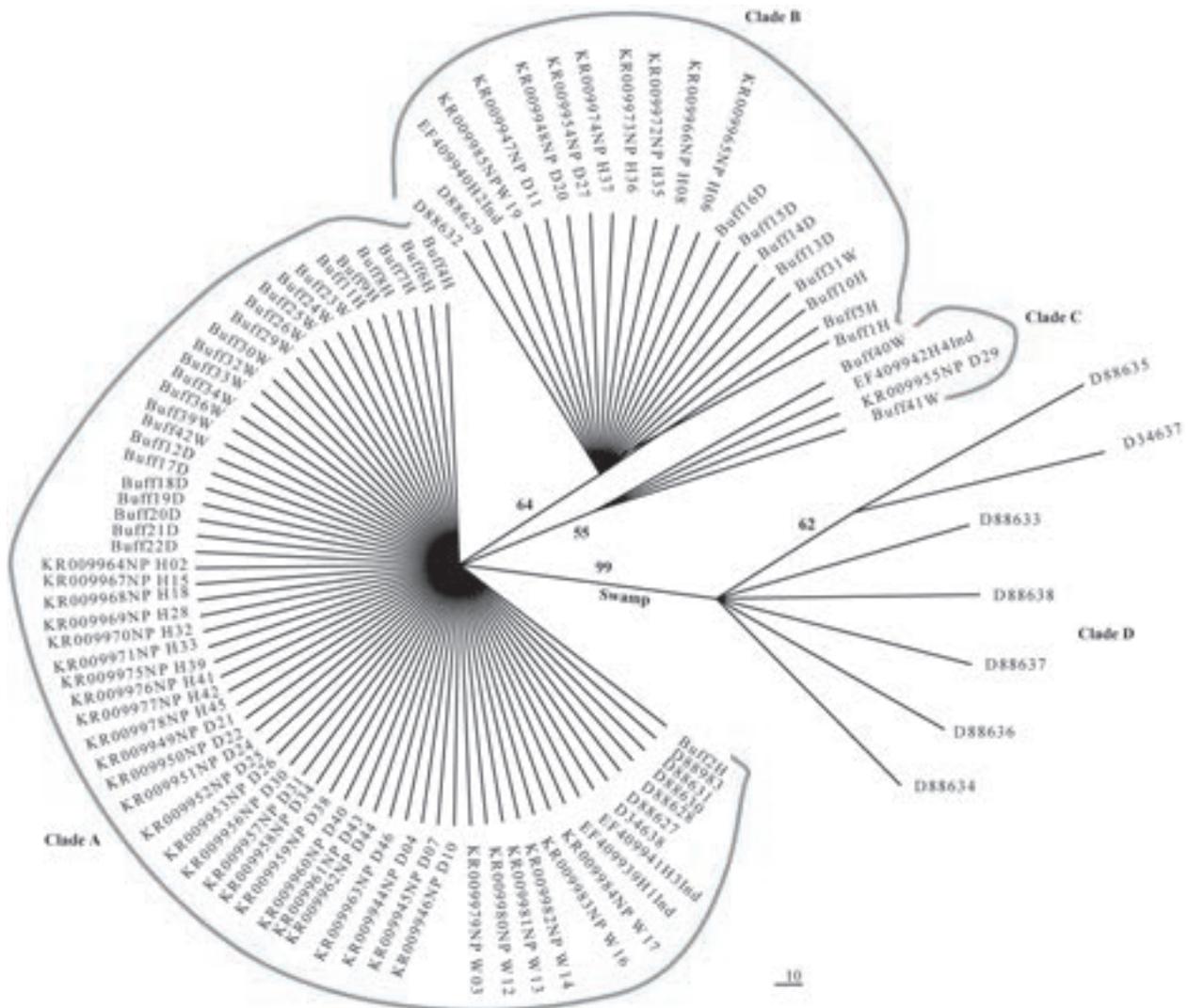
### Conservation status of Wild Buffalo population in Nepal

Nepal is home to a population of the Endangered Asiatic Wild Buffalo *Bubalus arnee*, the progenitor of domesticated water buffalo (Lei et al. 2007). Recent

**Table 4. Analysis of molecular variance (AMOVA) for the three groups of buffaloes based on partial cytochrome b region.**

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation indices ( $F_{ST}$ )
Among populations	2	0.716	0.00590	2.78237	0.02782*
Within populations	75	15.463	0.20618	97.21763	

\* $p > 0.05$



**Figure 3. Maximum parsimony tree constructed using 422bp partial Cytochrome b sequences of 36 buffaloes of Nepal (this study), 42 additional Nepalese sequences of Zhang et al. (2016), four sequences of Kumar et al. (2007), and 15 sequences of both river and swamp buffalo from Kikkawa et al. (1997). Numbers on the branches are bootstrap values.**

censuses of Wild Buffalo in KTWR revealed that the population increased to 433 individuals (KTWR 2016), an increase of 105 individuals compared to 2014 (Khatri et al. 2013 ). There were only 63 individuals at the time of establishment of KTWR in 1976 (Dahmer 1978). The historic range of this species extended further west within Nepal, and at least as far as Chitwan National

Park, however, Wild Buffalo have been restricted to KTWR for an estimated 60 years and they are under constant threat of extirpation from floods, habitat deterioration, hybridization, and the potential for diseases and parasites transmitted by domestic livestock (Heinen & Kandel 2006). Since Wild Buffalo have been eliminated from the greater part of their former

range, the Nepalese population is very important for the survival of the species globally (Beyers et al. 1995; Hedges 1995, 2001; Choudhury & Barker 2014).

Given the precarious existence of Wild Buffalo within KTWR, several wildlife conservationists have emphasized the need to translocate a sufficient number of individuals to sites within their indigenous range. Chitwan National Park had this species at least until the 1950s (Spillet & Tamang 1966; Aryal et al. 2011) and has extensive grassland areas, and much larger riverine habitats with sufficient upland areas that are not prone to flooding, compared to KTWR (Heinen & Paudel 2015). For these reasons 18 Wild Buffaloes were translocated to Chitwan National Park from KTWR recently, and more need to be moved in the near future (Shah et al. 2017; Kandel et al. 2018).

#### Identification of wild individuals in KTWR

Translocation of endangered species can restore species, protect populations from threats and reinstate the local ecosystem functions (Tarszisz et al. 2014). Adequate morphological and genetic studies should be carried out to distinguish putative wild from feral backcrossed animals for translocation programs. During translocation enough purely wild individuals must be moved to assure genetic variation in the founding population. Although the recent selection of individuals for translocation to Chitwan was based on phenotypic and behavioural characteristics widely recommended by Dahmer (1978), many individuals of mixed wild-domestic ancestry may not be correctly distinguished from wild animals (Flamand et al. 2003).

On the basis of the partial cytochrome b sequences, we were able to define only three haplotypes in Nepalese buffalo. None of the haplotypes were specific to wild, domestic and hybrid types identified here. These haplotypes had been identified by Zhang et al. (2007, 2016), Kumar et al. (2007), and Kikkawa et al. (1997) in their Nepalese, Indian, and wider samples (Pakistan, Bangladesh, Thailand and Sri Lanka). Although we have used the partial sequence of cytochrome b gene, the complete length (1,120bp) of this gene sequence reported from other studies (Kikkawa et al. 1997; Kumar et al. 2007) including 42 Nepalese samples from Zhang et al. (2016) did not show consistent distinctions between wild, hybrid, and domestic group of buffalos. Non-significant genetic variability observed between three groups of buffaloes and the highest, i.e., 97.21% total variations found within group in AMOVA analysis, clearly shows an evidence of gene flow between groups. In KTWR, most of the time wild, feral and domestic herds

share grazing areas, where crossbreeding between groups occurs frequently. Moreover, low genetic variation between groups is also attributed to local the farmers' practice of crossbreeding domestic females with wild males (Heinen 2001).

NJ and MP analysis performed (results not shown) with the complete length (1,120bp) cytochrome b sequences of Zhang et al. (2016, 42 Nepalese sequences), Kumar et al. (2007, four river buffalo haplotypes), and Kikkawa et al. (1997, seven river and eight swamp haplotypes) provided essentially the same topography of the tree but an addition of one more haplotype represented by Genbank accession KR009945NP\_D07 alone.

Lau et al. (1998) using partial cytochrome b sequence (303bp) and D-loop (158bp) sequence suggested that Wild Asian Water Buffalo (*Bubulus arnee*) in Assam, Nepal and Indo-China is the possible ancestor of both river and swamp buffalo. The study by Tanaka et al. (1996) also supports this hypothesis. Nepal's Wild Buffalo show swamp type phenotypic characteristics but Zhang et al. (2011) found them genetically closer to river buffalo. Our study is consistent with Zhang et al. (2011) in that our MP, NJ and Network analyses of swamp buffalo showed distinct variation with a bootstrap value of almost 100% and six nucleotide differences between these groups. Similar results were obtained by Mishra et al. (2015) in upper Assamese and Chilika populations of domestic buffalo, which show phenotypic similarities to swamp buffalo but are also genetically closer to wild-type buffalo in the region. Our multi-lineage MP and NJ trees and several previous studies (Tanaka et al. 1996; Lau et al. 1998; Zhang et al. 2011, 2016; Mishra et al. 2015) inferred the ancestral nature of Wild Water Buffalo including the remnant population in Nepal. In this context, to determine genetically pure wild individuals in KTWR detailed and advanced genetic research is necessary.

#### National capacity building and close collaborations for genetic translocation

Phylogenetic analysis of partial cytochrome b region revealed overlapping clusters of wild, feral and domestic buffalo types residing in KTWR. The findings of this study along with previous genetic studies on water buffalo populations distributed globally including Wild Buffaloes of KTWR suggest that, single marker or partially overlapping markers are not sufficient to show the level of admixture and introgression of domestic in wild stock at the local level. Genome level assessments of source population offers specific criteria and objective means for translocation of an appropriate

group of buffaloes. Over the past several years thousands of Single Nucleotide Polymorphisms (SNPs) have been identified in wild and farm animals (Ciani et al. 2014; Decker et al. 2014; Edea et al. 2014) including river and swamp buffalo (Imartino et al. 2017; Colli et al. 2018). Axiom® Buffalo Genotyping Array that includes about 90K SNP loci covering the water buffalo genome-wide was developed in collaboration with the International Buffalo Genome Consortium. This 90K “SNP-Chip” was tested in several river buffalo populations throughout its distribution and found to have about 70% high quality and polymorphic SNPs (Imartino et al. 2017). This chip provides tremendous opportunity for genome wide investigation of genetic diversity and population structure in wild and feral buffaloes, their genetic mapping and quantification of the level of domestic introgression into wild happening in different herds of KTWR. This ability will further help to select genetically pure wild individuals for a translocation. Few studies have proposed translocation protocols focusing on how many and what individuals should be selected representing different herds of putative wild stock or feral backcrossed (Heinen 2002; Heinen & Kandel 2006; Heinen & Paudel 2015) buffalo in KTWR. Before planning a translocation of buffaloes, the current protocol should be revised thoroughly to decide on the selection of an adequate number of appropriate individuals from each herd and for SNP genotyping throughout the whole genome of those selected individual buffaloes.

Sufficient genetic diversity of wild individuals or feral backcrossed as suggested by Heinen & Paudel (2015) should be represented from a source population of KTWR to the translocated population in the native area such as Chitwan, Bardiya or other appropriate sites (Heinen 2002) in Nepal. Founding genetic diversity of translocated population will be determined by the number of genetically variable wild individuals, the proportion of diverse pure stocks, those that contribute genetically to the next generation and number and frequency of polymorphic alleles that represent whole genomes of the source population. Translocated populations are mostly small in size therefore they are prone to loss of genetic diversity very rapidly through genetic drift (Frankham et al. 2012). Genetic assessment of source populations in advance of translocation (pre-translocation) helps to guide translocation plans and inform post translocation assessment or monitoring of genetic diversity in the founders (Groombridge et al. 2012). In addition to geneticists, active involvement of conservation biologists, wildlife experts, wildlife veterinarians, ecologists, physiologists and local people

in the translocation program can ensure longer-term welfare and wellbeing of the re-introduced population.

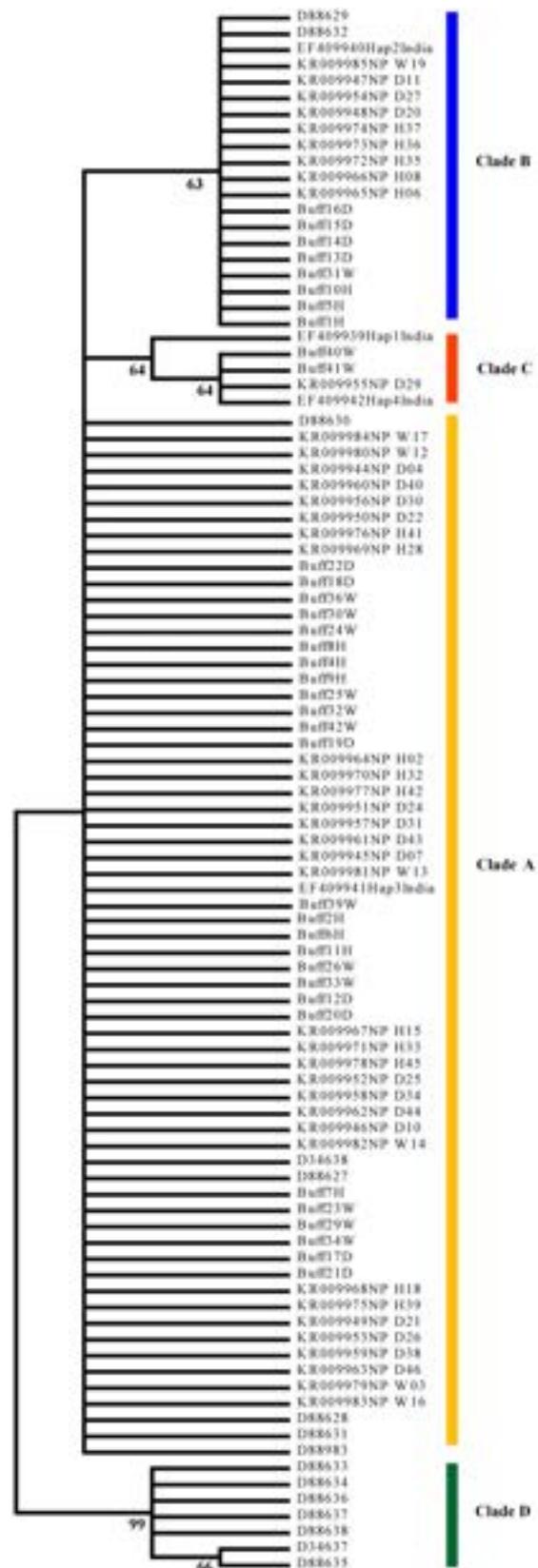
Before embarking on a genetic translocation program for the buffalo of KTWR, Nepal should upscale its laboratory facilities, design population-based advanced genetic research and take the initiative to build a DNA bank of all possible individuals counted in 2016. The DNA bank, reference DNA sequences and genotype database are crucial for research and conservation efforts, to enhance our understanding of genetic effects of introgression, the study of dietary patterns on different buffalo types and assess the status of pathogens affecting the buffaloes with different genetic backgrounds. Using the same blood samples collected during this study we have reported the prevalence of malaria parasites for the first time in buffaloes of KTWR (Kandel et al. 2019). Given the lack of highly technical laboratories and trained manpower in Nepal and the urgency to identify wild individuals reliably, collaborative research with international universities, research institutes and conservation partners on advanced molecular studies are to be jointly conducted. In conclusion this research sets a baseline to develop well defined action-oriented strategies that guide pre-translocation genetic study of wild buffaloes in KTWR and their monitoring through post-translocation genetic studies. Key actions highlighted in this paper such as collaboration between partners, establishment of DNA bank of all extant individuals in KTWR, involve experts from different disciplines, upscale and strengthen present laboratories and build capacity of available human resources for genomic level data management are important steps to be taken by the Ministry of Forest and Environment, Department of National Parks and Wildlife Conservation and its national and international conservation partners for genetic translocation of Wild Buffalo including other threatened species of Nepal.

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Appendix 1. Neighbour-joining tree for 97 partial Cytochrome *b* sequences of river and swamp Water Buffalo. Numbers on the nodes are percentage bootstrap values from 10000 replications. Color of the clade correspond to different haplotypes as depicted in Figure 2.

**Nepali abstract:** संकटापन्न सूचीमा रहेको जंगली भैसी "अर्ना" नेपालमा कोशीटप्पु वन्यजन्तु आरक्षमा पाइन्छ । मनसुत्का समयमा कोशी नदीमा आउने बाढी र अनेक प्राकृतिक रोग तथा विपदाका कारण अर्नाहरू उच्च जोखिममा रहेका छन् । बढ्दो जनसंख्या र जलवायु परिवर्तनका कारण हुन सक्ने अनेकौं जोखिमहरूको दिगो व्यवस्थापन र घरपालुवा भैसीको उत्पादकत्व बढाउनका लागि संरक्षित अर्नामा रहेको जंगली अनुवंशिक गुणहरूको संरक्षण हुनु आवश्यक छ । यसैसँगै नेपाल सरकार वन तथा यातायात मन्त्रालय, राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभागले कोशीटप्पुका केहि अर्नाहरूलाई नेपालमा बिरलमा अर्ना पाइने क्षेत्र मध्येको पिलठान राष्ट्रिय निकुञ्ज क्षेत्रमा स्थानान्तरण गरेको थियो । यसरी अर्नाहरूलाई स्थानान्तरण गर्दा संरक्षित अर्नाहरू मध्येबाट जंगली आनुवंशिक गुण र विलोपताहरू बोकेको शुद्ध जंगली अर्नाका माछ र पाहापाडीहरू छनैट प्नु भने धुनीतीपूर्ण हुदै आएको छ । कोशीटप्पु आरक्षको वरपर घना मानव वस्तीहरू रहेकाले संरक्षित अर्ना र घरपालुवा भैसी बिच यदाकदा हुने सम्बन्धले अर्नाको जंगली अनुवंशमा विस्तारै क्षयिकरण भईरहेको छ । अर्नाहरूको बाहिरी स्वरूपबाट आनुवंशिक रूपमा शुद्ध जंगली माछ र पाहापाडीहरू फला लगाउन संभव नभएकोले अर्नाको स्थानान्तरणका समयमा ३६ वटा भैसीबाट संकलन गरिएको रगत र गोबरका नमूनाहरूको डिएनएका साथै विगतमा नेपालबाटै गरिएका अनुसन्धान लगायत भारत, श्रीलंका, बंगलादेश, पाकिस्तान र इटालीका नदी र टलटल दुबै प्रजातिका १७ वटा भैसीको अनुवंश (४२२ बि.पि, साइटोक्रोम बि., डिएनए) को संयुक्त रूपमा विश्लेषण गरिएको थियो । यसरी विश्लेषण गर्दा सहज रूपमा नदी र टलटलीय प्रजातिका भैसीहरूको माइटोकण्ड्रियल डिएनए मा प्रस्ट भिन्नता पाइयो तर शुद्ध अर्ना, घरपालुवा र पि दुबैको सम्बन्ध बाट जन्मिएका मिश्रित नरहरू छुट्याउन सकिएन । डिएनएको विश्लेषणमा १७.२१७% अनुवंशिक विविधता आरक्षका बदान भित्रै रहेको र बाँकी २.७८२% आनुवंशिक विविधता फनक-फनक बदानहरू बिच रहेको पाइएको छ । यस आँकडाले कोशीटप्पुका संरक्षित अर्ना र आरक्ष भित्र छोडिएका केहि घरपालुवा भैसीका बीच विगत ४० वर्ष भन्दा बढी समय देखि लगभग ६-७ पुस्तासम्म दोहोरो समागम भई पाहापाडीहरू जन्मिरहेको क्षय पुष्टि गरिएता पनि कोशीटप्पुमा संरक्षित केहि जंगली अर्नाहरू घरपालुवा नदी प्रजातिका भैसीहरूको पूर्वज हुन सक्ने देखाएको छ । अनुसन्धानमा आधारित यस लेखमा कोशीटप्पुमा संरक्षित अर्नाको दिगो संरक्षण तथा व्यवस्थापनको सुनिश्चितताका लागि आनुवंशिक रूपमा शुद्ध अर्नाको पहिचान स्थापित गर्न सक्ने राष्ट्रिय प्रयोक्तालाई उपलब्ध पूर्वाधार र आवश्यक दक्षताको बृत्त्याङ्कन गरिएको छ । साथै यस अध्ययनमा विभिन्न राष्ट्रिय तथा अन्तर्राष्ट्रिय अनुसन्धान संस्था र जैविक विविधता संरक्षणका सरोकारवाला सरकारी तथा गैर सरकारी साझेदारी निकायहरू बिच समन्वय र सहकार्य हुनु पर्ने विषयलाई जोड दिई अर्नाहरूमा थपिस्वमा गरिनु पर्ने अझ विस्तृत र परीणामयुक्ती आनुवंशिक अनुसन्धानका लागि अर्नाको डिएनए बैंक र डिजिटल सिक्वेन्स डाटाबेस विकास गरिएको कुरा उल्लेख गरिएको छ ।

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**Author contribution:** RCK, RCP, MKC and GSS conceived the research idea. RCK, AS, PK, CPP and SBB collected field samples. RCP and AS performed DNA extraction, PCR, Sequencing and data analyses. All the authors contributed equally in preparing the manuscript.

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## A REVIEW ON STATUS OF MAMMALS IN MEGHALAYA, INDIA

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**Abstract:** In this paper we present an updated checklist of mammals found in Meghalaya. Using online databases and search engines for available literature, we provide the scientific names, accepted English names, conservation status as per IUCN Red List, Indian Wildlife (Protection) Act schedules, appendices in CITES, local distribution status, endemism, last reported sighting, an account of previous studies carried out relative to mammals and a tentative bibliography of the mammalian species found in Meghalaya. A total of 162 species were found to be existing in the state with Chiropterans forming the largest group and 27 species found to be threatened, seven Near Threatened and seven Data Deficient.

**Keywords:** Checklist, CITES, Indian Wildlife (Protection) Act, IUCN, mammalian species, northeastern India.

**Abbreviation:** BBL—Balpakram Baghmara Landscape; CEPF—Critical Ecosystem Partnership Fund; CITES—Convention on International Trade in Endangered Species of Wild Fauna and Flora (Appendix I, II and III); EGH—East Garo Hills; EJH—East Jaintia Hills; EKH—East Khasi Hills; GBIF—Global Biodiversity Information Facility; GH—Garo Hills; ISFR—Indian State Forest Report; IUCN—International Union for Conservation of Nature; IUCN Red List of Threatened Species: Categories (CR—Critically Endangered; EN—Endangered; VU—Vulnerable; NT—Near Threatened; LC—Least Concern; NA—Not Assessed); IWPA—Indian Wildlife (Protection) Act, 1972 (Schedule I, II, III, IV, and V); JH—Jaintia Hills; KH—Khasi Hills; MBSAP—The Meghalaya State Biodiversity Strategy and Action Plan; NP—National Park; RB—Ri-Bhoi; RF—Reserved Forest; SGH—South Garo Hills; WGH—West Garo Hills; WJH—West Jaintia Hills; WKH—West Khasi Hills; WS—Wildlife Sanctuary; WWF—World Wide Fund for Nature; ZSI—Zoological Survey of India.

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## INTRODUCTION

The global mammalian fauna is represented by 6,495 species, of which 6,399 are extant and 96 are extinct (Mammal Diversity Database 2019). India has a total of 423 species, which accounts for 7.81% of the global mammalian species (Sharma et al. 2015). A majority of these species are distributed in the four biodiversity hotspots of India—the Western Ghats and Sri Lanka, the Himalaya, Indo-Burma, and Sundalands biodiversity hotspots (Myers et al. 2000; CEPF 2016). The state of Meghalaya (25–26°N, 89.5–93°E; Figure 1) is part of the Indo-Burma biodiversity hotspot along with other parts of northeastern India south of the Brahmaputra River. This hotspot also includes parts of Bangladesh, Myanmar, Thailand, Vietnam, Cambodia, Laos PDR, southern China and touches a small portion of peninsular Malaysia and extends over two million square kilometers (Myers et al. 2000; Mittermeier et al. 2004). The Indo-Burma biodiversity hotspot has been considered to be one of the most important regions in the world and is ranked among the top 10 hotspots for irreplaceability due to high species diversity and endemism (Myers et al. 2000; Mittermeier et al. 2004). Its forests, freshwater and coastal ecosystems not only support many globally threatened species but also the region's human population. It houses about 430 mammalian species of which 73 species are endemic to the hotspot. Its natural habitat has been reduced to about 5% of its original extent due to human activities such as shifting cultivation, conversion to farmland, plantations, logging, deliberate forest fires, mining, damming and poaching (Tordoff et al. 2012). This has earned the hotspot a rank in the top five for most threatened (Mittermeier et al. 2004; Tordoff et al. 2012).

Meghalaya, nestled in the northeastern India biogeographic zone is a conflux of the Indo-Malayan and Indo-Chinese biogeographic realms (Palni et al. 2011) and as such is considered one of the richest habitats of Asia with a high diversity of mammals, birds, and plants (Rodgers & Panwar 1988, as cited in WWF 2019). Its diverse landscapes with gentle slopes in the north, steep slopes in the south forming deep valleys and a central plateau (Mani 1974; MBSAP 2017) has resulted in a diversification of its forests and wildlife. About 76.45% of its total geographic area is under forest cover (ISFR 2017) and is composed of tropical evergreen forests, tropical semi-evergreen forests, tropical moist and deciduous forests, grasslands and savanna, temperate forests and subtropical pine forests (Haridasan & Rao 1985). Meghalaya is also a part of the Meghalaya

subtropical forests ecoregion and has been described as the gateway to the Malayan fauna and as such houses closely related species with different distributional ranges such as the Indian and Chinese pangolins (WWF 2019). In recognition of its important position of harbouring diverse mammalian species, we attempt to update the list of mammalian species found in the region.

One of the earliest accounts of mammals in the state of Meghalaya dates back to the 19<sup>th</sup> century surveys that covered British India and other neighbouring regions (Harlan & Burrough 1834; McClelland 1841; Blyth 1852; Dobson 1874; Jerdon 1874; Sterndale 1884; Blanford 1888–91). Between 1847 and 1875, numerous collectors had also visited the region and documented the mammals found in the state (Alfred 1995). During the early decades of the 20<sup>th</sup> century, many mammalian specimens were collected from various parts of the state and a description of some of the species was done (Allen 1906; Kemp 1924; Hinton & Lindsay 1926). From the second half of the century, the Zoological Survey of India (ZSI) has also published numerous works on the fauna of the state (Alfred 1995). Apart from the work done by ZSI, other authors have also compiled lists of mammals found in Meghalaya as part of a wider effort to document mammals of the entire Indian subcontinent (Pocock 1939, 1941; Ellerman & Morrison-Scott 1951; Ellerman 1961; Prater 1965; Corbett & Hill 1992).

Between 1989–94, ZSI conducted a systematic survey of all fauna in the state including mammals through specimens available in its repository and secondary literature, and published a checklist of mammals (Das et al. 1995). A total of 139 mammal species were reported, representing 83 genera and 27 families in the state. This list, however, had also erroneously cited species that were not historically found in the region. Since then, many mammalian species have been discovered and an updated checklist is warranted. Recently, Choudhury (2013) compiled a comprehensive checklist and systematic review of all mammals found in northeastern India including Meghalaya based on primary as well as secondary sources, which serves as an important source of information for the mammals of northeastern India. Kakati and Kabra (2015) reported 51 mammalian species in Balpakram-Baghmara Landscape, Garo Hills, while Goswami (2015) documented 20 mammalian species in Jaintia Hills. Meghalaya's extensive karst topography provided the ideal settings for the diversification of bats with a tentative list of about 65 bat species having been reported from the state (Saikia et al. 2018). Considering all the new additions to the state in recent years

by surveys and records from all available literature, we provide an updated checklist of mammals for Meghalaya along with their distribution, conservation and management status.

## METHODS

We collected all published and gray literature available about the mammals of Meghalaya and thoroughly reviewed them to prepare a comprehensive list of mammals that have been reported to occur in Meghalaya. Online databases, web portals, websites and sites such as Google Scholar, ResearchGate, Biodiversity Heritage Library, Shodhganga, GBIF, and IUCN Red List were accessed for collecting the literature. Technical Reports and unpublished literature were also collected from the authors through personal communication. Conservation status as per IUCN Red List, schedule

category in Indian Wildlife (Protection) Act 1972 (IWPA), Appendices in Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and last reported sighting in the state were compiled to highlight the importance of the landscape for mammal conservation (Image 1). The taxonomic arrangement of the species follows Wilson & Reeder (2005) and Wilson & Mittermeier (2009).

## RESULTS AND DISCUSSION

A total of 162 species of mammals belonging to 31 families were reported from the state of Meghalaya (Table 1; Figure 2). Chiropterans formed the largest group of mammals with 65 species (40%). This was followed by rodents with 35 (22%) and carnivores with 34 species (22%). The rest of the groups constituted less than 20% of the total mammal diversity in the state

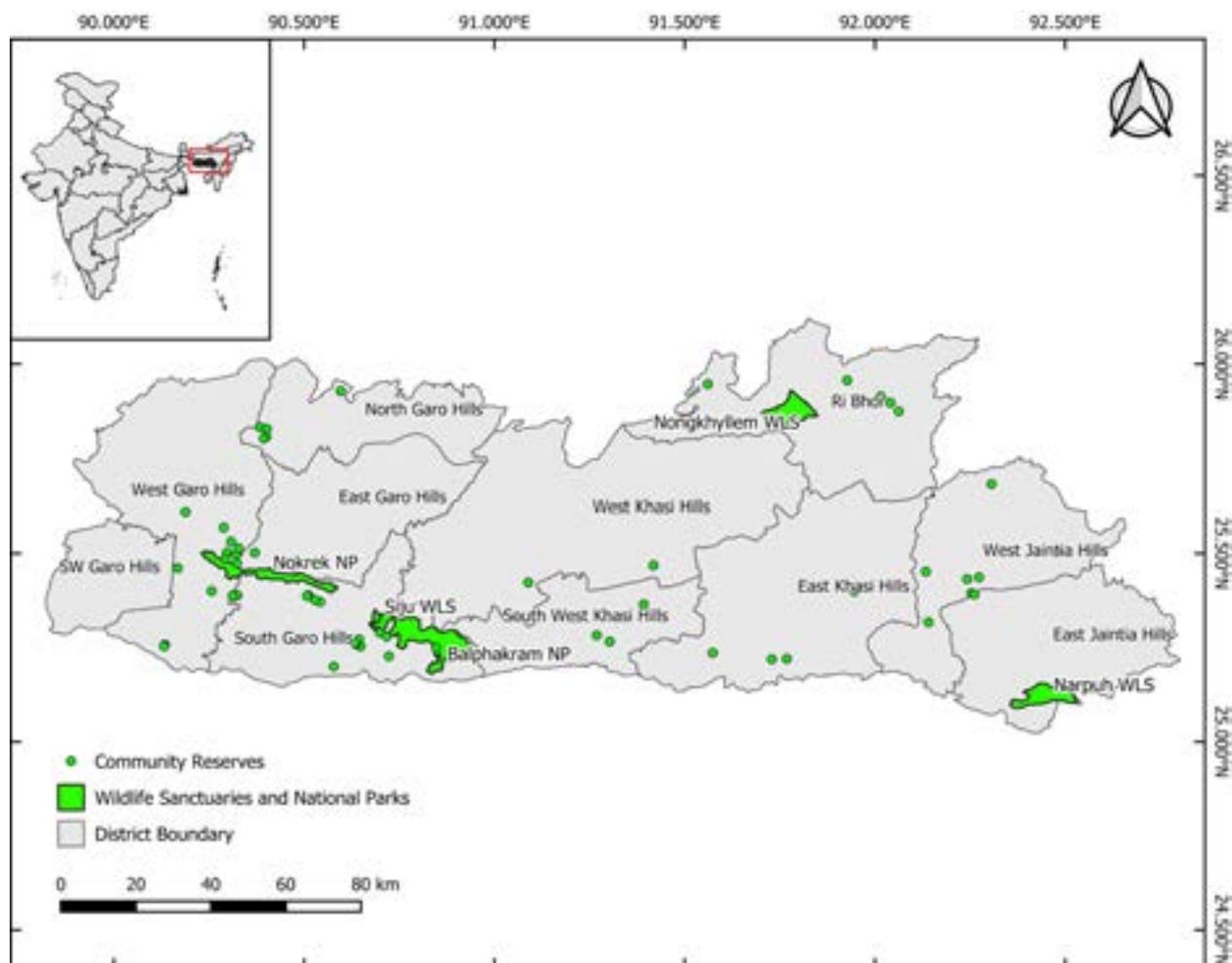


Figure 1. The state Meghalaya with protected areas.

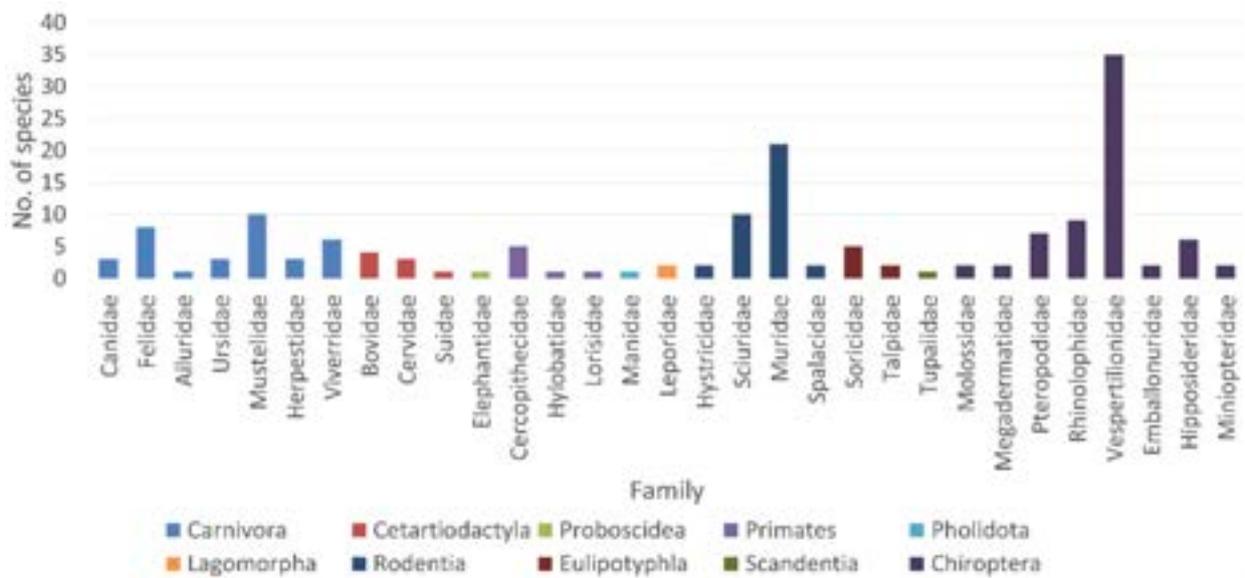


Figure 2. Mammal species richness in different families in Meghalaya.

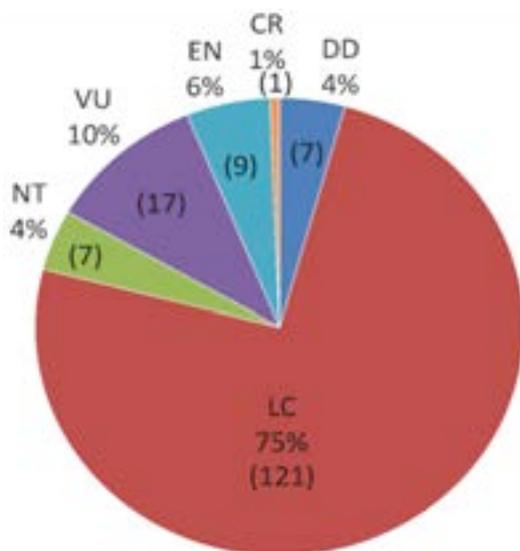


Figure 3. Percent mammals in IUCN Red List category.

(Figure 3). Evening bats formed the largest group at 35 species, followed by murids at 21, sciurid and mustelids at 10 each. Among the 162 species, 27 are threatened (one Critically Endangered, nine Endangered, 17 Vulnerable) and seven species each as Near Threatened and Data Deficient (Figure 2, Table 2). None of the species, however, are endemic to Meghalaya.

#### Mammals excluded from the Meghalaya list

A few species have been excluded from this list because they could be locally extinct, erroneously mentioned in the literature or have not been formally

recognized as a separate species. A description of their past and current distribution in northeastern India is also summarized here in support of their exclusion from this list.

**Barasingha** *Rucervus duvaucelii* was stated to be present in East Khasi Hills of Meghalaya (Das et al. 1995) although no record of its existence is available except for a hunting report in 1894 on three Barasinghas shot on the border of East Garo Hills of Meghalaya and Goalpara District of Assam (Choudhury 2001; Choudhury 2013). In northeastern India, the species' past range extended along the foothills of the Himalaya from upper Assam and were common in the Brahmaputra Valley with many having still existed in a few districts of Assam till as late as 1934 (Blanford 1888–91; Lydekker 1915; Bhadian 1934; Schaller 1967). Since then, however, their numbers have drastically dwindled and their current distribution in northeastern India is now restricted to only a few pockets of Assam in Kaziranga NP and Manas NP (Schaller 1967; Choudhury 2001a; Srinivasulu et al. 2012; IUCN 2019).

**Chital** *Axis axis* was stated to be present in Ri-Bhoi and East Khasi Hills districts of Meghalaya (Das et al. 1995). It is, however, unlikely to have occurred as there is no historical record of the species occurring south of the Brahmaputra River (Choudhury 2001a). In northeastern India, Chital is restricted to the western part of Assam, north of the Brahmaputra River (Choudhury 2001a; IUCN 2019) with its most recent record at Manas NP (Bhatt et al. 2018). Its easternmost record was at Dhunsiri River in Darrang District reported in 1935 by A.J.W. Milroy (De

1935).

**Chousingha** *Tetracerus quadricornis* was reported to be present in West Garo Hills although it could be a misidentified *Naemorhedus goral* (Das et al. 1995). This species is endemic to peninsular India and parts of lowland Nepal (Leslie et al. 2009; Srinivasulu et al. 2012; IUCN 2019).

**Himalayan Serow** *Capricornis thar* in this paper is reported as *Capricornis rubidus*. Due to the lack of information on the taxonomic status of the *Capricornis* sp. in northeastern India, some reports have considered them as *C. thar* (Srinivasulu et al. 2012; IUCN 2019) although others have reported it as *C. rubidus* (Choudhury 2013; Kakati et al. 2015).

**Indian Crested Porcupine** *Hystrix indica* was reported from Meghalaya (Goswami 2015); however, this species is not distributed in northeastern India (IUCN 2019).

**Dormer's Bat** *Scotozous dormeri* was reported by Sinha (1995) based on a damaged male specimen from Shillong but examination of its teeth and external characteristics suggested it to be of another species, *Hypsugo cadornae* (Saikia et al. 2018).

**Dusky Leaf-nosed Bat** *Hipposideros ater* reported in Meghalaya from a single record that is doubtful, and is excluded from this list (Kurup 1968; Choudhury 2013; Saikia et al. 2018).

A new species of bat, *Hipposideros khasiana* was reported in Meghalaya based on the differences in call frequencies of the bats from their closely related species *H. larvatus* (Thabah et al. 2006). Due to the lack of type material, however, this species has not yet been formally recognized as a distinct species from *H. larvatus* (Saikia 2018; Saikia et al. 2018).

Specimens collected from Siju Cave in Meghalaya and identified as the Eastern Bent-winged Bat (*Miniopterus fuliginosus*) (Sinha 1999) were found to be that of *M. magnater* (Saikia et al. 2018). *M. fuliginosus* although likely to be found in the state is yet to be formally identified (Saikia et al. 2018).

#### A note on natural history of other species

**Elephants:** Numerous studies on Asian Elephants *Elephas maximus* have been carried out in Meghalaya (Johnsingh 1996a,b; Choudhury 1999, 2004, 2007; Johnsingh & Williams 1999; Williams & Johnsingh 2004; Datta-Roy et al. 2008, 2009; Marak 2009; Kaul et al. 2010; Marcot et al. 2011; Goswami et al. 2014). Meghalaya is known to have one of the largest and densest populations of Elephants in India with a population of 1,811 as per 2008 records (Datta-Roy et al. 2008; Marcot 2011). The State Forest Department conducted the first

Elephant census in and around Balpakram National Park (NP) in 1981. The number of Elephants was estimated to be 2,333 (Gogoi & Choudhury, as cited in Williams & Johnsingh 1996a). The first state-wide Elephant census was conducted in 1993 which estimated Elephants to be numbering 1,850 (William & Johnsingh 1996a; Choudhury 1999). The number increased slightly to 1,868 Elephants in 2002 (Marcot 2011). Fourteen distinct populations of Elephants were identified throughout northeastern India (Choudhury 1999). Two of the populations are shared between Assam and Meghalaya. These two populations form a sizeable portion of the estimated 11,000 Elephants found in northeastern India (> 50%). One large population was found in Ri-Bhoi and Jaintia Hills districts and another in West Khasi Hills, Ri-Bhoi, and Garo Hills districts. Populations in western Khasi Hills are around more than 800 and in Garo Hills around 1,800, with the latter having a density of 0.74 km<sup>-2</sup>. A smaller population of Elephants exist in some parts of Jaintia Hills (Choudhury 1999). Areas in and around Nokrek NP and Balpakram-Baghmara Landscape in Garo Hills have been reported to have the highest potential for long term conservation of Elephants in the region (William & Johnsingh 1996a) and as such warrant greater conservation efforts. Three critical Elephant corridors were identified in Garo Hills, one of them faced tremendous anthropogenic pressure and another one was at risk of being overexploited for timber and coal as most of the corridor is private or community owned land and does not come under the control of the Forest Department (William & Johnsingh 1996b). In 2003, six corridors (five in Garo Hills and one in Jaintia Hills) were identified by the Wildlife Trust of India for the long-term conservation of Elephants in the state (Kaul et al. 2010).

Human-Elephant interaction is intense in some parts of Garo Hills. This is mainly attributed to disturbances caused by 'jhum' (slash and burn regime) and coal mining (William & Johnsingh 1996a). Retaliatory killing due to crops being raided and poaching of Elephants has also increased over the years (Johnsingh & William 2004). The presence of Elephants in human-occupied areas and intensity of conflicts with humans varies highly across the landscape. Various factors such as sowing and harvesting season of crops and availability of wild forage had an influence on the Elephant visits (Datta-Roy et al. 2008, 2009); however, in most cases, these Elephant visits were not negative in nature. Elephants have also been observed to use sites close to PAs more intensely than sites away from the PAs. The presence of humans further augmented this trend (Goswami et al. 2014). A few Elephants (40–50) from South Garo

Table 1. Checklist of mammals found in Meghalaya, India.

	Taxa	Common name	IUCN Red List status	Wildlife Protection Act, 1972 schedule/status	CITES	Distribution	Source
	<b>Order Proboscidea: Elephants</b>						
	<b>Family Elephantidae: Elephants</b>						
1	<i>Elephas maximus</i>	Asian Elephant	EN	Sch I (Part I)	I	WGH, EGH, SGH, RB, WKH, JH	1,2,3,4,5
	<b>Order Scandentia: Tree Shrews</b>						
	<b>Family Tupaiidae: Tree Shrew</b>						
2	<i>Tupaia belangeri</i>	Common Tree Shrew	LC	Sch II (Part I)	II	WGH, EGH, EKH	1,2,3,4
	<b>Order Primates: Primates</b>						
	<b>Family Lorisidae: Lorises</b>						
3	<i>Nycticebus bengalensis</i>	Bengal Slow Loris	VU	Sch I (Part I)	I	WGH, EGH, EKH	1,2,3,4
	<b>Family Cercopithecidae: Old World Monkeys</b>						
4	<i>Macaca arctoides</i>	Stump-tailed Macaque	VU	Sch II (Part I)	II	All districts	1,2,3,4
5	<i>Macaca assamensis</i>	Assamese Macaque	VU	Sch II (Part I)	II	All districts	1,2,3,4
6	<i>Macaca leonina</i>	Northern Pig-Tailed Macaque	VU	Sch II (Part I)	II	All districts	1,2,3,4
7	<i>Macaca mulatta</i>	Rhesus Macaque	LC	Sch II (Part I)	II	All districts	1,2,3,4,5
8	<i>Trachypithecus pileatus</i>	Capped Langur	VU	Sch I (Part I)	I	All districts	1,2,3,4,5
	<b>Family Hylobatidae: Gibbons</b>						
9	<i>Hoolock hoolock</i>	Western Hoolock Gibbon	EN	Sch I (Part I)	I	WGH, EGH, SGH, RB, WKH	1,2,3,4,5
	<b>Order Rodentia: Rodents</b>						
	<b>Family Muridae: Rats and Mice</b>						
10	<i>Bandicota bengalensis</i>	Lesser Bandicoot Rat	LC	Sch V	NA	EKH, JH, GH	1,2,3
11	<i>Bandicota indica</i>	Large Bandicoot Rat	LC	Sch V	NA	EKH, JH, GH	1,2,3
12	<i>Berylmys bowersi</i>	Bower's Rat	LC	Sch V	NA	Mawphlang	1,3,9
13	<i>Berylmys mackenziei</i>	Kenneth's White-toothed Rat	DD	Sch V	NA	Shillong	1,2,3
14	<i>Chiropodomys gliroides</i>	Pencil-tailed Tree Rat	LC	Sch V	NA	EKH, JH	1,2,3
15	<i>Leopoldamys edwardsi</i>	Long-tailed Giant Rat	LC	Sch V	NA	WGH, EGH, RB, JH	1,2,3
16	<i>Micromys minutus</i>	Harvest Mouse	LC	Sch V	NA	EKH	1,2,3
17	<i>Mus booduga</i>	Little Indian Field Mouse	LC	Sch V	NA	WGH, EGH, EKH, JH	1,2,3
18	<i>Mus cervicolor</i>	Fawn-coloured Mouse	LC	Sch V	NA	EKH, GH, JH	1,2,3
19	<i>Mus cookii</i>	Cooke's Mouse	LC	Sch V	NA	Khonshnon, Shangpung	1,3,9
20	<i>Mus musculus</i>	House Mouse	LC	Sch V	NA	All districts	1,2,3
21	<i>Mus pahari</i>	Sikkim Mouse	LC	Sch V	NA	WGH, EKH, JH	1,2,3
22	<i>Niviventer fulvescens</i>	Chestnut Rat	LC	Sch V	NA	WGH, EKH, RB, JH	1,2,3
23	<i>Niviventer confucianus</i>	Chinese White-bellied Rat	LC	Sch V	NA	WGH, EKH, RB, JH	3
24	<i>Niviventer niviventer</i>	White-bellied Rat	LC	Sch IV	NA	EKH, RB, JH	1,2,3,4
25	<i>Rattus andamanensis</i>	Indo-Chinese Forest Rat	LC	Sch V	NA	WGH, EKH, RB, JH	1,3,9
26	<i>Rattus nitidus</i>	White-footed Himalayan Rat	LC	Sch V	NA	WGH, EGH, SGH, EKH, RB, JB	1,2,3
27	<i>Rattus rattus</i>	House Rat	LC	Sch V	NA	All districts	1,2,3
28	<i>Rattus norvegicus</i>	Brown Rat	LC	Sch V	NA	EKH	2,3
29	<i>Rattus tanezumi</i>	Oriental House Rat	LC	Sch V	NA	All districts	1,3
30	<i>Vandeleuria oleracea</i>	Indian Long-tailed Tree Mouse	LC	Sch V	NA	EKH, JH	1,2,3

	Taxa	Common name	IUCN Red List status	Wildlife Protection Act, 1972 schedule/status	CITES	Distribution	Source
	<b>Family Sciuridae: Squirrels</b>						
31	<i>Ratufa bicolor</i>	Malayan Giant Squirrel	NT	Sch II (Part I)	II	All districts	1,2,3,4,5
32	<i>Belomys pearsonii</i>	Hairy-footed Flying Squirrel	DD	Sch II (Part I)	NA	GH	2,3
33	<i>Hylopetes alboniger</i>	Parti-coloured Flying Squirrel	LC	Sch II (Part I)	NA	EKH, JH	1,2,3
34	<i>Petaurista petaurista</i>	Red Giant Flying Squirrel	LC	Sch II (Part I)	NA	All districts	1,2,3
35	<i>Petaurista philippensis</i>	Indian Giant Flying Squirrel	LC	Sch II (Part I)	NA	GH, KH	1,2,3
36	<i>Callosciurus erythraeus</i>	Red-bellied Squirrel	LC	No mention of this in WPA	NA	All districts	2,3,4
37	<i>Callosciurus pygerythrus</i>	Hoary-bellied Squirrel	LC	Sch II (Part I)	NA	All districts	1,2,3,4,5
38	<i>Dremomys lokriah</i>	Orange-bellied Himalayan Squirrel	LC	Sch II (Part I)	NA	All districts	1,2,3
39	<i>Funambulus pennanti</i>	Himalayan Five-striped Palm Squirrel	LC	Sch IV	NA	KH, JH	2,3
40	<i>Tamiops macclellandii</i>	Himalayan Striped Squirrel	LC	No mention of this in WPA	NA	EKH	1,2,3,4
	<b>Family Spalacidae: Bomboo Rats</b>						
41	<i>Cannomys badius</i>	Bay Bamboo Rat	LC	Sch V	NA	EGH, EKH, JH	1,2,3
42	<i>Rhizomys pruinosus</i>	Hoary Bamboo Rat	LC	Sch V	NA	All districts	1,2,3
	<b>Family Hystricidae: Old-World Porcupines</b>						
43	<i>Atherurus macrourus</i>	Asiatic Brush-Tailed Porcupine	LC	Sch II (Part I)	NA	All districts	1,2,3,4
44	<i>Hystrix brachyura</i>	Himalayan Crestless Porcupine	LC	Sch II (Part I)	NA	All districts	1,2,3,4,5
	<b>Order Lagomorpha: Hares and Rabbits</b>						
	<b>Family Leporidae: Hares</b>						
45	<i>Caprolagus hispidus</i>	Hispid Hare	EN	Sch I (Part I)	I	Balpakram NP and Chenggni border SGH	3
46	<i>Lepus nigricollis</i>	Indian Hare	LC	Sch IV	NA	All districts	1,2,3,4
	<b>Order Eulipotyphla: Moles and Shrews</b>						
	<b>Family Soricidae: Shrews</b>						
47	<i>Crociodura fuliginosa</i>	Southeast Asian Shrew	LC	NA	NA	WGH, EKH	2,3
48	<i>Crociodura attenuata</i>	Asian Grey Shrew	LC	NA	NA	WGH, EKH, JH	1,2,3
49	<i>Suncus etruscus nudipes</i>	Pygmy White-toothed Shrew	LC	NA	NA	EKH, JH	1,2,3
50	<i>Suncus murinus</i>	Asian House Shrew	LC	NA	NA	EKH, WGH, JH	1,2,3
51	<i>Anourosorex assamensis</i>	Assam Mole Shrew	LC	NA	NA	EKH, JH	1,2,3
	<b>Family Talpidae: Moles</b>						
52	<i>Euroscaptor micrura</i>	Indian Short-tailed Mole	LC	NA	NA	EKH, JH, GH	1,2,3
53	<i>Parascaptor leucura</i>	White-tailed Mole	LC	NA	NA	EKH, JH	1,2,3
	<b>Order Chiroptera: Bats</b>						
	<b>Family Miniopteridae: Long-fingered Bats</b>						
54	<i>Miniopterus pusillus</i>	Lesser Bent-winged Bat	LC	No mention of this in WPA	NA	Umlingsha, EKH	7
55	<i>Miniopterus magnater</i>	Large Bent-winged Bat	LC	No mention of this in WPA	NA	SGH, EKH, EKH, WKH, RB.	7
	<b>Family Pteropodidae: Old World Fruit Bats</b>						
56	<i>Cynopterus brachyotis</i>	Lesser Short-nosed Fruit Bat	LC	Sch V	NA	EKH, WGH	1,3,7
57	<i>Cynopterus sphinx</i>	Greater Short-nosed Fruit Bat	LC	Sch V	NA	WGH, EKH, SGH, KH, EKH	1,2,3,7
58	<i>Eonycteris spelaea</i>	Lesser Dawn Bat	LC	Sch IV	NA	SGH, EKH, JH	1,2,3,7
59	<i>Macroglossus sobrinus</i>	Hill Long-tongued Fruit Bat	LC	Sch IV	NA	EKH, JH	1,2,3,7

	Taxa	Common name	IUCN Red List status	Wildlife Protection Act, 1972 schedule/status	CITES	Distribution	Source
60	<i>Megaerops niphanae</i>	Northern Tailless Fruit Bat	LC	Sch V	NA	WKH, EJH	1,2,3,7
61	<i>Pteropus giganteus</i>	Indian Flying Fox	LC	Sch V	II	EKH, RB, WGH	1,2,3,4,7
62	<i>Rousettus leschenaultii</i>	Leschenault's Rousette	LC	No mention of this in WPA	NA	WGH, EGH, SGH, EKH, JH	1,2,3,7
<b>Family Rhinolophidae: Horseshoe Bats</b>							
63	<i>Rhinolophus affinis</i>	Intermediate Horseshoe Bat	LC	NA	NA	EKH, JH	1,2,3,7
64	<i>Rhinolophus lepidus</i>	Blyth's Horseshoe Bat	LC	NA	NA	KH, JH, SGH	1,2,3,7
65	<i>Rhinolophus luctus</i>	Great Woolly Horseshoe Bat	LC	NA	NA	KH, JH	1,2,3,7
66	<i>Rhinolophus macrotis</i>	Big-eared Horseshoe Bat	LC	NA	NA	KH, EJH	1,3,7
67	<i>Rhinolophus pearsonii</i>	Pearson's Horseshoe Bat	LC	NA	NA	KH, JH, GH	1,2,3,7
68	<i>Rhinolophus pusillus</i>	Least Horseshoe Bat	LC	NA	NA	EKH, SGH, WGH, JH	1,2,3,7
69	<i>Rhinolophus siamensis</i>	Thai Horseshoe Bat	LC	NA	NA	EJH	7
70	<i>Rhinolophus sinicus</i>	Chinese Horseshoe Bat	LC	NA	NA	EKH, EJH	7
71	<i>Rhinolophus subbadius</i>	Little Nepalese Horseshoe Bat	LC	NA	NA	GH, EKH	1,2,3,7
<b>Family Hipposideridae: Old-World Leaf-Nosed Bats</b>							
72	<i>Coelops frithii</i>	Tailless Leaf-nosed Bat	LC	NA	NA	KH	1,2,3,7
73	<i>Hipposideros armiger</i>	Great Himalayan Leaf-nosed Bat	LC	NA	NA	KH, JH, GH	1,2,3,7
74	<i>Hipposideros cineraceus</i>	Least Leaf-nosed Bat	LC	NA	NA	EKH, JH, RB	1,2,3,7
75	<i>Hipposideros larvatus</i>	Horsfield's Leaf-nosed Bat	LC	NA	NA	WGH, KH, EJH, RB	1,2,3,7
76	<i>Hipposideros pomona</i>	Anderson's Leaf-nosed Bat	LC	NA	NA	SGH, EKH, RB, EJH	1,2,3,7
77	<i>Hipposideros lankadiva</i>	Indian Leaf-nosed Bat	LC	NA	NA	EKH, RB, EJH, SGH	2,3,7
<b>Family Megadermatidae: False Vampire Bats</b>							
78	<i>Megaderma lyra</i>	Greater False Vampire	LC	NA	NA	WGH, RB, EKH, EJH	1,2,3,7
79	<i>Megaderma spasma</i>	Lesser False Vampire	LC	NA	NA	BBL, RB, EKH	1,3,4,7
<b>Family Emballonuridae: Sheath-tail Bats</b>							
80	<i>Saccolaimus saccolaimus</i>	Bare-rumped Sheath-tail Bat	LC	NA	NA	Phulbari, WGH	1,3,7
81	<i>Taphozous melanopogon</i>	Black-bearded Tomb Bat	LC	NA	NA	WKH	1,7
<b>Family Molossididae: Free-Tailed Bats</b>							
82	<i>Chaerephon plicatus</i>	Wrinkle-lipped Free-tailed Bat	LC	NA	NA	EKH, WGH	1,2,3,7
83	<i>Otomops wroughtoni</i>	Wroughton's Free-tailed Bat	DD	Sch I (Part I)	NA	EKH, JH	1,3,7
<b>Family Vespertilionidae: Evening Bats</b>							
84	<i>Arielulus circumdatus</i>	Black-gilded Pipistrelle	LC	NA	NA	EKH	1,2,3,7
85	<i>Eptesicus pachyotis</i>	Thick-eared Bat	LC	NA	NA	KH	1,2,3,7
86	<i>Scotomanes ornatus</i>	Harlequin Bat	LC	NA	NA	EGH, EKH, JH	1,2,3,7
87	<i>Scotophilus heathii</i>	Asiatic Greater Yellow House Bat	LC	NA	NA	WGH, EKH	1,2,3,7
88	<i>Scotophilus kuhlii</i>	Lesser Asiatic Yellow House Bat	LC	NA	NA	GH, EKH	1,2,3,7
89	<i>Pipistrellus ceylonicus*</i>	Kelaart's Pipistrelle	LC	NA	NA	EJK	1,7
90	<i>Pipistrellus coromandra</i>	Indian Pipistrelle	LC	NA	NA	JH, EKH, RB, GH	1,2,3,7
91	<i>Pipistrellus javanicus</i>	Javan Pipistrelle	LC	NA	NA	KH	1,7
92	<i>Pipistrellus paterculus</i>	Mount Popa Pipistrelle	LC	NA	NA	EJH	1,7
93	<i>Pipistrellus mimus</i>	Least Pipistrelle	LC	NA	NA	WGH, EKH	1,2,3,7

	Taxa	Common name	IUCN Red List status	Wildlife Protection Act, 1972 schedule/status	CITES	Distribution	Source
94	<i>Pipistrellus kuhlii</i>	Kuhl's Pipistrelle	LC	NA	NA	EKH	3,7
95	<i>Barbastella leucomelas</i>	Eastern Barbastelle	LC	NA	NA	JH, KH	1,2,3,7
96	<i>Plecotus homochrous</i>	Long-eared Bat	LC	NA	NA	KH	1,2,7
97	<i>Hypsugo joffrei</i>	Joffre's Pipistrelle	DD	NA	NA	EKH	7
98	<i>Hypsugo savii</i>	Savi's Pipistrelle	LC	NA	NA	EKH	1,2,3,7
99	<i>la io</i>	Great Evening Bat	LC	NA	NA	KH, EJH	1,2,3,7
100	<i>Tylonycteris malayana</i>	Greater Bamboo Bat	LC	NA	NA	EJH	7
101	<i>Tylonycteris pachypus</i>	Lesser Bamboo Bat	LC	NA	NA	WGH, EKH	1,2,3,7
102	<i>Myotis altarium</i>	Szechwan Myotis	LC	NA	NA	EKH, EJH	7
103	<i>Myotis formosus</i>	Hodgson's Bat	LC	NA	NA	EKH	1,2,3,7
104	<i>Myotis horsfieldii</i>	Horsfield's Myotis	LC	NA	NA	JH	1,2,3,7
105	<i>Myotis laniger</i>	Chinese Water Myotis	LC	NA	NA	EKH	1,2,3,7
106	<i>Myotis longipes</i>	Kashmir Cave Bat	DD	NA	NA	SGH, EJH, EKH, WKH	1,2,3,7
107	<i>Myotis muricola</i>	Nepalese Whiskered Bat	LC	NA	NA	WJH, EKH	1,3,7
108	<i>Myotis pilosus</i>	Rickett's Big-Footed Myotis	NT	NA	NA	EKH	7
109	<i>Myotis siligorensis</i>	Himalayan Whiskered Bat	LC	NA	NA	JH, KH	1,2,3,7
110	<i>Harpiocephalus harpia</i>	Lesser Hairy-winged Bat	LC	NA	NA	EKH	1,2,3,7
111	<i>Murina aurata</i>	Little Tube-nosed Bat	LC	NA	NA	EKH	1,3,7
112	<i>Murina cyclotis</i>	Round-eared Tube-nosed Bat	LC	NA	NA	EKH, JH	1,2,3,7
113	<i>Murina huttoni</i>	Hutton's Tube-nosed Bat	LC	NA	NA	EKH, EJH	3,7
114	<i>Murina jaintiana</i>	Jaintia Tube-Nosed Bat	DD	NA	NA	EKH, EJH	7
115	<i>Murina pluvialis</i>	Rainy Forest Tube-nosed Bat	DD	NA	NA	EKH, EJH	7
116	<i>Murina tubinaris</i>	Scully's Tube-nosed Bat	LC	NA	NA	JH, EKH	1,2,3,7
117	<i>Kerivoula hardwickii</i>	Common Woolly Bat	LC	NA	NA	SGH, WJH	1,2,3,7
118	<i>Kerivoula kachinensis</i>	Kachin Woolly Bat	LC	NA	NA	EKH, EJH	7
	<b>Order Pholidota: Pangolins</b>						
	<b>Family Manidae: Pangolins</b>						
119	<i>Manis crassicaudata</i>	Indian Pangolin	EN	Sch I (Part I)	I	WGH, EGH, SGH	1, 2, 3
120	<i>Manis pentadactyla</i>	Chinese Pangolin	CR	Sch I (Part I)	I	SGH, EKH	1,2,3,5
	<b>Order Carnivora: Carnivores</b>						
	<b>Family Felidae: Cats</b>						
121	<i>Catopuma temminckii</i>	Asian Golden Cat	NT	Sch I (Part I)	I	EGH, SGH, JH	1,2,3,8
122	<i>Felis chaus</i>	Jungle Cat	LC	Sch II (Part I)	II	WGH, EGH, SGH	1,2,3,8
123	<i>Pardofelis marmorata</i>	Marbled Cat	NT	Sch I (Part I)	I	GH	1,2,3,4
124	<i>Prionailurus bengalensis</i>	Leopard Cat	LC	Sch I (Part I)	I/II	All districts	1,2,3,4
125	<i>Prionailurus viverrinus</i>	Fishing Cat	VU	Sch I (Part I)	II	All districts	1,3
126	<i>Neofelis nebulosa</i>	Clouded Leopard	VU	Sch I (Part I)	I	All districts	1,2,3,4
127	<i>Panthera pardus</i>	Leopard	VU	Sch I (Part I)	I	All districts	1,2,3,4,5
128	<i>Panthera tigris</i>	Tiger	EN	Sch I (Part I)	I	All districts	1,2,3,6

	Taxa	Common name	IUCN Red List status	Wildlife Protection Act, 1972 schedule/status	CITES	Distribution	Source
	<b>Family Viverridae: Civets</b>						
129	<i>Arctictis binturong</i>	Binturong	VU	Sch I (Part I)	III	All districts	1,2,3
130	<i>Arctogalidia trivirgata</i>	Small-toothed Palm Civet	LC	Sch II (Part I)	NA	BBL	1,3,4
131	<i>Paguma larvata</i>	Himalayan Palm Civet	LC	Sch II (Part I)	III	All districts	1,2,3,4
132	<i>Paradoxurus hermaphroditus</i>	Common Palm Civet	LC	Sch II (Part I)	III	All districts	1,2,3,4
133	<i>Viverra zibetha</i>	Large Indian Civet	LC	Sch II (Part I)	III	All districts	1,2,3,4
134	<i>Viverricula indica</i>	Small Indian Civet	LC	Sch II (Part I)	III	WGH	1,2,3,4
	<b>Family Herpestidae: Mongooses</b>						
135	<i>Herpestes javanicus</i>	Small Indian Mongoose	LC	Sch II (Part I)	III	All districts	1,2,3,4
136	<i>Herpestes edwardsii</i>	Indian Grey Mongoose	LC	Sch II (Part I)	III	WGH	1,2,3
137	<i>Herpestes urva</i>	Crab-eating Mongoose	LC	Sch II (Part I)	III	WGH, EGH	1,2,3,4,5
	<b>Family Canidae: Dogs and Foxes</b>						
138	<i>Canis aureus</i>	Golden Jackal	LC	Sch II (Part I)	III	WGH, EGH, SGH, JH	1,2,3,4
139	<i>Cuon alpinus</i>	Wild Dog	EN	Sch II (Part I)	II	All districts	1,2,3,4
140	<i>Vulpes bengalensis</i>	Indian Fox	LC	Sch II (Part I)	III	SGH	1,2,3
	<b>Family Ursidae: Bears</b>						
141	<i>Helarctos malayanus</i>	Malayan Sun Bear	VU	Sch I (Part I)	I	All districts	1,2,3
142	<i>Melursus ursinus</i>	Sloth Bear	VU	Sch II (Part I)	I	RB, EKH, GH	1,2,3
143	<i>Ursus thibetanus</i>	Asiatic Black Bear	VU	Sch II (Part I)	I	All districts	1,2,3,4
	<b>Family Mustelidae: Weasels, Badgers, and Otters</b>						
144	<i>Aonyx cinerea</i>	Asian Small-clawed Otter	VU	Sch I (Part I)	II	WGH, JH	1,2,3
145	<i>Lutra lutra</i>	Common Otter	NT	Sch II (Part I)	I	All districts	2,3
146	<i>Lutrogale perspicillata</i>	Smooth-coated Otter	VU	Sch II (Part I)	II	WGH	1,2,3,4
147	<i>Arctonyx albogularis</i>	Northern Hog Badger	LC	Sch I (Part I)	NA	All districts	1
148	<i>Arctonyx collaris</i>	Hog Badger	VU	Sch I (Part I)	NA	All districts	1,2,3,4
149	<i>Martes flavigula</i>	Yellow-throated Marten	LC	Sch II (Part I)	III	All districts	1,2,3,4
150	<i>Melogale moschata</i>	Small-toothed Ferret Badger	LC	Sch II (Part I)	NA	KH	1,2,3,4
151	<i>Melogale personata</i>	Burmese Ferret-badger	LC	Sch II (Part I)	NA	BBL, KH	1,2,3,4
152	<i>Mustela kathiah</i>	Yellow-bellied Weasel	LC	Sch II (Part I)	III	WKH, EKH	1,2,3
153	<i>Mustela strigidorsa</i>	Stripe-backed Weasel	LC	NA	NA	EKH, JH	1,3
	<b>Family Ailuridae: Red Panda</b>						
154	<i>Ailurus fulgens</i>	Red Panda	EN	Sch I (Part I)	I	WGH, EGH, SGH, WKH, EKH	1,2,3
	<b>Order Artiodactyla: Even-Toed Ungulates</b>						
	<b>Family Suidae: Pigs</b>						
155	<i>Sus scrofa</i>	Indian Wild Boar	LC	Sch III	NA	All districts	1,2,3,4,5
	<b>Family Cervidae: Deer</b>						
156	<i>Muntiacus vaginalis</i>	Indian Muntjac	LC	Sch III	NA	All districts	1,2,3,4,5
157	<i>Axis porcinus</i>	Hog Deer	EN	Sch III	I/III	RB, GH	2,3
158	<i>Rusa unicolor</i>	Sambar	VU	Sch III	NA	WGH, EGH, SGH, RB, EKH	1,2,3,4
	<b>Family Bovidae: Cattle, Antelopes, and Goats</b>						
159	<i>Bubalus arnee</i>	Wild Water Buffalo	EN	Sch I (Part I)	III	BBL, Siju WS, WKH	2,3
160	<i>Bos gaurus</i>	Gaur	VU	Sch I (Part I)	I	All districts	1,2,3,4

	Taxa	Common name	IUCN Red List status	Wildlife Protection Act, 1972 schedule/status	CITES	Distribution	Source
161	<i>Capricornis rubidus</i>	Red Serow	NT	Sch I (Part I)	I	All districts	3,4
162	<i>Naemorhedus griseus</i>	Chinese Goral	NT	Sch III	I	WGH	1,2,3

Sources: 1—IUCN Red List | 2—Das et al. (1995) | 3—Choudhury (2013) | 4—Kakati & Kabra (2015) | 5—Goswami (2015) | 6—Kumar et al. (2010) | 7—Saikia et al. (2018) | 8—Mukherjee et al. (2019) | 9—Molur et al. (2005). EGH—East Garo Hills | EJH—East Jaintia Hills | EKH—East Khasi Hills | GH—Garo Hills | JH—Jaintia Hills | KH—Khasi Hills | RB—Ri-Bhoi | RF—Reserved Forest | SGH—South Garo Hills | WGH—West Garo Hills | WJH—West Jaintia Hills | WKH—West Khasi Hills.

Hills have been reported to cross the border annually to Bangladesh and lone males in Jaintia Hills have also been observed to cross the International boundary occasionally (Choudhury 2007).

**Primates:** The distribution and status of different primate species were mapped, and the forest status and human pressures in northeastern India were assessed by Srivastava (2006). Extensive surveys were carried out throughout the region between 1994 and 1999. The survey reported the presence of Rhesus Macaque *Macaca mulatta*, Assamese Macaque *Macaca assamensis*, Northern Pig-Tailed Macaque *Macaca leonina*, Stump-Tailed Macaque *Macaca arctoides*, Capped Langur *Trachypithecus pileatus*, Western Hoolock Gibbon *Hoolock hoolock*, and Bengal Slow Loris *Nycticebus bengalensis* in Meghalaya. All the species were observed to occur in very low densities. Habitat loss and hunting were reported to be the main threats to the primates. In some cases, indiscriminate hunting had extirpated local populations despite the availability of large tracts of primary forest. Interactions with humans due to crop raiding have led to retaliatory killing of macaques and langurs (Srivastava 2006).

Stump-Tailed Macaque was reported to occur in Mawsynram area of the Khasi Hills, Balpakram NP and Nokrek RF while Pig-Tailed Macaque were reported in all districts including Mawsynram area of East Khasi Hills, West Garo Hills, and West Khasi Hills (Biswas 1977; Sati & Alfred 1990; Molur et al. 2003). Rhesus Macaque was reported to occur in Garo and Khasi hills, Assamese Macaque in Balpakram NP, Songsek Tasek RF and Siju WS, and Capped Langur in Garo Hills (Molur et al. 2003). Habitat destruction and hunting were stated to be the main threats for all the species.

Preliminary investigations have reported the occurrence of the Bengal Slow Loris in Meghalaya (Radhakrishna et al. 2006; Nandini et al. 2009; Radhakrishna et al. 2010). The species occurs in very low numbers and its population may have reduced over the years owing to threats such as forest fragmentation, vehicle collision, and hunting (Radhakrishna et al. 2006).

Other major threats to the species were hunting for bushmeat, capture for pets, man-made fires, mining and conversion of forests to plantations (Radhakrishna et al. 2010). The species has been sighted in Nongkhylllem WS, Narpuh RF, Baghmara RF, Balpakram NP, and Nokrek NP although its density is very low (Molur et al. 2003; Kakati et al. 2009; Radhakrishna et al. 2010).

The occurrence of Western Hoolock Gibbons has been reported in East Garo Hills, South Garo Hills, Ri-Bhoi, and Khasi Hill districts (Baskaran 1975; Molur et al. 2003). Hoolock Gibbon also occurs in 32 localities in West Garo Hills (Alfred & Sati 1990). Hoolock Gibbon populations in West Garo Hills had declined between 2007 and 1985–87 by 26.2% owing to human disturbances such as tree felling, jhum, livestock grazing, and poaching (Alfred & Sati 1990; Sati 2011). It was observed that the rate of survival might have been severely affected and establishment of new colonies was not happening.

**Rodents:** Rodents have hardly been studied in Meghalaya, except for a couple of studies that reported on the partial albinism of White-Bellied Rat *Rattus niviventer* (Rajagopal & Mandal 1965). A faunal account of all rodent species found in India was provided through an extensive collection available at the British Museum (Ellerman 1961). Accounts of all mammalian species including a few rodent species found in the Khasi, Jaintia, and Garo hills region was also provided by Hinton & Lindsay (1926).

**Chiropterans:** Bats have been well documented in Meghalaya. About 65 species have been reported in the state (Saikia et al. 2018). One of the first scientific records of bats in Meghalaya was of the description of *Scotomanes ornatus* done by Blyth in 1851 (Saikia et al. 2018). Since then bats have been documented throughout the Khasi, Jaintia, and Garo hills (Dobson 1871, 1872, 1874; Thomas 1921; Kemp 1924; Hinton & Lindsay 1926; Topal 1970; Lal 1977; Sinha 1990, 1994, 1995, 1999; Thabah & Bates 2002; Thabah et al. 2006, 2007; Ruedi et al. 2012a,b, 2014; Thong et al. 2017; Saikia et al. 2017, 2018; Korad 2018). Most of the studies reported only the taxonomic status and distribution of

**Table 2. Conservation status according to IUCN Red List, IWPA 1972 and CITES. Numbers show the number of species.**

<b>Indian Wildlife (Protection) Act, 1972</b>	
Schedule I	23
Schedule II	33
Schedule III	5
Schedule IV	9
Schedule V	23
Not assessed	65
<b>CITES (2017)</b>	
Appendix I	22
Appendix II	12
Appendix III	13

the bat species. One of the few studies that focused on the ecology of bats (Thabah et al. 2007) reported on the feeding and echolocation behavior of the Great Evening Bat *la io*. The authors found that the species preyed on birds, although coleopterans formed the main constituent of their diet.

Unregulated mining activities for limestone and coal near cave systems pose a threat to the caves and the fauna they harbour (Saikia 2018). Expansion of plantations, demand for firewood and deforestation associated with mining activities threaten the survival of bat species as well as all other forest-dwelling species (Ruedi et al. 2012a, 2014). Hunting of bats for a supplementary source of protein is another threat. Improvised techniques are employed for their capture from caves by locals, and the number of captured bats has been reported to go as high as 100 on a single night. Some of the bat meat is sold in local markets (Ruedi et al. 2012b; Saikia et al. 2018). This overharvesting has led to a decline in the bat population, with some colonies retreating to inaccessible cliffs and caverns (Saikia et al. 2018).

**Dhole:** A questionnaire-based survey reported that Dhole *Cuon alpinus* was still common in Garo Hills although throughout the state it had become very rare (Johnsingh 1985). The species was last sighted in Garo Hills (Kakati & Kabra 2015).

**Bears:** A few studies have reported the occurrence of three bear species in Meghalaya, viz., Asian Black Bear *Ursus thibetanus*, Sloth Bear *Melursus ursinus*, and Malayan Sun Bear *Helarctus malayanus* (Blanford 1888–91; Hinton & Lindsay 1926; Sathyakumar 2001; Johnsingh 2003; Sathyakumar & Choudhury 2007; Choudhury 2011; Kakati & Kabra 2015). An Asian Black Bear was photo-captured in Balpakram-Baghmara Landscape

in Garo Hills (Kakati & Kabra 2015). The occurrence of Sloth Bear in Meghalaya was confirmed through a specimen acquired from Khasi Hills and preserved at the Zoological Survey of India as well as by local hunters who were familiar with all three species (Choudhury 2011). Choudhury (2011) also reported the sighting of a Malayan Sun Bear pelt from Balpakram NP in the early 1980s; however, no systematic study has been carried out till date for any of the bear species.

The major threats faced by these species are habitat loss, construction of linear infrastructure and dams, coal mining and shortening of jhum cultivation cycles (Sathyakumar 2001; Choudhury 2011). Asiatic Black Bear also faces pressure from poaching for its bile (Choudhury 2011).

**Mustelids:** Specimens of Yellow-bellied Weasel *Mustela kathiah* and skins of Burmese Ferret-badger *Melogale personata* were acquired from Khasi and Jaintia hills (Pillai & Biswas 1971). The authors stated that the Khasis ascribed magical properties to the teeth of the weasel and used it to remove fish bone stuck in the throat. The Burmese Ferret-badger and Small-toothed Palm Civet *Arctogalida trivirgata* were camera-trapped for the first time by Kakati et al. (2014a, 2014b) in Garo Hills. The Burmese Ferret-badger had earlier only been recorded in Khasi Hills (Choudhury 2013).

**Red Panda:** A disjunct population of Red Panda *Ailurus fulgens*, locally known as Matchibel, was reported in Meghalaya through four skins of the species collected from Nokrek and Balpakram in Garo Hills in the 1960s and 1980s (Choudhury 1997, 2000a,b). The largest known specimen was shot in the early 1960s in Nokrek NP (Choudhury 2000a). This was the first record of the species in a tropical forest. It also holds the record for the lowest elevation reported for the species at 200m. This population was thought to have migrated through the Patkai and Naga ranges to the Garo Hills. It was also stated to be found in the Khasi Hills (Choudhury 2000b) although no evidence was provided.

**Ungulates:** Populations of Wild Water Buffalo *Bubalus arnee* have declined in Meghalaya as well as in the whole of northeastern India owing to the destruction of habitat through the conversion of elephant-grass jungles to farmland, hunting pressure and transmission of diseases by livestock. In Meghalaya, there currently exists only a small population in Balpakram NP (Choudhury 1994).

The Gaur *Bos gaurus* is mainly found in South Garo Hills and West Khasi Hills although a small population is also found in Ri-Bhoi District (Choudhury 2002).

## CONCLUSION

Meghalaya being part of the Indo-Burma Biodiversity Hotspot and also hosting a diverse array of Indo-Malayan species is an important landscape for the conservation of many of the mammalian species that exist in the subcontinent. With about 38% of all Indian mammals found in the state, it is worthwhile to emphasize the need for greater conservation efforts in this region. Although a decent number of studies have been conducted, most have focused only on Asian Elephants and only in the Garo Hills region while hardly a handful of studies have focused their attention on other species and in other parts of the state; most of the studies on other species have been limited to preliminary investigations and provide only a synoptic view of species distribution, occurrences and threats. Certain taxa such as the chiropterans have in recent years been well documented, but are restricted only to the taxonomic field. Other taxa such as the rodents, although representing the second largest group in the state, have hardly been studied. A wider concerted effort in conducting additional studies on other lesser appreciated groups and lesser known species and tackling both ecological as well as human-wildlife questions would provide a firm foundation for

undertaking holistic conservation actions to ensure the persistence of the mammalian fauna in the state.

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Image 1. Camera trap photos: A—Leopard | B—Clouded Leopard | C—Leopard Cat | D—Yellow-throated Martin. © GBPN/NMHS-2017-18/MG 32.

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## A COMPARATIVE ANALYSIS OF HAIR MORPHOLOGY OF WILD AND DOMESTIC UNGULATE PREY SPECIES OF LEOPARD *PANTHERA PARDUS FUSCA* (MAMMALIA: CARNIVORA: FELIDAE) FROM GOA, INDIA

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**Abstract:** Guard hairs were collected from four live domesticated ungulate species and shed guard hairs of six wild ungulate species from zoo captive animals from five individuals each. Photographic reference was prepared showing analytic features of hair characteristics. Study results were analysed and cuticle and medulla patterns were identified along with pigmentation features from the literature available for wild and domestic ungulates from India and abroad. Clear and easily distinguishable morphological characters of hair medulla and cuticle were used in the present study. Scat analysis of big cats used in this study is easy, speedy and efficient which can be used in routine investigations related to wildlife, crime forensics as well as human animal conflicts by studying carnivore feeding habits. In a majority of the animal species, the distal part of the hair showed maximum variation from the rest of the hair portions. The cuticle scales were imbricate in all tested animals. Scale position in almost all the tested animals was transversal except in goat (proximal part and medial part) and mouse deer (Distal part). Majority of the species showed smooth margins at proximal and medial part. Whereas the distal part scale margin was crenate and rippled in appearance the proximal part and medial part of hair of the majority of sampled animals showed a regular wave -type scale pattern whereas the distal part of hair showed irregular wave-type scale pattern in dominance. The composition of the medulla was multicellular in all the sampled deer species. Only the cow calf's hair medulla was unicellular and uniseriate in appearance. A comparison of the hair of the domestic pig with that of the wild boar and gaur hair with that of cow calf and buffalo calf hair was made for the first time in the present study. Similarly goat hair morphology can also be differentiated from other cervids in this study. Medulla and cuticle characters in combination with each other can help differentiate wild ungulate species from the domestic ones since these wild ungulate species are frequently involved in hunting crime investigations. Therefore, the photographic reference presented in this study can be used in wildlife forensic science as well as predator diet analysis as an appropriate reference for prey species identification.

**Keywords:** Carnivore prey identification, hair pigmentation, medulla, ungulate.

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**Author contribution:** BSPD collected the hair specimens, analyzed them and wrote the manuscript. AHD'C assisted with the analysis of the specimens by providing protocols and revision of the manuscript. SKS supervised the study and helped in the revision of the manuscript.

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## INTRODUCTION

The presence of hair on the body is one of the important characteristics of class Mammalia. Mammalian hair is structurally unique in different mammal species which is a result of adaptation and evolution in response to the environment. This characteristic is widely used to identify animals at species level (Menike et al. 2012). Identification of mammals using hair morphology has also been used in diet analysis of predator species using scat analysis. In southern India several studies have documented feeding habits of Leopard *Panthera pardus fusca* during the last two decades (Ramakrishnan et al. 1999; Athreya et al. 2014). All these studies suggest that wild as well as domestic animals form major components of leopard diet especially in areas of human dominated landscapes. Predation of livestock is one of the most important human-leopard conflict scenarios in India and is the primary cause for leopard persecution. This in turn can lead to major threats to leopard conservation goals. Therefore, understanding the leopard diet can play an important role for its conservation in human dominated landscapes of India.

Photographic references of the hair structures of wild and domestic ungulate prey species of leopard has effective practical applications in understanding leopard feeding habits. Hair morphology is an important tool that can be used to identify animal species (ENFSI 2015); however, identification of species from hair structure is not a straightforward process and practitioners need to develop expertise to identify and to be able to distinguish hair characteristics especially in closely related species. Thus, the understanding and comparison of hair structures is important to help distinguish one species from another.

Mammals have four types of body hair, of which guard hair are the most important in differentiation between various animal species (Tridico 2005; Knecht 2012). A typical mammal hair consists of a hair root and hair shaft. The root is embedded in the epidermis and the shaft is the part which extends above the epidermis as a cylindrical structure. The hair shaft is made up of three distinct morphological layers, i.e., medulla (central layer), cortex (intermediate layer) and cuticle (outer layer) (Deedrick & Koch 2004a; Debelica & Thies 2009; Knecht 2012). The medulla, which is the innermost layer of the hair shaft, is a honeycomb-like keratinous structure which can be continuous, discontinuous or fragmented with vacuoles in between (Deedrick & Koch 2004a). The cortex contains keratin fibers and pigment granules which are responsible for

the coloration of the hair. The cuticle, the outermost layer, consists of overlapping keratin scales (Deedrick & Koch 2004a). Two main patterns of cuticle scales are: (i) imbricate, and (ii) coronal. The distance between every two successive scale margins can be close, intermediate or wide, depending on the animal species (Debelica & Thies 2009). The pattern of the cuticle scales, the type and the diameter of the medulla and/or the characteristics of pigmentation can be used for animal species identification (Brunner & Coman 1974).

The presence of high content of cysteine-containing keratin and dead keratinocytes delays postmortem changes and chemical decomposition in mammal hair (Harkey 1993; Knecht 2012). This property of mammalian hair has helped in carrying out forensics investigations.

Although several studies have reported hair structure identification for wild and domesticated mammal hairs (Dharaiya et al. 2012), very few of them deal with ungulates. Further, such studies are also required to create database for different geographical areas. In the present study we provide a comprehensive comparative database about wild and domestic ungulate prey species of the leopard in Goa.

## MATERIALS AND METHOD

### Sample collection and preparation

For preparing the photographic reference of cuticle and medulla characters, shed hairs were collected from six wild ungulate species from the night shelter enclosures of the Bondla Zoo located at Bondla Wildlife Sanctuary, Ponda Goa and from four live domestic ungulate species from five individuals each from Goa during January 2016 to December 2016. Guard hairs were separated from other hairs based on their properties before analysis as given by De Marinis & Asprea (2006). Hairs were then immersed in 70% ethanol solution for 5–10 minutes to remove any debris and non-hairy sticky materials. The hair strands were then dried and cleaned on a blotting paper. Each hair length was divided into three parts: proximal (base), medial (middle), distal (apical). Longer hairs were cut into these three respective parts for comparative analysis of each strand at different lengths whereas shorter hairs were used as a whole.

### Examination of hair cuticle scale pattern

Cuticle scales of individual hairs were analyzed using the methodology of Mukherjee et al. (1994b). A 20% gelatin solution was prepared by boiling the gelatin powder in distilled water. The solution was cooled and

two drops of Leishman's stain was added to the solution to obtain a pale blue colour. One to two drops of this solution was used to prepare a smooth film on a clean dry glass slide and immediately cleaned hair shafts were superficially placed on the film. The glass slide was then covered with a glass petri plate and left for 15–20 minutes at room temperature. The hair shafts were then slowly separated from the gelatin film using forceps such that an imprint of the scales was formed on the glass slide. These imprints were observed and photographed under (400X) magnification using a light microscope (Olympus microscope BX 53).

### Examination of hair medulla and pigmentation

For hair medullary pattern and pigment analysis, hairs were immersed in xylene for 24 hours. The hairs were then dried and then mounted on glass slides using a drop of DPX and then covered with a coverslip (Mukherjee et al. 1994b). The slides were allowed to dry for an hour and then observed and photographed under a light microscope at 400X magnification.

## RESULTS

Hair structures from different species were identified and compared for their cuticle and medulla patterns along with pigmentation features from the literature available for wild and domestic ungulates from India and abroad (Mukherjee et al. 1994; Dharaiya & Soni 2012; Joshi et al. 2012; Ghallab et al. 2018).

### Comparative analysis of cuticle

Cuticle scale position, scale margin, scale margin distance and scale patterns were analyzed from guard hairs collected from six wild and four domestic animal hairs at different hair lengths (Table 1; Images 1–10). Analysis of hair at three different levels revealed considerable variations in scale margin, scale margin distance and scale patterns in all the sampled animals whereas scale position showed no major variations at different hair lengths. In a majority of the animal species, the distal part of the hair showed maximum variation from the rest of the hair portions. The cuticle scales were imbricate in all the species studied. Scale position in almost all the studied animals was transversal except in Goat *Capra aegagrus hircus* (proximal part and medial part) and Mouse Deer *Moschiola indica* (distal part).

Based on scale margin type, pattern and distance, the species of animals could be clearly differentiated.

Table 1. Comparative analysis of the Cuticle characteristics of wild and domestic ungulate species from Goa.

Species	Proximal part of hair			Medial part of hair			Distal part of hair			
	Scale position	Scale margin	Scale margin distance	Scale position	Scale margin	Scale margin distance	Scale position	Scale margin	Scale margin distance	Scale pattern
Cow calf	Transversal	Smooth	Distant	Transversal	Smooth	Near	Transversal	crenate	Near	Single chevron
Buffalo calf	Transversal	Smooth	Near	Transversal	Rippled	close	Transversal	Rippled, crenate	Near	Irregular wave
Pig	Transversal	Crenate	Near	Transversal	Rippled, crenate	Close	Transversal	Crenate	Close	Irregular wave
Goat	Intermediate	Crenate	Distant	Intermediate	Smooth	Distant	Transversal	Rippled	Near	Single chevron
Spotted deer	Transversal	Smooth	Near	Transversal	Smooth	Near	Transversal	Smooth, crenate	Distant	Regular, irregular wave
Wild boar	Transversal	Rippled	Close	Transversal	Rippled	Near	Transversal	Rippled	Near	Irregular wave
Sambar deer	Transversal	Smooth	Distant	Transversal	Smooth, Rippled	close	Transversal	Rippled	Near	Irregular wave
Mouse deer	Transversal	Smooth	Distant	Transversal	Smooth	Distant	Intermediate	smooth	Distant	Irregular mosaic
Barking deer	Transversal	Smooth	Near	Transversal	Smooth	Near	Transversal	Smooth, crenate	Distant	Regular wave
Gaur	Transversal	Rippled	Near	Transversal	Crenate	Close	Transversal	Crenate	Near	Single Chevron



Image 1. *Bos taurus* (calf) medulla; distal, medial and proximal part of the hair (400 X).

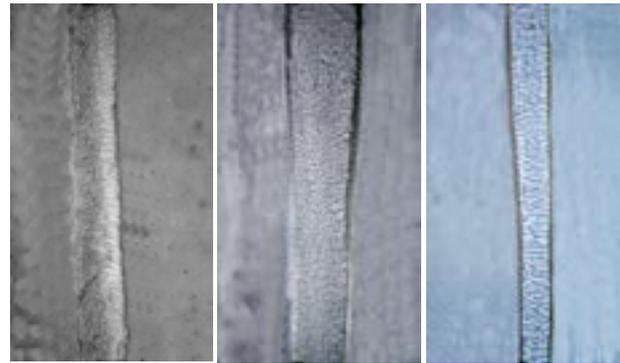


Image 2. *Bubalus bubalis* (calf) medulla; distal, medial and proximal part of the hair (400X).

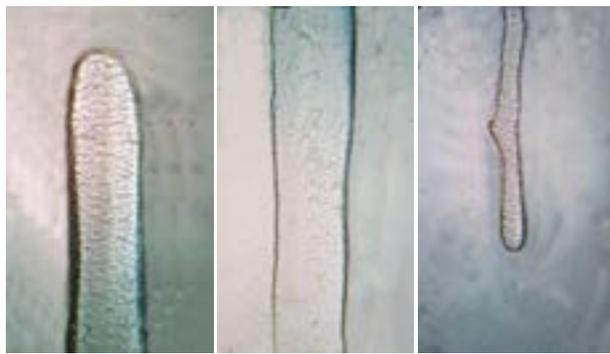


Image 3. *Sus scrofa domestica* (adult) medulla; distal, medial and proximal part of the hair (400 X).

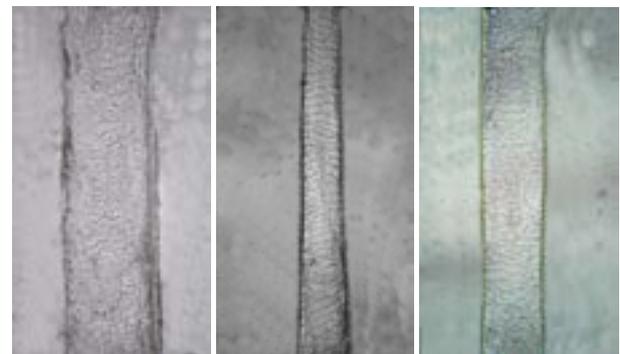


Image 4. *Capra aegagrus hircus* (adult) medulla; distal, medial and proximal part of the hair (400 X).

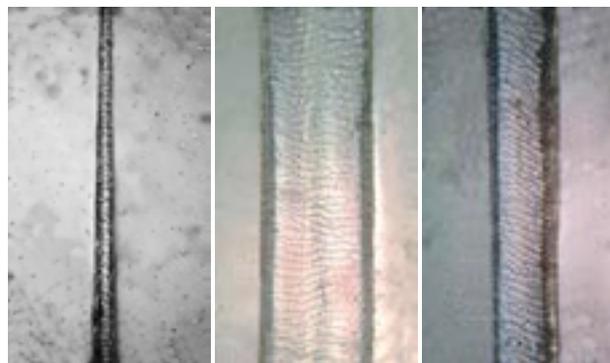


Image 5. *Axis axis* (adult) medulla; distal, medial and proximal part of the hair (400 X).

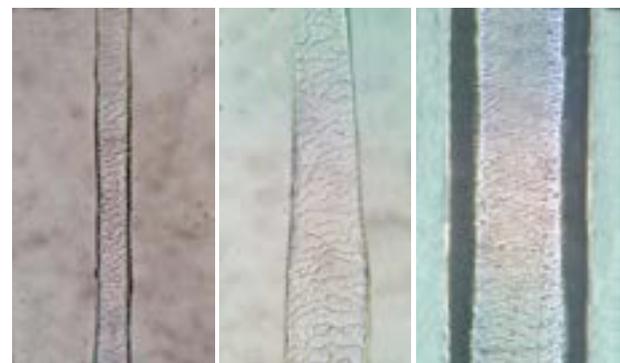


Image 6. *Sus scrofa* (adult) medulla; distal, medial and proximal part of the hair (400 X).

A majority of the species showed smooth margins at proximal and medial part, whereas the distal scale margin was crenate and rippled in appearance. The proximal and medial parts of the hair of a majority of the sampled animals showed regular wave type scale pattern whereas the distal part of hair showed irregular wave type of scale pattern. A single chevron type of scale pattern was seen in the medial part and distal

part of gaur hair (*Bos gaurus*) and distal part of cow calf (*Bos taurus*) and goat (*Capra aegagrus hircus*). Buffalo calf (*Bubalus bubalis*) and domestic pig (*Sus scrofa domestica*) both showed double chevron type of scale pattern in the medial part.

Therefore, the hair of Wild Boar *Sus scrofa* and domestic pig can be very well differentiated from the analysis of cuticle characteristics. Goat hair also can be

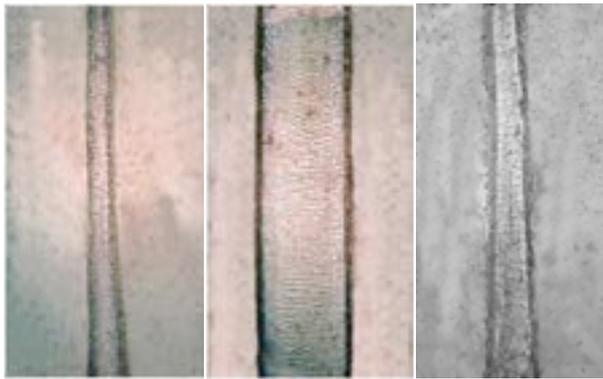


Image 7. *Rusa unicolor* (adult) medulla; distal, medial and proximal part of the hair (400 X).

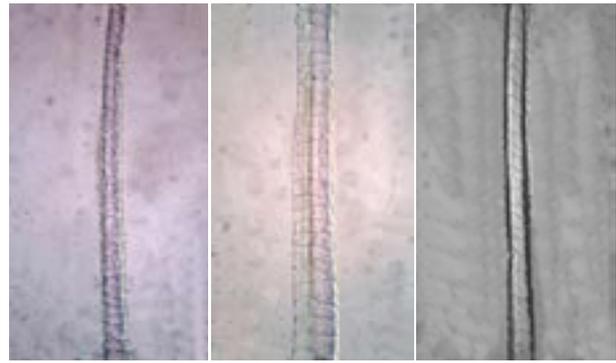


Image 8. *Moschiola indica* (adult) medulla; distal, medial and proximal part of the hair (400 X).

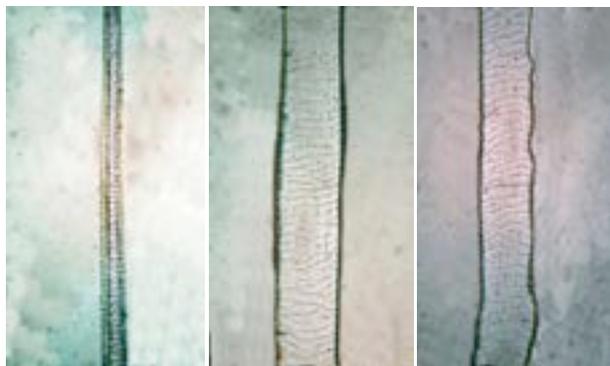


Image 9. *Muntiacus muntjac* (adult) medulla; distal, medial and proximal part of the hair (400 X).

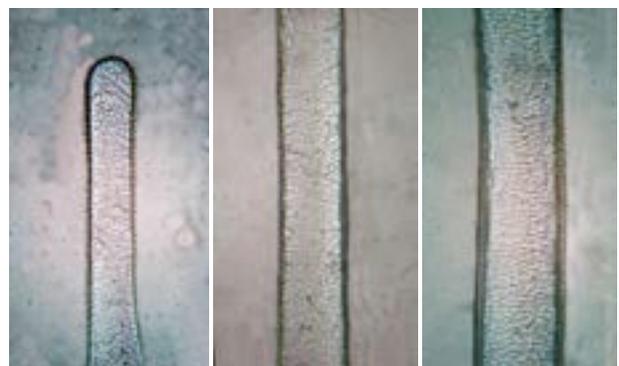


Image 10. *Bos gaurus* (adult) medulla; distal, medial and proximal part of the hair (400 X).

differentiated from other sampled deer species using cuticle pattern analysis. In case of the sampled deer species such as Sambar Deer *Rusa unicolor*, Spotted Deer *Axis axis* and Barking Deer *Muntiacus muntjac*, including Mouse Deer *Moschiola indica*—all exhibited very similar cuticular scale patterns.

#### Comparative analysis of hair medulla morphology and pigmentation

In addition to cuticle scale characteristics, hair medulla morphology, composition, structure, pattern and margins as well as the pigmentation has been used to identify and compare the sampled wild and domestic ungulate prey species of leopard. The composition of medulla was multicellular in all the sampled deer species. Only cow calf hair medulla was unicellular and uniseriate in appearance. Medulla cell type could not be differentiated in buffalo calf, domestic pig and goat. Amorphous type medulla structure was observed in buffalo calf, domestic pig, goat, Spotted Deer, Wild Boar and Gaur, whereas filled lattice type medulla was

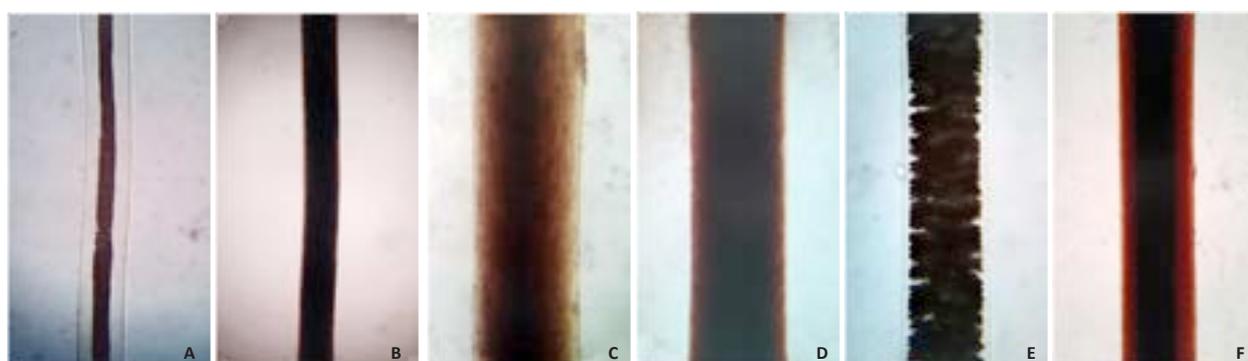
observed in Sambar Deer, Mouse Deer and Barking Deer. Vacuolated and fragmented medulla was observed only in case of spotted deer. A majority of the sampled ungulate hairs showed irregular type margin type except in case of Sambar Deer, Mouse Deer, Barking Deer and Gaur. Pigmentation was not observed in hair cortex of cow calf, Spotted Deer and Mouse Deer (Table 2; Images 11–12

#### Morphometric analysis of ungulate hair

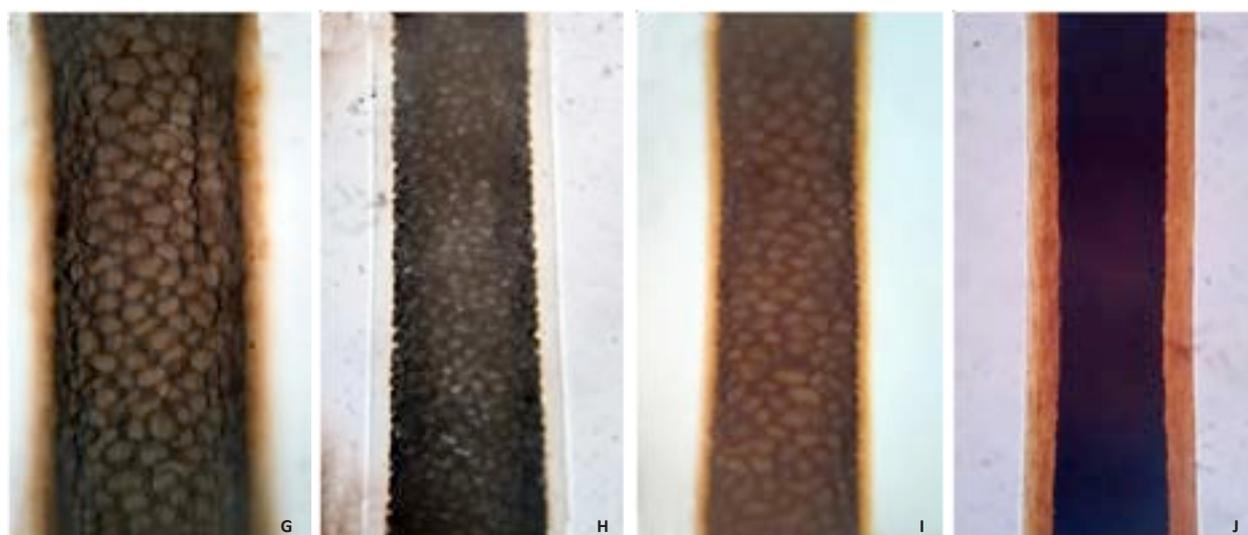
Total hair diameter as well as medullary thickness was measured using binocular microscope with camera attachment using ProgRes software. Total diameter of hair was maximum at the proximal part compared to the rest of the portion of the hair in all the sampled ungulate species, whereas thickness of medulla was maximum at the medial part in a majority of the species with the exception of cow calf, buffalo calf and goat. Medulla did not extend to the distal end in case of wild boar and to the proximal end in case of gaur whereas in case of cow calf and spotted deer medulla was absent at both the

**Table 2. A comparative analysis of medulla and pigmentation features of wild and domestic ungulate species from Goa.**

Comparative analysis of hair medulla morphology and pigmentation in different animal species					
Animal species	Composition	Structure	Pattern	Margin type	Pigmentation
Cow calf	Unicellular	Uniseriate	Continuous	Irregular	No pigments
Buffalo calf	Cells not visible	Amorphous	Continuous	Irregular	Granules and streak like pigments
Domestic pig	Cells not visible	Amorphous	Continuous	Irregular	Streak like pigments
Goat	Cells not visible	Amorphous	Continuous	Irregular	Granules and streak like pigments
Spotted Deer	Multicellular	Amorphous, vacuolated	Fragmented	Irregular	No pigments
Wild Boar	Cells not visible	Amorphous	Continuous	Irregular	Granules and streak like pigments
Sambar	Multicellular	Filled lattice	Continuous	scalloped	Streak pigments
Mouse Deer	Multicellular	Filled lattice	Continuous	Scalloped	No pigments
Barking Deer	Multicellular	Filled lattice	Continuous	Scalloped	Streak like pigments
Gaur	Multicellular	Amorphous	continuous	Scalloped	Streak like pigments



**Image 11. Morphology of the hair medulla in different ungulate prey species of Leopard.**  
 A—*Bos taurus* (calf) | B—*Bubalus bubalis* (calf) | C—*Sus scrofa domesticus* | D—*Capra aegagrus hircus* | E—*Axis axis* | F—*Sus scrofa* at medial part of the hair (400x).



**Image 12. Morphology of the hair medulla in different ungulate prey species of Leopard.**  
 G—*Rusa unicolor* | H—*Moschiola indica* | I—*Muntiacus muntjac* | J—*Bos gaurus* bison, at medial part of the hair (400x).

Table 3. Morphometric analysis of total hair and medulla of wild and domestic ungulate species from Goa.

	Morphometric analysis of ungulate animal Hair														Remarks
	Total hair thickness ( $\mu\text{m}$ )							Medulla thickness ( $\mu\text{m}$ )							
	Proximal part	SD	Medial part	SD	Distal part	SD	Proximal part	SD	Medial part	SD	Distal part	SD			
<b>Cow calf</b>	70.17	$\pm 16.88$	55.76	$\pm 2.78$	31.06	$\pm 9.37$	50.20	$\pm 7.49$	30.42	$\pm 2.50$	15.14	$\pm 2.57$	Medulla absent in distal end and proximal end		
<b>Buffalo calf</b>	266.88	$\pm 18.96$	181.19	$\pm 2.57$	37.21	$\pm 8.29$	151.89	$\pm 2.10$	149.68	$\pm 3.35$	26.94	$\pm 8.29$	-		
<b>Domestic pig</b>	120.08	$\pm 3.93$	185.86	$\pm 16.90$	38.26	$\pm 16.90$	57.79	$\pm 13.05$	163.85	$\pm 4.07$	0.00	$\pm 0.00$	Medulla absent at distal part		
<b>Goat</b>	153.75	$\pm 4.57$	137.97	$\pm 2.35$	34.03	$\pm 12.43$	111.85	$\pm 6.82$	106.70	$\pm 1.27$	14.78	$\pm 4.94$	-		
<b>Spotted Deer</b>	95.90	$\pm 19.73$	134.30	$\pm 2.63$	17.23	$\pm 4.99$	53.77	$\pm 32.48$	110.03	$\pm 4.03$	12.39	$\pm 4.48$	Medulla absent at proximal and distal end		
<b>Wild Boar</b>	363.80	$\pm 4.43$	372.30	$\pm 17.05$	41.16	$\pm 5.33$	278.08	$\pm 5.14$	313.18	$\pm 10.33$	29.99	$\pm 10.28$	Medulla absent at distal end		
<b>Sambar</b>	240.57	$\pm 6.31$	228.82	$\pm 1.55$	20.33	$\pm 4.75$	169.28	$\pm 37.04$	193.50	$\pm 2.88$	9.49	$\pm 1.56$	-		
<b>Mouse Deer</b>	135.77	$\pm 5.19$	159.04	$\pm 2.64$	13.94	$\pm 5.79$	97.03	$\pm 31.79$	124.26	$\pm 3.22$	9.19	$\pm 2.99$	-		
<b>Barking Deer</b>	118.15	$\pm 61.11$	180.22	$\pm 9.38$	23.21	$\pm 5.09$	129.02	$\pm 33.99$	166.23	$\pm 2.39$	25.12	$\pm 5.70$	-		
<b>Gaur</b>	120.68	$\pm 23.91$	162.74	$\pm 2.00$	46.15	$\pm 14.55$	46.77	$\pm 21.22$	104.22	$\pm 7.38$	23.86	$\pm 7.77$	Medulla absent at proximal end		

distal far and proximal end. Only domestic pig hair was devoid of medulla completely in the distal part (Table 3).

## DISCUSSION

Our results showing hair morphological characters of wild and domestic ungulates were similar to the available literature (Brunner & Coman 1974; Knecht 2012), but with a few exceptions. Our medulla analysis results in the case of Spotted Deer showed fragmented amorphous medulla. During the study period since there was no reports of leopard attacks on adult cow and buffalo in Goa, only the hairs of the young one of these domestic ungulates were used in the study. Cuticle as well as medulla characters of adult and young individuals of these species did not show any variation when compared with the literature (Ghallab et al. 2018). Hair characteristics of domestic pig have been compared with that of wild boar as well as gaur with that of cow calf and buffalo calf for the first time in the present study. Similarly goat hair morphology can also help differentiate it from other cervids in the present study. This information can be put to best use when identifying carnivore species in human-carnivore conflict situations where they attack livestock and other domesticated animals. Unlike hair cuticle character, medulla of all the studied species did not show variation along the hair length. Cuticle of hair at different lengths in all the studied species showed variation in scale position, pattern, spacing and margin. This is helpful in microscopic analysis of hair fragments which are usually the case when studying the diet of predators through scat analysis. Medulla and cuticle characters in combination can help differentiate wild ungulate species from the domestic ones since these wild ungulate species are mostly involved in crime investigations such as illegal hunting.

Characters of hair when used in isolation may not be of much value in species identification as they show high variation; however, when these characters are analyzed in combination these they may provide significant information for identification of species (Brunner & Coman 1974; Teerink, 1991). Several studies are available on the combined analysis of hair characters for species identification. Joshi et al. (2012) have done a comparative hair study only on the basis of the hair colour and medulla structures in Mouse Deer, Spotted Deer, Barking Deer and Sambar Deer. Dharaiya & Soni (2012) have documented characters of transverse section of Spotted Deer, Sambar Deer, buffalo, cow,

and goat, but have not explored cuticle and medulla characters. The remaining studies on ungulate hair are mainly based on the predator's scat-hair (diet) analysis.

Analysis and examination of animal hair plays a vital role in wildlife forensics investigation. Analysis of hair collected from the crime scene can provide essential information about the species involved (Soni et al. 2003). Hair analysis has even proved beneficial in tracing chemical poisoning cases in animals (Harkey 1993; Krumbiegel et al. 2014) and hence the same can be utilized in wildlife forensic investigation where the carcass has decomposed and tissues cannot be collected.

This photographic reference in the present study will help to identify the ungulate prey species of leopard and other wild carnivores from the scat analysis from such localities. Therefore, the photographic reference presented in this study can be used in wildlife forensic science as well as predator diet analysis, as an appropriate reference for prey species identification.

In conclusion, the present study provides a first-step towards preparation of local photo reference database of hair of wild and domesticated ungulate species which can be used in forensic investigations as well as to study human carnivore conflict scenarios arising out of livestock depredation. Further studies using more advanced techniques such as electron microscopy can be used to prepare a complete local atlas for all wild and domestic animal species' identification.

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## INTRODUCTION

With growing human population and concomitant increased demand for agricultural land and forest produce, the incidences of human-wildlife negative interactions and reclamation of forest land have increased, and thus implementation of effective wildlife conservation legislatures and policies are at bay. India is the second most populous country in the world with human population density of 323 people per km<sup>2</sup> (Census 2011). Fortunately, India also has the largest constitutional framework of law in the world for protecting the rights to live for people as well as wildlife. Among the government policies like, the National Forest Act (1988), and Schedule Tribes and other Forest Dweller Recognition Act (2006) have legitimized the rights of the people especially tribes, for the settlement inside or at the fringe of forest, and utilize its resources. Likewise, Forest Conservation Act (1980) prevents excessive lumbering or extraction of natural resources from reserve forests, wildlife sanctuaries, and national parks. With such policies in place and on the contrary, burgeoning human population, it is very difficult to implement conservation strategies effectively. This is mainly because sudden restriction in the use of forest resources or eviction from inside or vicinity of the protected areas may create conflict among forest dwellers and the government machineries (Mukherjee & Borad 2004). Attitude of the people living around or inside the forest is very significant in implementation of conservation policies or management actions (Winter et al. 2005). Attitude, however, vary inevitably depending upon several factors. While benefits from the forest (e.g., collection of timber and non-timber forest products) create positive attitude, loss of assets (e.g., crop foraging and depredation of livestock by wildlife) generates negative attitude (Walpole & Goodwin 2001; Talukdar & Gupta 2017). Moreover, education, awareness, age and socio-economic status can largely influence the attitudes (Karanth et al. 2008). Since people's perception and attitude towards forest and wildlife significantly influences effective wildlife conservation (Soto et al. 2001; Sundaresan et al. 2012), a thorough understanding of the factors influencing the perception is most important in developing management actions and implementing policies both at local and national levels. In addition, it promotes public awareness regarding the importance of forest and its resource (Gillingham & Lee 1999; Soto et al. 2001; Kaltenborn et al. 2006).

Keeping this in the backdrop, the present study was conducted in the Barail Wildlife Sanctuary (BWS)

in Assam, India, an eco-sensitive zone, to elucidate people's perception towards the forest and its wildlife. This sanctuary forms a part of the Barail range, in the Indo-Chinese sub-region and Indo-Burma biodiversity hotspot (Myers et al. 2000). It is one among the few remaining tropical forests of India (Pawar & Birand 2001; Choudhury 2013a), and the only protected area in southern Assam. A complex network of small and large streams along with diverse forest types makes the sanctuary an ideal habitat for mammalian fauna. The sanctuary is known to shelter a high diversity of primates and bears. This includes threatened species like Hoolock Gibbon *Hoolock hoolock*, Stump-tailed Macaque *Macaca arctoides*, Pig-tailed Macaque *M. nemestrina*, Capped Langur *Trachypithecus pileatus*, Bengal Slow Loris *Nycticebus bengalensis*, Assamese Macaque *M. assamensis*, and Rhesus Macaque *M. mulatta* (Choudhury 1997, 1988, 2005, 2013a, 2016; Mazumder 2014). Further, the Barail range and its adjoining areas, form an unique bear kingdom, with three out of eight globally known species (Choudhury 2011, 2013a,b, 2016), including Malayan Sun Bear *Helarctos malayanus*, Sloth Bear *Melursus ursinus*, and Asiatic Black Bear *Ursus thibetanus*. Besides, various species of small carnivorous, ungulates and rodents are also reported from the sanctuary (Choudhury 2013a). The adjoining areas of the BWS, however, are densely populated including habitations and agricultural fields, and thus the chances of exploitation are obviously higher (Pawar & Birand 2001). Thus, conserving the wildlife wealth of the sanctuary would be a difficult venture without the active participation of the locals.

In view of the above issues, we assumed that socio-economic factors, knowledge of the sanctuary, and forest management influence the perception of the locals towards conservation of the sanctuary. Further, we also tried to assess the perception towards mammals of this sanctuary, for which we assume that losses by animals, income status and knowledge of wildlife may largely influence their perception. Our endeavor had been to understand the perception of the local people towards the sanctuary and its wildlife; so that we can suggest some recommendations for effective long-term conservation.

## METHODS

### Study area

Barail Wildlife Sanctuary is located in the Cachar District of Assam, India. Sprawled over an area of 326.24km<sup>2</sup>, BWS is bounded by the Indian state Meghalaya in the west and north-west, the Dima Hasao District of Assam to the north-east, and Cachar District of Assam in the south and east. The course of the river Jatinga divides the sanctuary into two blocks namely western (Karimganj division) and eastern (Cachar division) blocks. The river Dolu runs from the eastern boundary and the river Boleswar runs from western boundary of BWS. Besides, a network of small rivulets and rapids are widely spread inside the sanctuary. The primary vegetation of the BWS is tropical evergreen, semi-evergreen forest and moist deciduous as well as barren grass blanks (Choudhury 2013a). Champion & Seth (1968) classified the vegetation as Cachar tropical evergreen forest, Cachar tropical semi-evergreen forest, and subtropical broadleaf hill forest.

The present study was conducted in eight sites within the radius of 2km from the sanctuary covering both the eastern and western blocks (Figure 1) as follows:

#### Eastern block:

1. Indranagar (24.986°N, 92.863°E): This village lies at the southeastern boundary of the sanctuary. The river Dolu runs north to south dividing the sanctuary from this village. Further, the area is characterized by monoculture of *Areca catechu* in the home gardens and *Tectona grandis* as the forest plantation. Perhaps this is the only site where a forest plantation was seen. Amaranagar and Nagar tea gardens surround the village.

2. Telacherra (24.972°N, 92.798°E): It is located to the south of BWS. In order to fulfill our criteria, we restricted the survey to one part of this village called Subangpunjee. The village is formed with the contiguous forest patch of the sanctuary that is vegetation constituted at the buffer zone. Forest patch is relatively dense with mixed forest, mainly bamboo. Home gardens are also common and a small stream called Subang-cherra flows from southeast to northwest along the village.

3. Marwacherra (24.972°N, 92.767°E): The village is located to the southwestern boundary of the sanctuary which is near the Silchar-Lumding highway (NH 27). The area is characterized by monoculture of *Areca catechu* and a few patches of bamboo; vegetable crops and paddy cultivation are prominent here.

4. Bandarkhal (25.057°N, 92.802°E): It is located to the northeastern boundary of the sanctuary and near

the Silchar-Lumding highway (NH 27). The area has large rocky stream and streamline forest, which is more dense in its interior. Besides, home gardens and monoculture of wild banana also occur in the area.

#### Western Block:

5. Daralcherra (24.969°N, 92.635°E): The village is at the south end of the boundary. The area is characterized by degraded forest patch and crop cultivation for home garden.

6. Lakhicherra (25.022°N, 92.487°E): It lies at the southeastern boundary of the sanctuary. The area is characterized by slopes with wild banana plants, *Areca catechu* and home gardens.

7. Isacherra (25.020°N, 92.524°E): This village also lies at the southeastern boundary of the sanctuary, adjacent to Lakhicherra. Fragmented patch of secondary forest along monoculture of *Areca catechu* and home gardens are common in this village.

8. New Malidhar (25.188°N, 92.706°E): This village is located at the western most limit of the sanctuary. The village is formed along the river Boleswar that flows in between BWS and Narpuh Wildlife Sanctuary of Meghalaya. This river demarcates the states of Assam and Meghalaya. Slopes are characterised by monocultures of *Areca catechu*, wild banana plants, and home gardens.

### Data Collection

Preliminary survey was conducted with forest officials in order to locate the fringe villages surrounding the BWS between December 2016 and January 2017. Thereafter, we restricted to the randomly selected eight villages which were located within 1–2 km radius from the boundary of the sanctuary. After selecting the villages, detailed survey regarding the perception of villagers towards the forest and the wildlife of the sanctuary was started from January 2017 and continued till February 2018. The purpose of the interview was explained to the respondents, and those who were willing to participate were interviewed. For the convenience, we used the vernacular language, Bengali. Each respondent represented a single household, which were selected randomly from the villages. In this manner, we interviewed at least 50% of the households from each village. Data were collected using close-ended as well as open-ended questionnaires targeting head of the households, people who regularly visit forest, and the local hunters. Majority of the respondents (>97%) were male aged more than 35–40 years. In terms of literacy, all the respondents were able to read and write their name. Most of them (93%), however, had



Figure 1. Location of study sites in Barail Wildlife Sanctuary, Cachar (Assam, India). The study sites are marked as 'square box'.

primary education, a few had secondary (4%), and a very few (3%) were graduates. Each of the responses was taken in as 'yes' or 'no'. We also used another ordinal measurement for perception in which, coding was done using 0-1-2 (very less-less-moderate) for income status and 3-2-1 (yes-neutral-no) for tourism.

**Data Analysis**

Logistic regression models were used to examine relationships between perceptions as dependent variables, and socio-economic factors, knowledge of the forest and wildlife, forest management and as independent variables. Each factor was grouped and codes were assigned for each attribute for the purposes of logistic regression (Table 1). We also assigned codes for each responses (yes/positive=1 and no/negative=0). Multicollinearity among independent variables was checked using tolerance tests (Htun et al. 2012) before running logistic models. Multicollinearity is considered high if the tolerance is lower than 0.2. Data sets were tested to get perception towards the BWS and conservation of the mammals using a hierarchical approach in which socio-economic factors were entered in step one (hereafter referred to as Model 1 and Model 2) and knowledge and forest management variables were entered in step two (hereafter referred to as Model 3 and Model 4) (Htun et al. 2012). For obtaining

Table 1. Respondent's socio-economic status and knowledge towards protection and management of the Barail Wildlife Sanctuary. [INR=Indian Rupees]

Independent variables	Attributes	Percent (n = 287 individuals)
Socio-economic		
Provide settlement	Positive	66.9
Loss by wildlife (Mammals)	Positive	53.3
Accessibility to main road	Good (Located beside the main road)	51.9
	Bad (not accessible directly by main road)	48.1
Income	Very less (<2,700 INR)	5.6
	Less (2,701–5,000 INR)	53.3
	Moderate (> 5,000–12,000 INR)	41.1
Knowledge of the sanctuary		
Aware about the protected area	Positive	57.5
Forest extraction are not allowed	Positive	48.1
Knowledge of forest management		
Aware about forest official activity	Positive	83.6
Relation with forest official	Positive	71.8

the perception about the mammals of BWS, we used two models (Model 5 and Model 6) containing socio-economic variables and knowledge of wildlife. Odds ratios of significant variables were checked to facilitate Model interpretation. Odds ratios greater than 1 indicated increase in the likelihood of the occurrence of the event, and odds ratios less than 1 as decrease in the likelihood of the occurrence of the event (Tabachnick & Fidell 2013).

**RESULTS**

**Perception towards BWS**

More than half of the respondents (66.5%) had positive perception with the establishment of the sanctuary. In Model 1, where we tested to run socio-economic factors, the Model was found statistically significant ( $\chi^2=20.01$ ;  $p=0.001$ ) and correctly classified 79.1% cases (respondents) who believe that the establishment of sanctuary provided legal land for settlement and cultivation around the sanctuary (Table 4). These respondents were likely to have positive perception. Respondents suffering crop loss due to the mammals, however, were associated with a reduction in the likelihood of exhibiting positive perceptions. In Model 2, we incorporated people’s knowledge about the sanctuary and forest management, along with Model 1. This Model is significant ( $\chi^2=60.20$ ;  $p=0.000$ ) and correctly classified 83.4% respondents to bear positive perception towards establishment of the sanctuary. In Model 2, the social-economic variable settlement/cultivation was positively correlated and significant (Table 4). Likewise, Model 2 also showed that people who were aware about the protection of the sanctuary and forest officials’ monitoring were approximately 8 times and 2.5 times more likely to have positive perception than those who did not. Loss by animals, however, was not significant in this Model.

Only 47.04% respondents had negative perception towards the establishment of the BWS. When socio-economic variables were entered in Model 3, the model was significant ( $\chi^2 =35.56$ ;  $p=0.000$ ) and classified 63.8% cases of negative perception (Table 4). The Model shows that people with ‘less income’ have approximately 17% more chance to have negative perceptions. With increasing crop loss from mammals, increase was the likelihood of negative perception. When the variables—knowledge of the sanctuary and forest management—were added, Model 4 was significant ( $\chi^2 =35.56$ ;  $p=0.000$ ) and classified 68.9%

**Table 2. Respondent’s knowledge towards the wildlife (mammals) and its protection law in India. [INR=Indian Rupees].**

Independent variables	Attributes	Percent (n = 287 individuals)
Socio-economic		
Loss by wildlife (Mammals)	Positive	53.3
Income	Very less (<2,700 INR)	5.6
	Less (2,701–5,000 INR)	53.3
	Moderate (> 5,000–12,000 INR)	41.1
Knowledge of wildlife		
Wildlife is beneficial for the forest	Positive	68.3
Wildlife is protected	Positive	80.5

**Table 3. Respondent’s perception towards Barail Wildlife Sanctuary and its wildlife (Mammals).**

Perceptions	Percent positive response (n = 287 individuals)
Barail Wildlife Sanctuary	
Are you happy with establishment of the sanctuary?	79.1
Do you think that the sanctuary does not offer any benefit?	47.0
Wildlife of Barail Wildlife Sanctuary	
Can humans and wildlife co-exist?	66.6
Do you think that wildlife is not beneficial for the people?	39.7

respondents with negative perception (Table 4). Thus, according to this model, people with ‘less income’ have approximately 19.4% more chance to exhibit negative perceptions, which is more than Model 3. Again, crop loss caused by mammals was positively correlated to negative perception. People with knowledge of the protected area were more likely to answer that its establishment had not brought any negative impact, but other knowledge variables including forest management were insignificant for the model. Both Models 3 and 4 showed that people having ‘bad accessibility to main road’ had 42.5% and 34% more chance of increasing likelihood of negative perception. Respondents were more or less satisfied with performance of forest officials. Further, respondents did agree that officials promote conservation, check illegal activity, frequently monitor the sanctuary, and help the locals.

**Perception for wildlife of BWS**

About two-third (66%) of the respondents had positive perception with respect to co-existence of human and wildlife in the sanctuary. When socio-

**Table 4. Predicting odd ratios of people’s perceptions (positive and negative) in Barail Wildlife Sanctuary. [Reference group in explanatory variable is not added. \*p<0.01; \*\*p<0.05; ‘+’ reference group]**

Variables	Happy with establishment of the sanctuary [positive]		The sanctuary does not offer any benefit [negative]		
	Model 1	Model 2	Model 3	Model 4	
<b>Socio-economic</b>					
Provide settlement (Yes)	3.026*	2.013*	0.802	0.857	
Loss by wildlife (Mammals) (Yes)	0.512**	0.782	2.688*	2.198*	
Accessibility to main road (Good)	0.838	0.889	1.425*	1.398*	
Income	Very less+				
	Less	0.610	0.665	1.170**	1.194**
	Moderate	0.561	0.571	0.736	0.708
<b>Knowledge of the sanctuary</b>					
Aware about the protected area (Yes)		8.030*		0.454*	
Forest extraction are not allowed (Yes)		0.990		1.458	
<b>Knowledge of forest management</b>					
Aware about forest official activity (yes)		2.597**		0.857	
Relation with forest official (Yes)		0.839		1.184	
<b>Percent correctly classified</b>	<b>79.1</b>	<b>83.4</b>	<b>63.8</b>	<b>68.9</b>	
<b>χ<sup>2</sup></b>	<b>20.001*</b>	<b>60.208*</b>	<b>35.561*</b>	<b>46.459*</b>	

economic factors and knowledge of wildlife were run in the model, the model was found statistically significant ( $\chi^2 = 203.46$ ;  $p=0.000$ ) and classified 75.9% cases of positive perception. This model (Model 5) shows that people with ‘less income’ had 90% chance to have positive perception (Table 5). Also, perception of the respondents having knowledge about the wildlife protection laws and knowledge about the beneficial role of wild animals were positively correlated and significant. Thus, increasing knowledge was associated with increase in the likelihood of showing positive perceptions.

Only 38.67% respondents considered that wildlife is not beneficial. Model 6 was run with socio-economic factors and knowledge of the wildlife, and was statistically significant ( $\chi^2=88.72$ ;  $p=0.000$ ), classifying 75.02% cases (Table 5). It thus represented that increasing loss by animals were more likely to increase the negative perception, while other variables like income and knowledge of wildlife was not significant, i.e., the loss of crop due to wild animals was the solo variable which determined the negative perception.

**Tourism**

From Models 3 and 5, it is evident that income status has significant influence on the positive and negative perceptions towards BWS and its wildlife. All respondents belong to economically backward class,

**Table 5. Predicting odd ratios of people’s perceptions (positive and negative) for the mammals of Barail Wildlife Sanctuary. [Reference group in explanatory variable is not added. \*p<0.01; \*\*p<0.05; ‘+’ reference group]**

Variables	Human and wildlife can co-exist [positive]	Wildlife is not beneficial for the people [negative]	
	Model 5	Model 6	
<b>Socio-economic</b>			
Loss by wildlife (Mammals) (yes)	1.063	4.455*	
Income	Very less+		
	Less	0.013*	1.513
	Moderate	0.490	2.217
<b>Knowledge of wildlife</b>			
Wildlife is protected area (yes)	9.840*	1.161	
Wildlife is beneficial for the forest (yes) allowed	6.298*	0.450	
<b>Percent correctly classified</b>	<b>75.9</b>	<b>75.02</b>	
<b>χ<sup>2</sup></b>	<b>203.46*</b>	<b>88.72*</b>	

with average monthly income ranging from 3736±877.01 (INR) to 6315±1720.49 (INR) (Mean±SD). Therefore, increasing revenue may eventually increase their socio-economic wellbeing, which in turn may help in reversing their negative perception. One of the common approaches is tourism. In order to find the perception towards tourism, we set a questionnaire in context to

**Table 6. People's perception on tourism in Barail Wildlife Sanctuary.**

Statement	Positive response (in percentage)								x <sup>2</sup>	p	Cramer's V
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8			
Heard about tourism before	35.4	30.9	38.4	32	75.7	50	25.7	82.2	8.04	0.04	0.116
Happy if tourism is encouraged	60.4	42.8	33.3	32	48.4	40	68.5	55.5	10.06	0.01	0.122
Tourism will increase source of income	56.2	30.9	41	32	60.6	45	57.1	35.5	13.51	0.00	0.14
Tourism will not hamper the aesthetic values	75	48	46.1	36	45.4	40	60	54.4	8.82	0.03	0.111

tourism (Table 6). Respondents were asked if they were aware of tourism, and it was found that the majority of the respondents (New Malidhar (82.22%), Lakhicherra (75.75%), Isacherra (50%), and Daralcherra (60.6%)) were aware of it. More than 60% of the respondents of the villages (like Indranagar, Telacherra, Marwacherra and Bandarkhal), however, were unaware of it. For respondents who were unaware, a thorough discussion was conducted about tourism. Then, in subsequent questionnaire session, it was found that the majority of the respondents from Indranagar (56.25%), Daralcherra (68.57%), and New Malidhar (55.55%) villages would be happy if tourism is promoted, while in case of other villages, majority (>40%) were neutral regarding the same. Respondents from Indranagar (56.25%), Lakhicherra (60.6%), and Daralcherra (57.14%) believed that tourism would eventually increase their source of income while more than 45% of the respondents of other three villages had a neutral response. Further, majority of the respondents from Indranagar (75%), Telacherra (59.52%), Marwacherra (56.41%), Bandarkhal (56%), and New Malidhar (64.44%) believe that tourism would cause no harm to their cultural taboos, while more than 54.54% respondents of the other two villages were neutral.

## DISCUSSION

Majority of the inhabitants living around the vicinity of the BWS came to this part of Assam from neighbouring hills of Meghalaya, Karbi Anglong, and Dima Hasao for settlement, and their primary source of their livelihood is agriculture. After the declaration of wildlife sanctuary (in 2004) many areas have been restored as protected areas. Consequently, there has been shrinkage of the lands for agriculture due to restriction of the fringe areas of the sanctuary. These settlements with the tribal-dominated population had been converted into revenue village under the provisions of the Schedule Tribes and Other Traditional Forest Dwellers (Recognition of Forest

Rights) Act, 2006. Thus, these local communities have positive attitudes towards the sanctuary as such they were benefitted with land for permanent settlement and cultivation, especially 'jhum' (slash & burn) cultivation. People are also of the opinion that some part of the sanctuary should be protected as it conserves resource and reduces hunting of wildlife in this part. Such resolution for settlement does not reduce their problems of living completely, as socio-economic condition of these people is poor. The land allocated to them for settlements and farming is not sufficient. Besides, poor road communication has deprived them from basic requirements. This is the reason why many respondents had negative perceptions, and were of the opinion that state or central government should spend money for the welfare of the people rather than investing on animals and the forest. Further, the respondents had very less choice of livelihood since the sanctuary provides no other opportunities, and in turn increases their dependence on the sanctuary. Therefore, many respondents condemned the decision to not allow the collection of forest products, and respondents are not in full agreement with the spirit of conservation.

In our hypothesis, we assumed that socio-economic factors, knowledge of the sanctuary and knowledge of forest management have large influence and our logistic model, showed the significance of these variables in influencing the perception towards the sanctuary. Similar finding has also been observed in previous studies from other protected areas (Kideghesho et al. 2007; Karanth & Nepal 2012; Htun et al. 2012; Dewu & Roskaft 2018). These results confirm that socio-economic benefit may lead to positive attitudes towards the protected areas while socio-economic problems may lead to negative attitudes. Our logistic model does show significant influence of the income status, measured as monthly income, on their perception; the same is quite low to fulfill their basic requirements. In fact, one of the persistence problems within the local is lacking of social-economic benefit and this is very important in achieving positive attitude for protection of the sanctuary (Oldekop

et al. 2016).

In our Model 5, people around the sanctuary believe that wildlife is beneficial for the forest, and they showed positive attitudes towards the wildlife (mammals) of this sanctuary. Having the traditional values of conservation ingrained in their ethos and belief, they believe in co-existence of both human and wildlife, and understand their importance as well. Further, they know about the wildlife and forest laws. All these factors influence their opinion that hunting is awful. Concurrently, losses caused due to some wild animals have led to negative attitudes. Their agricultural practice mainly includes jhum cultivation, crop production like paddy, potato, tomato, cabbage, and some other vegetables. Crops usually attract wild animals, especially primates like Rhesus Macaque, and others like Wild Boar *Sus scrofa*. Villagers also have monoculture plantations of *Areca catechu*. Species like Hoary-bellied Himalayan Squirrel *Callosciurus pygerythrus* usually nibble on fruits of *Areca catechu* thereby reducing production. Arboreal animals, like primates and squirrels, 'damage' *Piper betel*, and the locals believe that these animals spread a plant disease which dry the plant leaf and vines entirely (locally called 'Utram'—the disease occurs as dark brownish spots in leaf which spreads to the entire plant, ultimately killing the plant). This plant disease, however, occurs due to high rainfall and humidity (Akhter et al. 2013). All these give rise to negative perceptions about wildlife. In such a situation, some people are forced to get rid of these species, and thus, do anything (including killing) just to reduce crop damage. Poachers use such opportunities to kill animals and they also target animals other than crop foragers. Thus, 'problematic' species cause unfavourable attitudes of people for other species as well. Some mentions of the problematic species, in the villages are Wild Boar, Rhesus Macaque, Hoary-bellied Himalayan Squirrel, Indian Muntjac *Muntiacus muntjac*, Jungle Cat *Felis chaus*, Large Indian Civet *Viverra zibetha*, and Small Indian Civet *Viverricula indica*. In fact, the villagers are of the view that these problematic species have increased in number, which may be due to frequent encounters with these species as well as their conservation in the sanctuary. We assume that both awareness of wildlife law and losses by animals would influence the attitudes towards the wildlife of the sanctuary, which is supported by our logistic Model 5 and Model 6 as well. Thus, our findings are in complete agreement with other studies (Kideghesho et al. 2007; Karanth & Kudalkar 2017; Dewu & Roskaft 2018), that losses by animals may eventually lead to more negative perception. Such attitudes were more common to the respondents with more variety of

farming.

Tourism can offer significant benefits to this sanctuary in the form of revenue to be used for conservation and management. Simultaneously, it provides benefits for the local communities (Goodwin 1996; Walpole & Goodwin 2001). In the study area, the respondents showed almost unanimous support for tourism. Regardless of their positive attitudes towards tourism, a few local people believed that they would not benefit, as outsiders would take advantage. It is obvious to have such thoughts as people of this area are inexperienced to tourism, however, it also draws our attention to prepare a better plan before initiating this concept of tourism. The planning should support equitable benefits for local as well. Engagement of unemployed youths of the fringe villages in different activities like guiding tourist and researchers will enhance community well-being. Such participation in different field activities would eventually increase their knowledge on fauna and flora present in the sanctuary. These would generate alternative livelihood sources other than agricultural activities, and encourage local people to conserve wild animals. Further, tourism management should be done considering the sentiments of the local people. Overall, positive attitude may be attributable to the early stage of development of tourism locally (Walpole & Goodwin 2001).

The concept of tourism can be further flourished with the introduction of 'homestay'. In this, people offer food and lodging to the tourist in exchange for money. The concept has been recently popularized in many parts of India like Arunachal Pradesh, Sikkim, Nagaland, Assam, Kerala, Uttarakhand and neighbouring Nepal; in the vicinity of protected areas. It eventually catches the attention of many international tourists as they are fascinated with indigenous/ local lifestyle of the host (Wang 2007; Bhalla et al. 2016). Further, structural design of these small houses with vernacular and tradition looks makes them attractive (Singh 1991; Bhalla et al. 2016). Thus, homestays can be an effective step to provide alternative income opportunity for the villagers (Dutta 2012; Bhalla et al. 2016)

So far as the management is concerned, most of the forest officials perform their duties sincerely. Lack of work force and proper equipment, however, poses difficulties. Relations between forest villagers and forest officials is very crucial for implementing any management strategy, as negative relation often gives rise to disputes that may sometimes bring about negative perceptions on wild animals. Under the present scenario, forest officials maintain good relation with local community people and

often help them, this ensures good management.

## CONCLUSION

The BWS forms a basis of wildlife conservation in this entire northeastern region of India. The people surrounding the sanctuary had positive and negative perception towards the sanctuary as well as its wildlife. Their perceptions are significantly influenced by their socio-economic factors, knowledge of the sanctuary, and forest management. Losses by animals, income status and knowledge significantly influence their perception towards mammals of this sanctuary. In this context, if problems between the local community and the sanctuary can be resolved or if management strategies are planned to provide benefits to the locals, effective conservation can be done. Severe losses by animals may be mitigated to minimal loss. Such strategies would eventually stand with a hope to reverse the prevailing threats and premeditate for threats in the future. Further, the findings may be used as a model for formulating long-term and effective conservation strategies in other protected areas with similar scenario.

## Recommendation

⌚ **Alternative livelihood**—As jhum cultivation is the primary source of their livelihood which is done in the vicinity area of the sanctuary, it may pose a threat to the entire fauna and flora. Therefore, if it is replaced by alternatives like high yielding crop varieties, their income and social wellbeing may be improved, and jhum (slash and burn) cultivation practice may be reduced. The locals may be provided with vocational, technical and skill trainings.

⌚ **Protection to problematic species**—Protection needs to be focused for the ‘problematic’ species like Rhesus Macaque, Small Indian Civet, Large Indian Civet, Hoary-bellied Himalayan Squirrels, Wild Boar and Indian Muntjac as they are mostly targeted by the people. Negative interactions with these foraging animals can be stopped if the sanctuary management creates an area near the buffer zone of the sanctuary in which food plants are grown. This may reduce crop raiding and improve positive attitudes of the locals. Thus, the locals would not facilitate poachers or hunters.

⌚ **Employment of local people**—Inclusion of people belonging to local community in jobs in the Department of Forest (of both central and state governments) would serve several purposes. For instance, it would improve their socio-economic status thereby decreasing their dependence on BWS, develop

a positive perception towards the sanctuary, and importantly since these people are well aware of the area they would be better managers and protectors of the sanctuary.

⌚ **Encouraging Tourism**—Tourism should be encouraged, and funds for small houses for home-stay should be allocated, so that unemployed local people may get involved. This would not only give an alternative source of income but also inculcate the intent of conserving wildlife.

⌚ **Facility to forest officials**—Proper facilities, including arms and ammunitions, should be supplied to the forest officials and guards to enable them to better monitor.

⌚ **Awareness**—Mass awareness campaigns must be conducted involving locals, political leaders, media persons, NGOs and administration, and locals especially school-going children and youths should be made aware of the ecosystem services, wildlife laws, etc.

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**Author contribution:** ASC—design of the concept, writing of manuscript, analysis and data collection. RAB—data collection, design of the concept. PC—writing of manuscript, analysis of data.





## AN ASSESSMENT OF BIRD COMMUNITIES ACROSS UJJANI AND ITS FIVE SATELLITE WETLANDS IN SOLAPUR DISTRICT OF MAHARASHTRA, INDIA

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**Abstract:** Ujjani wetland is a potential Ramsar site in Maharashtra, India with several satellite wetlands associated with it. The present study contributes to single large or several small habitat conservation theories by assessing wetland bird communities. Aquatic bird communities were assessed using area search and point count methods at Kumbhargaoon (Ujjani), Bhadalwadi, Madanwadi, Palasdev, Pimple and Ravangaon wetlands between October 2011 and September 2012. These are representative satellite wetlands around Ujjani. One-hundred-and-ten species of wetland birds across 12 orders and 29 families were recorded. Out of these, 66 were resident and 44 were found to be migrants. These birds represent 23% mudflat feeder, 16% upland feeder, 14% marsh feeder, 12% bird of prey, 11% surface feeder and fish eaters, while divers and wet meadow feeders were represented with 8.5% and 5% of the species, respectively. Among the birds recorded, Woolly-necked Stork *Ciconia episcopus*, Common Pochard *Aythya farina*, and Greater Spotted Eagle *Clanga clanga* belong to the Vulnerable category; while Eurasian Curlew *Numenius arquata*, Oriental Darter *Anhinga melanogaster*, Black-tailed Godwit *Limosa limosa*, Black-headed Ibis *Threskiornis melanocephalus*, Lesser Flamingo *Phoeniconaias minor*, Painted Stork *Mycteria leucocephala*, River Tern *Sterna aurantia*, and Great Thick-knee *Esacus recurvirostris* represent Near Threatened category on the IUCN Red List. The presence of these bird species underlines the importance and conservation priorities of a major as well as smaller satellite wetlands. Anthropogenic activities such as cattle grazing, fishing, sand and soil mining, land encroachment, urban development and tourism were observed as some of the threats to this wetland ecosystem as well as bird communities.

**Keywords:** Aquatic birds, Ramsar site, SLOSS, wetland bird communities.

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**Author contribution:** SPK—contributed in research idea development and its implementation on field, sample collection and analysis. SVM—contributed in designing of experiments and provided critical remarks on manuscript. PAK—provided inputs for deciding study area and sampling sites. Also contributed in technical aspects for wetland mapping. PVA—contributed in wetland ecosystem monitoring, field data collection and identification of bird species.

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## INTRODUCTION

Wetlands are distinct zones intermediate between terrestrial and aquatic ecosystem where the water table is usually at or near the surface of the land and is covered by shallow water (Semeniuk & Semeniuk 1995). Wetlands are the most productive ecosystems in the world (Mitsch et al. 2009). Natural and man-made reservoirs form small heterogenous water patches in their vicinity. Such small wetlands associated with large streams are called satellite wetlands (Bedford 1996; Novitski et al. 1996; Patten et al. 2008).

Wetlands maintain the health of the environment and support a rich biodiversity. They complete habitat requirements of various water birds. Birds are excellent indicators of wetland health (Kumar & Gupta 2013). Habitat protection is important to conserve bird communities associated with it.

Large wetlands normally receive all the importance while smaller and isolated wetlands receive least attention and are often neglected from conservation priorities. Many studies have focused on major and larger wetlands while very few scientific investigations have been undertaken on comparatively small, isolated and not so well-known wetlands. In the absence of such studies, smaller and isolated wetlands are neglected from appearing in conservation priorities even if they are located in the vicinity of a major wetland.

On a theoretical conservation perspective, single large or several small (SLOSS) habitats debate is well known; many ecologists argue for one large habitat while an equal number of scientists advocate for many small habitats (Ma et al. 2010). It was argued that species richness increases with habitat area and hence larger block of habitat would support more species than any of the smaller blocks. Further, Simberloff & Abele (1976) contested that if the smaller protected area had unshared species, then it is possible that two smaller reserves could have more species than a single large reserve.

Before we take any stand on such perspectives, it is primarily required to initiate studies that compares a large wetland and many smaller ones. Such studies can help in deciding conservation priorities in fragmented habitats. In this context, the present study explores the status of bird communities harboured at one large wetland and several small satellite wetlands associated with it. Ujjani wetland and its satellite wetlands were assessed for this work.

The Ujjani Dam is an earthen-cum-masonry gravity dam located on river Bhima. The Bhima River is a

tributary of river Krishna that originates in the ranges of the Western Ghats. This dam is amongst the largest dams in Maharashtra, situated near Ujjani Village of Solapur District. The dam was primarily built for irrigating water-scarce fields. It is located at 18.299°N & 74.763°E, 465m. This region is a plain expanse with negligible slope and the reservoir is spread across 348 km<sup>2</sup> (Mahabal et al. 2011). It has more of shallow areas at the fringe parts of the reservoir, that provide a distinctive habitat for the avifauna. The Ujjani wetland is a potential Ramsar site in Maharashtra (Samant 2002; Islam & Rahmani 2008).

## MATERIAL AND METHODS

### Study area

The Ujjani wetland has a huge expanse and to overcome practical difficulty in sampling, Kumbhargaoon (18.266°N & 74.805°E, 494m), a representative location of Ujjani wetland was selected for sampling as it covers very shallow to very deep-water areas. As Ujjani wetland is dam backwater, similar type of five man-made satellite wetlands, viz., Bhadalwadi (18.234°N & 74.781°E, 511m), Madanwadi (18.285°N & 74.707°E, 515m), Ravangaon (18.330°N & 74.613°E, 556m), Pimple (18.247°N & 74.729°E, 516m), and Palasdev (18.211°N & 74.865°E, 507m) associated with Ujjani wetland was selected for the present study. In order to know the exact location of these wetlands, satellite imagery was superimposed on the toposheet. A map of these sites was prepared to indicate relative locations of these wetlands with respect to Ujjani with the help of QGIS 2.18 (Image 1).

### Bird survey

The study was conducted between October 2011 and September 2012. Counts were conducted near the wetland where all or most of the surface area and edge were visible (Bibby et al. 2000). The survey duration includes the time required to thoroughly scan a wetland. Sampling was conducted using area search and point count method. Point counts were taken for areas where visibility is obstructed like marshy area and upland vegetation. No two-point counts were taken within a distance of 200m. Care was taken to ensure that birds were recorded only once (Bibby et al. 2000, Weller 1999). Area search methods were used for areas with clear visibility like open water. The accessible edges of wetlands were walked around to detect any unseen birds. The birds were observed during the peak hours of their activity from sunrise to 10.00h and 16.00–18.00



Image 1. Ujjani and its satellite Wetlands (Source: Google Image 2011).

h using Olympus (8 x 40 mm, 10 x 50 mm) binoculars. Identification of birds was done using field guides (Ali & Ripley 1995; Grimmett et al. 2013), and only those species with confirmed identity were recorded and reported.

#### Data classification and analysis

Recording and listing of these birds were done using standard common and scientific names (Praveen et al. 2016). Residential status of the birds as resident and migrants had been assigned with reference to the study area on the basis of presence or absence method. The status of the recorded bird species was established on the basis of frequency of sightings (Kumar & Gupta 2009) as Abundant (A) recorded 9–10 times out of 10 visits, Common (C) recorded 7–8 times out of 10 visits, Frequent (F) recorded 5–6 times out of 10 visits, Occasional (O) recorded 3–5 times out of 10 visits, Rare (R) recorded 0–2 times out of 10 visits (Therivel & Morris 1995).

Birds were delineated in eight feeding categories as bird of prey, fish eaters, divers, mudflat feeder, marsh feeder, wet meadow, surface feeder ducks, and upland feeders (Gole 1993).

To compare wetlands, a cluster analysis was performed on the presence of bird communities. Cluster analysis was performed using Jaccard's similarity

measure and a paired group method by PAST 3 software (Field & McFarlane 1968; Day & Edelsbrunner 1984; Washington 1984; Hartzell et al. 2007). The conservation status of the observed species was listed using the IUCN Red List, 2016 ver3.1 (IUCN 2016).

#### RESULTS AND DISCUSSION

One-hundred-and-ten species of wetland bird genera belonging to 12 orders and 29 families were recorded from Kumbhargaoon (Ujjani) and its satellite wetlands between October 2011–September 2012.

The checklist of birds observed, identified and recorded in the region along with their family and resident status is provided in Table 1. It was observed that Anatidae (16 species) followed by Scolopacidae (14 species) were the most represented families of the study area (Figure 1); whereas, Anhingidae, Dicuridae, Falconidae, Gruidae, Meropidae, Pandionidae, Podicipedidae, Recurvirostridae, and Rostratulidae were represented by just a single genus and least represented.

Among the recorded species 66 species were resident and 44 migratory. Relative abundance studies revealed that six species were abundant, 21 species were common, 46 species were frequent, 25 occasional and 12 were rare. Woolly-necked Stork *Ciconia episcopus*,

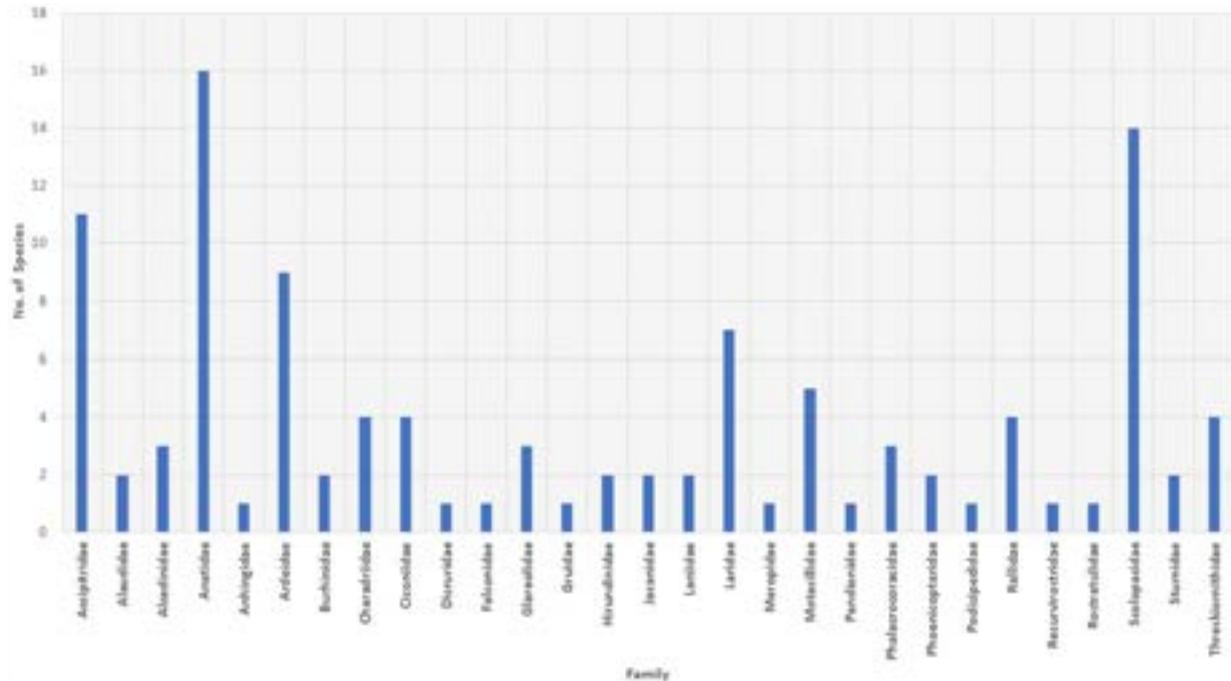


Figure 1. Distribution of bird species across families.

Common Pochard *Aythya farina* and Greater Spotted Eagle *Clanga clanga* were found to belong to the Vulnerable category of the IUCN and their number is found to be decreasing globally; while Eurasian Curlew *Numenius arquata*, Oriental Darter *Anhinga melanogaster*, Black-tailed Godwit *Limosa limosa*, Black-headed Ibis *Threskiornis melanocephalus*, Lesser Flamingo *Phoeniconaias minor*, Painted Stork *Mycteria leucocephala*, River Tern *Sterna aurantia*, and Great Thick-knee *Esacus recurvirostris* represent the Near Threatened (NT) category of IUCN Red List.

Feeding class-wise distribution of observed species indicated that mudflat feeders were most common with 23% species, followed by upland feeders with 16%, marsh feeders with 14%, bird of prey with 12%, surface feeders and fish eaters each with 11%, while divers and wet meadow feeders were represented with 8.5% and 5% of the species, respectively (Figure 2).

Relative abundance analysis indicates Common Coot *Fulica atra* as most abundant at Kumbhargoan (Ujjani) and Palasdev while Oriental Darter *Anhinga melanogaster* and Eurasian Curlew *Numenius arquata* were least abundant. The Painted Stork *Mycteria leucocephala* and Rosy Starling *Pastor roseus* were found to be most abundant at Bhadalwadi wetland and Eurasian Curlew *Numenius arquata*, Pheasant-tailed Jacana *Hydrophasianus chirurgus* were least abundant. At Madanwadi wetland Common Sandpiper

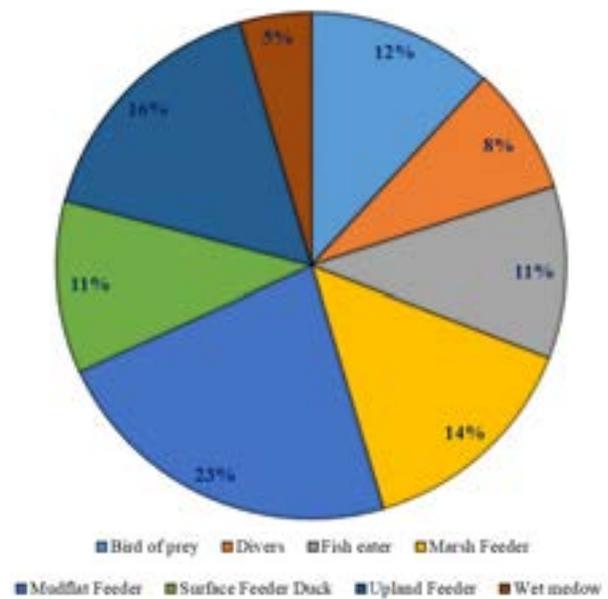


Figure 2. Representation of species (%) across feeding habitat class.

*Actitis hypoleucos*, Little Ringed Plover *Charadrius dubius* showed highest relative abundance and Eurasian Thick-knee *Burhinus oedipnemus*, Great Cormorant *Phalacrocorax carbo* recorded the lowest. The Common Coot *Fulica atra* and Brahminy Starling *Sturnia pagodarum* were found to be most abundant at Pimple and Ravangaon wetland respectively while Great Egret *Ardea alba* and Common Moorhen *Gallinula chloropus*

Table 1. Status of birds recorded at Ujjani and its satellite wetlands, Maharashtra.

	Family & Species	Wetland	Month	Resident status	Abundance	Red List Status
	<b>Accipitridae</b>					
1	Black-winged Kite <i>Elanus caeruleus</i>	Uj, Bh, Ma, Pa, Ra	Jan–Dec	R	F	LC
2	Black Kite <i>Milvus migrans</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	F	LC
3	Brahminy Kite <i>Haliastur indus</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
4	Greater Spotted Eagle <i>Clanga clanga</i>	Uj	Feb	M	O	VU
5	Crested Serpent Eagle <i>Spilornis cheela</i>	Uj, Bh, Pa, Ra	Jan–Dec	R	F	LC
6	Western Marsh Harrier <i>Circus aeruginosus</i>	Uj, Bh, Pa	Dec–Feb	M	F	LC
7	Montagu's Harrier <i>Circus pygargus</i>	Uj, Pa	Dec–Feb	M	R	LC
8	Bonelli's Eagle <i>Aquila fasciata</i>	Uj, Ra	Nov–Feb	R	O	LC
9	Tawny Eagle <i>Aquila rapax</i>	Uj, Ma, Ra	Jan–Dec	R	O	LC
10	Short-toed Snake Eagle <i>Circaetus gallicus</i>	Uj, Bh, Pa, Ra	Jan–Dec	R	O	LC
11	Shikra <i>Accipiter badius</i>	Uj, Pa	Sep–Dec	R	O	LC
	<b>Alaudidae</b>					
12	Indian Bushlark <i>Mirafra erythroptera</i>	Uj, Ma, Pa, Ra	Jan–Dec	R	C	LC
13	Rufous-tailed Lark <i>Ammomanes phoenicura</i>	Uj, Ma	Jan–Dec	R	F	LC
	<b>Alcedinidae</b>					
14	Common Kingfisher <i>Alcedo atthis</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
15	Pied Kingfisher <i>Ceryle rudis</i>	Uj, Bh, Pa	Jan–Dec	R	F	LC
16	White-throated Kingfisher <i>Halcyon smyrnensis</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	F	LC
	<b>Anatidae</b>					
17	Indian Spot-billed Duck <i>Anas poecilorhyncha</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
18	Comb Duck <i>Sarkidiornis melanotos</i>	Uj, Pa	Oct–Mar	R	R	LC
19	Lesser Whistling Duck <i>Dendrocygna javanica</i>	Uj, Bh, Pa	Jan–Dec	R	C	LC
20	Tufted Duck <i>Aythya fuligula</i>	Uj, Bh, Pa	Nov–Feb	M	O	LC
21	Eurasian Wigeon <i>Mareca penelope</i>	Uj, Bh, Pa	Nov–Mar	M	O	LC
22	Gadwall <i>Mareca strepera</i>	Uj, Bh, Pa	Nov–Feb	M	F	LC
23	Garganey <i>Spatula querquedula</i>	Uj, Bh, Ma, Pa, Pi, Ra	Oct–Mar	R	F	LC
24	Bar-headed Goose <i>Anser indicus</i>	Uj, Pa	Nov–Mar	M	F	LC
25	Cotton Teal <i>Nettapus coromandelianus</i>	Uj, Bh, Ma, Pa	Oct–Jan	R	O	LC
26	Northern Pintail <i>Anas acuta</i>	Uj, Bh, Pa	Nov–Feb	M	F	LC
27	Northern Shoveler <i>Spatula clypeata</i>	Uj, Bh, Pa	Nov–Mar	M	O	LC
28	Common Pochard <i>Aythya ferina</i>	Uj, Bh, Pa	Jan–Feb	M	R	VU
29	Red-crested Pochard <i>Netta rufina</i>	Uj, Pa	Jan–Feb	M	R	LC
30	Ruddy Shelduck <i>Tadorna ferruginea</i>	Uj, Bh, Ma, Pa	Nov–Mar	M	F	LC
31	Common Teal <i>Anas crecca</i>	Uj, Bh, Pa, Pi	Nov–Feb	R	F	LC
32	Common Shelduck <i>Tadorna tadorna</i>	Uj, Bh, Ma, Pa	Dec–Jan	M	R	LC
	<b>Anhingidae</b>					
33	Oriental Darter <i>Anhinga melanogaster</i>	Uj, Bh, Ma, Pa	Oct–Feb	R	R	NT
	<b>Ardeidae</b>					
34	Cattle Egret <i>Bubulcus ibis</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
35	Great Egret <i>Ardea alba</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
36	Intermediate Egret <i>Ardea intermedia</i>	Uj, Bh, Ma, Pa	Jan–Dec	R	F	LC
37	Little Egret <i>Egretta garzetta</i>	Uj, Bh, Ma, Pa, Pi	Jan–Dec	R	C	LC

	Family & Species	Wetland	Month	Resident status	Abundance	Red List Status
38	Black-crowned Night Heron <i>Nycticorax nycticorax</i>	Uj, Bh, Ma, Pa	Jan–Dec	R	O	LC
39	Grey Heron <i>Ardea cinerea</i>	Uj, Bh, Ma, Pa, Pi, Ra	Oct–May	R	C	LC
40	Indian Pond Heron <i>Ardeola grayii</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
41	Striated Heron <i>Butorides striata</i>	Uj, Bh, Ma, Pa, Pi	Jan–Dec	R	F	LC
42	Purple Heron <i>Ardea purpurea</i>	Uj, Bh, Ma, Pa	Jan–Dec	R	F	LC
	<b>Burhinidae</b>					
43	Eurasian Thick-knee <i>Burhinus oedicnemus</i>	Uj, Ma, Pa, Pi	Jan–Dec	R	O	LC
44	Great Thick-knee <i>Esacus recurvirostris</i>	Uj, Bh, Pa	Jan–Dec	R	R	NT
	<b>Charadriidae</b>					
45	Red-wattled Lapwing <i>Vanellus indicus</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	F	LC
46	Yellow-wattled Lapwing <i>Vanellus malabaricus</i>	Uj, Ma, Pa	Jan–Dec	R	O	LC
47	Kentish Plover <i>Charadrius alexandrinus</i>	Uj, Ma	Nov–Feb	R	O	LC
48	Little Ringed Plover <i>Charadrius dubius</i>	Uj, Ma, Pa, Pi	Jan–Dec	R	A	LC
	<b>Ciconiidae</b>					
49	Asian Openbill <i>Anastomus oscitans</i>	Uj, Bh, Pa	Jan–Dec	R	F	LC
50	Painted Stork <i>Mycteria leucocephala</i>	Uj, Bh, Pa	Jan–Dec	R	C	NT
51	European White Stork <i>Ciconia ciconia</i>	Uj, Pa	Nov–Jan	R	F	LC
52	Woolly-necked Stork <i>Ciconia episcopus</i>	Uj, Bh, Ma, Pa	Oct–Dec	R	O	VU
	<b>Dicruridae</b>					
53	Black Drongo <i>Dicrurus macrocercus</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	C	LC
	<b>Falconidae</b>					
54	Common Kestrel <i>Falco tinnunculus</i>	Uj, Pa, Ra	Nov–Feb	M	C	LC
	<b>Glareolidae</b>					
55	Collared Pratincole <i>Glareola pratincola</i>	Uj, Ma	Oct–May	M	F	LC
56	Oriental Pratincole <i>Glareola maldivarum</i>	Uj, Ma	Oct–May	M	F	LC
57	Little Pratincole <i>Glareola lactea</i>	Uj, Ma, Pa	Jan–Dec	R	A	LC
	<b>Gruidae</b>					
58	Demoiselle Crane <i>Grus virgo</i>	Uj	Jan	M	O	LC
	<b>Hirundinidae</b>					
59	Barn Swallow <i>Hirundo rustica</i>	Uj, Bh, Ma, Pa, Pi, Ra	Oct–Jan	M	C	LC
60	Wire-tailed Swallow <i>Hirundo smithii</i>	Uj, Bh, Ma, Pa, Pi, Ra	Oct–Jan	M	F	LC
	<b>Jacanidae</b>					
61	Bronze-winged Jacana <i>Metopidius indicus</i>	Uj, Bh	Jul	R	R	LC
62	Pheasant-tailed Jacana <i>Hydrophasianus chirurgus</i>	Uj, Bh	Aug	R	R	LC
	<b>Laniidae</b>					
63	Bay-backed Shrike <i>Lanius vittatus</i>	Uj, Bh, Ma, Pa, Pi	Jan–Dec	R	C	LC
64	Long-tailed Shrike <i>Lanius schach</i>	Uj, Pa, Ra	Jan–Dec	R	C	LC
	<b>Laridae</b>					
65	Black-headed Gull <i>Chroicocephalus ridibundus</i>	Uj, Pa	Nov–Feb	M	F	LC
66	Brown-headed Gull <i>Chroicocephalus brunnicephalus</i>	Uj, Pa	Nov–Feb	M	F	LC
67	Pallas's Gull <i>Ichthyophaga ichthyophaga</i>	Uj, Pa	Dec–Jan	M	O	LC
68	Caspian Tern <i>Hydroprogne caspia</i>	Uj, Pa	Dec–Mar	M	F	LC
69	Gull-billed Tern <i>Gelochelidon nilotica</i>	Uj, Pa	Dec–Mar	M	F	LC
70	River Tern <i>Sterna aurantia</i>	Uj, Pa	Dec–Mar	M	F	NT

	Family & Species	Wetland	Month	Resident status	Abundance	Red List Status
71	Whiskered Tern <i>Chlidonias hybrida</i>	Uj, Pa	Jan–Mar	R	F	LC
	<b>Meropidae</b>					
72	Green Bee-eater, <i>Merops orientalis</i>	Uj, Bh, Pa, Pi, Ra	Jan–Dec	R	C	LC
	<b>Motacillidae</b>					
73	Citrine Wagtail <i>Motacilla citreola</i>	Uj, Ma,	Nov–Feb	M	F	LC
74	Grey Wagtail <i>Motacilla cinerea</i>	Uj, Ma, Pa	Nov–Feb	M	C	LC
75	White-browed Wagtail <i>Motacilla maderaspatensis</i>	Uj, Ma, Ra	Jan–Dec	R	F	LC
76	Western Yellow Wagtail <i>Motacilla flava</i>	Uj, Pa	Nov–Feb	M	C	LC
77	White Wagtail <i>Motacilla alba</i>	Uj	Jan–Feb	M	F	LC
	<b>Pandionidae</b>					
78	Osprey <i>Pandion haliaetus</i>	Uj, Pa	Dec–Feb	M	O	LC
	<b>Phalacrocoracidae</b>					
79	Great Cormorant <i>Phalacrocorax carbo</i>	Uj, Bh, Ma, Pa,	Oct–Jan	R	F	LC
80	Indian Cormorant <i>Phalacrocorax fuscicollis</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	A	LC
81	Little Cormorant <i>Microcarbo niger</i>	Uj, Bh, Ma, Pa, Pi, Ra	Jan–Dec	R	A	LC
82	Greater Flamingo <i>Phoenicopterus roseus</i>	Uj, Pa	Jan–Feb	M	O	LC
83	Lesser Flamingo <i>Phoeniconaias minor</i>	Uj, Pa	Feb–Mar	M	R	NT
	<b>Podicipedidae</b>					
84	Little Grebe <i>Tachybaptus ruficollis</i>	Uj, Bh, Ma, Pa, Pi	Jan–Dec	R	A	LC
	<b>Rallidae</b>					
85	Common Coot <i>Fulica atra</i>	Uj, Bh, Pa, Pi	Jan–Dec	R	A	LC
86	Common Moorhen <i>Gallinula chloropus</i>	Uj, Bh, Ma, Pa, Pi	Jul–Oct	R	F	LC
87	Purple Swamphen <i>Porphyrio porphyrio</i>	Uj, Bh, Ma, Pa, Pi	Jan–Dec	R	F	LC
88	White-breasted Waterhen <i>Amaurornis phoenicurus</i>	Uj, Bh, Ma, Pa, Pi	Jul–Oct	R	F	LC
	<b>Recurvirostridae</b>					
89	Black-winged Stilt <i>Himantopus himantopus</i>	Uj, Ma, Pa, Ra	Jan–Dec	R	C	LC
	<b>Rostratulidae</b>					
90	Greater Painted-snipe <i>Rostratula benghalensis</i>	Uj, Pa	Jan–Dec	R	O	LC
	<b>Scolopacidae</b>					
91	Eurasian Curlew <i>Numenius arquata</i>	Uj, Bh	Nov–Mar	R	R	NT
92	Black-tailed Godwit <i>Limosa limosa</i>	Uj, Pa	Nov–Feb	M	F	NT
93	Common Greenshank <i>Tringa nebularia</i>	Uj, Ma, Pa	Nov–Mar	M	F	LC
94	Common Redshank <i>Tringa totanus</i>	Uj, Ma, Pa	Oct–Mar	R	F	LC
95	Spotted Redshank <i>Tringa erythropus</i>	Uj, Pa	Oct–Par	R	O	LC
96	Ruff <i>Calidris pugnax</i>	Uj, Pa	Nov–Dec	R	R	LC
97	Common Sandpiper <i>Actitis hypoleucos</i>	Uj, Ma, Pa	Nov–May	M	F	LC
98	Green Sandpiper <i>Tringa ochropus</i>	Uj, Pa	Nov–May	M	F	LC
99	Marsh Sandpiper <i>Tringa stagnatilis</i>	Uj, Pa	Nov–Jan	M	O	LC
100	Wood Sandpiper <i>Tringa glareola</i>	Uj, Pa	Jan–Apr	M	O	LC
101	Common Snipe <i>Gallinago gallinago</i>	Uj, Bh, Ma, Pa	Nov–Feb	M	F	LC
102	Pintail Snipe <i>Gallinago stenura</i>	Uj, Pa	Nov–Feb	M	O	LC
103	Little Stint <i>Calidris minuta</i>	Uj, Ma, Pa, Pi	Oct–Mar	M	F	LC
104	Temminck's Stint <i>Calidris temminckii</i>	Uj, Bh	Jan–Feb	M	O	LC

	Family & Species	Wetland	Month	Resident status	Abundance	Red List Status
	<b>Sturnidae</b>					
105	Rosy Starling <i>Pastor roseus</i>	Uj, Bh, Pa	Dec–Feb	M	F	LC
106	Brahminy Starling <i>Sturnia pagodarum</i>	Uj, Ma, Pa, Ra	Jan–Dec	R	C	LC
	<b>Threskiornithidae</b>					
107	Black-headed Ibis <i>Threskiornis melanocephalus</i>	Uj, Bh, Ma, Pa	Sep–Oct	R	F	NT
108	Glossy Ibis <i>Plegadis falcinellus</i>	Uj, Bh, Ma, Pa	Oct–Jan	M	O	LC
109	Indian Black Ibis <i>Pseudibis papillosa</i>	Uj, Bh, Ma, Pa, Pi	Jan–Dec	R	F	LC
110	Eurasian Spoonbill <i>Platalea leucorodia</i>	Uj, Bh, Pa	Jan–Dec	R	F	LC

Uj—Ujjani | Bh—Bhadalwadi | Ma—Madanwadi | Pa—Palasdev | Pi—Pimple | Ra—Ravangaon | R—Resident | M—Migratory | LC—Least Concerned | NT—Near Threatened | VU—Vulnerable | A—Abundant | C—Common | F—Frequent | O—Occasional | R—Rare.

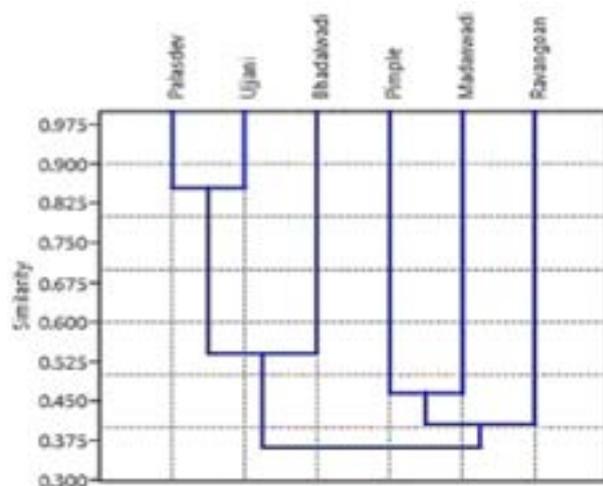


Figure 3. Similarity between satellite wetlands and Ujjani. Comparison was made using Jaccard's similarity index (Paired group-UPGMA).

were found to be least abundant.

When Shannon-Weiner diversity and evenness across satellite wetlands were calculated, it was found to be 3.5, 0.37 at Kumbhargoan (Ujjani) followed by 3.5, 0.38 at Palasdev; 3.1, 0.42 at Madanwadi, 2.6, 0.45 at Pimple, 2.5, 0.22 at Bhadalwadi, and 2.2, 0.33 Ravangaon wetland at  $p < 0.05$ .

Jaccard's similarity index was calculated from the record of occurrence of the bird species across these wetlands. It was observed that, Kumbhargoan (Ujjani) and Palasdev were most similar wetlands with index value of 0.88, while Kumbhargoan (Ujjani) and Ravangaon were most dissimilar in their species composition with index value of 0.36. Kumbhargoan (Ujjani) and Bhadalwadi was more similar with index value of 0.59 followed by Kumbhargoan (Ujjani) and Madanwadi with reported value of 0.49. Kumbhargoan (Ujjani) and Pimple to were

among least similar wetlands with value of 0.4. It reveals that, out of five associated wetlands of Ujjani reservoir; Palasdev, Bhadalwadi and Madanwadi wetlands show high resemblance and similarity for the inhabitation of wetland bird communities. Bird communities harboured by Ujjani and Ravangaon wetland were fairly distinct. Detailed cluster analysis paired (UPGMA) of Jaccard's similarity Index of each wetland was shown in Figure 3.

Present study provides checklist of wetland bird communities at Ujjani as well as its five satellite wetlands. Of the 110 bird species recorded at Kumbhargoan (Ujjani) wetland, 94 were recorded at Palasdev, while 59, 54, 31, and 29 species were reported at Bhadalwadi, Madanwadi, Pimple, and Ravangaon wetlands, respectively. Vital base line information on the presence and abundance of bird communities based on sightings were collected. Ujjani and other wetlands have shallow water expanse resulting in rich abundance and diversity of bird communities. The wetland bird communities are in general heterogeneous in their feeding habitat (Kumar & Gupta 2013). The diversity of the wetland birds observed at other satellite wetlands may indicate a presence of a wide spectrum of feeding niches. In the present study agriculture fields surrounding the Kumbhargoan (Ujjani) wetland and satellite wetlands with scattered plants, viz., *Acacia* species, *Zizyphus* species, and *Tamarindus* species, probably provide diverse roosting and foraging habitation grounds to the bird communities.

A comparison of bird communities of main wetlands with its satellite wetlands revealed that, some of the satellite wetlands support an almost equal number of bird species to that of the main wetland. Also, all these satellite wetlands together share, more than 95% of the total bird species composition of Ujjani wetland. Presence of the threatened bird species highlights the significance of the wetland as an important

conservation site (Islam & Rahmani 2004, 2008) and wintering ground for wetland birds. From conservation priorities both large as well as small wetlands are important. Reduction in existing anthropogenic activities like cattle grazing, fishing, sand and soil mining, land encroachment, urban development, and tourism would improve conservation status of bird communities. Detailed studies on physical characterization of wetlands and habitat preference by bird communities are necessary to understand the role of satellite wetlands in the conservation of avifauna.

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## GROWTH RATE OF CAPTIVE GHARIALS *GAVIALIS GANGETICUS* (GMELIN, 1789) (REPTILIA: CROCODYLIA: GAVIALIDAE) IN CHITWAN NATIONAL PARK, NEPAL

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**Abstract:** Gharials *Gavialis gangeticus* have been reared in ex situ facilities in the Gharial Conservation and Breeding Center (GCBC) in Chitwan National Park of Nepal since the 1980s. There remains a paucity of detailed information concerning their growth rates, particularly with respect to season. We randomly selected 20 gharials (45 months old) in the GCBC, tagged them, and recorded total length (TL) and weight over three warm (April–September) and two cold (October–March) seasons between 01 April 2013 and 30 September 2015. We also recorded amounts of fish consumed by these gharials every month over the 30-month period. On average per season, the gharials grew by  $9.48 \pm 3.63$  cm ( $1.58$  cm/month) in length and gained  $2.61 \pm 1.14$  kg ( $0.43$  kg/month). Growth rates were significantly higher during warm seasons. The highest increase in both length (mean =  $21.2 \pm 8.61$  cm) and weight (mean =  $5.59 \pm 2.12$  kg) occurred during the first warm season (April 2013–September 2013) of the study, and annual growth rate was also highest during the first year. Our data indicated strong correlation between mean length and body weight. A total of 2,103.9 kg fish was consumed by 20 gharials over 30 months, for a mean consumption of 3.5 kg fish per individual per month. Mean fish consumption was also significantly higher during warm ( $96.99 \pm 37.35$  kg) versus cold ( $29.83 \pm 17.09$  kg) seasons. Survival rate was 100%. Our findings establish baseline data for growth and feeding rates of captive gharials that will be useful in making management decisions in captive breeding and rearing facilities.

**Keywords:** Captive breeding, feeding, hatchlings, Narayani River, Rapti River, total length.

**Abbreviations:** GCBC—Gharial Conservation and Breeding Center | NP—National Park | TL—Total length.

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**Author contribution:** BK and AB conceptualized and designed the study. BK collected data and AB performed data analysis. BK and AB prepared the manuscript.

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## INTRODUCTION

Gharials *Gavialis gangeticus* are Critically Endangered crocodylians currently distributed in among 14 widely-spaced locations in India and Nepal (Lang et al. 2019). In Nepal this species occurs in the Karnali and Babai rivers in Bardia National Park (NP) and the Narayani and Rapti rivers in Chitwan NP (Fig. 1). Major threats are habitat destruction and alteration, water extraction, construction of dams and barrages, mortality in fishing nets and pollution (Lang et al. 2019). Gharial populations were reduced by more than 90% throughout their range, including Nepal, between 1930 and 1980 (Stevenson & Whitaker 2010). In response to this crisis, the Government of Nepal initiated an ex situ conservation program in Chitwan NP by establishing the Gharial Conservation and Breeding Center (GCBC) in 1978 (Fig. 1). The main goal of the GCBC has been to reinforce and maintain viable populations of gharials in situ (Maskey 1989; Khadka 2010). Scientific information on every aspect of captive breeding, incubating and rearing is critical to ensure efficient conservation programs (Maskey 1989; Ballouard et al. 2010). Although updated information is available on some aspects of nesting and reproduction of gharials in the GCBC (Ballouard et al. 2010; Khadka 2010, 2013), there is a knowledge gap concerning growth and feeding rates of captive animals

in Nepal. Historic information on survival, growth and feeding of hatchlings was reported by Maskey (1989), but there is no recent information available. To address this knowledge gap and inform management actions, we investigated and established baseline on growth and feeding rates of captive gharials in GCBC (Images 1 & 2).

## MATERIALS AND METHODS

### STUDY AREA

This study was conducted at GCBC facilities in Chitwan NP (27.400–27.813 °N and 83.880–84.830 °E; datum= WGS84; Fig. 1). Chitwan NP covers an area of 953km<sup>2</sup> and is located in Chitwan and Nawalparasi districts in south-central Nepal. It has a sub-tropical climate that can be broadly divided into three seasons: warm (March–May), monsoon (June–September), and cold (October–February) (Maskey 1989). Chitwan NP is drained by two major rivers (the Narayani and the Rapti), in which both Gharials and Mugger Crocodiles *Crocodylus palustris* occur.

### METHODS

GCBC collects Gharial eggs from the Narayani and Rapti rivers annually for incubation in semi-natural conditions. Hatchlings are reared for up to five years



Image 1. Group of Gharials *Gavialis gangeticus* feeding on fish at the Gharial Conservation and Breeding Center, Chitwan National Park, Nepal. Photograph by Bed Bahadur Khadka taken on 4 June 2015.



Image 2. A close-up picture of tagged Gharial feeding on fish at the Gharial Conservation and Breeding Center, Chitwan National Park, Nepal. Photograph by Bed Bahadur Khadka taken on 4 April 2013.

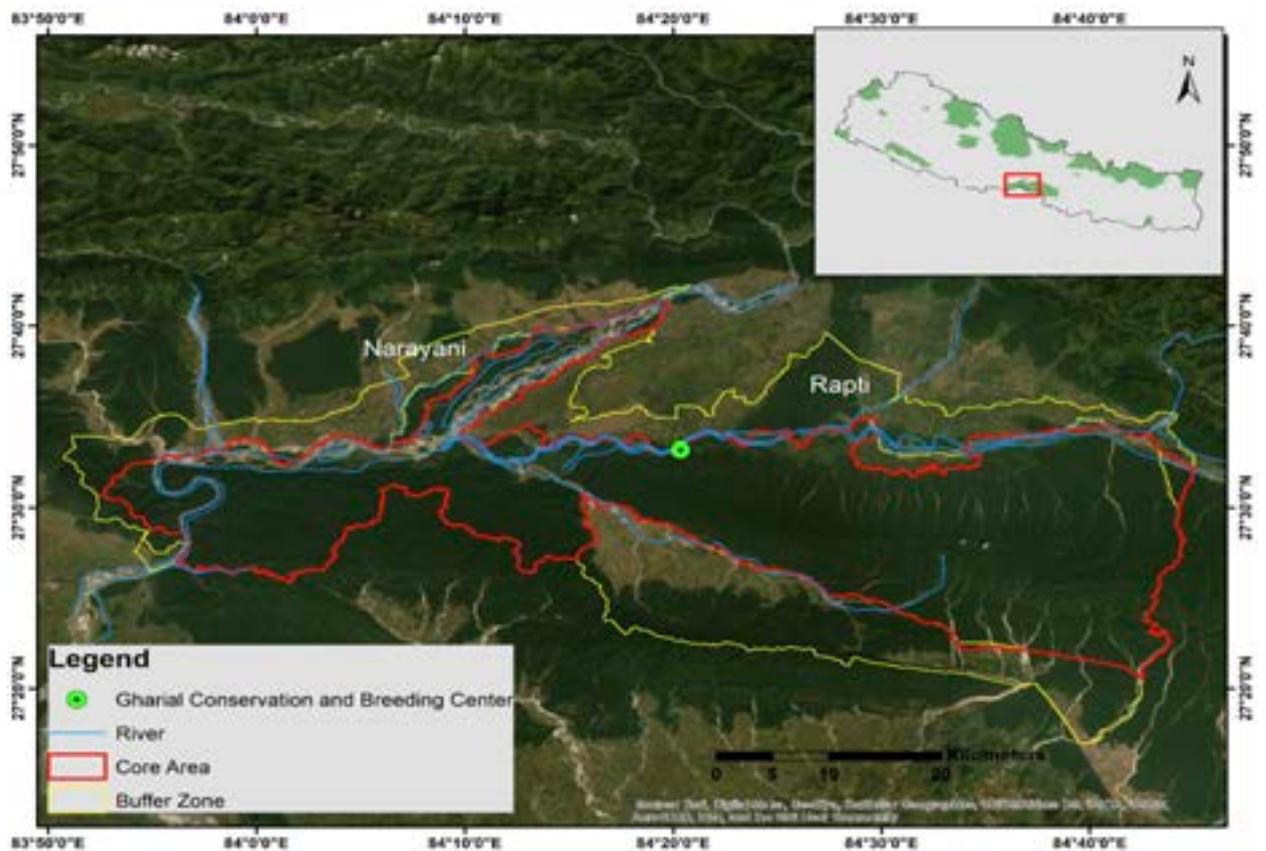


Figure 1. Map of Chitwan National Park showing location of the Gharial Conservation and Breeding Center.

until they attain around 1.5 m total length (TL; distance from anterior tip of the snout to the posterior tip of the tail), and eventually released into various rivers within their geographic range. GCBC has released 1,465 Gharials between 1981 and March 2019 in the Narayani, Rapti, Karnali, Babai, and Koshi rivers (Bed Dhakal, pers. comm. 06.vii.2019).

We randomly selected 20 Gharials which had hatched between the first and second week of June 2009, and tagged them with uniquely numbered plastic cattle tags attached to one of the vertical tail scutes. Since our objective was not to estimate growth rates between sexes, we did not identify sex of these Gharials. Gharials were thus 45 months old when the study started on 01 April 2013. We reared the selected Gharials together in a separate enclosure of 9 x 7 m. The enclosure was bedded with sand, and contained a pond (6m long, 3m wide and 1.25m deep) and did not have any shade. For the purposes of this study, and taking into account the effect of temperature on crocodylian metabolism, we divided 12 month-periods into two seasons—warm (April–September) and cold (October–March). Fish, primarily comprised of *Oreochromis* spp., *Puntius* spp., *Cirrhinus mrigala*, and *Gudusia chapra* was fed to the experimental group for the duration of the study (01 April 2013–30 September 2015). The amount of fish offered to the group was based on decades of experience of rearing Gharials in GCBC. The amount of fish fed out was weighed, and amount that was uneaten was deduced to estimate amount of fish consumed by Gharials. We determined average fish consumption over a month by dividing the weight of consumed fish in that month by total number of Gharials. In April and September each year, we measured TL using a flexible measuring tape to the nearest cm, and recorded body weight using a spring balance to the nearest gram.

We performed data analysis using Deducer package (Fellows 2012) in R (R Core Team 2018) and presented mean values along with standard deviation. We performed paired t-test and student's t-test wherever applicable to test for statistical significance. We also performed linear regression analysis to test for association between TL and weight of Gharials. We prepared the map on ArcGIS 10.3.

## RESULTS

At the start of the study (01 April 2013) Gharials ranged 140–167 cm in TL (mean= 150.3±8.09 cm) and 5.6–10.5 kg in weight (mean= 7.49±1.35 kg) (Table 1; Fig.

2). Considering mean TL of 43.71cm and mean weight of 100g of 100 one-month old Gharial hatchlings (Bed Khadka unpub.), they had thus grown around 96.29–123.29 cm in TL (mean= 106.59 cm) and 5.5–10.4 kg in weight (mean= 7.39 kg) since hatching, equivalent to mean growth rates of around 2.13–2.73 cm/month (mean= 2.36 cm/month) and 0.12–0.22 kg/month (mean= 0.16 kg/month) in TL and weight, respectively. Similarly, by the end of the study (30 September 2015) Gharials ranged from 169–229 cm in TL (mean= 197.7±18.15 cm) and 11.5–30 kg in weight (mean= 20.55±6.29 kg) (Table 1; Fig. 2) and they had thus grown around 29–62 cm in TL (mean= 47.40±18.18 cm) and 5.9–19.5 kg in weight (mean= 13.06±5.71 kg) in 30 months duration (Table 2), equivalent to mean growth rates of around 1.58 cm/month and 0.43 kg/month in TL and weight, respectively.

We measured seasonal growth in Gharials in unequal numbers of warm (n=3) and cold (n=2) seasons. On

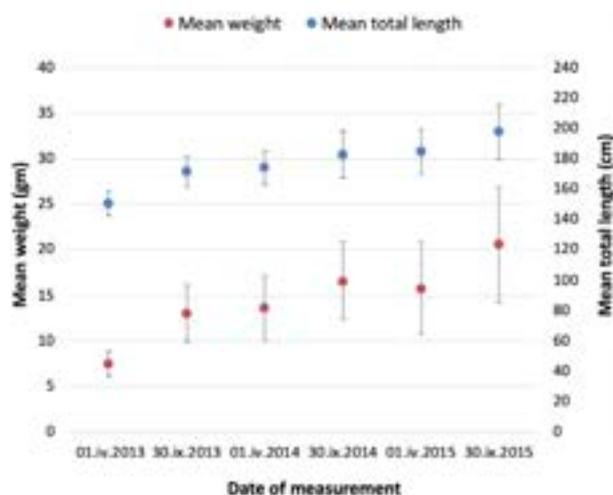


Figure 2. Mean total length and weight of 20 Gharials recorded with respect to warm and cold seasons from April 2013–September 2015. Bars represent standard deviation.

Table 1. Mean total length and weight of 20 Gharials recorded with respect to warm and cold seasons from April 2013–September 2015.

Date of measurement	Total length (cm)		Weight (kg)	
	Mean	Range	Mean	Range
01.iv.2013	150.3±8.09	140–167	7.49±1.35	5.6–10.5
30.ix.2013	171.5±10.01	156–189	13.07±3.12	9–20
01.iv.2014	174.1±11.17	158–194	13.65±3.62	8.5–22
30.ix.2014	182.65±15.76	159–208	16.6±4.23	10–25
01.iv.2015	184.35±14.77	163–208	15.8±5.02	8.5–27.5
30.ix.2015	197.7±18.15	169–229	20.55±6.29	11.5–30

average, Gharials grew by  $9.48 \pm 3.63$  cm per season (Table 2) and mean increase in TL was higher during warm than cold season (One tailed t-test;  $t = 9.53$ ,  $df = 19$ ,  $P < 0.001$ ). On average, Gharials gained weight by  $2.61 \pm 1.14$  kg per season (Table 2) and gained more weight during warm than cold season (One-tailed t-test;  $t = 10.64$ ,  $df = 19$ ,  $P < 0.001$ ). Our data also allowed annual growth to be calculated for at least two different 12-month periods (Table 2), each including one warm and one cold season. The annual growth rate was 1.98 cm/month and 0.51 kg/month for length and weight respectively for 2013–2014. Similarly, annual growth rate was 0.85 cm/month and 0.17 kg/month for length and weight respectively

for 2014–2015. Mean change in TL of Gharials either increased or remained constant between seasons, while mean change in weight decreased in some Gharials during cold seasons (Table 2). There was a strong correlation between mean TL and body weight ( $r^2 = 0.95$ ;  $F_{1,18} = 327.1$ ,  $P < 0.001$ ). Twenty Gharials consumed a total of 2,103.9 kg fish over 30 months period with a mean of 3.5 kg fish consumed per Gharial per month (Table 3). Mean fish consumption was significantly higher (One tailed t-test;  $t = 6.65$ ,  $df = 25.47$ ,  $P < 0.001$ ) for warm than cold periods; fish consumption during warm season was more than threefold higher than for cold. While Gharials exhibited considerable variation in their growth, survival rate was 100% throughout the duration of the study.

**Table 2.** Mean change in total length and weight of 20 Gharials with respect to warm and cold seasons from April 2013–September 2015.

Duration	Season	Change in total length (cm)		Change in weight (kg)	
		Mean	Range	Mean	Range
iv.2013–ix.2013	Warm	$21.2 \pm 8.61$	3–36	$5.58 \pm 2.12$	2.8–10
x.2013–iii.2014	Cold	$2.60 \pm 2.19$	0–8	$0.57 \pm 1.11$	-1.5–2.5
iv.2014–ix.2014	Warm	$8.55 \pm 7.42$	1–30	$2.95 \pm 2.13$	0–8
x.2014–iii.2015	Cold	$1.70 \pm 1.89$	0–7	$-0.8 \pm 2.47$	-6.5–7.5
iv. 2015–ix.2015	Warm	$13.35 \pm 6.77$	2–24	$4.75 \pm 2.90$	1–10.5
iv.2013–iii.2014		$23.80 \pm 9.84$	5–39	$6.16 \pm 2.75$	2.3–12
iv.2014–iii.2015		$10.25 \pm 6.79$	1–30	$2.15 \pm 3.27$	-2–14.5
Warm seasons		$4.30 \pm 2.8$	0–11	$-0.22 \pm 2.87$	-6.5–8.5
Cold season		$43.10 \pm 17.97$	18–81	$13.28 \pm 4.92$	7.5–21.5
Overall		$47.40 \pm 18.18$	22–83	$13.06 \pm 5.71$	5–22.5

## DISCUSSION

Growth rates have been studied on some species of crocodylians, but similar information on Gharials is largely lacking. Although historic accounts on growth and feeding rates for captive Gharials in Nepal is provided by Maskey (1989), we did not find any similar studies on Gharials in India, to the best of our knowledge. Maskey (1989) reported growth rate in both weight and TL of Gharial hatchlings in captivity was higher in warm than in cold periods. The same study also reported a strong correlation between the rates of increase in body weight and TL of Gharial hatchlings (Maskey 1989). Similarly, fish consumption rates of Gharials were reported to be up to 2.5 times greater in warm than in cold by Maskey (1989). These findings on growth and feeding rates of Gharials corroborate with our finding. Growth is a product of food intake and bioenergetic needs which in turn is mediated by temperature (Maskey 1989).

**Table 3.** Amount of fish consumed by 20 Gharials in warm and cold seasons from April 2013–September 2015.

Duration	Season	Fish consumed(kg)			N*
		Total	Mean per month	Range	
iv.2013–ix.2013	Warm	525.9	$87.65 \pm 38.65$	32.4–138	6
x.2013–iii.2014	Cold	161.5	$26.91 \pm 23.42$	8.5–64	6
iv.2014–ix.2014	Warm	522.5	$87.08 \pm 34.06$	41.5–145	6
x.2014–iii.2015	Cold	196.5	$32.75 \pm 8.58$	24.5–45.5	6
iv. 2015–ix.2015	Warm	697.5	$116.25 \pm 37.71$	69.5–162.5	6
Cold seasons		358	$29.83 \pm 17.09$	8.5–64	12
Warm seasons		1745.9	$96.99 \pm 37.35$	32.4–162.5	18
Overall		2103.9	$70.13 \pm 45.26$	8.5–162.5	30

\*Number of months

Various studies have suggested that growth rate in crocodylians can vary among species as well as within the same species from different geographical regions, age groups and sex (Thorbjarnarson 1988; Gorzula & Seijas 1989; Saalfeld et al. 2008; Barrios-Quiroz et al. 2012; Balaguera-Reina et al. 2015). We estimated growth rate in TL between 0.73–2.76 cm/month (mean= 1.58 cm/month) for Gharials. Growth rate in TL of Spectacled Caimans *Caiman crocodilus* was reported between 2–2.6 cm/month during the first year of life (Gorzula & Seijas 1989). Growth rate in TL of American Crocodile *Crocodylus acutus* hatchlings in wild was reported to vary between Haiti (3.9 cm/month) (Thorbjarnarson 1988) and in Panama (0.9–4.8 cm/month) (Balaguera-Reina et al. 2015). Similarly, growth rate in TL of American Alligators *Alligator mississippiensis* was also reported to vary between 2.7 cm/month (for individuals with TL<50 cm when captured) and 2.3 cm/month (for individuals with TL between 50–125 cm when captured) in wild (Saalfeld et al. 2008). Growth rate is also observed to vary between different age groups in Gharials in captivity (Bed Khadka Unpub.; Maskey 1989). One month old Gharial hatchlings (mean TL= 43.71±1.40 cm; range=40.1–47 cm; n=100) showed mean growth rate of 2.7cm/month over the period of 109 days (Bed Khadka Unpub.) which was higher than the mean growth rate reported in this study. It should, however, also be noted that growth rates in crocodylians are typically higher in captivity than in wild. Relation between temperature and growth rate is reported in other species of crocodylians as well. For instance, Joanen & McNease (1987) showed that growth rates in American Alligators could be doubled by optimizing temperature throughout the year. Similarly, Webb et al. (1978) showed that growth rate in Saltwater Crocodiles *Crocodylus porosus* was higher in the wet-hot season than in the dry-cold season in Australia and growth rate decreased as body size increased.

Gharials are reared in captive facilities across Nepal and India and held at zoos throughout the world. Our findings establish baseline growth and feeding rates for captive Gharials. Such information will be helpful in optimizing feeding and rearing practice for Gharials in captivity.

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## AMPHIBIAN ABNORMALITIES AND THREATS IN PRISTINE ECOSYSTEMS IN SRI LANKA

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**Abstract:** Amphibian abnormalities are caused by numerous etiologies prevailing in the environment. Since amphibians are good bio indicators of the environment, amphibian abnormalities are popularly known as a veritable ecological screening tool to assess ecosystem health. The present study was carried out encompassing within and outside the Horton Plains National Park areas, from January to November 2017. Distribution of amphibian morphological abnormalities were assessed in and around the five lentic water bodies through gross visual encounter. Six quadrates of 1m×2m were randomly placed in each sampling site. Frequency and composition of amphibian abnormalities were assessed in a total of 694 amphibians, belonging to four families and 11 species. Thereby, 4.5% and 80.87% abnormality indexes were accounted for respectively within and outside the park, comprehended surficial abnormalities, ectromelia and femoral projection abnormality types. Surficial abnormalities were the most predominant in both localities, generally occurring at the hind limb region of pre-mature stages of *Taruga eques*. Two lentic water bodies were identified as “abnormality hotspots” within and outside the Horton Plains National park; however, a multiplicity of possible combinations of potential causes of abnormalities were present in the environment. Hence, finding the exact causes of amphibian abnormalities are an extremely difficult exercise in the field.

**Keywords:** Abnormality, Horton Plains National Park, morphological, *Taruga eques*.

**Abbreviations:** DWC—Department of Wildlife Conservation | GPS—Global Positioning System | HPNP—Horton Plains National Park | IUCN—International Union for Conservation of Nature | OHPNP—Outside the Horton Plains National Park | RH—Relative Humidity | T amb—Ambient temperature | Tw—Water temperature.

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**Author contribution:** All authors contributed equally. GKVPTS was the main researcher on this research project, field sampling, data collection, data analysis and preparation of the paper. WADM was the supervisor of this piece of research and provided immense guidance and support from the initiation of the project until the completion, with her valuable experience and knowledge. AdS provided some important relevant literature and valuable suggestions with his expertise in field studies, which contributed a lot to improve parts in the methodology section and to prepare the manuscript.

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## INTRODUCTION

“Abnormality” refers to “any deviation from normal morphology, independent of whether its origin was developmental or acquired after proper development” (Lunde & Johnson 2012). Both amphibian malformations and deformities are included in amphibian abnormalities (Reeves et al. 2008). Amphibian abnormalities can be classified mainly as surficial abnormalities (infectious diseases/cysts and wounds), skeletal abnormalities and eye abnormalities (Linder 2003; Reeves et al. 2008). Amphibian abnormalities have interconnected with many factors including, chemical contaminants (Bridges et al. 2004; Lunde & Johnson 2012), trematode, cestode, and nematode parasites (Ankley et al. 2004; Imasuen & Ozemoka 2012), predators (Johnson et al. 2006; Johnson & Bowerman 2010) and UV (Ultraviolet) radiation (Blaustein & Johnson 2003; Lannoo 2008).

Anthropogenic activities have been recognized as a main element for the modification of aquatic habitats (Johnson & Chase 2004). Especially nutrient loading in freshwater ecosystems (particularly ponds) directs to the acceleration of eutrophication that result in shifting the community composition. This results in various amphibian abnormalities.

Helminth infections are considered as a major governing factor for existing amphibian abnormalities and linked with numerous factors. Most of the trematode infections in amphibians are recorded from the habitats associated with agriculture and cattle farms. Trematodes in the genus *Ribeiroria* occur in lentic aquatic water bodies (Johnson et al. 2004). Aquatic snails in lentic aquatic water bodies serve as intermediate host for *Ribeiroria* parasite. When high nutrient levels are present the snail densities increase exponentially. Free swimming cercariae emerge from infected snails then penetrate and encyst as metacercariae in the second intermediate host (Jayawardena et al. 2010b) often around the developing limb buds of amphibian larvae, leading to improper limb development (Blaustein & Johnson 2003). In addition to that frequent exposure of amphibians to chemical contaminants accumulated in lentic water bodies leads to a reduction of immunity strength. As a result of that amphibians become highly susceptible to parasitic infections (Kiesecker 2002; Budischak & Belden 2009; Lunde & Johnson 2012). Cestodes are one of the major parasite groups which infect the amphibians. Cestodes commonly occur within the musculature of the body and in the hind limbs of adult and juvenile frogs (Gilliland & Muzzall 2002) and a number of metacercariae of helminthes has been recorded in the leg musculature of

deformed amphibians (Gilliland & Muzzall 1999).

There is a considerable effect on anuran abnormalities from predators existing in natural environments (Johnson & Bowerman 2010). Most of these amphibian abnormalities are caused by aquatic predators such as dragonfly larvae (Bowerman & Johnson 2010; Johnson & Bowerman 2010), small fishes, crabs, crayfishes (Johnson et al. 2001a, 2006), diving beetles, predatory odonate nymphs and water scorpions (Ballengee & Sessions 2009). Smaller aquatic predators (insect larvae, small fishes) attack the exposed portions of larval and metamorphosis stages of anurans such as the tail or limbs (Bowerman & Johnson 2010). A traumatic loss of an entire limb (Lannoo 2008), however, and wounds of amphibians can be seen after the metamorphosis which are produced by the attack of large vertebrate predators (Bowerman & Johnson 2010).

Leech attack causes some of the abnormalities of amphibians and many abnormality studies have proved that leech attacks cause a high prevalence of missing limbs (ectromelia) or parts of the limbs (Johnson et al. 2001a, 2002, 2006; Ballengee & Sessions 2009; Bowerman & Johnson 2010).

Considering worldwide abnormality studies it can be seen that most of them have been carried out as laboratory studies, related with chemical inductions (Burkhart et al. 1998; Lajmanovich et al. 2003) and parasitic inductions (Johnson et al. 2001a; Stopper et al. 2002) to frog embryos and tadpoles in various limb bud stages. Only a few studies, however, have been conducted to investigate abnormalities of amphibians in the field (Johnson et al. 2001b; Peltzer et al. 2011). As regards the fact that Sri Lanka is a biodiversity hotspot, only four studies have been mentioned (Rajakaruna & Samarawickrama 2007; de Silva 2009, 2011; Meegaskumbura et al. 2011). Though, abnormality surveys were based on selected regions of the country, it's more valuable to extract data from pristine ecosystems with the purpose of identifying the actual threats of amphibian's survival. Therefore, the present research was undertaken to assess the amphibian abnormalities and their possible predators which cause amphibian abnormalities encircling the area in and around the lentic water bodies within and outside the World Heritage Horton Plains National Park as a comparison of threats and abnormality types possessed by amphibians against two different localities to ultimately fulfill the knowledge gap of field studies pertinent to amphibian abnormalities.

## MATERIALS AND METHODS

### a) Study site

The study was conducted within and outside the Horton Plains National Park (HPNP) from January to November 2017. HPNP is located between 6.802 northern latitudes and 80.807 eastern longitudes (Green 1990) which occupies an area of 3,160ha and is contiguous with the Peak Wilderness Sanctuary to the west. It is in the eastern extremity of the Nuwara Eliya District in Sri Lanka within the range of 2,100–2,300 m elevation (DWC 2007). Tropical montane cloud forests and wet pathana grasslands are the two distinct habitats in the park (Gunatilleke & Gunatilleke 1990) with a narrow ecotone belt of shrubs and herbs between the two.

Most of the lentic water bodies are surrounded by three main grass types (*Chrysopogon nodulibarbis*, *Andropogon polyptychos*, and *Garnotia exaristata*) and one bamboo species (*Arundinaria densifolia*) in the grassland habitat (DWC 2007). The sampling was conducted in selected five lentic water bodies on four days per month, providing equal effort to each sampling site. Three lentic water bodies (A, B and C) were selected within the HPNP and two (D and E) were selected outside the HPNP based on the availability of amphibians (Figure

1A). Sampling sites, outside the HPNP were located within a range of 1,170.9–1,864.7 m elevation and mainly associated with forested area. GPS (Global positioning system) points of these sampling sites were recorded using Garmin etrex Euro hand held GPS receiver.

Six quadrates each 1 x 2 m were placed within each sampling site for the sampling of amphibians. Three quadrates were randomly placed in the area 1m from the pond bank outside the pond and three quadrates were randomly placed inside the pond in the area of 1m from the pond bank (Faruk et al. 2013). Anuran species (larvae, metamorphs, juveniles, and adults) and leeches in these quadrates were surveyed from morning to afternoon (08.00–16.00 h) and during the night (18.00–20.00 h). Moreover, vegetation within each plot was searched even when slight movement was detected. Head lamps and torches were used for nocturnal searches. Larval amphibians were captured using active sweeps (Dodd 2010), metamorphic anurans were captured with a dip net or by hand (Ouellet et al. 1997) and adult amphibians were captured with a dip net or by hand (Wheeler & Whelsh 2008; Urbina & Jenny 2009). A small amount of water was added to the container to prevent overheating and desiccation (Wheeler & Whelsh 2008). Overcrowding based on the container size and Tamb was avoided (Lunde & Johnson 2012). At the end

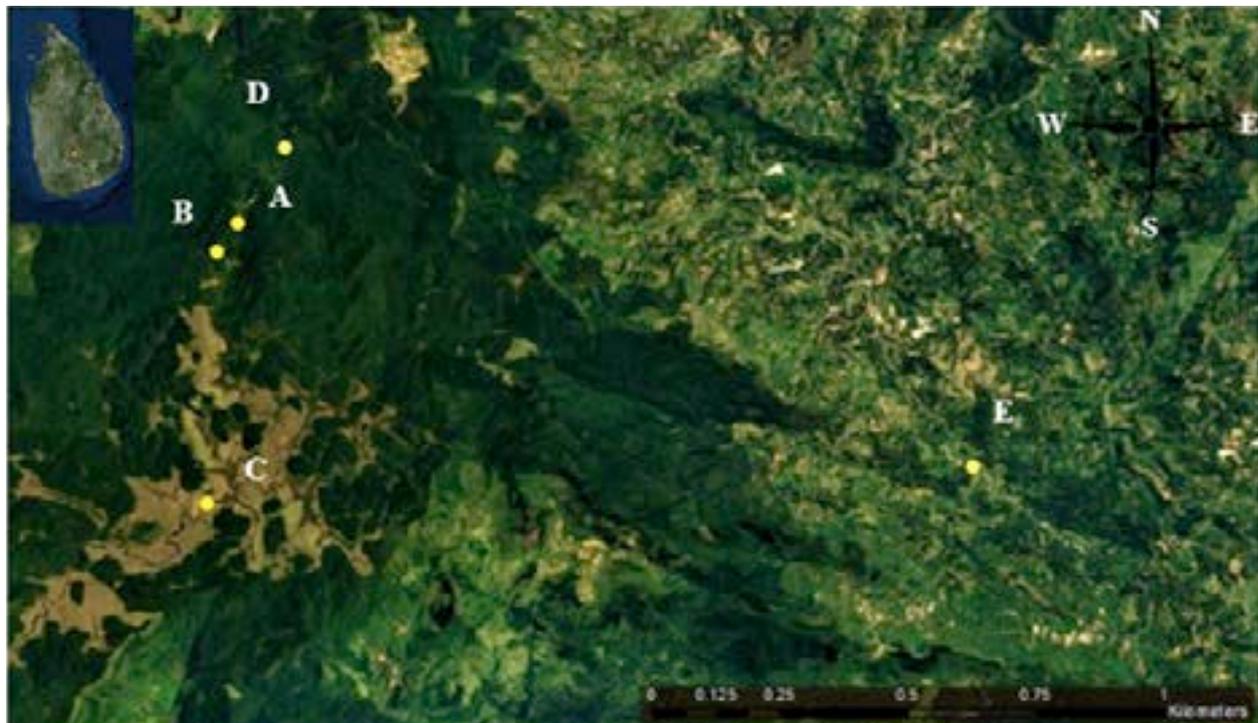


Figure 1. (A). Locations of sampling sites within and outside the Horton Plains National Park. [Lentic water bodies within the HPNP]. A (6.839°N and 80.812°E), B (6.834°N and 80.809°E), C (6.801°N and 80.807°E), D (6.850°N and 80.818°E), E (6.805°N and 80.908°E).

of the survey they were released back to their habitat after removing the ectoparasites from the attached skin surface of the amphibians. Amphibian species and their stages were identified using the amphibian guides of Dutta & Manamendra-Arachchi (1996), Manamendra-Arachchi & Pethiyagoda (2006), and de Silva (2007). When an amphibian was captured, (a) it was identified to species level, (b) life stage noted, (c) presence or absence of the abnormalities assessed (in larvae, metamorphs, juvenile and adult stages) (If abnormalities were present, they were recorded with the count, according to the abnormality classifications of guides) and recorded.

#### **b) Identification of amphibian abnormalities**

Amphibian physical abnormalities (assessed the abnormalities which appeared externally) were associated with the loss of body parts or organs. External abnormalities were identified through gross visual inspections (both dorsal and ventral sides of the amphibian body) and classified based on Meteyer (2000), Johnson et al. (2001b), Johnson et al. (2002), Lannoo (2008), Rajakaruna et al. (2008), Reeves et al. (2008), Johnson & Hartson (2009), and Peltzer et al. (2011) guides. Surficial abnormalities (cysts) were identified by careful examination of the external body surface (dorsal side, ventral side, around the fore limbs and hind limbs) and using the external morphological features of the infection. Cysts of infected amphibians are found as clear or brownish colour (Johnson et al. 2004), round, swelling nodules of the musculature (Ostler 2004). Moreover, Johnson et al. (2004), Ostler (2004), de Silva (2007, 2009), and Jayawardena et al. (2010a, b) were referred to for the identification of cysts. Based on the number of cysts (surficial), severity of amphibian infections were classified as mild and moderate. Mild infectious amphibians possess 1–3 nodules and moderate infectious amphibians possess 4–6 nodules (de Silva 2009).

#### **c) Identification and sampling of possible predators of amphibians**

Leeches were identified by careful examination of the external body surface of the anuran larvae, metamorphs, juveniles, adults and also the grasses and shrubs which were adjacent to the bank of the water body. Possible aquatic invertebrate predators of the amphibian life stages (larvae, metamorphs, juveniles and adult) were sampled by using the 0.36 × 0.22 m dip net, performing 2m sweeps at 11 evenly spaced points around each pond's perimeter (Bowerman & Johnson 2010). Captured aquatic predatory varieties were identified, counted, recorded and released to the same lentic water

body. Amphibian invertebrate predators in the ponds were classified as either abundant (<100 individuals trap per day) or rare (<10 individuals trap per day) based on density capture per day according to Bowerman & Johnson (2010). Possible large vertebrate predators (aquatic birds) of amphibians were assessed based on observations in and around the lentic water bodies.

Aquatic invertebrate predators of amphibians were identified based on the morphological characteristics (nature of the abdomen, presence of exoskeleton and arrangement of gills) with the help of Curtis (2011) and Quek et al. (2014). Possible large vertebrate predators (aquatic birds) of amphibians were identified (colouration of feathers and beak and foot type) using Harrison (2011), a bird guide. All types of abnormalities, aquatic invertebrates and large vertebrate predators as possible threats of amphibians (which cause amphibian abnormalities) were photographed using a digital camera.

#### **d) Determination of the environmental variables**

Environmental variables of lentic water bodies were measured to find out the habitat suitability for amphibians (Hamer & Lane 2002; Urbina & Jenny 2009; Dodd 2010; Sparling 2010). The atmospheric data temperature (°C) and relative humidity (RH) were assessed. Ambient temperature (Tamb) was recorded 2m above the water surface / ground (using Kestrel 4000 weather meter, USA) and relative humidity (RH) data were recorded 2m above the ground. In addition to that soil pH values were obtained using Kelwey soil tester, under the soil chemical data. Furthermore, dissolved oxygen (DO) (YSI 550A Dissolved Oxygen Instrument), water temperature (Tw), pH (YSI Eco Sense pH 100A meter), and conductivity (YSI Eco Sense EC300A Conductivity meter) were measured using analysis of water physiochemical data. Soil chemical data and water physiochemical data were recorded once a month in each plot (as mentioned above) using a standardized data sheet.

#### **e) Data analysis**

The Minitab version 17.0 statistical software was used to calculate the mean and standard deviation (SD) values of environmental variables and possible predators of amphibians in each sampling site. Graphical representations were created using Microsoft Excel 2013 software. Abnormality index (AI) was calculated using the equation,

$$AI = (\text{Total number of abnormal amphibians} / \text{Total number of amphibians inspected}) \times 100\%$$

## RESULTS

### a) Abundance of amphibian species

A total of 694 amphibians belonging to four families and 11 species were recorded during the study in the five lentic water bodies studied. Five-hundred-and-eleven individuals were examined inside the HPNP and 183 individuals were examined outside the HPNP (Table 1).

### b) Amphibian abnormalities

Out of the 511 amphibians examined inside the HPNP, only 23 (4.50%) had abnormalities; however, 80.87% of amphibians outside the HPNP, possessed abnormalities. *Taruga eques* had the most number of abnormalities in both localities—22 inside the HPNP and 147 outside the HPNP. This was 99.30% of AI outside the HPNP and 95.70% of AI inside the HPNP with respect to *T. eques*. Surficial abnormalities (Image 1) were more dominant than ectromelia and femoral projection abnormality types in both localities, thereby mild infections (60.00%) (Image 1A and 2B) and moderate infections (77.55%) (Image 1C) were predominant respectively within and outside the HPNP (Figure 2A). Cysts were recorded only in *T. eques*, accounted for 5.76% within the HPNP and 100.00% outside the HPNP (Figure 2B). Ectromelia was recorded in both *T. eques* (Image 2A) (0.58%) and *M. greenii* (Image 2B) (0.74%) species. Femoral projection was found only in *F. cf. limnocharis* (Image 2C) which accounted for 5.56% frequency of the abnormalities. Comparing the abnormality index with amphibian life stages (Figure 2C), no abnormal larvae were found in

both localities. Abnormal metamorphs were found only inside the HPNP which accounted for 4.35% of the abnormalities. A high AI, however, was observed in the juvenile stage than in adults in both localities—56.52% and 77.70% within and outside the HPNP. Moreover, most of the abnormalities were found at the hind limb region of amphibians (Figure 2D). When comparing the AI of amphibians in each sampling site, no abnormal amphibians were recorded in pond C. Amphibians with ectromelia were only recorded within the HPNP in sampling site B; however, 1.16%, 14.39%, 100%, and 2.78% abnormality percentages were recorded in A, B, D, and E ponds, respectively (Table 2). With reference to national conservation states (determined by IUCN), 94.71% of the amphibians found in the HPNP were endangered, thereby 4.55% had abnormalities, whereas in contrast 100% of the amphibians were abnormal outside the HPNP.

### c) Comparison of amphibian predatory density in each sampling site

Damselfly larvae *Elattonneura leucostigma* were the most abundant possible predatory type of the amphibians. Damselfly larvae (Image 3A) were recorded in A, B and C sampling sites within the HPNP. Water scorpions (Image 3C), dragonfly larvae *Orthetrum glaucum* (Image 3B), Pond Herons *Ardeola grayii* (Image 3E) were recorded only in pond A. Leeches (Image 3D) were recorded in A and B sampling sites. Crabs *Perbrinckia glabra* (Image 3D) were only found in sampling site C. No possible predators of amphibians were recorded in pond E. Aquatic beetles were only recorded in pond D (Table 3).

**Table 1. Amphibian abundance in and around the lentic water bodies within and outside the Horton Plains National Park.**

Locality type	Family & Species	Abundance	Abnormality Index (%)
HPNP	<b>Dicoglossidae</b> <i>Minervarya greenii</i>	135	0.74
	<b>Microhylidae</b> <i>Microhyla zeylanica</i>	11	00
	<b>Microhylidae</b> <i>Uperodon palmatus</i>	03	00
	<b>Rhacophoridae</b> <i>Pseudophilautus alto</i>	02	00
	<i>Pseudophilautus frankenbergi</i>	01	00
	<i>Pseudophilautus microtypanum</i>	12	00
	<i>Taruga eques</i>	347	6.34
OHPNP	<b>Bufonidae</b> <i>Duttaphrynus melanostictus</i>	06	00
	<b>Dicoglossidae</b> <i>Euphyctis cyanophlyctis</i>	04	00
	<i>Minervarya kirtisinghei</i>	08	00
	<i>Fejervarya cf. limnocharis</i>	18	5.55
	<b>Rhacophoridae</b> <i>Taruga eques</i>	147	100

### d) Environmental variables of sampling sites

Highest conductivity was recorded in sampling site E. It may be due to the fact that the water was contaminated with agro-chemicals. Moreover, D lentic water body recorded the least water pH value which indicates acidic conditions and that more anthropogenic stressors are present in the water body (Table 4).

## DISCUSSION

Both amphibian malformations and deformities are included in amphibian abnormalities (Reeves et al. 2008). Some of the abnormalities are external physical abnormalities and others are internal (Blaustein & Johnson 2003; Spolyarich et al. 2011). The present study did not find any abnormalities in the family Bufonidae coinciding with the studies of Lannoo (2008) who

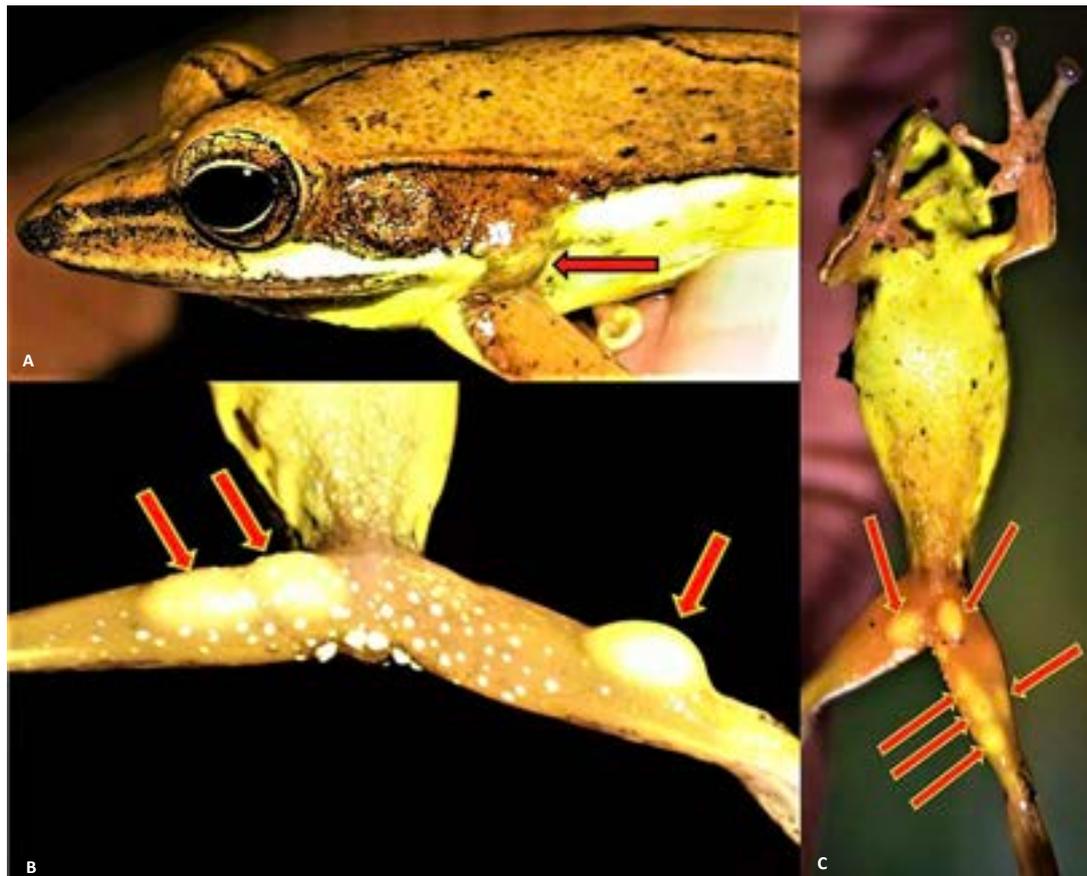


Image 1. A and B—mild infection: infected *Taruga eques* amphibians with clear colored 1–3 cysts (surficial abnormalities) as round swelling nodules of the musculature and subcutaneous tissue of the skin | C—moderate infection- Infected *Taruga eques* amphibians with 4–6 cysts. © G.K.V.P.T. Silva.



Image 2. A—Ectromelia in *Taruga eques* | B—Ectromelia in *Minervarya greenii* metamorph | C—femoral projection abnormality in *Fejervarya* cf. *limnocharis* | D—leeches attached to the external body surface of *Taruga eques*. © G.K.V.P.T. Silva.

**Table 2. Different abnormality types and abnormality indices recorded in each sampling site.**

Locality type	Sampling site	Ectromelia	Infections	Femoral projections	Abnormality index (%)
HPNP	A	-	4	-	01.16
	B	3	16	-	14.39
	C	-	-	-	00.00
OHPNP	D	-	147	-	100.00
	E	-	-	1	02.78

**Table 3. Comparison of density of amphibian possible predators in each sampling site number of predators trapped/observed per day.**

Predator	Locality type				
	HPNP		OHPNP		
	A	B	C	D	E
Aquatic beetles	1.89 ±0.93	-	-	-	-
Crustaceans	-	1.44±1.01	-	-	-
Damselfly larvae	1.78±1.92	104.33±31.89	1.22±1.09	-	-
Dragonfly larvae	11.56±6.73	-	-	-	-
Leeches	01.56±2.01	5.44±2.74	-	-	-
Pond herons	02±0.87	-	-	-	-
Water scorpions	1.56±1.01	-	-	-	-
Aquatic beetles	-	-	-	01±1.12	-

**Table 4. Environmental variables in and around the lentic water bodies in each sampling site.**

Site	Environmental variables (Mean ± SD)						
	T <sub>amb</sub> (°C)	RH	Soil pH	DO (mg/L)	T <sub>w</sub> (°C)	Water pH	Conductivity (µS/cm)
A	16.29 ± 0.30	94.11±2.57	5.90±0.38	4.67±0.19	15.73±0.53	8.14±0.68	18.56±5.68
B	16.15±0.58	95.05±2.06	5.91±0.50	4.55±0.26	15.75±0.45	7.93±0.62	17.07±5.79
C	15.88±0.32	94.50±2.77	5.88±0.45	4.65±0.23	16.01±0.66	8.30±0.66	20.95±7.13
D	16.80±0.67	95.03±2.66	4.49±0.18	4.52±0.16	16.21±0.77	6.89±0.90	18.94±4.03
E	15.41±0.89	91.95±8.14	5.15±0.45	4.66±0.19	16.99±0.38	6.95±0.93	25.65±4.50

observed that the Bufonidae family is less susceptible to abnormalities.

Previous studies have indicated that less than 5% abnormality prevalence of the population in a particular area or site is normal (Johnson et al. 2002; Kiesecker et al. 2004; Piha & Pekkonen 2006; Lunde & Johnson 2012). The abnormality prevalence within the HPNP was 4.50%, which is within the accepted range; however, abnormality prevalence outside the HPNP was 86.55% and should be considered as abnormally high (Piha & Pekkonen 2006). Lentic body wise, pond B inside the HPNP had 14.5% abnormality prevalence and pond D had abnormality prevalence of 100%. These high abnormality prevalent sites were classified as “hotspots” with respect

to abnormalities (Johnson & Bowerman 2010). B lentic water body was located within the pristine ecosystem of HPNP. Moreover, all the water quality parameters were also within the standard levels for amphibian survival. Therefore, further experiments must be carried out to find out the exact cause for abnormalities in B water body. In contrast, D lentic water body was located on the way to HPNP and most visitors use it as a garbage dumping site (which may be the reason for reporting less DO and soil pH values). As a result of that, this high nutrient content of the water body provides a better environment to increase the parasitic density. It might be the major reason for recording 100% of surficial abnormalities in D lentic water body. Since, observed

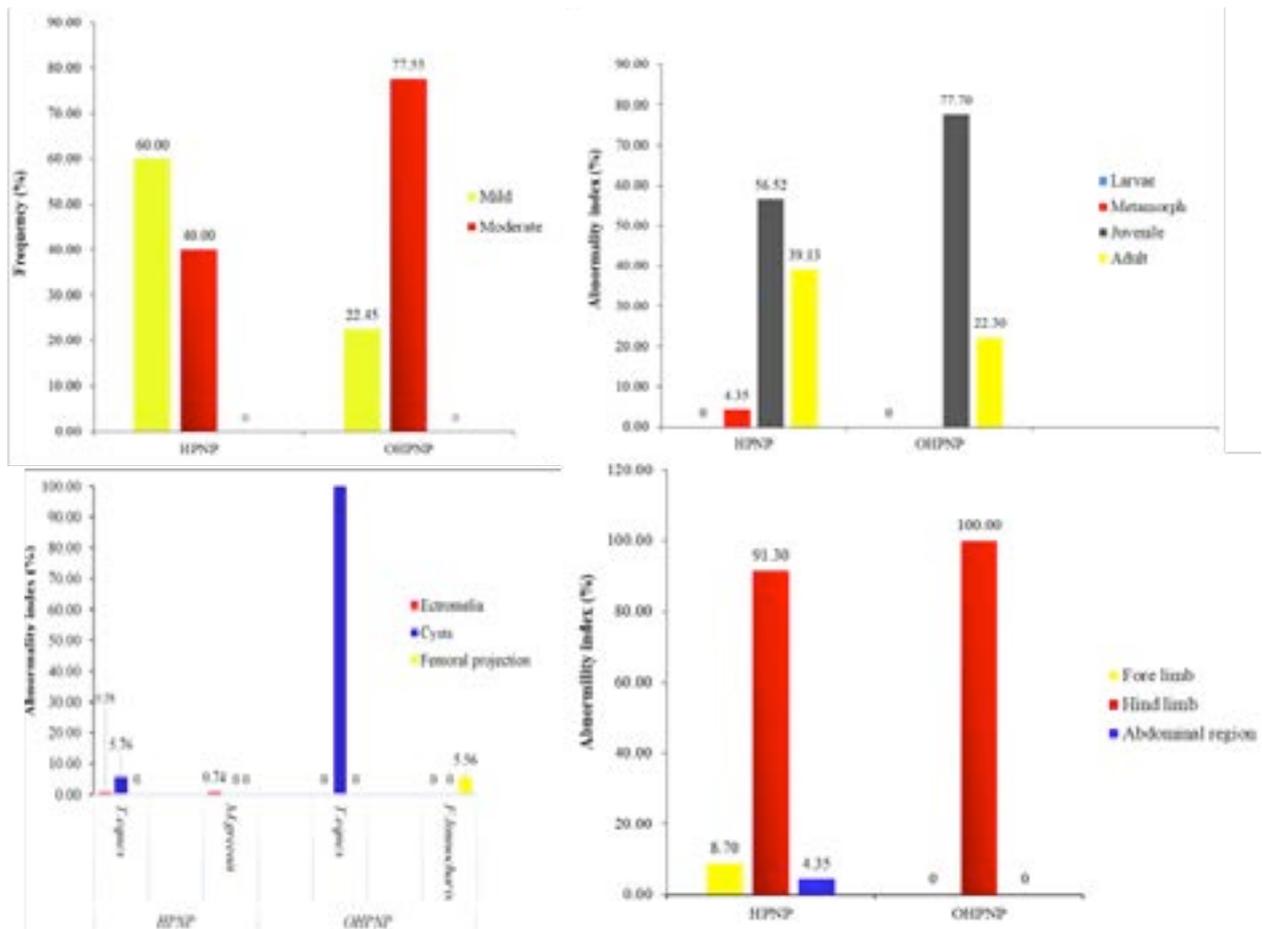


Figure 5. A—comparison of the infection severity within and outside the Horton Plains National Park (HPNP) | B—comparison of abnormality types recorded in different amphibian species found within and outside the HPNP | C—comparison of Percentages of abnormality indices recorded from different amphibian life stages within and outside the HPNP | D—comparison of abnormalities based on the body region where the abnormalities were recorded.

abnormality prevalence of B and D lentic water bodies were greater than the expected baseline range, further investigations are warranted to find out the locality specific causes (Lunde & Johnson 2012).

Highest number of amphibian abnormalities were reported in pre-matured stages of amphibians which may be the reason that either adult populations of amphibians are less susceptible for abnormalities against different environmental stressors or they have reduced survivorship. The survival of abnormal amphibians declines due to a high predation pressure and inability to capture its prey (Lunde & Johnson 2012). Since abnormal adult amphibians are unable to survive long periods of time in the environment, frequently a smaller number of adult amphibian populations are discovered with abnormalities (Goodman & Johnson 2011; Lunde & Johnson, 2012). Previous abnormality studies have also observed that most of the amphibian abnormalities are associated with hind limbs (Ouellet et al. 1997;

Johnson et al. 2002; Piha & Pekkonen 2006), which may be resulted by the attack of natural predators when they try to avoid the escape of amphibians. Results of the present study also tallies with these findings as most of the abnormalities found as cysts arise within the musculature found in the hind limbs.

Surficial abnormalities (cysts) were the predominant abnormality type recorded in this study. Even the HPNP comprises optimal conditions for growth of *Batrachochytrium dendrobatidis*, in the most observed cysts may be caused by parasitic helminths infected after limb bud stages of amphibians since they were located within musculature, mostly around limb structures and were easily visible to the naked eye as swelling round nodules; in contrast pathogenic cysts are microscopic and confined only to the epidermis (Lunde & Johnson 2012). Lunde & Johnson (2012) also recorded that the high number of amphibians inhabiting lentic water bodies are commonly infected with many trematode species that



**Image 3. Possible predators of amphibians which cause amphibian abnormalities. A—Damsel fly larva | B—Dragonfly larva | C—Water scorpion | D—Crustacean (crab) | E—Pond Heron. © G.K.V.P.T. Silva.**

form cysts under the skin within musculature.

Amphibian abnormalities due to trematode infections depend on the stage of limb development at which infections occurred (Schothoefter et al. 2003). Therefore, timing of the infection is a critical determinant in forming abnormalities (Jayawardena et al. 2010b). Infection coincides at pre-limb bud developmental stage of the tadpoles which results in high mortality rate with axial abnormalities. Trematode infections acquired at limb bud stage also produces high abnormality rate including ectromelia (Schothoefter et al. 2003; Jayawardena et al. 2010b). Further more, there is no any effect to limb development and survival of tadpoles when they are infected at the paddle stage (Schothoefter et al. 2003; Jayawardena et al. 2010b). However after the infection at the paddle stage of amphibians, encysted parasites are able to remain viable even at the adult stage of amphibians (Imasuen & Ozemoka 2012). This was also observed in the amphibians in the present study. Existence of amphibian predators in ponds generally increase the trematode infection of amphibians (Thiemann & Wassersug 2000; Lunde & Johnson, 2012). Trematodes commonly occur in the lentic water bodies with dragonfly larvae (Bowerman & Johnson 2010;

Lunde & Johnson, 2012). These two factors may have contributed to amphibian abnormalities in the lentic water bodies that were studied.

All the amphibian abnormalities cannot be explained away as parasitic infections. Lannoo (2008) and Lunde & Johnson (2012) observed that high level of ectromelia are present in frogs even when parasites are absent. In addition to parasites, amphibian predators (vertebrates and invertebrates) play a major role in limb abnormalities in amphibians (Bowerman & Johnson 2010). Larval amphibians are attacked by small predators including aquatic invertebrates; however, traumatic loss and injuries in limbs of amphibians (after metamorphosis) are mostly caused as a result of failed predatory attempts by large vertebrate predators (Bowerman & Johnson 2010). Bowerman & Johnson (2010) observed a direct relationship between the leech density and the abnormality level in field studies and laboratory induction of leeches. Leeches act as predators at amphibian larval stages and act as parasites of both larval and adult stages (Schalk & Forbes 2002; McCallum et al. 2011). Damsel fly larvae was the abundant possible predators recorded in site B and all the ectromelia were observed in the same pond. Therefore, there is a high possibility of these predators causing the ectromelia observed in site B.

Water quality values of all sampling sites were included to the estimated optimum ranges (Sparling 2010) for amphibian growth and development; however, with respect to soil pH values, even moderate acidic condition is identical to HPNP (Chandrajith et al. 2009), strongly acidic conditions can't be expected within the sampling sites outside the HPNP, which indicate that both sampling sites outside the HPNP may have exposed to some anthropogenic activities.

Multiplicity of possible combinations of potential causes of abnormalities are present in the environment. Hence, finding the exact causes of amphibian abnormalities are an extremely difficult exercise in the field. The fact that abnormalities are linked with ecology, epidemiology, and developmental biology further increases the complexity of this problem.

## CONCLUSIONS

*Taruga eques* was the most susceptible amphibian species to abnormalities irrespective of the locality type, indicated that amphibians may have been exposed to some stress conditions linked with multiple factors. Present research findings revealed that toads are less susceptible to amphibian abnormalities. Since, B and

D lentic water bodies were discovered as abnormality hotspots, further experiments are crucial at those particular lentic water bodies, for determining the site-specific cause for amphibian abnormalities. The present study clearly discloses further that Sri Lankan tree frogs are in a critical state. Since *Taruga eques* is confined only to the central hill regions of the country with declining population and all the amphibians which belong to endangered conservation state had abnormalities outside the HPNP, it is clear that mandating urgent measures to carryout extensive research-based conservation work before including it to critically endangered state is needed.

The present research data reveals that potential predators of amphibians are provided with excellent environmental conditions for their existence within the HPNP as a protected area in contrast to areas outside the HPNP. The present research, however, suggests that exigency of extensive research-based studies for the identification of complex causes for abnormalities in amphibians integrated with many disciplines, including ecology, toxicology, parasitology, and developmental biology. These studies will be important not only as ecological or evolutionary influences, but as significant implications for conservation and contemporary concerns over widespread amphibian population losses.

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## DIVERSITY AND DISTRIBUTION OF ORCHIDS OF GOA, WESTERN GHATS, INDIA

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**Abstract:** The Botanical Survey of India launched a pilot project during 2015–2017 on exploration of orchids of Goa State covering all the protected areas. A total of 68 orchid species belonging to 28 genera were documented of which 42 are epiphytic and 26 are terrestrial. Twenty-eight species are endemic, of which 23 species are strictly endemic to the Western Ghats, two are endemic to peninsular India, and three are endemic to the country. Distribution of orchids in Goa is concentrated mainly in the Western Ghats region, which accounts for 86% of the total orchid species richness of the state. The most favourable habitat was found to be semi-evergreen forests followed by moist-deciduous forests. Among all the protected areas, the Mhadei Wildlife Sanctuary was found to be rich in orchid diversity (39 species) followed by Bhagwan Mahavir Wildlife Sanctuary, and Cotigaon Wildlife Sanctuary. The MaxEnt data shows the highly suitable area for orchids in Goa is approximately 1,005km<sup>2</sup>, which is 27% of the total geographic area of the state.

**Keywords:** Endemic, MaxEnt, peninsular India, protected area.

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## INTRODUCTION

The Western Ghats region of Goa lies in the extreme east of the state and consists of a wide belt of rich forest with abundant biodiversity. This area is extensively protected by national parks and wildlife sanctuaries. It covers almost 600km<sup>2</sup> and has an average elevation of about 800m. Though, Goa State occupies just 2% area of the Western Ghats, it harbours c. 7% of the endemic flowering plant species of the Western Ghats (Joshi & Janarthanam 2004). Garcia de Orta described some interesting medicinal plants from this region way back in 1565 which marks the initiation of the botanical studies in the region, but, thereafter, for almost three centuries there was no contribution to the botany of Goa. Roxburgh (1820) occasionally referred to plants of this region as belonging to Konkan. Graham (1839) in his Catalogue of Bombay Plants often referred to Lush, who had earlier collected plants from Goa. Dalzell & Gibson (1861) and Nairne (1894) have also referred to plants from this region in their works. Most of these previous workers were often referred to by Hooker (1872–1897) in his majestic work on the Flora of British India in the context of plants occurring in Konkan and Goa in particular. Dalgado (1898) enumerated plants occurring in Goa and Sawantwadi based on earlier published reports but there was no orchid included in the enumeration. Subsequently, several botanists have made extensive plant collections in adjacent parts of Goa. Vartak (1966) reported 65 species of orchids belonging to 30 genera from Karnataka and Maharashtra including a few orchids from Goa, followed by Rao (1986) 21 species from Goa, Parab (2009) 26 species belonging to 20 genera from Goa and Mandar & Lakshminarasimhan (2013) presented the floristic account of Molem National Park which includes 34 species of orchids. Despite all these, the state of Goa is poorly explored in terms of orchid diversity. As such, the Botanical Survey of India launched a pilot project on exploration of orchids of Goa State covering all the protected areas (PAs), which resulted in several new distributional records of orchids for Goa (Jalal et al. 2015a,b; Jalal & Jayanthi 2016a,b; Jalal 2017). During the survey, taxonomic inventory of orchids was carried out in different PAs, and the status of orchids was documented. In the present work, an attempt has also been made to predict the suitability of potential orchid rich area based on MaxEnt (maximum entropy) niche approach (Phillips et al. 2006; Phillips & Dudik 2008).

## MATERIAL AND METHODS

### Study area

The state of Goa is located on the Indian west coast nestled between Maharashtra and Karnataka. The total geographical area of the state is approximately 3,702km<sup>2</sup>. The state stretches out to a length of 105km from north to south and 60km wide from east to west and is divided into two districts, North Goa and South Goa (Figure 1). North Goa district comprises of six talukas, namely, Pernem, Bardez, Tiswadi, Bicholim, Ponda, and Sattari while the South Goa district comprises of six talukas, namely, Dharbandora, Mormugao, Salcete, Quepem, Sanguem, and Canacona (Figure 1). Physiographically, Goa is divided into three main regions, viz., i) the eastern Sahyadris—sub-region of the Western Ghats, and covering ~43% of the total state area, ii) the central uplands—the tract between the coast and the Ghats, consisting of rolling hills, slopes and valleys, which covers ~35% of the state area, and iii) the western coastal plains—the coastal belt which accounts for ~22% of the total area of the state. As per Champion & Seth (1968) classification of forest types of India, the forests of Goa fall in the following types: i) estuarine vegetation consisting of mangrove species along narrow muddy banks of rivers, ii) strand vegetation along the coastal belts, iii) plateau vegetation confined especially in low altitudes, and iv) semi-evergreen and evergreen forests. It has a tropical monsoon climate and the region is generally warm and humid throughout the year. The temperature ranges from 20°C to 34°C, and atmospheric humidity ranges from 60% to 90% throughout the year due to the proximity of the state to the Arabian Sea. The average annual rainfall received in the state is about 3200mm. Over 90% of annual rainfall occurs during monsoon months of June to September (Ibrampurkar 2012). Goa is the only state in India which has protected the complete Western Ghats section within the state. The state has one national park (Bhagwan Mahavir) and six wildlife sanctuaries, which contributes about 52% of the forested area.

### Data collection

After obtaining the survey permission from Forest Department of Goa, botanical explorations were undertaken from August 2015 to 2017, in different districts of Goa covering all the protected areas. Orchid species in flowering and fruiting stages were collected and photographed. All macro-morphological characters, such as vegetative and floral structures, were likewise recorded in the field. Species in the non-flowering stage were collected and maintained as living collections for

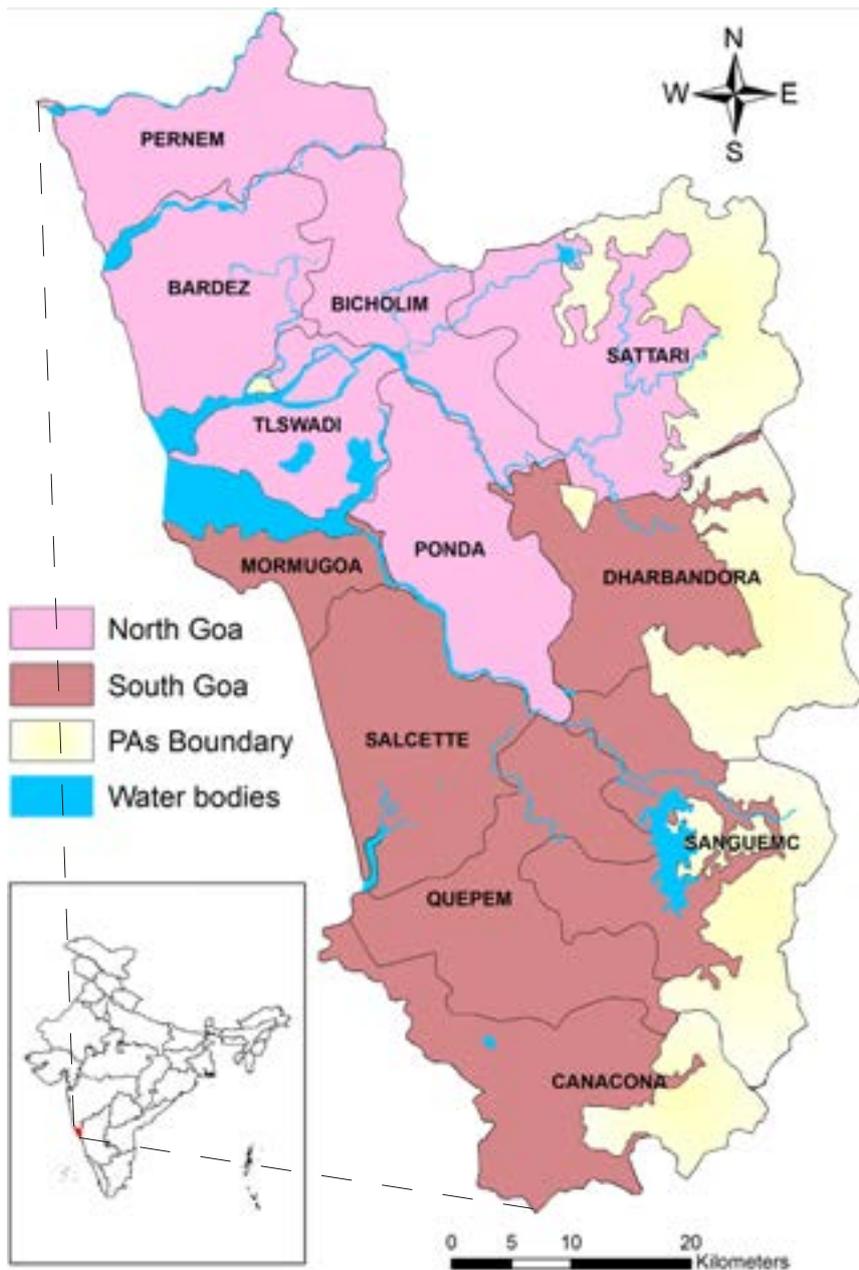


Figure 1. Study area map.

further studies. The geographical co-ordinate of each orchid was recorded using Garmin GPS etrex30. The herbarium specimens were processed following Jain & Rao (1977). Collected specimens were identified using Cooke (1967), Santapau & Kapadia (1966) and Abraham & Vatsala (1981). All the herbarium specimens collected, were deposited in the Herbarium of the Botanical Survey of India, Pune (BSI).

A total 552 GPS records were collected during the field survey for species distribution model. This study was restricted only to species with five or more occurrence records, thus 49 species were taken for

modeling purposes. Nineteen bioclimatic variables (Hijmans et al. 2005) derived from climatic data from the 1950–2000 period were used. All variables were reduced to a grid resolution of 30 arc-seconds or  $0.008333^{\circ}$  (approximately  $1\text{km}^2$ ) for the analysis. MaxEnt ver. 3.3 was used for species prediction modeling as it works with presence-only data (Phillips et al. 2006). The analysis was performed using the default parameters: maximum iterations to 500 and using convergence threshold in  $1.0\text{E}-5$ . Duplicate presence records were removed by the program prior to model development. For the potential habitat map cells reported by MaxEnt, cell values of 1

are considered the most suitable, whereas closer to zero regarded as less suitable. The probability values that were equal or greater than a threshold value of 0.5 indicates the potential suitable habitat of a species (Phillips et al. 2006). The MaxEnt produces continuous outputs of potential habitat suitability ranging from 0 to 1, which were further reclassified into two classes—suitable and unsuitable. The threshold was selected as per ‘minimum training presence’ threshold technique limits. The priority areas were calculated by summing up the thresholded binary maps of all 49 species and reclassifying the grid cells. The priority zones were further divided into low, medium and high zones for the sake of convenience.

## RESULTS AND DISCUSSION

The present outcome is based on intensive field surveys as well as information collected from different herbaria and literature. A total of 68 species of orchids belonging to 28 genera were documented, of which 42 are epiphytic and 26 are terrestrial (Table 1) (Images 1–3). In the present survey presence of 58 species were confirmed and the remaining 10 species were included based on herbarium records and literature (Images 4–67). *Habenaria*, *Dendrobium* and *Oberonia* were the most species rich genera in the study area. Twenty-eight species were endemic, of which 23 species are restricted to the Western Ghats, while two are endemic to peninsular India and three are Indian endemic. Three broad habitats were categorized for orchids, viz., semi-evergreen forests, moist-deciduous forests, and plateaus (Fig 2). The most favourable habitat was found to be semi-evergreen forests hosting 40 species followed by moist-deciduous forests (37 species). Very few terrestrial orchids were reported from plateaus (6 species), which are mainly found at higher elevations. These plateaus are threatened due to local grazing. In Goa, these plateaus are known as ‘sada’.

Distribution of orchids in Goa is concentrated mainly in the Western Ghats region, which accounts for 86% of the total orchid diversity of the state (Fig. 3). The high undulating mountains are covered with semi-evergreen forests and provide suitable habitats for many endemic orchids. Half a dozen important rivers, e.g., Mandovi, Mhadei, and Zuari, flow between these mountains and maintain high humidity levels throughout the year which is an important factor for the growth and development of epiphytic orchids.

The entire Western Ghats of the state is covered

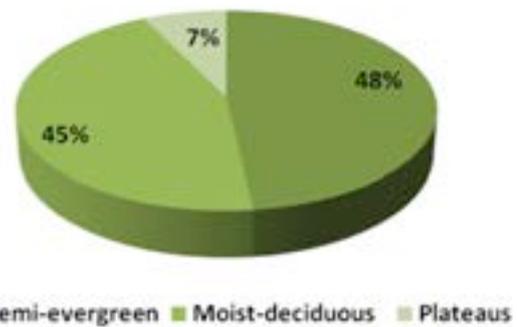


Figure 2. Different habitat types of orchids in Goa.

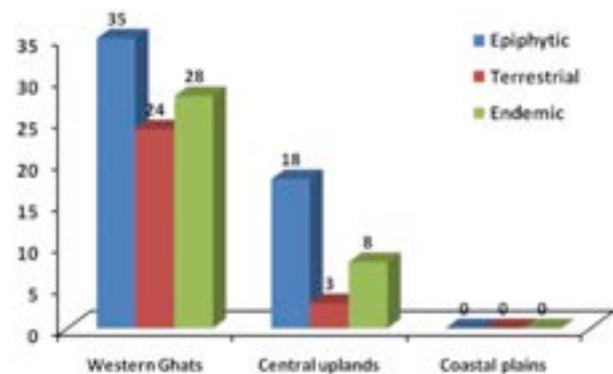


Figure 3. Distribution of orchids in different phyogeographic zones of Goa.

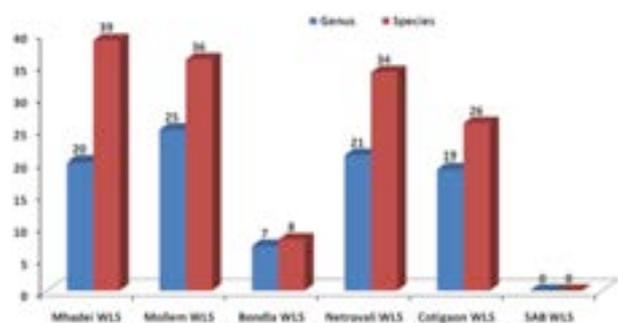


Figure 4. Orchid species richness in different PAs of Goa.

under protected areas and there is no direct threat to these orchids; however, a few human settlements still exist inside the wildlife sanctuary as a result of which some pockets of these natural habitats are disturbed due to lopping of forests and from cattle grazing. The central upland is a transition zone between the Western Ghats and the coastal plains. The area, adjoining the Western Ghats, has semi-evergreen and moist-deciduous forests at the base of the hills which provide a very favorable habitat for epiphytic as well as terrestrial orchids. These forests are moderately dense and receive less

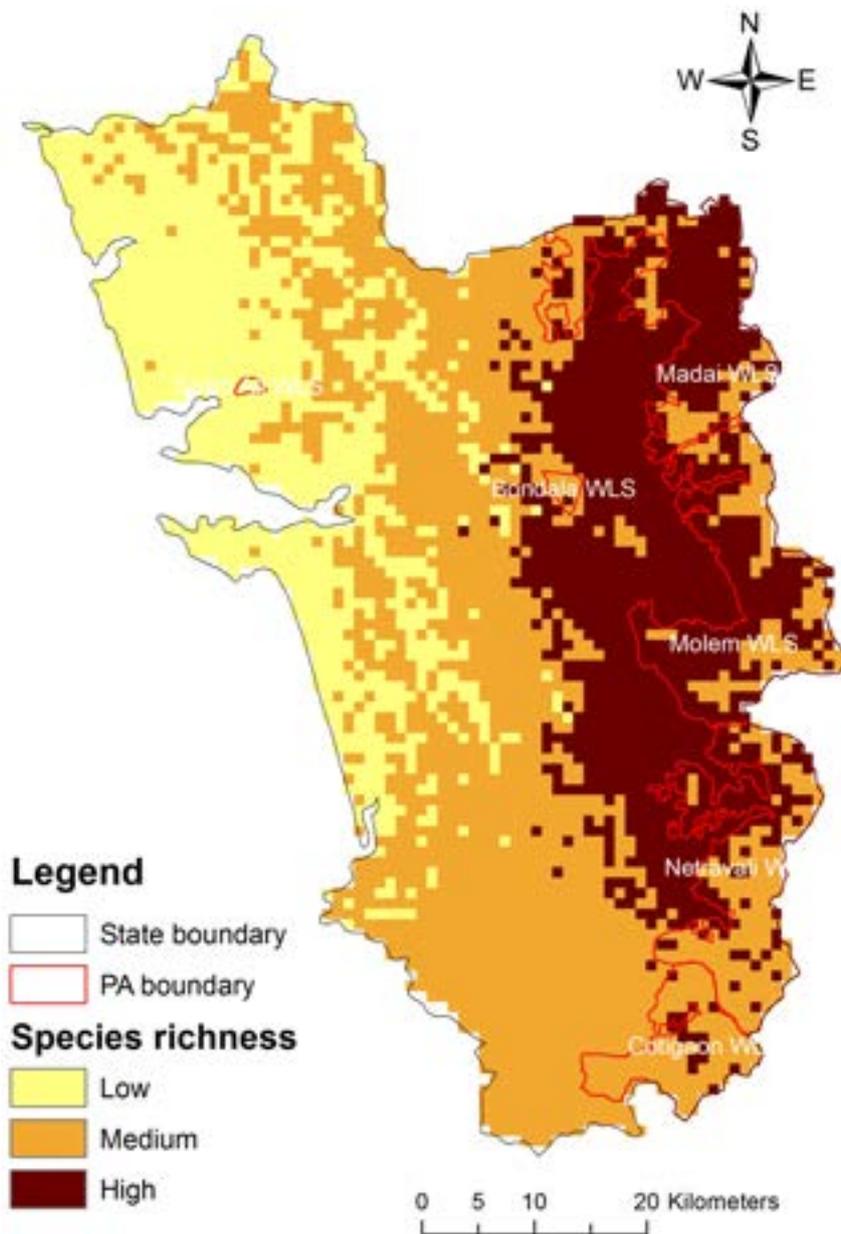


Figure 5. Potential orchid richness map.

intense sunlight and have high humidity as well. It has an average height ranging 30–100 m and most of the landscape is covered with coconut palms, paddy fields, betelnut farms, commercial establishments, human settlements, and many active iron ore mines. A total of 18 species are distributed in this part of which eight are endemic. Threats to the orchids are high in this part as many forest patches are being cleared for developmental purposes and new mining activities. Large forest patches are private or community land for which mining clearance is not necessary. The rampant encroachment is destroying the pristine forest habitat. Species such as *Acampe praemorsa*, *Bulbophyllum sterile*, *Cymbidium*

*bicolor*, *Dendrobium ovatum*, and *Rhynchostylis retusa* are encountered mostly in such disturbed habitats, along roadside and forests edges.

The coastal plains have no orchid presence. This is a narrow stretch of low-lying area dominated by estuarine mangroves.

In the present survey all the protected areas of Goa were also surveyed to know the orchid diversity and to identify the best protected area for in situ conservation. The findings reveal that Mhadei Wildlife Sanctuary has orchid richness (Fig. 4). This sanctuary covers most of the northeastern portion of Goa and the average rainfall in this region is above 3,200mm.



Image 1. A—*Acampe praemorsa* | B—*Aerides maculosa* | C—*Aerides ringens* | D—*Bulbophyllum sterile* | E—*Cleisostoma tenuifolium* | F—*Cottonia peduncularis* | G—*Cymbidium bicolor* | H—*Dendrobium aqueum* | I—*Dendrobium barbatulum*. © Jeewan Singh Jalal.

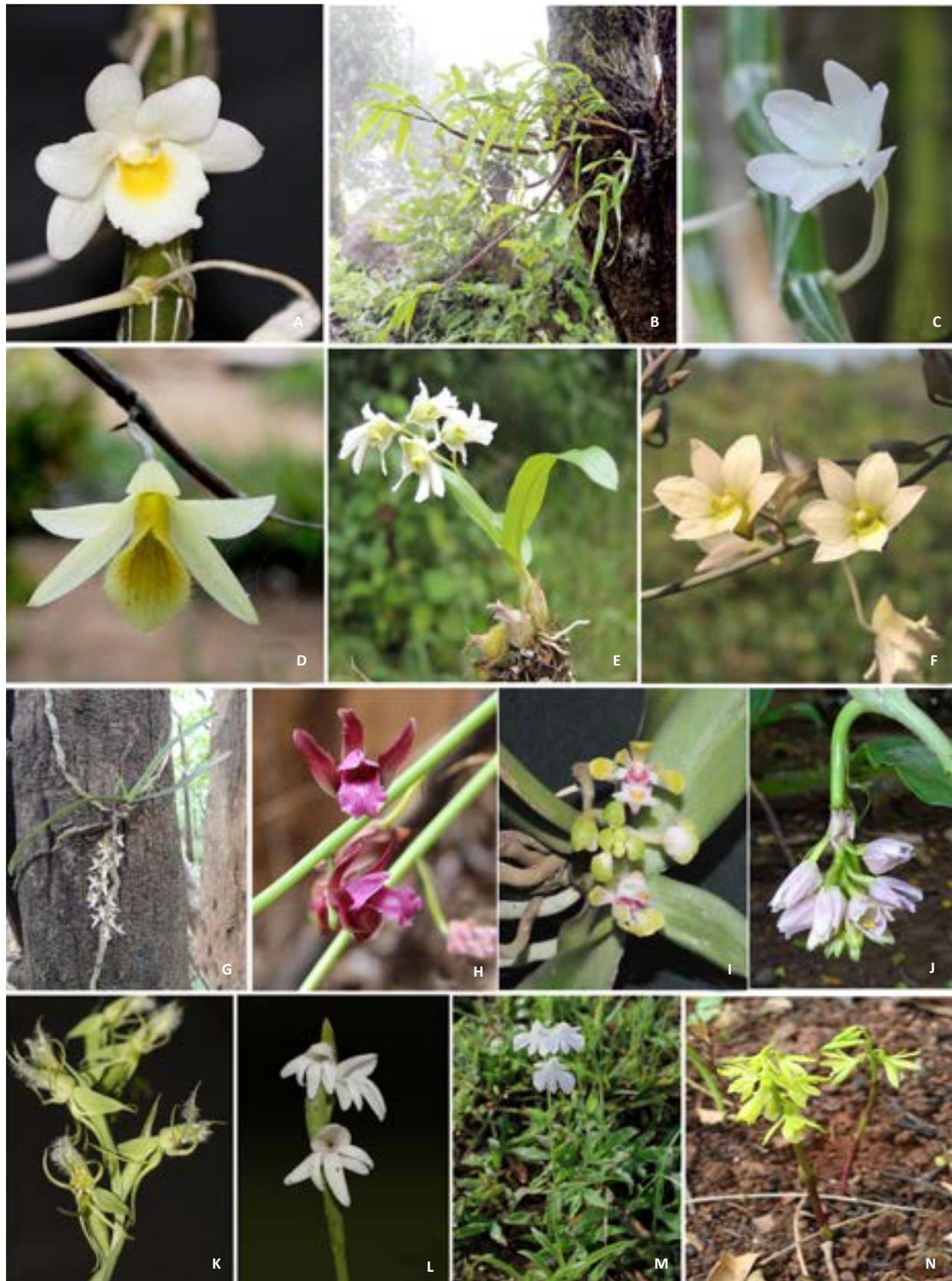


Image 2. A—*Dendrobium crepidatum* | B—*Dendrobium herbaceum* | C—*Dendrobium lawianum* | D—*Dendrobium macrostachyum* | E—*Dendrobium nanum* | F—*Dendrobium ovatum* | G—*Diplozentrum congestum* | H—*Eulophia spectabilis* | I—*Gastrochilus flabelliformis* | J—*Geodorum densiflorum* | K—*Habenaria elwesii* | L—*Habenaria heyneana* | M—*Habenaria suaveolens* | N—*Nervilia concolor*. © Jeewan Singh Jalal.

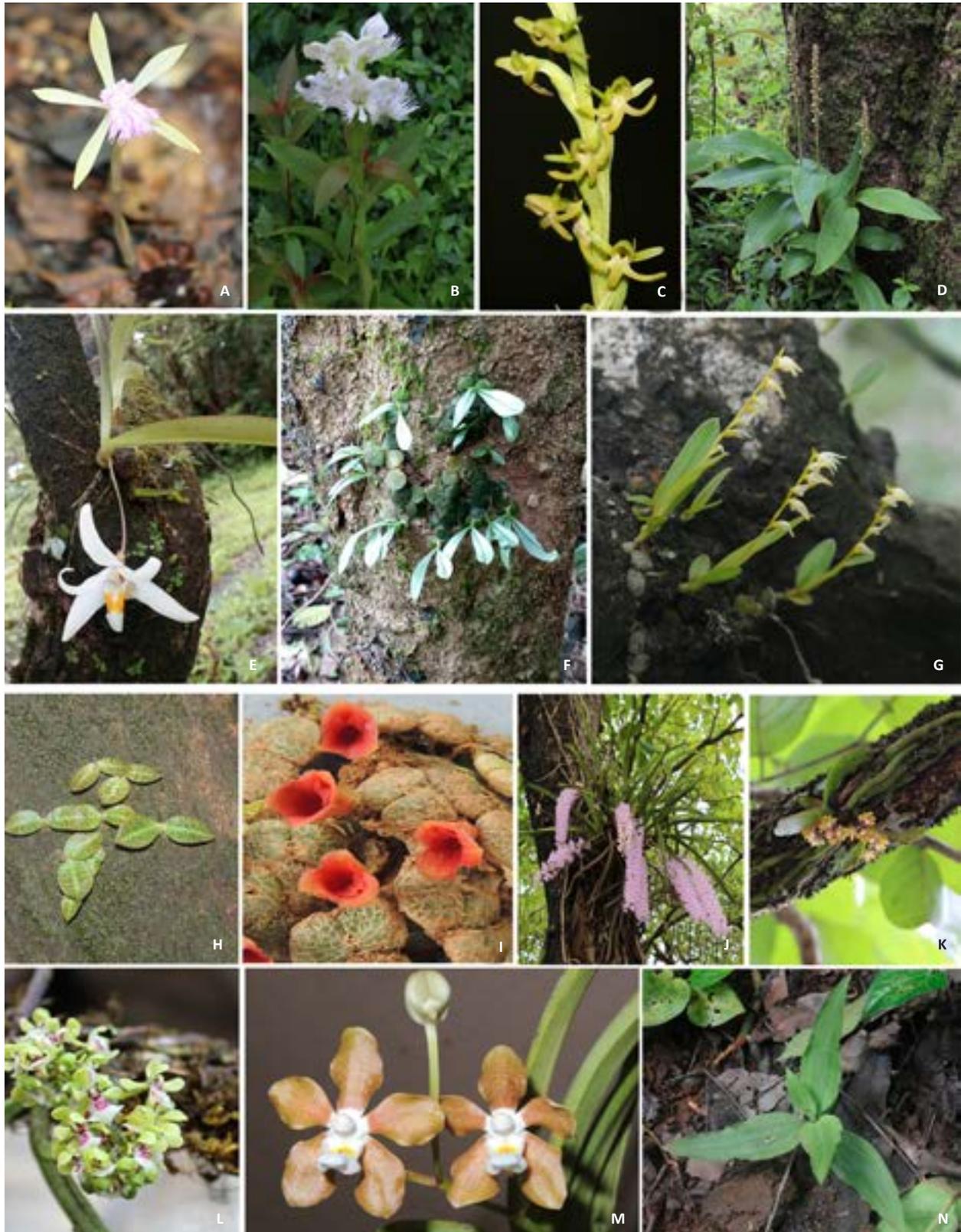


Image 3. A—*Nervilia crociformis* | B—*Pecteilis gigantea* | C—*Peristylus densus* | D—*Peristylus plantagineus* | E—*Pinalia reticosa* | F—*Porpax exilis* | G—*Porpax filiformis* | H—*Porpax jerdoniana* | I—*Porpax reticulata* | J—*Rhynchostylis retusa* | K—*Smithsonia straminea* | L—*Smithsonia viridiflora* | M—*Vanda wightii* | N—*Zeuxine longilabris*. © Jeewan Singh Jalal.

Table 1. List of orchids of Goa.

	Name of species	Habit	Phenology	Endemic	Habitat	Herbarium number	Occurrence in different protected areas				
							Mhadei	Mollem	Bondla	Netravali	Cotigaon
1	<i>Acampe praemorsa</i> (Roxb.) Blatt. & McCann	E	Apr–Dec		MDF	JSJ 203603	+	+	+	+	+
2	<i>Aerides crispa</i> Lindl.	E	May–Jun		SEF	JSJ 203637	+	+		+	
3	<i>Aerides maculosa</i> Lindl.	E	May–Jul	EPI	MDF, SEF	JSJ 203745	+	+	+	+	
4	<i>Aerides ringens</i> (Lindl.) C.E.C.Fisch.	E	Mar–Jul.		MDF	JSJ 203698A		+			+
5	<i>Bulbophyllum sterile</i> (Lam.) Suresh	E	Dec–May	WG	MDF	JSJ 203682	+	+	+	+	+
6	<i>Cleisostoma tenuifolium</i> (L.) Garay	E	Jun–Nov		MSD, SEF	JSJ 203615	+	+		+	+
7	<i>Cottonia peduncularis</i> (Lindl.) Rchb.f.	E	Mar–Apr		MDF	JSJ 203601	+	+		+	+
8	<i>Cymbidium bicolor</i> Lindl.	E	May–Jun		MDF	JSJ 203687		+		+	+
9	<i>Dendrobium aqueum</i> Lindl.	E	Sep–Dec	WG	SEF	JSJ 203667	+				
10	<i>Dendrobium barbatulum</i> Lindl.	E	Jan–May	WG	MDF, SEF	JSJ 203613	+	+	+	+	+
11	<i>Dendrobium crepidatum</i> Lindl.	E	Jan–Mar		MDF	JSJ 203697					+
12	<i>Dendrobium herbageum</i> Lindl.	E	Feb–Mar		SEF	JSJ 203630	+				
13	<i>Dendrobium lawianum</i> Lindl.	E	Mar–Apr	WG	SEF	JSJ 203634					+
14	<i>Dendrobium macrostachyum</i> Lindl.	E	May–Jun		MDF, SEF	JSJ 203707				+	+
15	<i>Dendrobium microbulbon</i> A.Rich.	E	Dec–Jan	WG	SEF	JSJ 203666	+				
16	<i>Dendrobium nanum</i> Hook.f.	E	Jul– Aug	WG	SEF	JSJ 203632	+				
17	<i>Dendrobium ovatum</i> (L.) Kranzl.	E	Sep–Feb	WG	MDF	JSJ 203696	+		+	+	+
18	<i>Dendrobium nodosum</i> Dalzell	E	Jul–Aug	WG	SEF	JSJ 203641	+				
19	<i>Dendrobium peguanum</i> Lindl.	E	Oct–Dec		MDF	JSJ 203743					
20	<i>Diplocentrum congestum</i> Wight	E	Apr–May	WG	MDF, SEF	JSJ 203754		+			
21	<i>Eulophia spectabilis</i> (Dennst.) Suresh	T	May–Jun		MDF, SEF	JSJ 203628	+			+	
22	<i>Gastrochilus flabelliformis</i> (Blatt. & McCann) C.J. Saldanha	E	Apr–Jun	WG	SEF	JSJ 203742		+			
23	<i>Geodorum densiflorum</i> (Lam.) Schltr.	T	Jun–Jul		MDF	JSJ 203610	+				
24	<i>Habenaria crinifera</i> Lindl.	T	Jul–Aug		SEF	JSJ 203640	+				
25	<i>Habenaria diphylla</i> (Nimmo) Dalzell	T	Aug–Sep		MDF	MND 186774		+			
26	<i>Habenaria elwesii</i> Hook.f.	T	Aug–Sep	WG	SEF	JSJ 203765				+	
27	<i>Habenaria foliosa</i> A.Rich.	T	Jul–Aug	WG	SEF	JSJ 203665	+				
28	<i>Habenaria grandifloriformis</i> Blatt. & McCann	T	May–Jul	EPI	PL	JSJ 203757					
29	<i>Habenaria heyneana</i> Lindl.	T	Jul–Sep	WG	PL	JSJ 203673		+			
30	<i>Habenaria longicorniculata</i> Graham	T	Aug–Sep		PL	JSJ 203779	+	+		+	
31	<i>Habenaria marginata</i> Colebr.	T	Sep–Oct		SEF	RSR 103443		+			

	Name of species	Habit	Phenology	Endemic	Habitat	Herbarium number	Occurrence in different protected areas				
							Mhadei	Mollem	Bondla	Netravali	Cotigaon
32	<i>Habenaria multicaudata</i> Sedgw.	T	Aug–Sep	WG	SEF	NPS124207		+			
33	<i>Habenaria plantaginea</i> Lindl.	T	Aug–Sep		MDF	***		+			
34	<i>Habenaria rariflora</i> A.Rich.	T	Jul–Aug	WG	PL	JSJ 203768				+	
35	<i>Habenaria suaveolens</i> Dalzell	T	Jul–Sep	WG	PL	JSJ 203668	+				
36	<i>Liparis deflexa</i> Hook.f.	T	Aug–Sep		MDF	MND179179		+			
37	<i>Liparis odorata</i> (Willd.) Lindl.	T	Jun–Sep		MDF, SEF	JSJ 203762	+	+		+	+
38	<i>Luisia tenuifolia</i> Blume	E	Mar–Apr		MDF, SEF	MND179064		+			
39	<i>Luisia zeylanica</i> Lindl.	E	May–Jun		MDF	JSJ 203621	+			+	+
40	<i>Malaxis versicolor</i> (Lindl.) Abeyw.	T	Jul–Aug		MDF, SEF	JSJ 203769	+	+	+	+	+
41	<i>Nervilia concolor</i> (Blume) Schltr.	T	May–Aug		MDF, SEF	JSJ 203674		+		+	+
42	<i>Nervilia crociformis</i> (Zoll. & Moritzi) Seidenf.	T	Jun–Aug		SEF	JSJ 203647	+				
43	<i>Oberonia brachyphylla</i> Blatter & McCann	E	May–Jun		SEF	V&SR1528		+			
44	<i>Oberonia brunoniana</i> Wight	E	Feb–Mar	WG	MDF	***	+			+	
45	<i>Oberonia mucronata</i> (D. Don) Ormerod & Seidenf.	E	Sep–Oct		MDF	JSJ 203715				+	
46	<i>Oberonia recurva</i> Lindl.	E	Sep–Oct		SEF	JSJ 203770				+	
47	<i>Oberonia verticillata</i> Wight	E	Sep–Oct	WG	MDF	JSJ 203708				+	
48	<i>Pecteilis gigantea</i> (Sm.) Raf.	T	Sep–Oct		SEF	JSJ 203766		+		+	
49	<i>Peristylus aristatus</i> Lindl.	T	Jul–Sep		SEF	JSJ 203629	+				
50	<i>Peristylus densus</i> (Lindl.) Santapau & Kapadia	T	Jul–Sep		PL	JSJ 203643	+			+	
51	<i>Peristylus plantagineus</i> (Lindl.) Lindl.	T	Jul–Sep		MDF, SEF	JSJ 203645	+	+		+	+
52	<i>Peristylus stocksii</i> (Hook.f.) Kraenzl.	T	Jul–Sep	IE	SEF	JSJ 203646	+				
53	<i>Phalaenopsis deliciosa</i> Rchb.f.	E	Aug–Sep		SEF	CRJ184871					+
54	<i>Pholidota imbricata</i> Lindl.	E	Jun–Jul		MDF	JSJ 203614	+	+		+	+
55	<i>Pinalia reticosa</i> (Wight) Kuntze	E	May–Jun	IE	SEF	JSJ 203649	+				
56	<i>Porpax exilis</i> (Hook.f.) Schuit., Y.P.Ng & H.A.Pedersen	E	Oct–Dec	WG	SEF	JSJ 203642	+			+	
57	<i>Porpax filiformis</i> (Wight) Schuit., Y.P.Ng & H.A.Pedersen	E	Jul–Aug	WG	MDF, SEF	JSJ 203604	+		+	+	+
58	<i>Porpax jerdoniana</i> (Wight) Rolfe	E	Jun–Jul	IE	MDF, SEF	JSJ 203606	+	+	+	+	+
59	<i>Porpax microchilos</i> (Dalzell) Schuit., Y.P.Ng & H.A.Pedersen	E	Jul–Aug	WG	MDF, SEF	JSJ 203648	+	+		+	+
60	<i>Porpax reticulata</i> Lindl.	E	Apr–Jun		MDF, SEF	JSJ 203755	+	+			
61	<i>Rhynchostylis retusa</i> (L.) Bl.	E	Jun–Jul		MDF	JSJ 203729		+		+	+
62	<i>Smithsonia straminea</i> C.J.Saldanha	E	May–Jun	WG	MDF	JSJ 203756	+			+	+
63	<i>Smithsonia viridiflora</i> (Dalzell) C.J.Saldanha	E	May–Jun	WG	SEF	JSJ 203721		+		+	+

	Name of species	Habit	Phenology	Endemic	Habitat	Herbarium number	Occurrence in different protected areas				
							Mhadei	Mollem	Bondla	Netravali	Cotigaon
64	<i>Tropidia angulosa</i> (Lindl.) Blume	T	Jan–Feb		SEF	MND187515		+			
65	<i>Vanda tessellata</i> (Roxb.) Hook. ex G. Don	E	May–Jun		MDF	JSJ 203741	+	+			+
66	<i>Vanda testacea</i> (Lindl.) Rchb.f.	E	May–Jun		MDF	JSJ 203607	+	+		+	+
67	<i>Vanda wightii</i> Rchb.f.	E	Sep–Oct		MDF	JSJ 203700		+			
68	<i>Zeuxine longilabris</i> (Lindl.) Benth. ex Hook.f.	T	Feb–Mar		SEF	JSJ 203611	+	+			

E—Epiphytic | T—Terrestrial | EPI—Endemic to Peninsular India | WG—Western Ghats | IE—Indian Endemic | SEF—Semi-evergreen Forest | MDF—Moist Deciduous forest | PL—Plateau | \*\*\*—Included from published record.

Figure 5 represents summation of potential orchid distribution of 49 orchid species in Goa (constituting 72% of total orchids of Goa) which was predicted using MaxEnt algorithm. The output has been further categorized into areas with high (21–34 species), medium (8–21 species) and low (less than 8 species) richness or suitability zones. The high richness zone is dominated by moist-deciduous forests interspersed with semi-evergreen forests and open plateaus, which are suitable for orchids. Many endemic species are restricted to the evergreen forest habitat pointing towards high habitat specificity and also towards the ecological importance of these habitats. Approximately, 1,005km<sup>2</sup> is found to be highly suitable for orchids, which is 27% of the total geographic area of Goa State.

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Image 4. *Acampe praemorsa* (203603)



Image 5. *Aerides crispa* (203637)



Image 6. *Aerides maculosa* (203745)



Image 7. *Aerides ringens* (203698A)





Image 11. *Dendrobium aqueum* (203667)



Image 12. *Dendrobium barbatulum* (203697)



Image 13. *Dendrobium crepidatum* (203697)



Image 14. *Dendrobium herbaceum* (203630)



Image 15. *Dendrobium lawianum* (203634)



Image 16. *Dendrobium macrostachyum* (203707)



Image 17. *Dendrobium microbulbon* (203666)



Image 18. *Dendrobium nanum* (203632)



Image 19. *Dendrobium nodosum* (203641)



Image 20. *Dendrobium ovatum* (203696)



Image 21. *Dendrobium peguanum* (203743)



Image 22. *Diplozentrum congestum* (203754)



Image 23. *Eulophia spectabilis* (203628)



Image 24. *Gastrochilus flabelliformis* (203742)



Image 25. *Geodorum densiflorum* (203610)



Image 26. *Habenaria crinifera* (203640)





Image 31. *Habenaria heyneana* (203673)



Image 32. *Habenaria longicorniculata* (203779)



Image 33. *Habenaria marginata* (103433)



Image 34. *Habenaria multicaudata* (124207)



Image 35. *Habenaria rariflora* (124207)



Image 36. *Habenaria suaveolens* (203668)



Image 37. *Liparis deflexa* (179179)



Image 38. *Liparis odorata* (203762)



Image 39. *Luisia teunifolia* (179064)



Image 540. *Luisia zeylanica* (203621)



Image 41. *Malaxis versicolor* (203769)



Image 42. *Nervilia concolor* (203674)

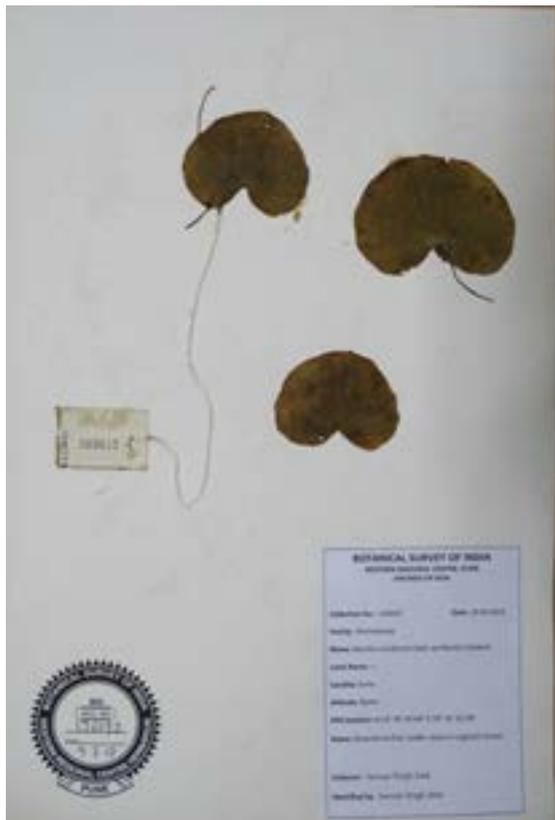


Image 43. *Nervilia crociformis* (203647)



Image 44. *Oberonia mucronata* (203715)



Image 45. *Oberonia recurva* (203770)



Image 46. *Oberonia verticillata* (203708)



Image 47. *Pecteilis gigantea* (203766)



Image 48. *Peristylus aristatus* (203629)



Image 49. *Peristylus densus* (203643)



Image 50. *Peristylus plantagineus* (203645)



Image 51. *Peristylus stocksii* (203646)



Image 52. *Phalaenopsis deliciosa* (184871)



Image 53. *Pholidota articulata* (203614)



Image 54. *Pinalia reticosa* (203649)



Image 55. *Porpax exilis* (203642)

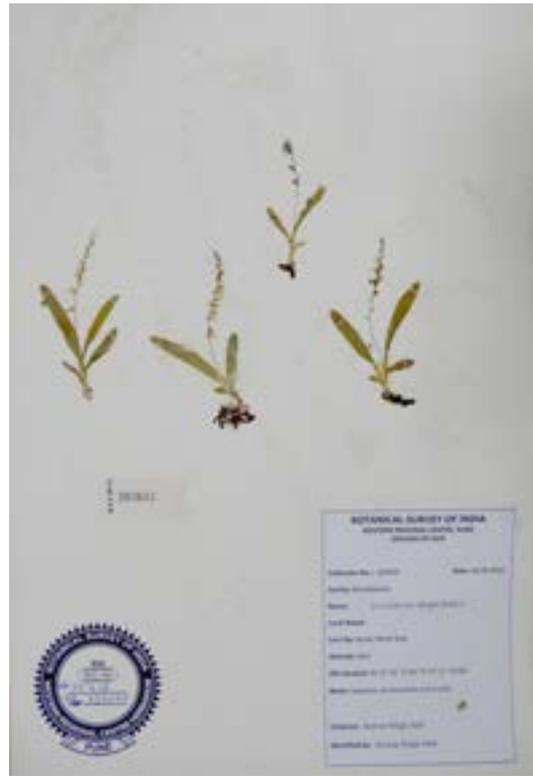


Image 56. *Porpax filiformis* (203604)



Image 57. *Porpax jerdoniana* (203606)



Image 58. *Porpax microchilos* (203648)



Image 59. *Porpax reticulata* (203755)



Image 60. *Rhynchostylis retusa* (203729)



Image 61. *Smithsonia straminea* (203756)



Image 62. *Smithsonia viridiflora* (203721)



Image 63. *Tropidia angulosa* (187515)

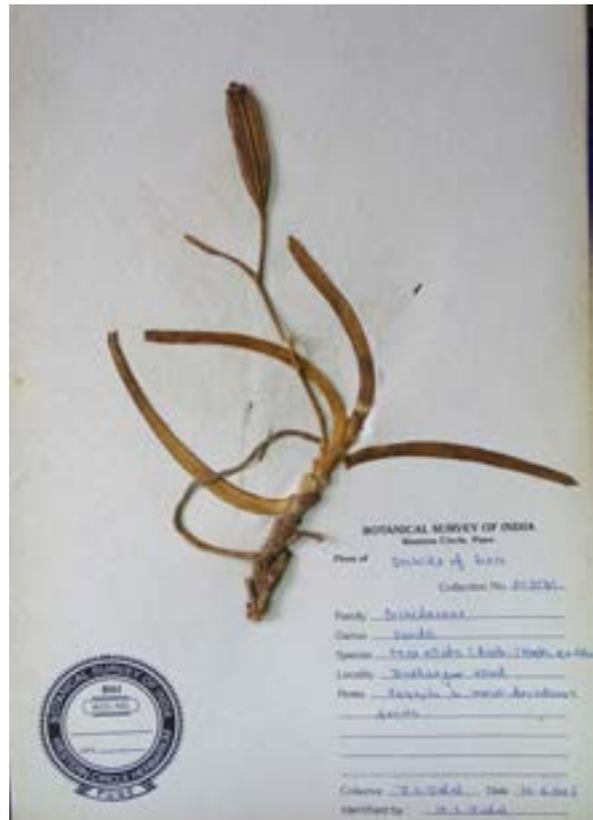


Image 64. *Vanda tessellata* (203741)



Image 65. *Vanda testacea* (203607)



Image 66. *Vanda wightii* (203700)



Image 67. *Zeuxine longilabris* (203611)





## EFFICACY OF OXYCLOZANIDE AND LEVAMISOLE TREATMENT ON THE GASTROINTESTINAL PARASITES IN CAPTIVE LIONS *PANTHERA LEO*

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**Abstract:** A study was carried out to determine the efficacy of anthelmintics on gastrointestinal parasites in lions under captivity at Bannerghatta Biological Park (Bengaluru), Sri Chamarajendra Zoological Garden (Mysuru) and Tiger-Lion Safari Tyavarekoppa (Shivamogga) during the period from January to June, 2018. Out of 20 faecal samples subjected to qualitative and quantitative methods, 66.6% were found positive for eggs of *Ancylostoma* spp., 60.0% for *Toxascaris leonina*, 20.0% for *Spirometra* spp. and 13.3% for *Balantidium coli* cysts with an overall infection rate of 75.0%. A combination of oxcyclozanide 6% w/v and levamisole 3% w/v (Neozide plus) at the rate of 1ml per 4kg body weight revealed egg per gram counts for *Ancylostoma* spp., *T. leonina* and *Spirometra* spp. to be reduced from 100, 11,450 ± 11,250 and 100 to zero respectively on subsequent 3, 7, 10 and 21 days post treatment and proved to be cent per cent effective.

**Keywords:** Anthelmintics, efficacy, gastrointestinal parasites, Lions.

In captive wild carnivores, the change in the environment and living conditions influences the ecology of the animal and might increase the susceptibility to many of the diseases, viz., bacterial, viral, parasitic and rickettsial diseases (Goossens et al. 2005). Especially, carnivores kept in captivity in zoos usually suffer from several parasitic infections, such as from nematodes, cestodes, trematodes, and protozoans. In particular,

among nematodes ascarids constitute the major parasitic infection in wild carnivores and are established as a problem in most of the zoos throughout the world (Sayid & Mohammed 1997–1998).

Among parasitic diseases, particularly helminthic infections have a greater ramification and significant impact on host survival, growth and reproduction through direct and indirect pathological effects. The subclinical infections may not cause any immediate alarming signs of disease but in the long course, they would render the animals susceptible to other concurrent infections (Muraleedharan et al. 1990). In addition, gastrointestinal parasites of wild carnivores include zoonotic species to humans and may raise public health concern (Acharjyo 2004).

Though helminthic diseases are a major constraint to zoo animals, the occurrence of parasitic infections may vary depending on the type of husbandry practices, viz., nutritional status, physiological condition, implementation of disease control programmes, and treatment administered (Singh et al. 2006). In most of the zoological gardens, the prevention and control of

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gastrointestinal parasitic infections is mainly dependent on the short term deworming programmes. A range of antiparasitic drugs has been used to reduce parasitism in wild animal populations. In particular, most drug treatment experiments using anthelmintic has been carried out by targeting nematode infections (Pedersen & Fenton 2015). The commonly used anthelmintic includes thiabendazole, piperazine citrate and adipate, pyrantel pamoate, albendazole, fenbendazole, levamisole, and ivermectin. Many studies have provided information on the effects of treatment on the target parasite, assessed either in terms of the prevalence of infection (proportion of hosts infected), mean parasitic abundance (mean number of parasites or parasitic eggs shed per host), or mean parasite intensity (mean number of parasite eggs shed per infected host).

The regular examination of faeces, assessment of parasitic load, and the assessment of drug efficacy, however, are not frequently carried out in many zoological parks or gardens. Therefore, the present study was undertaken to determine the efficacy of anthelmintic on the gastrointestinal parasites in captive lions.

## MATERIAL AND METHODS

### Study area

The study area included Bannerghatta Biological Park (BBP) which is located 22km south of Bengaluru in the hills of the Anekal range with 26356.16 hectares area with zoo, a pet corner, an animal rescue centre, a butterfly enclosure, an aquarium, a snake house, and a safari park. Sri Chamarajendra Zoological Garden (SCZG) is around 63.53 hectares located near the palace in Mysuru, is one of the oldest zoos in India and is home to a wide range of species (168). Tiger-Lion Safari Tyavarekoppa (TLST), Shivamogga is Karnataka's second safari park, after BBP with an area of 250ha.

### Collection of samples

During this study, a representative faecal sample of about 10g was collected from each enclosure and the methodology was followed as per Soulsby (1982) and Taylor et al. (2015). However, in Dehuri et al. (2013), 2g of faeces was collected and also for the methods the reference Soulsby (1982) is cited. A total of 20 faecal samples were collected from lions under captivity at BBP (12), SCZG (4), and TLST (4) during the period January–June 2018. The faecal samples were examined macroscopically and were subjected to microscopic examination by using qualitative (direct and concentration) and quantitative (Mc Master's) methods

to assess the severity of different parasitic infections (Soulsby 1982; Taylor et al. 2015). The parasitic eggs/larvae/cysts/oocysts were identified based on the standard morphological characters (Soulsby 1982; Zajac & Conboy 2012; Bowman 2014).

### Determination of anthelmintic efficacy

An anthelmintic efficacy against gastrointestinal parasitic infections was determined based on the eggs per gram (EPG) of faeces using Mc Master's method. During this study, a combination of oxyclozanide 6% w/v and levamisole 3% w/v (Neozide plus) @ 1ml per 4kg body weight in meat was administered during May 2018. The EPG was carried out during pretreatment and on 3<sup>rd</sup>, 7<sup>th</sup>, 10<sup>th</sup>, and 21<sup>st</sup> days post treatment. The percentage of efficacy of drug was determined by the following formula:

$$\text{Efficacy \%} = (\text{Pre treatment EPG} - \text{Post treatment EPG} / \text{Pre treatment EPG}) \times 100$$

### Statistical analysis

The statistical analysis of data was carried out by Fisher's exact and one way ANOVA tests using graph pad prism software, version 5.01.

## RESULTS

During this study, out of 20 faecal samples examined by direct and concentration methods, 15 samples were found to be positive for helminth infections of *Ancylostoma* spp. (66.6%), *T. leonina* (60.0%), *Spirometra* spp. (20%), and *Balantidium coli* (13.3%) cysts, with an overall infection rate of 75%. In BBP, SCZG, and TLST, 10 (83.3%), four (100%), and one (25.0%) samples were found to be positive for gastrointestinal parasitic eggs/cysts, respectively (Table 1). The statistical differences between the infections in the different locations were found to be nonsignificant at  $P < 0.05$ . The mixed infections of *Ancylostoma* spp. and *T. leonina* (5), *Ancylostoma* spp. and *Spirometra* sp. (2), and *Ancylostoma* spp. and *B. coli* (2) were commonly observed with an overall infection rate of 70%.

### Anthelmintic efficacy

The average mean pretreatment EPG counts was found to be  $100 \pm 0$  for *Ancylostoma* spp., 11,450  $\pm$  11,250 for *T. leonina* and  $100 \pm 0$  for *Spirometra* spp. Subsequently, after 3, 7, 10 and 21 days post treatment, the EPG counts were reduced to zero. An examination of faecal samples was negative for gastrointestinal parasitic infections with cent per cent efficacy on 21 days post treatment.

**Table 1. Number of faecal samples positive for gastrointestinal parasites in lions at different locations.**

	Locations	No. of animals examined	No. of animals infected	No. of positive samples			
				<i>Ancylostoma</i> spp.	<i>T. leonina</i>	<i>Spirometra</i> spp.	<i>Balantidium coli</i>
1	Bannerghatta Biological Park, Bengaluru	12	10 (83.3%)	8 (80.0%)	6 (60.0%)	3 (10.0%)	2 (20.0%)
2	Sri Chamarajendra Zoological Garden, Mysuru	4	4 (100.0%)	2 (50.0%)	2 (50.0%)	0	0
3	Tiger-Lion Safari Tyavarekoppa, Shivamogga	4	1 (25.0%)	0	1 (25.0%)	0	0
	Total	20	15 (75.0%)	10 (66.6%)	9 (60.0%)	3 (20.0%)	2 (20.0%)

## DISCUSSION

During this study, one faecal sample from TLST and two from SCZG were found to be positive for eggs of *Ancylostoma* spp. and *T. leonina* with very low parasitemia (EPG = 0). Therefore, anthelmintic efficacy was carried out at BBP. All the animals irrespective of the infection and whether the animals were positive or negative were administered a combination of oxcyclozanide (6% w/v), a broad spectrum antitrepatodal drug and levamisole (3% w/v) which has activity against nematodes and an immunostimulant at the rate of 1ml per 4kg body weight. In the present study, subsequently after 3<sup>rd</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 21<sup>st</sup> day post treatment, the EPG counts were reduced to zero per cent and the faecal samples were negative for infections indicating 100% efficacy. During this study, though the *Spirometra* infection was recorded, the animals were not administered with anticestodal drugs. Therefore, based on the present findings zoo veterinarians received the suggestion that wild carnivores under captivity should be regularly dewormed with anthelmintic only after an examination of faecal samples for the presence or absence of gastrointestinal parasitic infections to avoid unnecessary dosing with anthelmintic thereby reducing the cost of treatment and development of resistance in future. Many authors, however, have reported varied efficacy with different modes of action of anthelmintic— Sayid & Mohammad (1997–1998) at Khartoum Zoo, Sudan reported complete clearance of infection with piperazine but incomplete clearance of parasites on 28<sup>th</sup> day post treatment with thiabendazole in lions, leopards, jackal, and dwarf mongoose infected with *T. leonina*; Sur et al. (2000) treated lions infected with *Toxocara* and *Ancylostoma* with ivermectin injection (Ivomec) @ 1ml per 50kg body weight and recorded an absence of eggs on the 7<sup>th</sup> day post treatment; Kumar et al. (2005) treated 22 lions which showed the occurrence of *Toxocara* eggs in the faeces with piperazine @ 220mg per kg orally at M.C. Zoological Park, Chhatbir, Patiala District, Punjab

and later, with ivermectin at the dose rate of 1ml per 50kg body weight subcutaneously twice at one week intervals for piperazine resistant *T. cati* and observed the egg count to be reduced by 98.71%; Moudgil et al. (2017) reported that fenbendazole @ 10mg per kg body weight once daily for three consecutive days was ineffective to eliminate the infection in Asiatic Lions infected with *T. leonina* and observed eggs reduction by 69.35% at day 3 post treatment with three consecutive treatment schedule, however, extended period of time with fenbendazole for five days and ivermectin @ 100µg per kg body weight once daily for three alternative days resulted in eggs reduction by 95.34% and 95.74%, respectively, and proved to be effective.

The differences in the efficacy of each anthelmintic drug may be attributed to the fact that the vehicle of drug to captive wild animals (especially wild felines) play an important role in administering exact dosage the reason could be that the total dosage of anthelmintic drug cannot be calculated according to their body weight (Moudgil et al. 2017).

In conclusion, though helminthic and protozoan infections are known to occur in captive wild carnivores, the control measures undoubtedly would depend upon several factors. Further, the rationale behind the control of parasitic infections in wild carnivores of Indian zoos presumes that in as much as each carnivore is infected and that the zoo environment cannot be changed frequently because of space confinement, overcrowding, and the movement of keepers from one enclosure to another and the presence of stray dogs and cats which may act as a source of infection (Acharjyo 2004). The present findings indicated that quarterly deworming (once in three months) of all the animals and examination of faecal samples before and after deworming should be followed regularly to confirm the efficacy of treatment. In addition, a change of anthelmintic should be instituted from time to time to avoid drug resistance in captive wild carnivores.

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## FIRST RECORD IN 129 YEARS OF THE TAMIL TREEBROWN *LETHE DRYPETIS TODARA* MOORE, 1881 (LEPIDOPTERA: NYMPHALIDAE: SATYRINAE) FROM ODISHA, INDIA BY FRUIT-BAITING

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**Abstract:** The first record of a butterfly species, the Tamil Treebrown *Lethe drypetis todara* Moore, 1881 (Nymphalidae: Satyrinae) is described from Deomali Hill range of southern Odisha, in Eastern Ghats of India, collected by fruit-baiting. This is the first record from Odisha almost 129 years after its first report from Khurda in coastal Odisha by Taylor & de Niceville in the year 1888. The ecology and sampling of the species are described in this study.

**Keywords:** Bait trap, butterfly, Eastern Ghats, ecology, Koraput.

The subfamily Satyrinae, with about 2,500 described species, is one of the most diverse groups of butterflies (Peña & Wahlberg 2008), comprising over a third of the Nymphalidae diversity and found on all continents except Antarctica (Ackery et al. 1999; DeVries 2000). The group is particularly diverse in the Neotropics, with approximately 1,200 species in 137 genera (Lamas et al. 2004), occurring in all habitats with vegetation from sea level to the highlands of the Andes (DeVries 1987). Satyrinae butterflies in India are represented by 190 species under five tribes and 18 genera (Varshney & Smetacek 2015) and 30 species in peninsular India (Kunte 2000).

The Tamil Treebrown *Lethe drypetis todara* Moore, 1881, is a butterfly belonging to the subfamily Satyrinae and the family Nymphalidae. The global distribution

of the taxon is Sri Lanka, southern India to Pachmarhi, Kashmir to Assam and Myanmar (Mathew & Soumya 2013). Older literature report it from Sri Lanka to peninsular India (Bingham 1905; Antram 1924), common, widespread and endemic to these regions (Gaonkar 1996). It is locally very common in the low jungles of the Western Ghats and the Nilgiris (Wynter-Blyth 1957). Talbot (1947) reported this species as “not-rare” from peninsular India to Odisha region. Gaonkar (1996) reported it from Kerala, Tamil Nadu, Karnataka, Goa, Gujarat, and Maharashtra. In recent times, it has been reported from Kotagiri in the Nilgiris, the moist-deciduous forests in Biligiriranga Hills and Wyanad, the Nadgani Ghat and Silent Valley (Mathew & Soumya 2013). The species has also been reported from Kalakad-Mundanthurai Tiger Reserve, Tamil Nadu (Devy & Davidar 2001). There are quite a few reports of its occurrence from various parts of southern India particularly from the states of Karnataka (Districts of Chikkamagaluru, Kodagu, Shivamogga, Uttara Kannada, Hassan, Mysore, and Dakshina Kannada), Kerala (Districts of Idukki, Palakkad, Malappuram, Wayanad, and Kozhikode), Maharashtra (Sindhudurg District) and Tamil Nadu (Districts of Theni, Nilgiri, and Dindigul) (Ogale 2019).

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From central India, the species was reported from the Kanger Valley National Park and from Bilaspur, Chhattisgarh (Chandra 2006; Chandra et al. 2007) as well as from Pench Tiger Reserve, Madhya Pradesh (Tiwari et al. 2010). The first record of the Tamil Treebrown in Eastern Ghats is from Maredumilli and Jalatarangini waterfalls of Papikonda National Park in northern Eastern Ghats of Andhra Pradesh (Goswami et al. 2018). The species was recorded from wet riparian vegetation near waterfalls.

The Eastern Ghats hill range of southern Odisha is one of the biodiversity rich areas in Odisha (Dash et al. 2015). Although several studies on butterfly diversity has been carried out in different parts of the Eastern Ghats (Nandi 1987; Nair 2007; Paria et al. 2018), southern Odisha remains poorly explored except for some recent faunal inventories (Mohapatra et al. 2014; Debata et al. 2015; Purohit et al. 2017; De & Palita 2018; Debata & Palita 2018; Mahata et al. 2018). There is, however, no record of this species from Odisha except that of a report on its occurrence from Khurda (Figure 1) by Taylor & de Nicéville (1888), 129 years ago. In the present study, the first record of Tamil Treebrown from Odisha since its last sighting is described from the Deomali Hill range of

southern Odisha, in Eastern Ghats of India, trapped by fruit-baiting.

### Materials and Methods

For the past two and half years (September 2015 to March 2018), we have been studying the diversity and distribution of butterflies in different parts of Koraput District. During our surveys, we used the fruit-baiting technique in Deomali Hills (18.644–18.681 °N and 82.968–83.016 °E) to capture nymphalid butterflies (Figure 1). Deomali is the highest peak of Odisha (1,672m). The fauna and flora show marked similarity with high altitude species of the Himalayan and Western Ghats regions. The vegetation of the hill is tropical moist deciduous type. The valleys and slopes are covered with riparian semi-evergreen forests (Image 1), where species like *Diospyros malabarica*, *Mangifera indica*, *Ficus* spp., *Rubus ellipticus*, *Pittosporum wightii*, *Chionanthus ramiflorus*, *Neolitsea cassia*, *Zanthoxylum armatum* and *Zanthoxylum rhetsa* are dominant. The plateau is covered with grassland and meadows with several species of grasses of Poaceae and Cyperaceae families such as *Cyperus leucocephalus*, *Fimbristylis pierotii*, *Arundinella holocoides*, *Themeda mooneyi* and

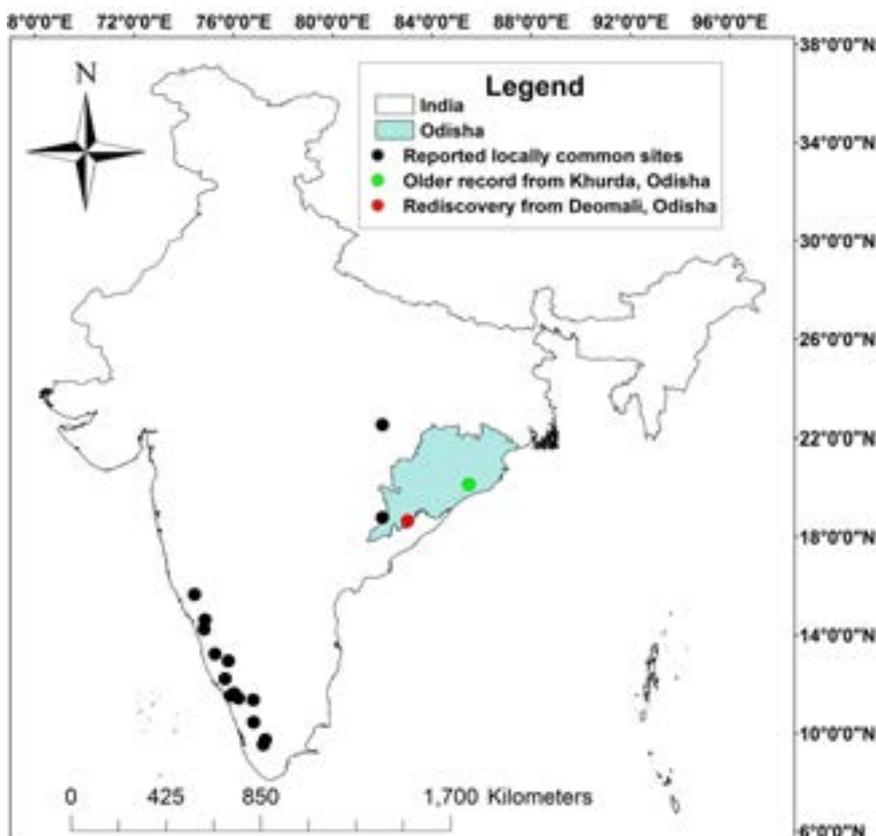


Figure 1. Map showing the distribution of *Letho drypetis todara* in India and its first record in Odisha from Deomali Hills, Southern Odisha, Eastern Ghats, India.



Figure 2. Placement of butterfly traps on both side of the transect line in three altitudinal bands (800–1,000 m, 1,000–1,200 m and 1,200–1,400 m) at Deomali Hill range, southern Odisha.



Image 1. Habitat of the study sites at Deomali Hill range, southern Odisha, Eastern Ghats, India.

*Themeda triandra*. Wide varieties of bryophytes (around 43 species) and pteridophytes (45 species) have been recorded from this region (Dash et al. 2015). Bamboo brakes are found in riparian regions of Deomali. Rainfall is heavy and well distributed. It covers an altitudinal range of 800–1,672 m and above 1,400m it is devoid of arboreal species.

### Butterfly sampling

Butterfly sampling was carried out by both transect counting and fruit-baiting technique once a month from September 2015 to March 2018 at four different sites. For butterfly trapping, we used a homemade butterfly trap (Shuey 1997) and prepared fruit based bait for capturing Nymphalid butterflies. We placed a total of six



Image 2. Home-made butterfly trap used for baiting of Nymphalidae butterflies in Deomali Hill range, southern Odisha.

traps; two traps each in 200m altitude bands, e.g., 800–1,000 m, 1,000–1,200 m, and 1,200–1,400 m. In each altitude band, two traps were placed at the midpoint of the 500m transect on both sides with a distance of 5m from the midpoint (Figure 2). The base of the trap

was placed one meter above the ground (Image 2). We placed the trap at 16:00h for 24 hours.

Transects and baited traps were placed with the help of GPS (GPSMAP® 64s, Garmin, US). Microclimate parameters, such as temperature, humidity, and wind speed were measured through digital anemometer (AVM-06, HTC, India). Canopy cover was measured through GLAMA application (Lubomír Tichý, Dept. of Botany and Zoology, MU Brno, Czech Republic, 2014–2015), installed in Smart phone (Moto G4 plus, 4<sup>th</sup> generation, Lenovo, China). The picture of the vegetation was taken by a 16 MP rear inbuilt camera of the smart phone at breast height and analysed by the app (Navarro-Martínez et al. 2017; Mahata et al. 2018). Light intensity was measured by Digital Light meter (LX-103, Lutron, India). Morphological features of the captured Tamil Treebrown (Image 3) were studied after Mathew & Soumya (2013).

#### Bait preparation

We prepared bait for six traps from the following.

1. Four medium-sized (10–14 cm length and 9–12 cm diameter) overripe bananas were sliced into two centimetres thick pieces and kept in a wide mouth 500ml beaker with a lid.

2. Sugar syrup was prepared with 50g of sugar in 200ml of water in another beaker and was boiled. It was stirred until dissolved.

3. The sugar syrup was added to the wide mouth beaker and to this 10ml of beer (United Breweries, Bangalore, India) was added and stirred.

4. It was left for 24 hours for fermentation.

#### Results and Discussion

Out of six traps, a male of *Lethe drypetis todara* was captured only in one trap at the GPS location of 18.648°N & 83.009°E on 18.03.2017 at 15:35h at an altitude of 1,296m. Along with this, three Common Bushbrown *Mycalesis perseus*, two individuals of Dark-brand Bushbrown *Mycalesis mineus* and two Bamboo Treebrown *Lethe europa*, all satyrine butterflies, were also captured in the same trap. The trap site was beside

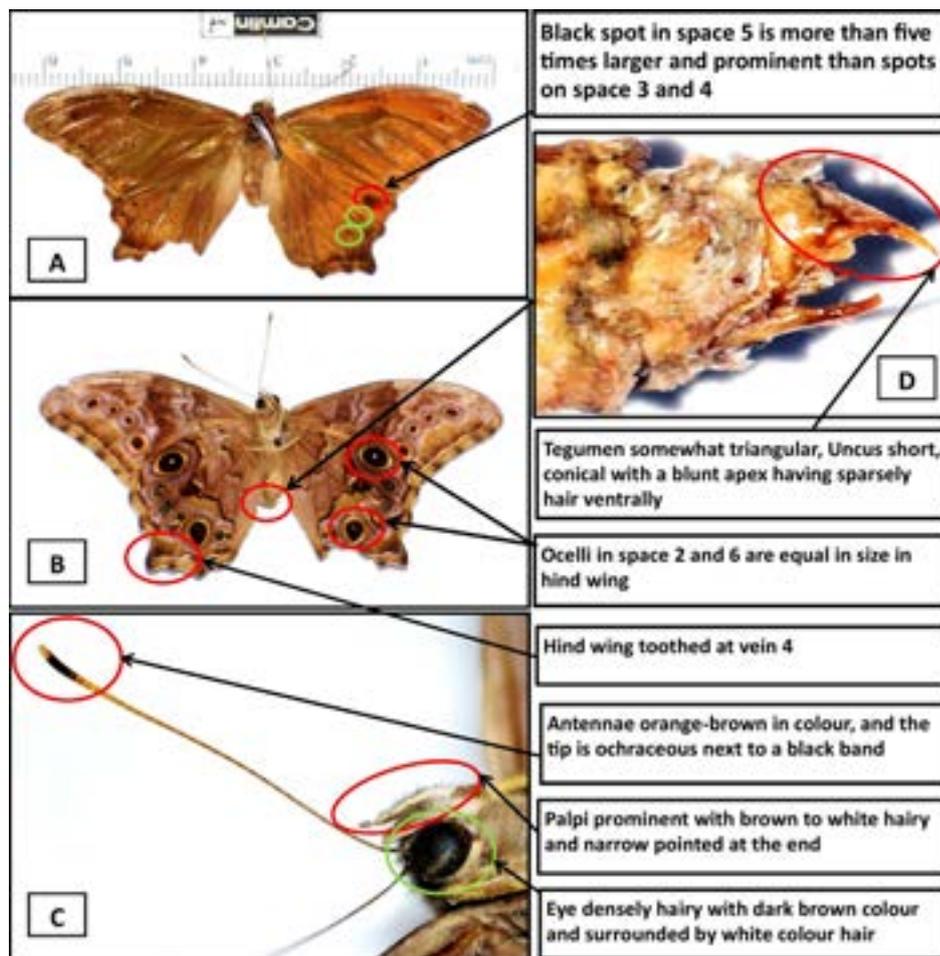


Image 3. *Lethe drypetis todara*. A—upperside view of male | B—underside view of male | C—head with antennae | D—male genitalia lateral view. © Anirban Mahata.

a perennial hill stream and the major vegetation was moist deciduous along with bamboo brakes. It was a sunny day with an average ambient temperature of 32.38°C, relative humidity 35.48%, wind speed 1.98 m/s, light intensity- 899.66 × 100 lux and tree canopy cover of 43.50%.

The Tamil Treebrown is generally found in forests having bamboo brakes on which their larvae develop (Mathew & Soumya 2013). Its larvae feed mostly on bamboos (*Bambusa arundinacea*) (Sevastopulo 1973) but they also seem to feed on other grasses. The eggs are laid singly on the underside of leaves (Mathew & Soumya 2013). In the present study at Deomali in Odisha, Tamil Treebrown was captured through fruit-baiting from riparian regions close to bamboo brakes.

The present report of Tamil Treebrown *Lethe drypetis todara* from Deomali Hills of Koraput after 129 years of its report from Khurda, Odisha in the year 1888, is the first record from Odisha and second from the Eastern Ghats of India in recent times. The earlier recorded locations of this species from Kanger Valley National Park of Chhattisgarh (Chandra 2006) and Papikonda National Park of Andhra Pradesh (Goswami et al. 2018) are geographically close to the current site. Of these locations Papikonda and Deomali are in the northern Eastern Ghats (Figure 1). As an endemic, common and widespread species from Sri Lanka and southern India (Gaonkar 1996), its range has extended from southern India towards Central India (Chandra 2006). The findings of the present study, further connect the distribution link of this species to Odisha (Figure 1). The rediscovery of *Lethe drypetis todara* from Deomali Hills indicates that this species is very habitat specific. We are of the opinion that distribution of this species can be best understood with more sampling studies through fruit-baiting along the Eastern Ghats hill ranges.

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## A REVIEW OF THE LEAFHOPPER TRIBE AGALLIINI (HEMIPTERA: CICADELLIDAE: MEGOPHTHALMINAE) WITH A REVISED KEY TO THE KNOWN PAKISTANI GENERA AND SPECIES

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**Abstract:** The tribe Agalliini is reviewed from Pakistan with additional description and illustration of a new record *Anaceratagallia pararobusta* (Pruthi) 1936. An updated checklist and keys are given to distinguish all the genera and species of the tribe Agalliini from Pakistan.

**Keywords:** Cicadomorpha, new record, taxonomy.

The leafhopper tribe Agalliini of the subfamily Megophthalminae includes 37 genera and 650 species worldwide (Gonçalves & Dietrich 2009; Viraktamath 2011; Viraktamath et al. 2012). Most of the species are grass, herb or leguminous crop feeders (Viraktamath 2011) and 13 species are known to be vectors of plant diseases (Wilson & Turner 2010). Agalliine species can be distinguished from other Cicadellidae by combination of the following characters: head short and broad, usually wider than pronotum; ocelli on face close to

dorsal margin; forewing with appendix very narrow or absent; hind wing with four apical cells closed; hind tibial macrosetae on AV starting from midlength of tibia, metabasitarsomere with one or two platellae on distal transverse row of setae; male subgenital plates short, often fused at base and male style usually forked caudally. Viraktamath (2011) provided a detailed study of the tribe Agalliini from the Oriental and Australian regions and compiled all of the available literature from those regions. Soon thereafter, Viraktamath et al. (2012) studied the Chinese Agalliini fauna and added four new genera and 10 new species, bringing the Chinese Agalliini to a total of 14 genera and 41 species.

Pruthi (1930, 1936) described two species of Agalliini from Pakistan, namely *Agallia robusta* and *Durgades idiocera* from Murree Hills. Later, Mahmood (1979) reported the presence of the genera *Aceratagallia*

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Kirkaldy, *Agallia* Curtis, *Agalliopsis* Kirkaldy, *Anaceratagallia* Zachvatkin, *Ceratagallia* Kirkaldy, *Nehela* White, *Peragallia* Ribaut and *Austroagallia* Evans, but no material has been found to confirm those identifications. Viraktamath (1973, 2004) described *Austroagallia nitobei* (Matsumura) and the new species *Durgades sympatrica* Viraktamath from Pakistan. Subsequently, Khatri et al. (2010) studied six species of Agalliini providing their re-descriptions and illustrations. Later, Viraktamath (2011) transferred all three Pakistani species of *Agallia* Curtis to *Anaceratagallia* Zachvatkin. In this paper, we report a new record of *Anaceratagallia pararobusta* (Pruthi, 1936) from Pakistan. The tribe Agalliini comprises three genera and 11 species from Pakistan. The specimens examined are deposited in the Entomological Museum, Northwest A&F University, Yangling, Shaanxi, China (NWFU).

#### Checklist of tribe Agalliini from Pakistan

*Anaceratagallia aciculate* (Horváth, 1894)  
*Anaceratagallia cuspidate* Dlabola, 1957  
*Anaceratagallia pararobusta* (Pruthi, 1936) **n. rec.**  
*Anaceratagallia robusta* Pruthi, 1930  
*Austroagallia fagonica* Sawai Singh & Gill, 1973  
*Austroagallia nitobei* (Matsumura, 1912)  
*Austroagallia sarobica* (Dlabola, 1964)  
*Austroagallia robusta* Sawai Singh & Gill, 1973  
*Austroagallia sinuate* (Mulsant & Rey, 1855)  
*Durgades idiocera* Pruthi, 1930  
*Durgades sympatrica* Viraktamath, 2004

*Note.* Detailed descriptions of known genera and species have been already provided by Viraktamath (2011) and hence are not repeated here except for the locality records and remarks.

#### Key to the genera of Agalliini from Pakistan

1. Hind margin of crown not curved or, if so, evenly curved behind eyes ..... 2
- Hind margin of crown sinuately curved behind eyes; base of aedeagus not sunken into dorsal apodeme, asymmetrical; anal collar well developed, with hooks or distally dentate; ocelli located in rather deep pits...*Austroagallia* Evans
2. Pronotum transversely rugose; aedeagus without subapical finger-like processes surrounding gonopore ..... *Anaceratagallia* Zachvatkin
- Pronotum not rugose; aedeagus with subapical finger-like processes surrounding gonopore ..... *Durgades* Distant

#### Genus *Anaceratagallia* Zachvatkin

*Anaceratagallia* Zachvatkin 1946: 159–161.

Type-species: *Cicada venosa* Fourcroy, 1785 by original designation.

**Distribution:** Palaearctic, Afrotropical and Oriental regions

#### Key to the species of *Anaceratagallia* from Pakistan (modified from Viraktamath 2011)

1. Anal collar process tridentate; aedeagal shaft with pair of subapical tooth-like processes ..... *A. cuspidata*
- Anal collar process with one or two subacute projections distally; aedeagus lacking processes ..... 2
2. Anal collar process with ventral subacute and dorsal acute projections, caudal margin between them either smooth or crenulated ..... *A. robusta*
- Anal collar process with single subacute projection ..... 3
3. Aedeagal shaft with one subapical tooth on dorsal margin ..... *A. aciculata*
- Aedeagal shaft devoid of subapical tooth on dorsal margin ..... *A. pararobusta*

#### *Anaceratagallia aciculata* (Horváth)

*Agallia venosa* var. *aciculate* Horváth 1894: 186

*Agallia aciculata* Vilbaste 1962: 134

*Anaceratagallia aciculata*; Metcalf 1966: 79;

Viraktamath 2011: 16.

**Material examined:** Not available.

**Remarks:** Khatri et al. (2010) reported this species from Pakistan and illustrated the male genitalia.

**Distribution:** Pakistan, Palaearctic region

#### *Anaceratagallia cuspidate* Dlabola

*Anaceratagallia cuspidate* Dlabola 1957: 298–299, figs 106–110; Viraktamath 2011: 16, figs 100–105;

*Agallia pseudorobusta* Rao & Ramakrishnan 1978a: 236–237, figs. 1 a–l.

**Material examined:** Not available.

**Remarks:** This species is similar to *A. robusta* in morphology but can be distinguished by tridentate anal collar process and aedeagal shaft with tooth-like paired projections (Viraktamath 2011).

**Distribution:** India, Pakistan, Palaearctic region

#### *Anaceratagallia pararobusta* (Pruthi, 1936)

Image 1A, Figure 1A–C

*Agallia pararobusta* Pruthi 1936: 104–105, fig. 119,

pl. VIII, fig. 4; *Anaceratagallia pararobusta* Viraktamath, 2011: 17, figs 3, 4, 14, 106–108.

**Measurement:** Body length: Male. 3.1mm. Vertex width including eyes, 1mm; vertex length, 0.24mm; pronotum width, 0.93mm; pronotum length, 0.5mm; scutellum width, 0.5mm; scutellum length, 0.39mm.

**Material examined:** Hm035141–Hm035145, 5 males, 10.viii.2017, Pakistan: Azad Jammu & Kashmir: Rawalakot, 33.858°N, 73.765°E, 1638m, coll. Hassan Naveed.

**Diagnosis.** Vertex with a couple of rectangular black spots obliquely placed. Face Ochraceous, with a few fuscous markings, fronto-clypeus with dark brown spots at lateral margin. Pronotum with black spots on anterior margin, median longitudinal spot larger than lateral spots on both sides on posterior margin. Basal triangles of scutellum with two black spots, posterior half brown. Forewings ochraceous with fuscous veins, cells mostly infuscated.

Male genitalia. Pygofer caudo-ventrally produced into spine-like process of unequal width. Aedeagal shaft mostly slender with slender dorsal apodeme. Anal collar with finger-like process, dorsal process directed ventrally.

Female genitalia. Hind margin of seventh sternite broadly concave.

This species closely resembles *A. laevis* (Ribaut) but differs in aedeagal shaft more slender.

**Distribution:** India, Pakistan

#### *Anaceratagallia robusta* (Pruthi, 1930)

*Agallia robusta* Pruthi 1930: 10–12, text figs 10–12; *Agallia delhiensis* Rao & Ramakrishnan 1978b: 241, fig. 3 a–l; *Agallia robusta* Khatri et al. 2010: 36, plate I, Fig. 4; *Anaceratagallia robusta* Viraktamath 2011: 17, figs 3, 4, 14, 23, 41–44, 109–120, 563, 577, 592.

**Material examined:** Not available

**Remarks:** This species is similar to *A. laevis* (Ribaut) but can be differentiated by the shape of the anal collar process with dorsal finger-like projection, and caudal margin oblique, crenulate to smooth (Viraktamath 2011).

**Distribution:** India, Pakistan

#### Genus *Austroagallia* Evans

*Austroagallia* Evans, 1935: 70. Type-species: *Austroagallia torrid* Evans, by monotypy.

*Peragallia* Ribaut, 1948: 59. Type species: *Bythoscopus sinuatus* Mulsant and Rey, by original designation; synonymy by Le Quesne 1964: 73.

**Distribution:** Australian, Afrotropical, Oceanic,

Oriental and Palaearctic regions

#### Key to the species of *Austroagallia* from Pakistan (modified from Viraktamath 2011)

1. Forewing with brownish reticulate venation; crown and pronotum with minute dot-like marks ..... *A. robusta*
- Forewing venation not reticulated; crown with prominent spots ..... 2
2. Aedeagal shaft with basal stout, elongate process ..... *A. sarobica*
- Aedeagal shaft lacking basal process, or reduced, tooth-like ..... 3
3. Aedeagal shaft with laminate process surrounding gonopore; anal collar hook spindle-shaped ..... *A. nitobei*
- Aedeagal shaft neither laminately expanded nor with laminate process or may be slightly expanded medially ..... 4
4. Aedeagus with a pair of finger-like processes at apex ..... *A. fagonica*
- Aedeagus without a pair of finger-like processes at apex ..... *A. sinuata*

#### *Austroagallia fagonica* Singh & Gill, 1973

Image 1B, Figure 1D–J

*Austroagallia fagonica* Singh & Gill 1973, in Bindra, 1973: 12–14, pl. 3, figs. 1–11; Viraktamath and Sohi 1980: 287, figs 17–21; Viraktamath 2011: 28, Figs. 45–48, 134–140.

**Measurement:** Body length: Male. 3.8mm. Vertex width including eyes, 1.1mm; vertex length, 0.14mm; pronotum width, 1mm; pronotum length, 0.54mm; scutellum width, 0.6mm; scutellum length, 0.4mm.

**Material examined:** Hm35266–Hm35271, 6 males, Hm35272–Hm35282, 10 females, 11.viii.2017, Pakistan: Azad Jammu & Kashmir: Rawalakot, 33.858°N, 73.765°E, 1,638m, coll. Hassan Naveed.

**Remarks:** This species has considerable color variation as described by Viraktamath 2011, but the male genitalia characters are consistent, such as the aedeagal shaft slightly broadened at the basal half (it maybe slender in the same species), with two finger-like processes directed on one lateral side and the anal collar process without a dorsal marginal tooth. These variations are interpreted as intraspecific variation. *Austroagallia fagonica*, as discussed by Viraktamath and Sohi (1980) resembles the Egyptian species *Austroagallia canopus* Linnavuori (1969) from which it differs in having a simple anal collar process compared to the branched process found in *A. canopus*.

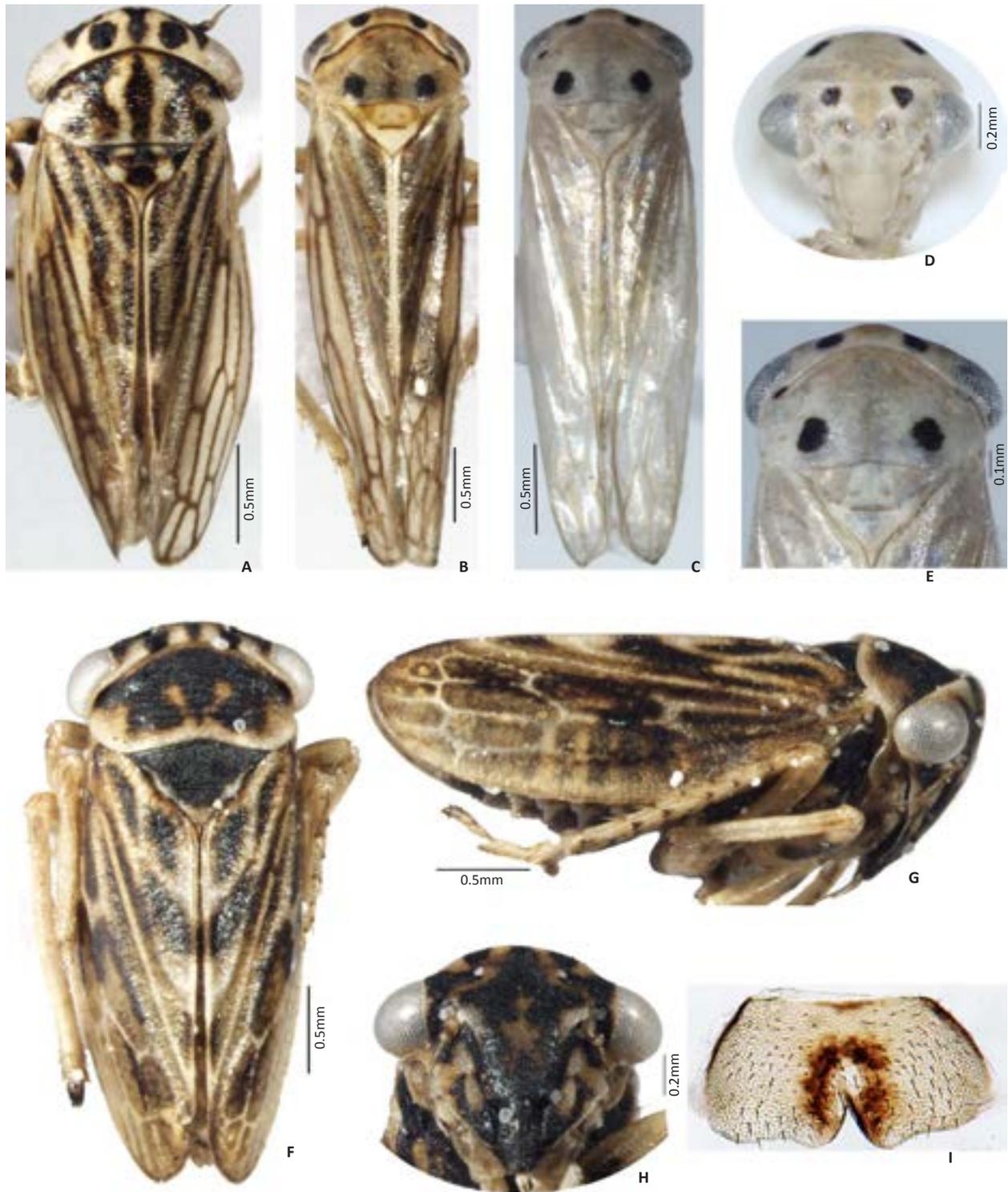


Image 1. A—*Anaceratagallia pararobusta* (Pruthi, 1936) habitus, dorsal view | B—*Austroagallia fagonica* Sawai Singh & Gill, 1973 habitus, dorsal view | C—*Austroagallia sinuata* (Mulsant & Rey, 1855): C—habitus, dorsal view | D—face | E—head | F—*Durgades sympatrica* Viraktamath, 2004: F—habitus, dorsal view | G—habitus, lateral view | H—face | I—female seventh sternite, ventral view. © Hassan Naveed.

**Distribution:** India, Pakistan.

***Austroagallia nitobei* (Matsumura, 1912)**

*Agallia nitobei* Matsumura 1912: 316;

*Austroagallia nitobei* (Matsumura), Viraktamath 1973: 307–308, figs 1, 2; Viraktamath & Sohi 1980: 285, figs. 1–4; Viraktamath 2011: 29, Figs. 141–143.

**Material examined:** Not available.

**Remarks:** Viraktamath (1973) recognized variation in populations of this species from Sri Lanka, Thailand, Vietnam, Pakistan and India. This species is similar to the Palearctic species *A. avicula* (Ribaut) in external features but can be distinguished by the aedeagus with a basal fold-like tubercle, shaft slender, and apical process elongate and oval with a crenulated lower border (Viraktamath 2011).

**Distribution:** India, Pakistan, Sri Lanka, China (Taiwan), Thailand, Vietnam.

***Austroagallia robusta* Singh & Gill in Bindra, 1973**

*Austroagallia robusta* Singh & Gill 1973, in Bindra 1973: 14–15, pl 4: figs 1–11; Viraktamath & Sohi 1980: 287, figs 11–16; Khatri et al. 2010: 35, pl 1b, fig. 2; Viraktamath 2011: 33, Figs. 36, 148–151.

**Material examined:** Not available.

**Remarks:** The aedeagus of *A. robusta* is about the same as that of the *A. sinuata*, but it differs in having the anal collar process slightly stout and with a curve. It can also be differentiated from other species of *Austroagallia* by the pair of very small speckle-like spots on the crown and pronotum and reticulate piceous venation on the disc of the corium (Viraktamath 2011).

**Distribution:** India, Pakistan.

***Austroagallia sarobica* (Dlabola, 1964)**

*Peragallia sarobica* Dlabola 1964: 246;

*Austroagallia sarobica* (Dlabola): Dlabola 1972: 218, generic placement; Bindra 1973: 4; Viraktamath & Sohi 1980: 289, figs 29–33; Khatri et al. 2010: 35, pl 1c, Fig. 3; Viraktamath 2011: 34, Figs. 152–155.

**Material examined:** Not available

**Remarks:** This species is similar to *A. robusta* in crown and pronotum having round spots, but differs in having the male genitalia with anal collar tridentate, aedeagal shaft with a basal process making it strongly asymmetrical, and forewings without subdivided antepical cells (Viraktamath 2011).

**Distribution:** India, Pakistan, Afrotropical and Palearctic regions

***Austroagallia sinuata* (Mulsant & Rey, 1855)**

Image 1C–E, Figure 2A–C

*Bythoscopus sinuatus* Mulsant & Rey 1855: 222;

*Agallia quadrisignata* Flor 1861: 557, synonymy by Fieber 1868: 462;

*Agallia homeyeri* Kirschbaum 1868: 32, synonymy by Fieber 1872: 32;

*Agallia fieberi* Vismara 1878: 41, synonymy by Löw 1885: 346;

*Austroagallia afganistanensis* Rao, Ramakrishnan & Ghai 1979: 655–656;

*Austroagallia sinuate* Khatri et al. 2010: 35, pl 1a, Fig. 1; Viraktamath 2011: 34, Figs. 24, 49–51, 156–158.

**Measurements.** Body length: Male. 3mm. Vertex width including eyes, 0.99mm; vertex length, 0.1mm; pronotum width, 0.85mm; pronotum length, 0.44mm; scutellum width, 0.6mm; scutellum length, 0.4mm.

**Material examined:** Hm032752–Hm032754, 3 males, Hm032751, 1 female, 4.viii.2016, Pakistan: Khyber Pakhtunkhwa, Abbottabad, 34.168°N, 73.221°E, 1,256m, coll. Hassan Naveed.

**Remarks:** This species is widely distributed in the southern Palearctic, Afrotropical and western Oriental and Neotropical regions. It shows great variation in the structure of both the anal collar process and aedeagus but usually is without reticulate venation of the forewing.

**Distribution:** India, Pakistan, Afrotropical, Palearctic regions and Neotropical regions.

**Genus *Durgades* Distant**

*Durgades* Distant 1912: 608; 1916: 237; Viraktamath 2004: 365–366.

Type-species: *Durgades nigropictus* Distant, by original designation.

**Distribution:** Foot hills of the Himalayas.

**Key to the species of *Durgades*** (modified from Viraktamath 2011)

- Aedeagal shaft with four finger-like processes surrounding gonopore ..... *D. idiocera*
- Aedeagal shaft with three finger-like subapical processes ..... *D. sympatrica*

***Durgades idiocera* Pruthi, 1930**

*Durgades idiocera* Pruthi 1930: 13–15, figs. 15–17, pl. II, figs. 1, 1a, 2; Viraktamath 2004: 369–370, figs 24–25; Viraktamath 2011: 46, Figs. 196–197.

**Material examined:** Not available.

**Remarks:** This species was collected by Pruthi (1930) from Murree Hills. This species can be distinguished from the other *Durgades* species in lacking a cross vein

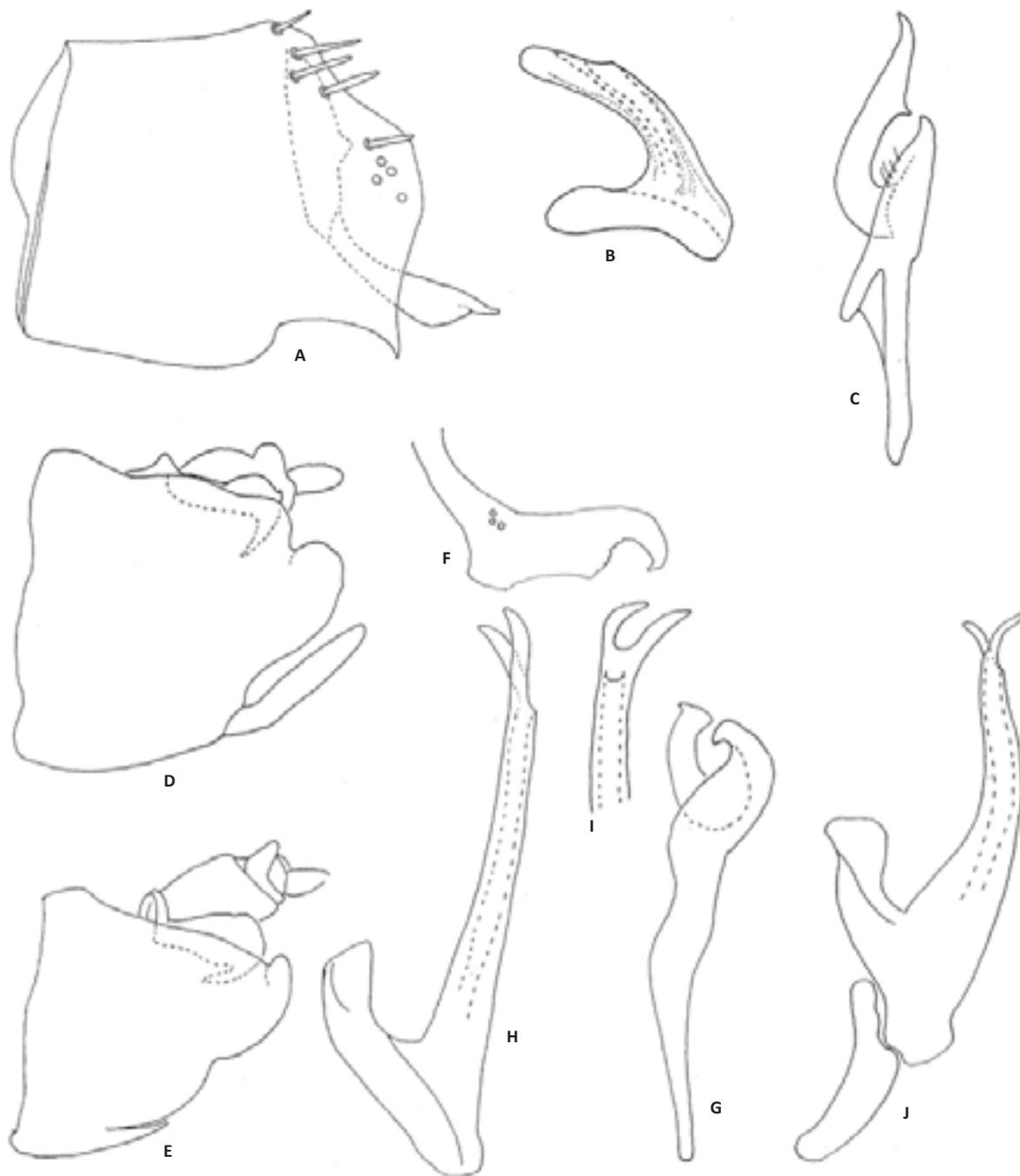


Figure 1. A–C—*Anaceratagallia pararobusta* (Pruthi, 1936): A—pygofer, lateral view; B—aedeagus, lateral view; C—style, dorsal view | D–J—*Austroagallia fagonica* Sawai Singh & Gill, 1973: D–E—pygofer, lateral view | F—anal collar process, lateral view | G—style, dorsal view | H—aedeagus, lateral view | I—apex of aedeagus, anterior view | J—connective and aedeagus, lateral view. (All reproduced from Viraktamath 2011).

between the claval veins of the forewings and having the aedeagal shaft with three curved finger-like processes and one straighter process surrounding the gonopore (Viraktamath 2011).

**Distribution:** Pakistan.

***Durgades sympatrica* Viraktamath, 2004**

Image 1F–I, Figure 2D–J

*Durgades sympatrica* Viraktamath 2004: 374, Figs. 57–65; Viraktamath 2011: 49, Figs. 229–237.

**Measurements.** Body length: Male. 3.2mm. Vertex width including eyes, 1.4mm; vertex length, 0.1mm;

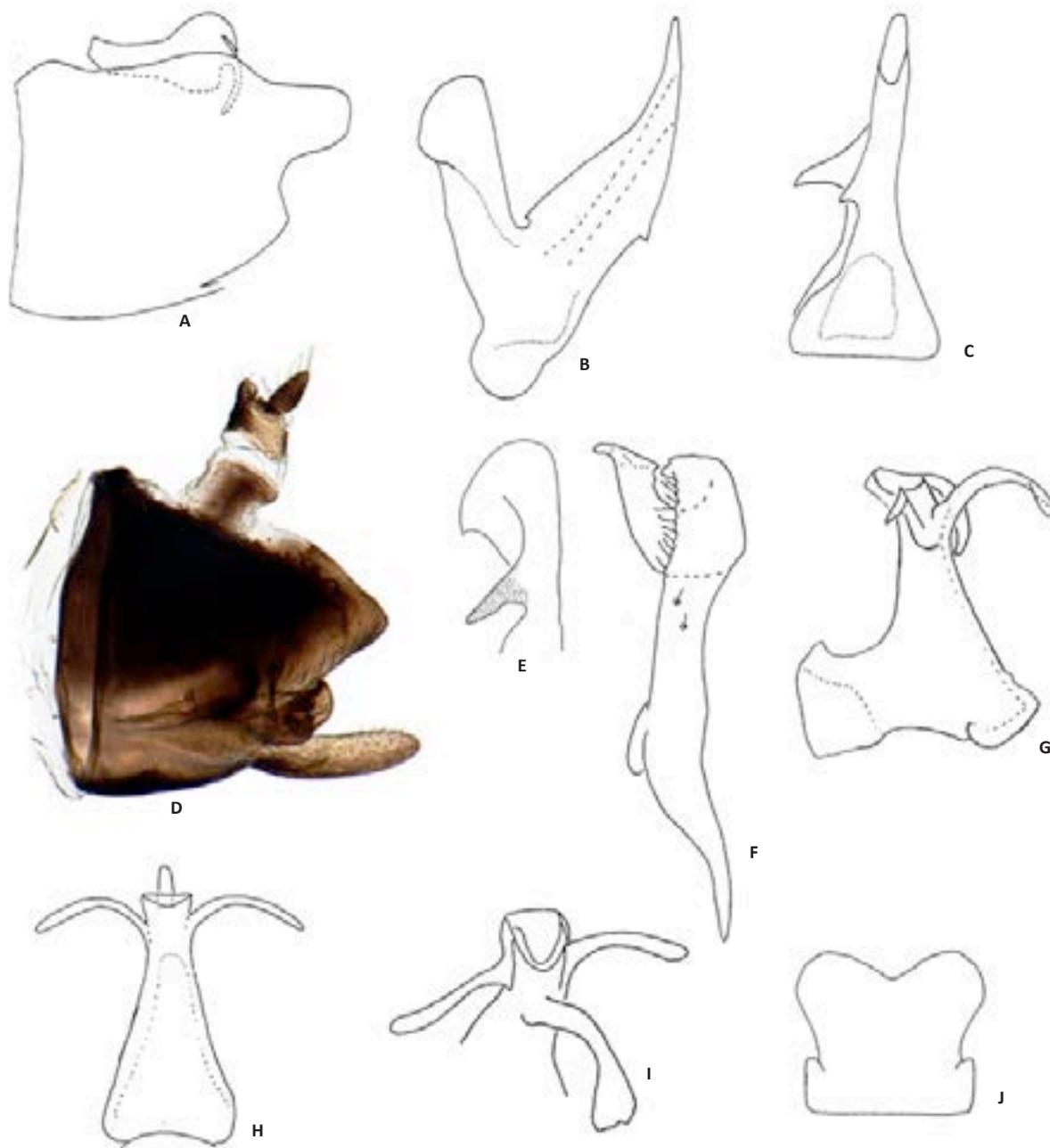


Figure 2. A–C. *Austroagallia sinuata* (Mulsant & Rey, 1855) (reproduced from Viraktamath 2011): A—pygofer, lateral view | B—aedeagus, lateral view | C—aedeagus, posterior view | D–J—*Durgades sympatrica* Viraktamath, 2004: D—pygofer, lateral view (reproduced from Viraktamath 2011) | E—mesal process of pygofer | F—style, lateral view | G—aedeagus, lateral view | H—aedeagus, dorsal view | I—apex of aedeagal shaft, posterior view | J—connective.

pronotum width, 1.1mm; pronotum length, 0.5mm; scutellum width, 0.8mm; scutellum length, 0.59mm.

**Material examined:** Hm35291–Hm35298, 8 males, Hm35288–Hm35290, 3 females, 25.viii.2017, Pakistan, Punjab, Murree Hills, 33.907°N, 73.394°E, 2291m, coll. Hassan Naveed.

**Remarks:** This species closely resembles *D. idiocera*

in coloration, external appearance and markings but can be readily distinguished by the three subapical aedeagal processes, of which one is distally bilobed with a serrated margin. The species can be brachypterous to macropterous.

**Distribution:** Pakistan.

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## THE WINDOWPANE OYSTER FAMILY PLACUNIDAE RAFINESQUE, 1815 WITH ADDITIONAL DESCRIPTION OF *PLACUNA QUADRANGULA* (PHILIPSSON, 1788) FROM INDIA

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**Abstract:** The Bivalvia family Placunidae Rafinesque, 1815 in India is reviewed in this paper based on previous literature and records. Additionally, the species *Placuna quadrangula* is described from the Indian sub-continent. Being an economically important family in this geographic region, this paper can be regarded as a baseline for further ecological, management and policy-related studies pertaining to *Placunidae* and other exploited species.

**Keywords:** India, *Placuna quadrangula*, Placunidae, Taxonomy.

**Abbreviations:** ANI—Andaman & Nicobar Islands | GOM—Gulf of Mannar | GOK—Gulf of Kutch | MBRC—Marine Biological Research Centre | NZC—National Zoological Collections | PB—Palk Bay | GBR—Great Barrier Reef | QGIS—Quantum Geographic Information System | ZSI—Zoological Survey of India | RUMF—Ryukyu University Museum, Fujukan.

**Tamil Abstract:** இந்தியாவில், 1815ல் இருந்த இருவழிபாட்டு குடும்பத்தினர். பிளாகுனிடே ரபின்ஸ்க் பற்றிய விபரம் இந்த கட்டுரையில் முந்தைய கால இலக்கியம் மற்றும் ஆவணங்களின் அடிப்படையில், மறு ஆய்வு செய்யப்படுகிறது. கூடுதலாக, பிளாகுனா குவாட்ரன்சுலா என்ற இனம் பற்றி இந்திய தீவகற்பத்திலிருந்தவாறு, விவரிக்கப்படுகிறது. இந்த புவிமியல் சார்ந்த நிலப்பரப்பில் இருந்த, பொருளாதாரத்தில் முக்கியத்துவம் பெற்ற குடும்பமாக இருப்பதால், இக்கட்டுரையானது பிளாகுனிடே மற்றும் பிற அழிக்கப்பட்ட இனம் சம்பந்தமான, இனி தொடரும் சுற்றுச்சூழல் பராமரிப்பு மற்றும் சொன்கை சார்ந்த படிப்புகளுக்கு, ஒரு உறுதியான அடித்தளமான விஷயம் என கருதப்படலாம்.

The family Placunidae Rafinesque, 1815 is comprised of the genera *Placunanomia* and *Placuna*, the latter with seven accepted living species (Huber 2010). Distributed mostly within the Indo-West Pacific region

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(Matsukuma 1987), the genus *Placuna* Lightfoot, 1786 has a long documentary history, as it is commercially exploited for pearl production, food, lampshades and shell-craft items (Gallardo et al. 1995). From Indian waters, *Placuna placenta* (Linnaeus, 1758) (Image 1) is the only species that has been thoroughly studied due to its economic value (Laxmilatha 2015a). *Placuna sella* (Gmelin, 1791) now *P. ehippium* (Philipsson, 1788) was first reported from the Andaman & Nicobar Islands, India by Prashad (1932), followed by Gulf of Mannar, southeastern coast of India (Rao & Dey 2000; Venkataraman et al. 2004) and was later revised by Stella (2010) (Image 2) from the Mandapam coast (Adjacent to GOM). *Placuna ehippium* is also exploited, especially in the Bay of Banate, Philippines and its surrounding areas, and mainly known for its food value (Gallardo 1994). Currently, as per published reports, two species of *Placuna*, *P. ehippium* and *P. placenta* are reported from the Indian coast (Rao 2017) (Image 3). In this study we confirm the presence of a third species from India, *P. quadrangula* (Philipsson, 1788). This paper further attempts to distinguish all three species from the Indian coast based on morphology.

#### MATERIALS AND METHODS

Five valves of *P. quadrangula* were collected from three different locations along the eastern coast of India (Table 1). The collected dry specimens were cleaned and preserved in zip-lock covers for further assessment. Identification to species level was made based on the characters provided by Lynge (1909) and Huber (2010). Specimens were deposited in the National Zoological Collections (NZC) repository in the Marine Biological Research Centre (MBRC), Zoological Survey of India (ZSI), Chennai. A single specimen of *P. placenta* deposited at (ZSI/MBRC) was assessed additionally. Measurements of specimens were recorded with Yuri Digital Calliper 200x0.01mm. Study area map was created using QGIS 3.6.3 Noosa.

#### SYSTEMATICS

Order Pectinida Gray, 1854

Superfamily Anomioidea Rafinesque, 1815

Family Placunidae Rafinesque, 1815

Genus *Placuna* Lightfoot, 1786

*Placuna* is monomyarian, with low umbones, V-shaped crurae and pallial line, often obscured. Valves circular to sub-circular and laterally compressed. All the characters are common in the species observed from the Indian subcontinent, and a detailed species wise description is given below.

#### *Placuna placenta* (Linnaeus, 1758) (Image 1)

**Description:** Shell thin, very flat, roughly circular or subcircular in shape, inequivalve, periostracum absent. Inner surface smooth, outer surface lamellate, growth lines present. Transparent when juvenile, turning opaque with age. Lacking radial lines on the external surface. Crurae below the umbones, unequal in size, adductor muscle scar slightly anterior of midline. Pallial line obscure and non-sinuated. Specimen examined has a damaged or broken outline.

**Distribution:** Extends from Gulf of Aden in the west to Taiwan in the east (Matsukuma 1987; Huber 2010).

#### *Placuna ehippium* (Philipsson, 1788)

**Description:** Shell saddle-shaped with curved dorsal margin without periostracum. Growth lines visible. External colour purple brown/brownish with black shades to large red-purplish-blackish spots on the interior surface. Lacking radial lines on the external surface. Crurae prominent, equal in size and wideset. Single adductor muscle scar in the center of midline; purplish in colour.

**Distribution:** India to Australia (Matsukuma 1987; Huber 2010).

**Remarks:** See Discussion.

Table 1. Materials examined.

Locality	Coordinates (decimal)	No. of valves	Dimensions Lx WxH (mm)	Date of collection	Collected by	Deposition no.
Kottivakkam (Chennai)	12.966°N, 80.265°E	2	80.38 x 78.77 x 8.23; 57.83 x 49.48 x 4.88	28.ii.2015	GS	ZSI/MBRC/M.2004; ZSI/MBRC/M.2005
Serenity Beach, Kottakuppam (Puducherry)	11.976°N, 79.845°E	1	92.09 x 91.30 x 9.59	19.x.2015	RRD	ZSI/MBRC/M.2006
Kasimedu, Royapuram (Chennai)	13.123°N, 80.297°E	2	71.94 x 61.37 x 7.32; 50.55 x 47.71 x 4.91	13.iii.2016	RRD; GS	ZSI/MBRC/M.2007; ZSI/MBRC/M.2008



Image 1. Dorsal and ventral side of *Placuna placenta* collected from Mandapam (southern India) (Coll. by R. Rajkumar, ZSI/MBRC/M.1718/5854) (© Deepak Samuel).

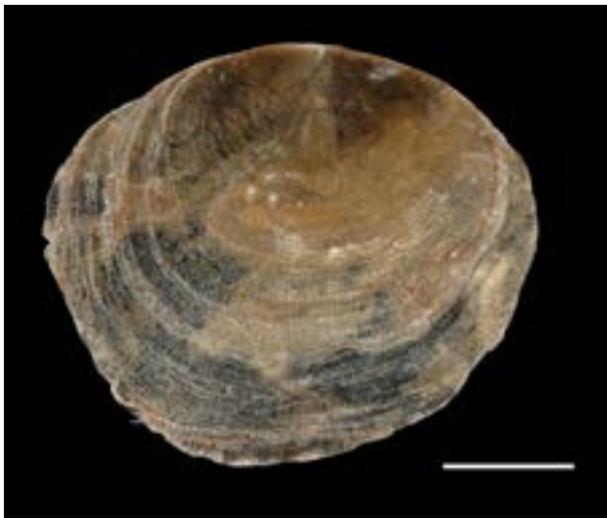


Image 2. Dorsal side of *Placuna ephippium?* collected from Mandapam (southeastern coast, India) (Scale = 2cm) (© C. Stella).

***Placuna quadrangula* (Philipsson, 1788)**  
(Image 3. A–J)

**Description:** Shell thin, brittle, papery, laterally compressed but slightly concave (Image 3. A–J). Outline quadrangular, periostracum inconspicuous. Surface smooth, lamellate, growth lines fine with closely arranged radial threads. Externally pinkish to whitish with non-uniform white radial rays originating from umbones; internally pinkish to whitish. Prominent crurae of equal size. Adductor muscle scar centrally situated, rounded. Pallial line obscure and non-sinuuated. Internal margins smooth. Internal and external surface of the specimens with attached fouling organisms.

**Distribution:** Present study – Tamil Nadu, Puducherry (eastern coast of India), Mergui Archipelago, Thailand, Indonesia, Philippines and Australia (Matsukuma 1987; Sanpanich 2011).



Image 3. (A–J) Specimens of *Placuna quadrangula* examined (Scale = 1cm). The white radial lines can be clearly seen in the dorsal side of the specimen A and G. (© Rocktim Ramen Das).

**Remarks:** The five examples collected from three different localities possess the typical radially rayed pattern.

## DISCUSSION

### Comparative analysis among the *Placuna* species from India with some notes on other Indo-pacific species

The radial colour patterns originating from the umbones (Image 3) a typical character for *P. quadrangula* is emphasized in Huber 2010. Although confused with *P. ehippium* which is larger, the specimen described from Mandapam has no mention of purple muscle scar nor the color predominance in the shell as seen in Huber 2010. Rather it is described as “almost transparent” along with light brownish nature with black patches (Image 3) (see Stella 2010). The transparent nature fits well with *P. placenta* juvenile as observed in the collections of the Ryukyu Museum (RUMF-ZM-03693) and in Kouri Shell Museum, Okinawa (Rocktim Ramen Das, pers. obs.) but is known to turn white and shiny after maturity. The presence of brown radial rays in the latter from southeastern Asian specimens (see Matsukuma 1987) might be misleading and needs reassessment. *P. ehippium* lacks such brown radial rays and possesses large red, purplish-black spots on the interior region (Henk Dekker pers. comm.).

### Life history of genus *Placuna*

A review of literature revealed that there is a limited amount of research based on the life history of genus *Placuna* with exclusive information available only for *P. placenta*. Adam Young (1980), who did an extensive study on the larval growth and development of *P. placenta*, revealed that the shells remain inequivalve and transparent from a very early stage. The author also revealed that from fertilized egg to the formation of spat, it takes about 10–11 days and the final sedentary phase is reached when around 600µm in size is reached during which several key morphological changes take place, viz., active foot appearance during larval metamorphosis. Narasimham (1984), who later studied the biology of *P. placenta* from the eastern coast of India (Kakinada Bay) (Figure 1) mentioned about the biannual spawning strategy of the species and based on the gonadal appearance and morphology divided the maturity into four stages, viz.: active, ripe, partially spawned and spent/resting. Interestingly, a recent observation from the coast of Sonmiani (Balochistan) indicated that *P. placenta* spawns all-round the year (Parveen et al. 2018) which contradicts the findings of Hornell (1909) and Moses (1939) whose studies were

from a not so distant area of Okha, Gujarat, India (~400 km). This probably indicates the local environmental parameters like temperature, salinity and monsoonal characteristics can play an important role at regional scales (Ladja 2002).

### Status of genus *Placuna* in India

Along the coast of India, the windowpane oyster (*P. placenta*) was initially reported by Hornell (1909a,b). In the 1970s, Narasimham documented its utilization due to its high economic value while Laxmilatha (2015) reviewed the economic value. Though the species is reported from various places of the Indian sub-continent (Table 3), it is commercially exploited only from specific areas along the coastline (Table 3). Exploitation of the species in areas of Gulf of Kutch was mainly for pharmaceutical purposes (Alagaswami & Narasimham 1973; Narasimham et al. 1993). Presently the exploitation levels are low in the Gulf of Kutch. In Kakinada Bay, *P. placenta* is regarded as one of the most important bivalve resources, but the stock is under threat due to overexploitation (Rao & Somayajulu 1996; Laxmilatha 2015b). Nauxim Bay in Goa had a minor fishery where the meat was locally consumed (Narasimham et al. 1993). Apart from the above-mentioned locations, the collection of windowpane oyster from the coastal waters of Tamil Nadu mainly for the pearl and shell craft industries. Vellapatti fishing hamlet near Tuticorin is the hub for the utilization of *P. placenta* in the cosmetic and paint industry. Rameswaram is famous for the windowpane oyster lampshades and mirrors. It is important to highlight that due to such activities, previous densely populated areas of *P. placenta* are lost (Tripathy & Mukhopadhyay 2015). The saddle-shaped oyster *P. ehippium* collected in Mandapam, Gulf of Mannar is also used in the production of a variety of curios/souvenirs, viz., trays, lampshades (Stella et al. 2010). The third species *P. quadrangula*, reported here is either invasive or has been overlooked over the past decade. It is important to highlight that Iredale in the scientific reports published related to the GBR expedition refers to a publication from the Bolten Museum dating back to 1798 which mentions *Ehippium anomia* (synonym *P. quadrangula*) from Tranquebar (now Tharangambadi, ~230km from Chennai). Further in-depth analysis revealed the information is related to its morphological characters, leaving the information regarding its geography being rather vague (see Iredale 1939; Röding 1906).

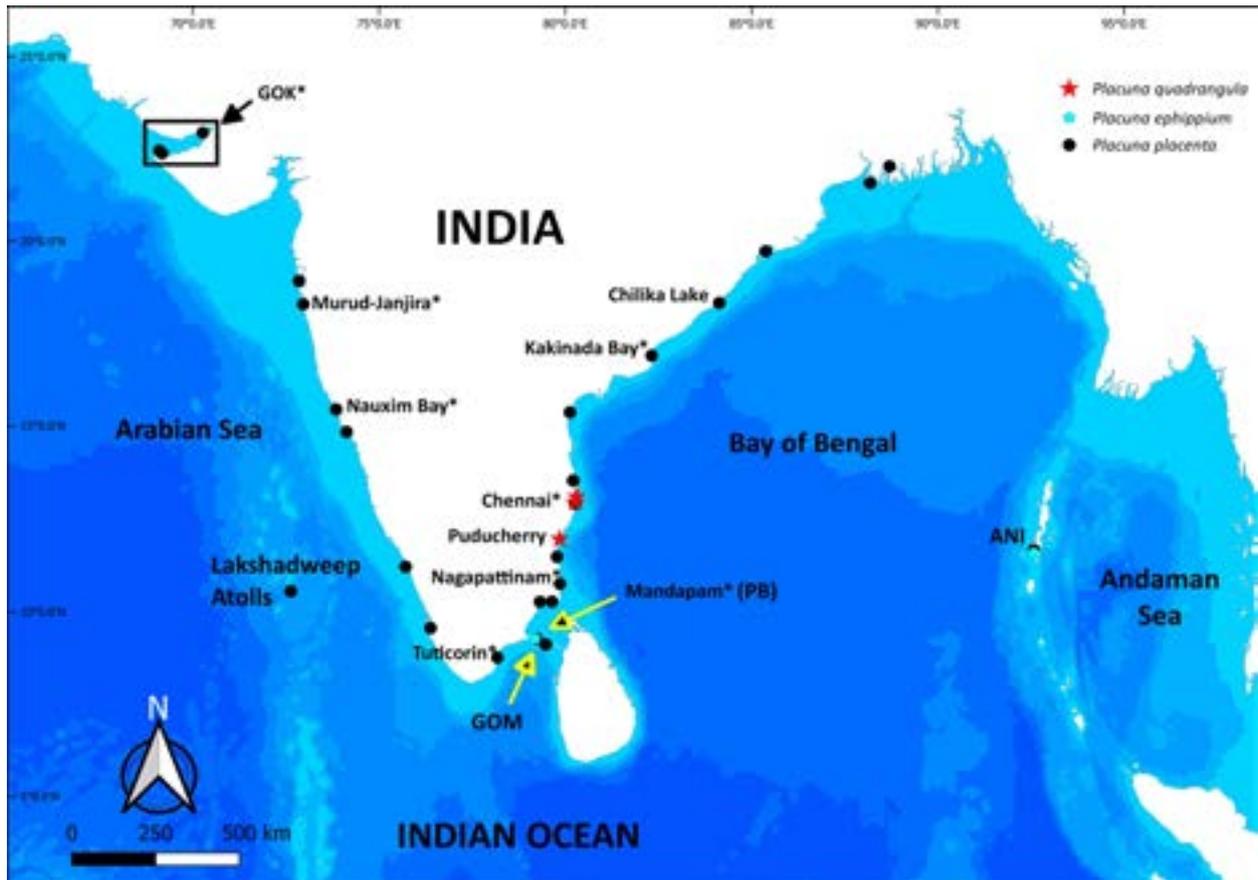


Figure 1. Distribution of genus *Placuna* along the Indian subcontinent. GOK=Gulf of Kutch; GOM=Gulf of Mannar; PB=Palk Bay; ANI=Andaman & Nicobar Islands; \* Exploited Regions.

Table 2. Summary of important morphological features among the Indian *Placuna* species.

	<i>Placuna placenta</i>	<i>Placuna ehippium</i>	<i>Placuna quadrangula</i>
Morphology (Figure 2)	Roughly circular	Saddle shape with curved dorsal margin	Quadrangular, thin, papery
Colour	Semi-transparent (juvenile); opaque and white (adult).	Purplish, large spots in the interior region (Red, Purple or Black)	Pinkish to whitish, with prominent white radial lines.
Crurae (Figure 2)	V-shaped, narrow and unequal	V-shaped, wide apart and equal	V-shaped, wide apart and equal
Radial lines	Absent	Absent	Irregular radial lines originating from umbones
Pallial Line	Obscure	Obscure	Obscure
Posterior adductor muscle	Monomyarian	Monomyarian	Monomyarian
Adductor muscle scar	Slightly anterior of midline	Center of midline, purplish	Center of midline, rounded
Umbones	Low	Low	Low



Figure 2. Sketch of the V-shapes crurae and hinge shape: A—*P. quadrangula* | B—*P. placenta* | C—*P. ehippium*.

Table 3. Reports of genus *Placuna* from various parts of the Indian subcontinent\*

Source	Locality/Region	Species
Hornell 1909b	Balapur Harbour, Beyt Island and Okha (Gujarat); Ennore (Tamil Nadu); Buckingham canal (Tamil Nadu); Pulicat lake (Andhra Pradesh)	<i>Placuna placenta</i>
Prashad 1932	Andaman Islands	<i>Placuna sella</i> *
Rai 1933	Bombay coast (Maharashtra)	<i>Placuna placenta</i>
Alagaswami & Narasimham 1973	Gulf of Kutch (Gujarat); Malabar coast, Vembanad lake (Kerala); Tuticorin**, Nagapattinam**, Kakinada bay** (Andhra Pradesh)	<i>Placenta placenta</i> **
Narasimham et al. 1993	Nauxim Bay** (Goa)	<i>Placenta placenta</i> **
Hameed & Somasundaram 1998	Gulf of Mannar (Tamil Nadu)	<i>Placenta placenta</i> **
Rao & Dey 2000	West Bengal	<i>Placuna placenta</i>
Venkataraman et al. 2004	Andaman & Nicobar Islands	<i>Placuna placenta</i>
Samuel et al. 2005	Dhanuskodi (Tamil Nadu)	<i>Placenta placenta</i> **
Stella et al. 2010	Mandapam** (Tamil Nadu)	<i>Placuna ephippium</i>
Boominathan et al. 2012, 2014	Kali River; Uttara Kannada district (Karnataka)	<i>Placuna placenta</i> , <i>Placenta placenta</i> **
Murugesan et al. 2013	Parangipettai (Tamil Nadu)	<i>Placenta placenta</i> **
Prabhu et al. 2013	Mallipattinam (Tamil Nadu)	<i>Placenta placenta</i> **
Thilagavathi et al. 2013	Muthupettai (Tamil Nadu)	<i>Placenta placenta</i> **
Bijukumar et al. 2015	Kavaratti Island (Lakshadweep)	<i>Placuna placenta</i>
Tripathy & Mukhopadhyay 2015	Murud-Jinjira** (Maharashtra); Pouchitra**, Raida**, Goomara** (GOK, Gujarat), Chennai**	<i>Placuna placenta</i>
Mahapatro et al. 2016	Chilika Lake (Odisha)	<i>Placuna placenta</i>
Rao 2017	Pindara Bay (Gujarat); Baitkal cove and Pavin halla (Karnataka); Pambam, Kundugal point (Tamil Nadu); Eatimukkala and Kalingapatnam (Andhra Pradesh); Jharkali and Jambu Island (West Bengal)	<i>Placuna placenta</i>
Ravinesh et al. 2018	Navi Mumbai (Maharashtra)	<i>Placuna placenta</i>
Present study	Chennai (Tamil Nadu); Kottakuppam (Puducherry)	<i>Placuna quadrangula</i>

\*The information may be non-exhaustive +*Placuna sella* is the synonym of *Placuna ephippium* ++*Placenta placenta* is a group under which *Placuna placenta* was assigned by Gray 1849, thus can be regarded as a synonym in this context, \*\*Exploited Areas

## CONCLUSION

*Placuna placenta* is the only species under the genus that is listed (as *Placenta placenta*) under Schedule IV of the Indian Wildlife Protection Act, 1972. As mentioned in the act, "No person shall hunt any wild animal specified in Schedules I, II, III, and IV except as provided under section 11 and section 12". Furthermore, Section 11 and 12 allows for hunting only in special cases and with proper documents and permissions from the concerned government authority; however, surreptitious fishing of this species continues even in protected areas, e.g., Kakinada Bay (Coringa Wildlife Sanctuary) (Laxmilatha 2015a,b) apart from other areas as mentioned above. Moreover, the recent news of *P. placenta* being smuggled to western Asian and South American countries in alternate forms (Ravinesh et al. 2018) further highlights the urgent need to assess the genus. As our study highlights the morphological aspects of this genera, a thorough comparative assessment of the internal organs and the application of molecular

methods should provide essential insights. On the other hand, biogeographical assessment of *P. quadrangula*, its ecology and its implications on *P. placenta* distributions is urgent. As the Indian Ocean is home to large and unknown malacofauna (see Das et al. 2017), continuous surveys to discover these understudied resources remains imperative.

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PLATINUM  
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The Mountain Tortoiseshell *Aglais rizana* (Moore, 1872) has a distribution extending from Safed Koh (Pakistan), from Chitralup to Sikkim (inner ranges in India), where it is believed to be 'rare' and found in the 'alpine' region of 2,400–4,500 m during May–August' (Evans 1932; Wynter-Blyth 1957; Haribal 1992; Kehimkar 2016). Its distribution range outside India extends up to the Pamirs-Altai mountains through Afghanistan and adjoining Pakistan (<https://www.nic.funet.fi/>). The species is absent in Nepal (Smith 1989, 2006; van Gasse 2017). Specimens of *A. rizana* have been collected from "Cheeni ('Chini' or Kalpa in Himachal Pradesh) at 2,759m, middle Kunawur (upper Kinnaur District in Himachal Pradesh), N.W. Himalayas" (Moore 1872); "Gulmarg (Kashmir), Kunawur (Himachal Pradesh) and Thibet" (Tibet) (3,000m) where "it appears to be rare" (DeNiceville 1886). While Wynter-Blyth (1940) reported it as a "rare butterfly of the high inner hills" with specimens collected from Shipki Pass in Kinnaur District in Himachal Pradesh along the Indo-China border, at 4,500m in July. The species has only been reported once from the Kumaon region of Uttarakhand by Hannyngton (1910) from the "inner ranges above 3,000m in July-August". Four specimens from Hari-Ki-Dun (Uttarkashi District) in northern Garhwal were collected by P.W. Mackinnon in June, 1907 (pers. obs.), while two more specimens were collected by S.N. Chatterjee in October, 1912 from the same area (pers. obs.). A male and a female were later collected from Kashmir (3,000m), India by O.C. Ollenbach on 07.viii.1915 that are all kept at National Forest Insect Collection (NFIC) at Forest Research Institute, Dehradun

## RECENT RECORDS OF THE RARE MOUNTAIN TORTOISESHELL *AGLAIS RIZANA* (MOORE, 1872) (LEPIDOPTERA: NYMPHALIDAE) IN THE UPPER GARHWAL, WESTERN HIMALAYA, INDIA, AFTER 100 YEARS

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(Images 1–4). There are also recent reports of the occurrence of this species from the upper reaches of Kumaon in Uttarakhand bordering Nepal by Trilok Singh Rana and Emmanuel Theophilus (Sondhi & Kunte 2018). There are, however, no recent occurrences of this species from the Garhwal Himalaya (Bhardwaj et al. 2012; Uniyal et al. 2013; Singh & Sondhi 2016) (Fig. 1). The species is currently protected and is listed in Schedule II, Part II, of the Indian Wildlife Protection Act, 1972 (Anonymous 2006).

Two individuals of *A. rizana* were recorded (Image 6) from a "sub-alpine" patch of vegetation along the Badrinath-Mana road, Chamoli District on 06.vii.2019 (3,151m; 30.760°N & 79.499°E) in the company of *Aglais caschmirensis* from 11.00h to 12.00h (temperature: 24.7°C & relative humidity: 52%). The vegetation here was composed mainly of *Rosa sericea* Lindl., *Tanacetum mubigenum* Wall.ex. DC, *Urtica* sp. along with herbs and grasses on rocky slopes by the side of the River Alakananda.

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Images 1 & 2. Male (left) and female (right) Mountain Tortoiseshell, *Aglais rizana* (Moore, 1872) collected from Kashmir, India by O.C. Ollenbach (07.viii.1915; National Forest Insect Collection (NFIC), Forest Research Institute, Dehradun, India).



Figure 1. Map depicting the present record of Mountain Tortoiseshell *Aglais rizana* (Moore, 1872) in Badrinath-Mana area, Chamoli District, Uttarakhand (Garhwal Himalaya) in relation to past confirmed records of the species across other areas in the Himalaya in India and Pakistan.



Images 3 & 4. Mountain Tortoiseshell, *Aglais rizana* (Moore, 1872) collected from Hari-ki-Dun, northern Garhwal, India by Mackinnon (vi.1907; kept at National Forest Insect Collection (NFIC), Forest Research Institute, Dehradun, India).



Image 5. Indian Tortoiseshell *Aglais caschmirensis* (Kollar, [1844]) (Badrinath-Mana road, Chamoli District, Uttarakhand, India (6.vii.2019)), for comparison with *A. rizana*.

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*A. rizanais* similar in appearance to the Indian Tortoiseshell *Aglais caschmirensis* (Kollar, [1844]), as the former is characterized by wings being broad and squarish, forewing cut off at the tip and not produced to apex but produced at v6 as compared to the latter where the forewing is narrow and more produced and the hind wings are quite prominently toothed at v.4 (Image 5). The upper hind wing blue spots are not inwardly brown

in *A. rizana* as in the *A. caschmirensis* (Evans 1932; Wynter-Blyth 1957; Kehimkar 2016). It is to be noted, however, that the “black discal spots in space 2 and 3” on the upper forewing of the specimens examined from northern Garhwal (Images 3–5) are much bigger in size than that of specimens collected from Kashmir Valley (Images 1,2).

The current sightings confirm the occurrence of this



**Image 6. Mountain Tortoiseshell *Aglais rizana* (Moore, 1872) photographed between Badrinath-Mana Village (3,151m), Chamoli District, Uttarakhand, India (6.vii.2019).**

rare species in the Garhwal Himalaya, after a gap of more than a 100 years.

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The Collembola are small, entognathous, wingless hexapods which poses a spring like jumping organ under the fourth abdominal segment known as furcula due to which they are called springtails. The presence of antennae and absence of cerci distinguishes it from other entognathous hexapods—Protura (with antennae

and cerci absent) and the Diplura (with antennae and cerci or pincers present).

There are 9,037 described species of Collembola present worldwide (Bellinger et al. 2019). In India the collembolan fauna constitutes about 342 species of 113 genera belonging to 20 families in which the family Paronellidae consists of 69 species of 15 genera (Mandal 2018). The member of the subfamily Paronellinae Borner distinguished from other entomobryids by straight unringed dentes lacking spines but with terminal bladder like projection with a short and blunt mucro which is quite different from other entomobryids (Mandal & Suman 2016). In the subfamily Paronellinae the genus *Dicranocentroides* consists of five species in India (Hazra & Mandal 2015) and 21 species in the world (Bellinger et al. 2019).

The earlier distribution of the species *Dicranocentroides indica* Handschin in India was from Assam, Meghalaya, Manipur, Mizoram, Nagaland, Arunachal Pradesh, Sikkim, Uttar Pradesh, Andhra Pradesh, Chattishgarh (Mandal 2018). Here, the first report details and description of *Dicranocentroides indica* was made from Sundargarh, Odisha (Figure 1).

## FIRST REPORT OF *DICRANOCENTROIDES INDICA* (HANDSCHIN, 1929) (COLLEMBOLA: PARONELLIDAE) FROM ODISHA, INDIA

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### Systematic Accounts (As per Mandal & Suman 2016)

Family: Paronellidae Borner, 1913

Subfamily: Paronellinae Borner, 1913

Genus: *Dicranocentroides* Imms, 1912

Species: *Dicranocentroides indica* Handschin, 1929

Materials examined: ENT/225/2018, Z.S.I. Lot No. 89/2018, 3 exs, 9.xii.2018, 21.763°N, 85.111°E, elevation 152m, Khandadhar Waterfall, Sundargarh, Odisha, India (Image 1), coll. Ashirwad Tripathy.

Diagonistic Characters: Colouration: Whole body golden brown in colour with faint purple blue pigment. The antennal segment I, II and III with a distinct distal bluish ring and IV mostly with bluish pigment. Legs with dark brown pigment; furca with diffused blue pigment. Clothing: Body covered with scales, cervix and anterior margin of II thoracic segment is covered with a collar of acuminate setae; macrachaetae obliquely truncated on II, III thoracic segment and I, II and III abdominal segments. At the posteromedial part the segment IV, V and VI contains acuminate setae. Head: Two dark ocellar field, each having eight ocelli in two longitudinal parallel rows. Antennal segment I and II were stouter; segment IV superficially annulated, apical sense knobs not distinct. Thorax: Relative length index of segments II:III :: 12:9, legs similar; unguis with paired basal and medial unpaired

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Figure 1. Distribution of *Dicranocentroides indica* in India showing old and new records.

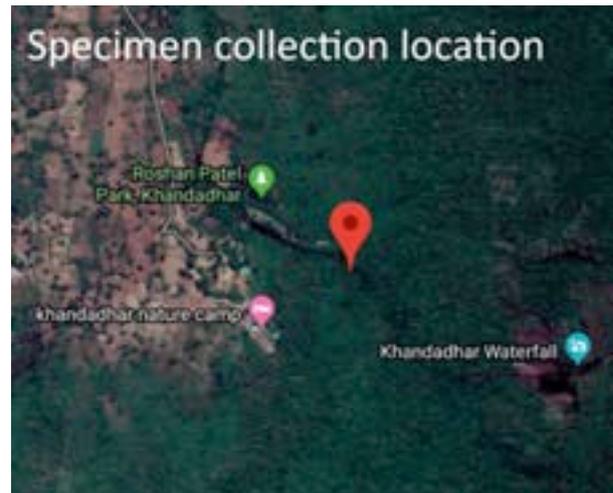


Image 1. Khandadhar Waterfall, Sundargarh District, Odisha (Google Maps)

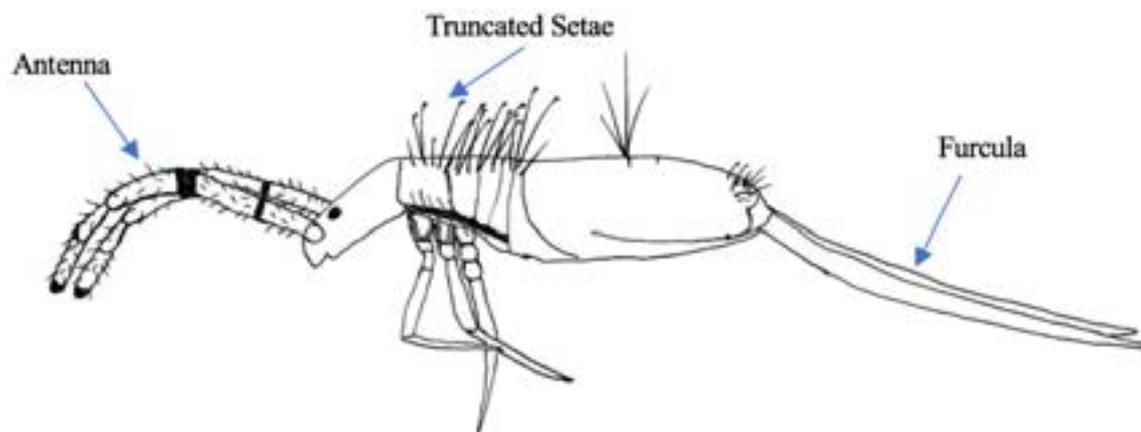


Figure 2. *Dicranocentroides indica* (Handschin, 1929) illustration. © Ashirwad Tripathy.

teeth, unguiculus lanceolate in shape; tenant hair long narrow and clavate. Abdomen: long ventral tube with protrusible vesicle retracted. Manubrium: Mucrodens 22:35, short mucro quadrangular with six striated teeth (Figure 2).

Remarks: Handschin (1929) first described this species in *Aphysa* genus from Europe. In course of revision of Indian collembola fauna, Mitra (1975) transferred the generic status to *Dicranocentroides* on the basis of the character of the genus present in the insect.

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Tenebrionidae is the versatile group, found almost in all habitats throughout the world—in rotten wood, under bark, stones and logs, feeding on decaying vegetation, in dung, seeds, cereals, fungi, roots, and dead animal matter. They are varied in shape and size measuring 2–35 mm in length. These forms are very often apterous,

or have vestigial wings, and the elytra are frequently immovable. Many of the wood feeding species have ample wings. The representatives of this family are hard to differentiate but can be identified by these characters (Hegde & Lal 2016). Body hard, antennal insertion hidden under frons, elytra usually completely covering abdomen, abdomen with five visible sternites and first three segments connate, front coxal cavities closed behind, heteromerous tarsi, tarsal segments and claws simple. Even though, there are some studies on the Tenebrionidae of West Bengal (Hegde & Vasanthakumar 2018) and some northeastern states like Manipur (Hegde & Lal 2016) Arunachal Pradesh (Hegde 2019), there is no comprehensive study on the Tenebrionidae fauna of Meghalaya state. As the various hills comprising the state of Meghalaya lies between the plains of Bangladesh and the Brahmaputra Valley of Assam, the topography is markedly different than the surrounding regions, which is why the fauna found in the State show richness in biodiversity and endemism. As the State lacks a detailed catalogue of Tenebrionidae, hence, an attempt has been made to prepare a systematic account of this group of Coleoptera.

Specimens present in the National Zoological Collection, collected by different survey parties of Zoological survey of India, Kolkata were identified and

## ADDITIONS TO THE KNOWLEDGE OF DARKLING BEETLES (COLEOPTERA: TENEBRIONIDAE) FROM THE INDO-BURMA BIODIVERSITY HOTSPOT, MEGHALAYA, INDIA

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classified and distribution records were as per Bouchard et al. (2005), Becvar and Purchart (2008), Lobl & Smetana (2008), Masumoto et al. (2011), Merkl (1990, 1991), Schawaller (2003, 2005, 2012, 2016). The identification is mainly based on the differences in the morphological characters and the structure of the genitalia. The registration numbers are also given for the material examined. The specimens from the old collections were in fragile conditions and hence the images were not given. The species reported elsewhere (outside India) are also included in distribution.

### 1. Subfamily: Lagriinae Latreille, 1825 (1820)

Tribe: Lupronini Ardoin, 1958

#### *Luprops kaszabi* Schawaller, 1997

1997. *Luprops kaszabi* Schawaller, Entomologische Zeitschrift 107: 295–298

Distribution: India [Uttarakhand, Assam and Meghalaya (Tura)], Nepal, West Malaysia.

#### *Spinolyprops himalayicus* Kaszab, 1965

1965. *Spinolyprops himalayicus* Kaszab, Miscelania Zoologica 2: 107–130.

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Distribution: INDIA [Uttarakhand, West Bengal, Meghalaya (Tura), Maharashtra and Tamil Nadu], Myanmar, Nepal, Bhutan, Laos, Thailand, Vietnam and Indonesia (Java and Bali).

**Tribe: Lagriini Latreille, 1825 (1820)**

***Bothynogria meghalayana* Merkl, 1990**

1990. *Bothynogria meghalayana* Merkl, Acta Zoologica Hungarica 36(3–4): 284.

Distribution: India [Meghalaya (Cheerapunji)].

Remark: Endemic

***Xanthalia martensi* Merkl, 1991**

1991. *Xanthalia martensi* Merkl, Stuttgarter Beitr, Naturk. 470(18): 12.

Distribution: India [Meghalaya (Mawphlang)], Nepal.

**2. Subfamily: Tenebrioninae Latreille, 1802**

**Tribe: Ulomini Blanchard, 1845**

***Uloma prehimalayana* Kaszab, 1975**

1975. *Uloma prehimalayana* Kaszab, Entomologia Basiliensia 1: 325

Distribution: India [Assam and Meghalaya (Cheerapunji)].

***Uloma rubripes* (Hope, 1831)**

*Uloma rubripes* Hope, 1831, The Zoological Misc. 1: 31.

Distribution: INDIA [Uttarakhand, Sikkim, West Bengal, Assam and Meghalaya].

***Uloma scita* Walker, 1858**

1858. *Uloma scita* Walker, Ann. Mag. Nat. Hist. (3)2: 284.

1894. *Uloma scita* Fairmaire, Ann. Soc. Ent. Belg. 38: 37.

Distribution: INDIA [Kashmir, Himachal Pradesh, Uttarakhand, West Bengal and Meghalaya (Garo hills)], Pakistan, Nepal and Bhutan.

**Tribe: Amarygmini Gistel, 1856**

***Amarygmus speciosus* Dalmann, 1823**

1823. *Amarygmus speciosus* Dalmann, Analecta Entomologica: 61.

Distribution: India [Meghalaya (Tura)], China (Yunnan), Myanmar, Nepal and Thailand.

**Tribe: Opatrini Brulle, 1832**

***Gonocephalum depressum* (Fabricius, 1801)**

1801. *Opatrum depressum* Fabricius: 117. – Steven 1829: 95.

1801. *Hopatum depressum* Fabricius – Gemminger 1870: 1931; Fairmaire 1893: 20, 1894: 17.

1801. *Gonocephalum depressum* (Fabricius). – Gebien

1906: 213.

1858. *Opatrum contrahens* Walker, 284. – Blair 1921: 269.

1858. *Hopatum contrahens* Walker – Gemminger 1870: 1931.

1858. *Gonocephalum contrahens* (Walker). – Gebien 1910b: 322, 1939: 447; Kaszab 1952a: 681.

Material examined: Reg. No. 3873-3880/H4A, 08 ex., 13.ii.1961, Shillong, Meghalaya [25.569 N; 91.884 E; 1503m], coll. S.N. Prasad.

Distribution: India [Kashmir, Himachal Pradesh, Arunachal Pradesh, Sikkim, West Bengal, Meghalaya (Shillong), China (Yunnan), Taiwan, the Philippines, Afghanistan, Pakistan, Nepal, Myanmar, Bhutan, Vietnam, Laos, Indonesia, Sri Lanka and New Guinea.

**3. Sub Family: Diaperinae Latreille, 1802**

**Tribe Scaphidemini Reitter, 1922**

***Basanus flaviventris* Blair, 1937**

1937. *Basanus flaviventris* Blair, The Entomologist Monthly Magazine 73: 35-37

Distribution: India [Sikkim, West Bengal and Meghalaya (Nokrek-Daribokgiri)], Vietnam; Laos.

***Ceropria induta induta* Wiedemann, 1819**

1819. *Helops indutus* Wiedemann, Zool. Mag., 1(3): 164.

1831. *Ceropria subocellata* Castelnau et Brulle, Anns. Soc. nat. Paris, 23: 398.

1982. *Ceropria kinugasai* Masumoto, Ent. Rev. Japan, 36: 151.

Material examined: Reg. No. 3881-3882 /H4A, 02 ex., 11.i.1962, Shillong, Meghalaya [25.569N; 91.884E; 1503m], coll. C. B. S.

Distribution: India [Arunachal Pradesh, West Bengal, Assam, Meghalaya (Shillong), Chhattisgarh and Andaman Islands]; Japan (Ryuku Is.); Korea, China, Taiwan, the Philippines (Engano Is.), Myanmar, Vietnam, Thailand, Malaya Peninsula, Indonesia (Borneo, Java, Halmahera, Nia Is., Simalur Is., Sulawesi, Sunda Is. and Sumatra).

**Tribe: Leiochrinini Lewis, 1894**

***Crypsis bimaculatus* Kaszab, 1946**

1946. *Crypsis bimaculatus* Kaszab, Ungarisches Naturwissenschaftliches Museum, 221: 191.

Distribution: India [Arunachal Pradesh, Sikkim and Meghalaya (Tura)], Nepal.

***Crypsis violaceipennis* Waterhouse, 1877**

1877. *Crypsis violaceipennis* Waterhouse, The Entomologist Monthly Magazine 14: 72-75.

Distribution: India [Uttarakhand, Sikkim, West Bengal

and Meghalaya (Tura)], Nepal and Laos.

***Platydema aurimaculatum* Gravely, 1915**

1915. *Platydema aurimaculatum* Gravely, *Rec. Ind. Mus.* 8: 523.

Distribution: India [Arunachal Pradesh (Hegde, 2019) and Meghalaya (Rongrengiri in East Garo Hills)], Myanmar, Laos and Thailand.

***Platydema capreolum* Chevrolat, 1877**

1877. *Platydema capreolum* Chevrolat, *Petites Nouvelles Entomologiques* 2: 170.

Distribution: India [Meghalaya (Garo Hills), Tamil Nadu], Nepal, Myanmar, Vietnam, Laos, Thailand and Sri Lanka.

***Platydema chalconeum* Gebien, 1925**

1925. *Platydema chalconeum* Gebien, *The Philippine J. Science* 27: 539-595.

Distribution: India [Meghalaya (Mawphlang in East Khasi Hills)], Myanmar, Thailand and Indonesia (Java and Borneo).

***Platydema flavopictum* Gebien, 1913**

1913. *Platydema flavopictum* Gebien, *Archiv. fur Naturgeschichte* (1914) A 79 (9): 17.

Distribution: India [Meghalaya (Tura in West Garo Hills)], Taiwan, Myanmar, Laos and Thailand.

***Platydema haemorroidale* Gebien, 1913**

1913. *Platydema haemorroidale* Gebien, *Archiv. fur Naturgeschichte* (1914) A 79 (9): 16

Distribution: India [West Bengal and Meghalaya (Songsak in East Garo Hills)], China, Taiwan, Nepal and Vietnam.

***Platydema shiva* Schwallier, 2003**

2003. *Platydema shiva* Schwallier: p. 263.

Distribution: INDIA [Meghalaya (Norkek National Park in Garo Hills)].

***Platydema vishnu* Schwallier, 2003**

2003. *Platydema vishnu* Schwallier, : p. 263.

Distribution: India [Meghalaya (Ronrengiri in East Garo Hills)].

***Derispia indica* Kaszab, 1946**

1946. *Derispia indica* Kaszab, *Ungarisches Naturwissenschaftliches Museum*, 221: 79.

Distribution: India [West Bengal and Meghalaya (Cherrapunjee in East Khasi Hills)], Nepal, Myanmar and Bhutan.

***Derispia shillonga* Schwallier, 2016**

2016. *Derispia shillonga* Schwallier, *Stuttgarter Beitragezur Naturkunde A Neue series*, 9: 199.

Distribution: India [Meghalaya (Khasi Hills)].

***Derispiola assamensis* Kaszab, 1946**

1946. *Derispiola assamensis* Kaszab, *Ungarisches Naturwissenschaftliches Museum*, 221: 116.

Material examined: Reg. No. 10648 /H4A, 01 ex. 07. iv.1927, Khasi Hills, Meghalaya, coll. Gopi Ram.

Distribution: India [Assam and Meghalaya (Khasi Hills)].

***Derispiola darjeelingiana* Kaszab, 1946**

1946. *Derispiola darjeelingiana* Kaszab, *Ungarisches Naturwissenschaftliches Museum*, 221: 117.

Distribution: India: Sikkim, West Bengal and Meghalaya (Tura in West Garo Hills)], Nepal.

***Derispiola fruhstorferi* Kaszab, 1946**

1946. *Derispiola fruhstorferi* Kaszab, *Ungarisches Naturwissenschaftliches Museum*, 221: 118.

Distribution: India [Meghalaya (Khasi Hills)], China (Sichuan), Vietnam and Thailand.

***Leiochrinus metallicus* Schwallier, 2016**

2016. *Leiochrinus metallicus* Schwallier, *Stuttgarter Beiträgezur Naturkunde A Neue series*, 9: 202.

Distribution: India [Sikkim and Meghalaya (Tura in West Garo Hills)].

**4. Subfamily: Stenochiinae, Kirby, 1837**

**Tribe Cnodalonini Oken, 1843**

***Andocamaria malgorzatae* Masumoto et al. 2011**

2011. *Andocamaria malgorzatae* Masumoto et al., *Annales Zoologici (Warszawa)* 61(2): 237-239.

Distribution: India [Meghalaya (Jaintia Hills)].

***Danodema subcalvum* Gebien, 1925**

1925. *Danodema subcalvum* Gebien, *The Philippine J. Science* 27: 355.

Distribution: India [West Bengal, Meghalaya (Tura in West Garo Hills), Tamil Nadu].

***Derosphaerus exularis* (Gebien, 1913)**

1913. *Derosphaerus exularis* (Gebien), *Archiv. fur Naturgeschichte* (1914) A 79 (9): 17.

Distribution: India [Assam, West Bengal, Meghalaya (Tura in West Garo Hills)].

***Derosphaerus rugosus* Gravelly, 1915**

1915. *Derosphaerus rugosus* Gravelly, Rec. Indian Mus., Calcutta, 8: 528.

Distribution: India [Uttar Pradesh, Arunachal Pradesh, Sikkim, Assam, West Bengal, Meghalaya (Tura in West Garo Hills)], Nepal.

***Foochounus assamicus* Kaszab, 1965**

1965. *Anobriomaia assamicus* Kaszab, Miscelanea Zoologica 2: 127.

Material examined: Reg. No. 10712/H4A, 01 ex., 30.viii.1917, Tura, West Garo Hills, coll. S. Kemp.

Distribution: India [Arunachal Pradesh (Hegde, 2019) and Meghalaya (Tura in West Garo Hills)], Nepal.

***Hexarhopalus jendeki* Bacvar and Purchart, 2008**

2008. *Hexarhopalus jendeki* Bacvar and Purchart, Annales Zoologici (Warszawa), 58(1): 57.

Distribution: India: Meghalaya

**Tribe: Stenochiini Kirby, 1837*****Strongylium aratum* Fairmaire, 1896**

1896. *Strongylium aratum* Fairmaire, Ann. Soc. Ent. Belg. XL: p. 35.

Distribution: India [Uttarakhand, Uttar Pradesh, Sikkim, West Bengal, Assam and Meghalaya], China (Yunnan), Nepal.

***Strongylium angusticolle* Maklin, 1864**

1864. *Strongylium angusticolle* Maklin, Monographie: 333

Distribution: India [Uttarakhand, Sikkim, West Bengal, Assam, Meghalaya], China (Yunnan), Nepal.

***Strongylium angustissimum* Pic. 1922**

1922. *Strongylium angustissimum* Pic., Mel. exo. ent. 37:27

Distribution: India [Uttar Pradesh, Sikkim, West Bengal, Assam and Meghalaya], China (Yunnan), Nepal.

***Strongylium cultellatum* Maklin, 1864**

1864. *Strongylium cultellatum* Maklin, Monographie: 345.

Distribution: India [West Bengal, Assam and Meghalaya], Japan, South Korea and China (Hongkong), Nepal.

***Strongylium stevensi* Gravelly, 1915**

1915. *Strongylium stevensi* Gravelly, Rec. Indian Mus., Calcutta, 8: 534

Distribution: India [Arunachal Pradesh (Hegde 2019), Assam and Meghalaya].

The northeastern states of India are sandwiched between the eastern Himalaya and Indo-Burma biodiversity hotspots. A few works on the Tenebrionidae fauna were reported from this region. Hegde (2019) has compiled the Tenebrionidae of Sikkim and Arunachal Pradesh from the Eastern Himalaya Biodiversity hotspots, while from the Indo-Burma biodiversity hotspot, Hegde & Lal (2018) worked on the fauna of this group from Manipur. In this paper, efforts are made to document the Tenebrionidae fauna of another state coming under Indo-Burma biodiversity hotspot, Meghalaya, where a total of 37 species of 20 genera belonging to nine tribes of four major sub families are found.

While the Eastern Himalaya region represents a large number of Tenebrionidae species (106 species from Sikkim and 63 species from Arunachal Pradesh), the diversity of the same is markedly less in the Indo-Burma region (13 species from Manipur and 37 species from Meghalaya) (Hegde 2019; Hegde & Lal 2016). The genus like *Laena*, currently known from high altitude areas of the Himalaya in the country, are very much present in Sikkim and Arunachal Pradesh, while there has been no report of the same from the relatively low altitude Indo-Burma region. Another genus, *Gonocephalum*, which is widely adapted to a number of habitats, generally dry conditions and have a large population comprising of numerous species in India (Hegde 2018), is represented by only five species from the relatively wet climatic conditions of the Indo-Burma region (Hegde & Lal 2016). However, the current paper is adding three species (*Ceropria induta induta*, *Derispiola assamensis* and *Foochounus assamicus*) under three genera, two tribes and one sub-family as new record to the Indo-Burma region, while along with these, one more species (*Gonocephalum depressum*) is added to the state fauna of Meghalaya.

The reports of Tenebrionidae from Manipur and Meghalaya represent a small geographic area under the Indo-Burma regions; further studies from Nagaland, Mizoram, Tripura and the southern banks of river Brahmaputra in Assam in India may reveal further additions to the knowledge of this group of Coleoptera. Comprehensive work on this group across the border in Myanmar and beyond that form the part of Indo-Burma region is still lacking.

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### Corrigenda

**Tanshi, I., A.E. Ogbeibu & P.J.J. Bates (2019).** Complementary bat (Mammalia: Chiroptera) survey techniques uncover two new country records for Nigeria. *Journal of Threatened Taxa* 11(14): 14788–14801. <https://doi.org/10.11609/jott.5294.11.14.14788-14801>

Page 14797: Under *Glauconycteris beatrix*, read 'New records: Okomu National Park' as 'New records: Okomu National Park (Image 9)'.

Page 14797: Under *Mimetillus moloneyi*, read 'New record: Emu (Image 9)' as 'New record: Emu'.

Page 14798: In Image 9 caption, read '*Mimetillus moloneyi* (Thomas, 1891)' as '*Glauconycteris beatrix* Thomas, 1901'.

The authors are grateful to Alexandre Hassanin for noting and pointing out the errors.

**BHUTAN ASIABELL *CODONOPSIS BHUTANICA*  
LUDLOW (ASTERALES: CAMPANULACEAE):  
A NEW ADDITION TO THE INDIAN FLORA**

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The genus *Codonopsis* Wall. [Campanulaceae] comprises of about 55 species distributed in central, eastern, and southern Asia, with Indochina region as the primary centre of speciation (Hong 2015; Dash 2018; Mabberley 2018). The members of the genus are characterized by perennials herbaceous ascending herbs or twiners, with solitary and large campanulate flowers; and with a peculiar foetid odour. C.B. Clarke (1881) reported 10 species of *Codonopsis* from British India; Kanjilal et al. (1939) reported only two species in Flora of Assam; Haridasan & Mukherjee (1996) dealt with 13 species of *Codonopsis* in Fascicles of Flora of India; and recently Dash & Mao (2011) described a new species *Codonopsis vadsea* S.S. Dash & A.A. Mao from Vadse Hills of Arunachal Pradesh, while Mao & Barbhuiya (2014) reported a *Codonopsis tubulosa* Kom. from Dzukou Valley of Manipur. The present estimate shows that the genus is represented by 15 species in India, primarily

distributed in the Himalayan region and adjacent areas (Dash 2018).

During our field exploration in Tawang District, Arunachal Pradesh in August 2017, some interesting plants of *Codonopsis* were collected from the sub-alpine area of the Bumla region (Images 1 & 2, Figure 1). A thorough study of the live material, available relevant literature (Clarke 1881; Kanjilal et al. 1939; Haridasan & Mukherjee 1996; Clement 2001; Giri et al. 2008; Hong et al. 2011; Dash & Mao 2011; Panday & Sinha 2012; Mao & Barbhuiya 2014; Hong 2015) and comparison of herbarium specimens and images with ASSAM, BM, CAL and K, the identity of the taxa was confirmed as *Codonopsis bhutanica* Ludlow. The species has not been reported from India so far, and thus reported here as an addition to the Indian flora. A detailed taxonomic description along with a photo collage illustrating different features of the plant, and a map showing the locality is provided here.

***Codonopsis bhutanica* Ludlow,**

J. Roy. Hort. Soc. 97: 127. 1972; R.A. Clement in Grierson & D.G. Long (eds.), Fl. Bhutan 2(3): 1385. 2001; Deyuan et al. in Z.G. Wu, P. Raven & D.Y. Hong (eds.), Fl. China 19: 523. 2011. *C. thalictrifolia* sensu Kanwal et al., J. Threat. Taxa 11(9): 14229. 2019 (non. Wall. 1824: 106).

Type: Bhutan, northeastern Bhutan, Shingbe Me La, 27.966°N & 91.650°E, 3810m, 02.vii.1949, F. Ludlow, G. Sherriff & J.H. Hicks. 20786 (BM000996411!).

Plants herbaceous. Stems procumbent, 25–45 cm,



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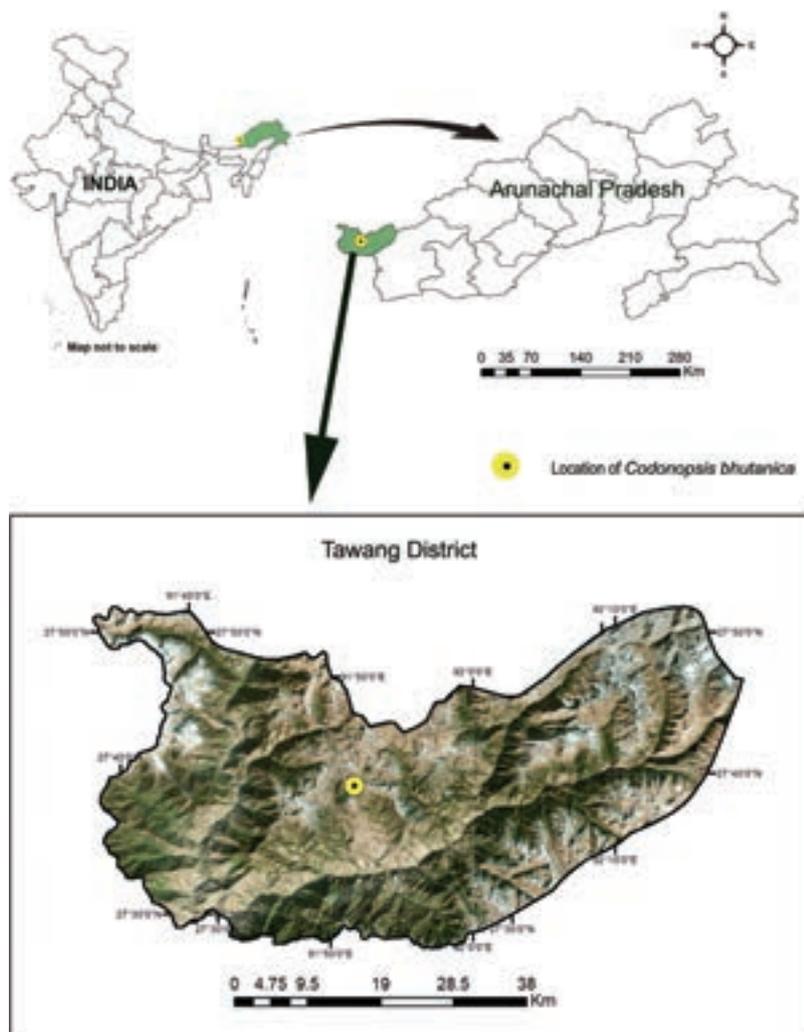


Figure 1. Locality map of *Codonopsis bhutanica* in Bumla area of Tawang District, Arunachal Pradesh, India.

with several slender branches at lower part; branches with purple spots, faintly hairy. Leaves alternate or nearly opposite, ovate-oblong or narrowly oblong, cordate, 7–22 × 6–18 mm, entire or thickened at margin, often recurved, obtuse at apex, sparsely hispidulous on both surface, more along the midrib on ventral surface; sessile or shortly petiolate, petiole 2–4 mm long. Flowers solitary, terminal on the main stem and sometimes on upper branches, rachis 6–10 cm long, often with purple patches; calyx adnate to ovary up to middle, lobes linear, or narrowly ovate, 6–11 × 3–5 mm, glabrous or hispidulous. Corolla tubular, 12–16 mm, deeply purple, violet at base; outer surface of corolla pale blue or purplish, inner surface of corolla whitish with purple spots; corolla lobe 5, rounded. Stamens 5; filaments dilated at base, pale yellow, glabrous, 3–4 mm long; anthers basifixed, yellow, 2–2.5 mm. Gynoecium 8–12 mm long, lower half of style with ovary deep violet, upper half with stigma pale violet in colour; ovary

inferior, 3-locular with numerous ovules; stigma 3-fid, c. 2mm across. Capsules conical, green, 12–15 × 8–12 mm. Seeds numerous, ellipsoid.

**Flowering & Fruiting:** July–October.

**Habitat and ecology:** Rarely found in grassy slopes or thickets, 3,500–4,500 m.

**Distribution:** Bhutan, Nepal, India (Arunachal Pradesh—this report).

**Specimen examined:** 87971 (CAL!), 31.viii.2017, India, Arunachal Pradesh, Tawang District, Bumla, Near Nagula Lake, 27.651°N & 91.861°E, 4,100m, coll. V. Kumar & S. Panday (Image 3).

**Associated species:** The species is found associated with *Meconopsis simplicifolia* (D. Don) Walp., *Pedicularis siphonantha* D. Don, *Codonopsis foetens* Hook.f. & Thomson, *Juncus cephalostigma* Sam., *Gypsophila cerastoides* D. Don etc.

**Notes:** The species was previously reported only from Nepal and Bhutan and considered as endemic to

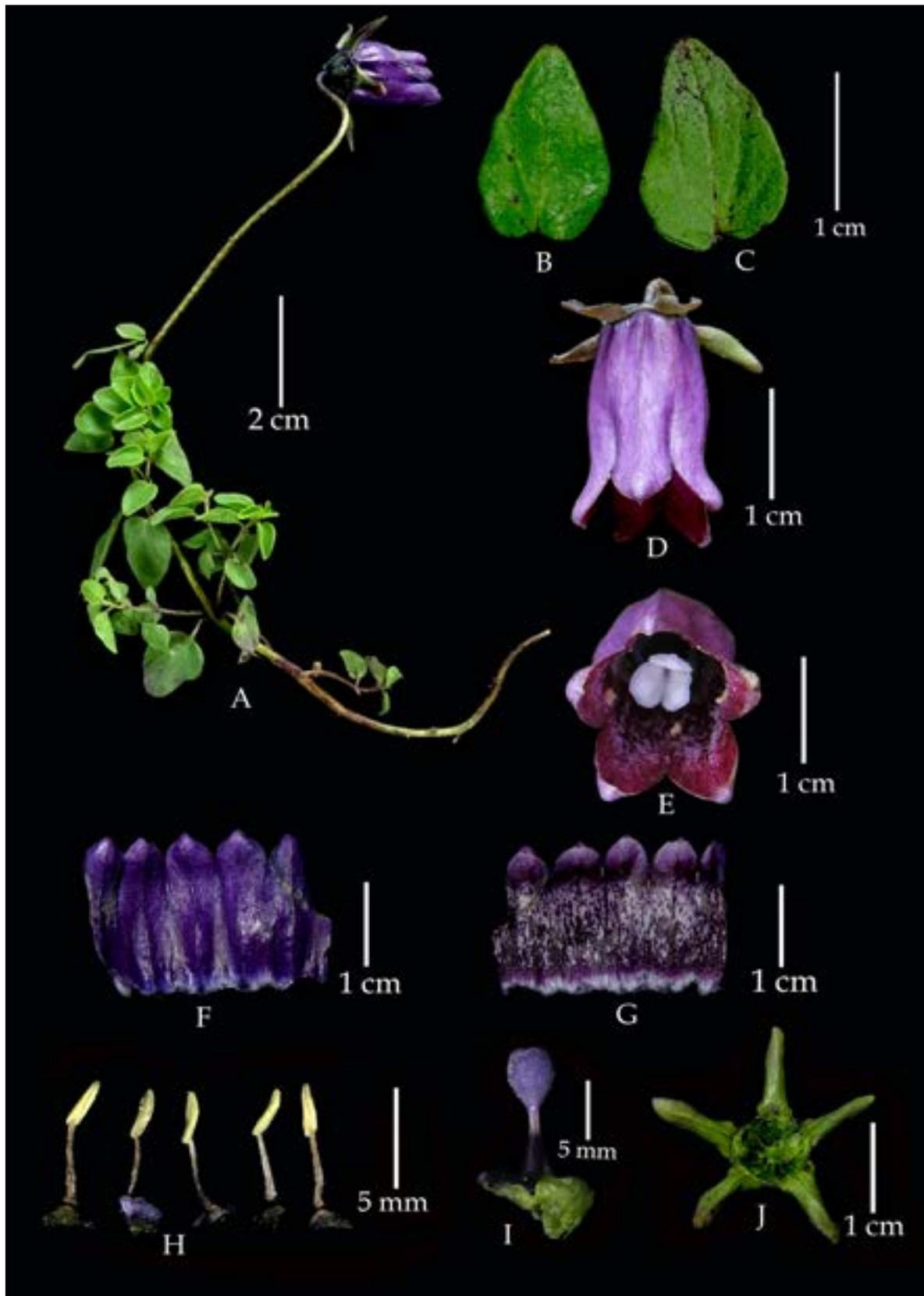


Image 1. *Codonopsis bhutanica*: A—plant habit | B—leaf – ventral surface | C—leaf – dorsal surface | D—lateral view of a flower | E—front view of a flower | F—opened corolla tube showing outer surface | G—opened corolla tube showing inner surface | H—stamens | I—gynoecium | J—fruit. © Samiran Panday.



Image 2. Habit of *Codonopsis bhutanica*.

central and eastern Nepal and Bhutan (Hong 2015); however, the collection of this species from the Bumla area of Arunachal Pradesh confirms its occurrences in India and shows an eastern extension from its type locality. Kanwal et al. (2019) have erroneously reported *Codonopsis thalictrifolia* as a new distributional record for Arunachal Pradesh. The herbarium and coloured image provided by Kanwal et al. (2019) shows that the flowers are campanulate, deep reddish-purple, corolla c. 1.4 cm long, corolla lobes triangular-ovate in shape and all these characters refer to the species *C. bhutanica* instead of *C. thalictrifolia* in which flowers are tubular, flared at mouth, pale blue, corolla 2.5–5.5 cm long, corolla lobes broadly oblong in shape.

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Image 3. Herbarium image of *Codonopsis bhutanica* (CALI, 87971).

**GENTIANA URNULA HARRY SM.  
(GENTIANACEAE), A NEW RECORD FOR THE  
FLORA OF ARUNACHAL PRADESH, INDIA**

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The genus *Gentiana* L. (Gentianaceae) consists of around 400 species that are mainly distributed in the alpine regions of the world, but also occur in temperate regions of Asia, Europe and the Americas (Ho & Liu 2001; Struwe & Albert 2002; Mabberley 2008; Favre et al. 2016). The Qinghai–Tibet Plateau (QTP) of the Himalaya is considered to be the main centre of diversity for *Gentiana*, hosting around 250 species (Ho & Pringle 1995). The name *Gentiana* is given by Linnaeus after Gentius, the King of Illyria. Gentianas are important medicinal plants in traditional Chinese medicine, and have been used for over 2,000 years for curing various ailments like hypotension, rheumatic pains, fevers and allergic inflammations (Gupta et al. 2012). In India, the genus is mainly distributed in temperate, sub-alpine, and alpine regions of the Himalaya. A total of 73 taxa (66 species, 4 subspecies and 3 varieties) of *Gentiana* are recognised from India, out of which 31 taxa are recorded

from the eastern Himalayan region whereas 27 taxa are confined to the western Himalaya and only five taxa are described from southern India (Sasidharan 2004; Gupta et al. 2012; Maity 2014; Shabir et al. 2017; Maity & Dey 2017; Maity et al. 2018).

A floristic survey was carried out in Tawang District of Arunachal during 2016–17 for the assessment of floral diversity of high altitude areas. During the collection, *Gentiana urnula* Harry Sm. was recorded from Nagula wetland complex area (27.647°N and 91.861°E at an altitude of 4,000m) of Tawang. The Nagula wetland area is very rich in high altitude floral diversity and little explored at present. This species is very rare and endemic to the eastern Himalaya. *Gentiana urnula* is an important medicinal plant and mostly used in Tibetan medicinal system for the treatment of diarrhoea, dysentery, food poisoning and common cold. The identification of the species was confirmed through the consultation of type specimens, the protologue description of the species and consultation of literature (Hooker 1882; Hara 1965, 1975; Polunin & Stainton 1984; Garg 1987; Stainton 1988; Hajara et al. 1996; Ho & Liu 2001; Giri et al. 2008; Chowdhery et al. 2009; Gupta et al. 2012; Maity 2014; Favre et al. 2016; Maity & Dey 2017; Shabir et al. 2017; Shabir et al. 2018; Maity et al. 2018). Furthermore the Herbarium specimens of the Botanical Survey of India (BSI), Itanagar (ARUN) and State Forest Research Institute (SFRI), Itanagar were consulted. International online herbaria and the Global Biodiversity Information Facility



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(GBIF) were also explored for species identification and distribution records. *G. urnula* has not been reported earlier from Arunachal Pradesh. The voucher specimen was deposited in the herbarium of the G.B. Pant National Institute of Himalayan Environment and Sustainable Development, (GBP) and Botanical Survey of India (BSI), Itanagar (ARUN) for future references.

***Gentiana urnula* Harry Smith**

Bull. Misc. Inform. Kew. 15: 51. 1961. Ho & Liu, Worldwide Monogr. *Gentiana*: 2001 (Image 1 A, B).

Holotype: Bhutan, Nelli la near Lingshi Dzong, 4,500m, 13 October 1949, Ludlow, Sherriff & Hicks 17458 (BM holotype; EUPS isotypes).

Perennial herbs, sometimes mat forming, 1.5–2.0 cm high. Stems simple or rarely branched, 1 or 2, glabrous. Basal leaves reddish-green, not rosette; cauline leaves crowded upward; petioles 1–1.5 mm long, membranous; lamina truncate-flabelliform, 5–8 mm, truncate or emarginate at apex, abruptly contracted at base, slightly cartilaginous a long margin, papillate near base only; mid-vein cartilaginous and crested, vein 1, upper most pair of leaves often sessile. Flowers terminal, solitary or 2, subsessile. Calyx tubular, obconic; lobes 5, leafy, leathery, orbicular; tube 4–6 mm long, membranous; lobes 3–4 mm long, similar to leaves. Corolla pale bluish-purple to pale yellow with blue streaks, campanulate, 2–3 cm long; lobes broadly ovate, 3–4.5 × 2.5–3.5 mm, apex rounded and cuspidate, entire at margins; plicae broadly ovate to subtruncate, 1–2 mm,

entire at margins or denticulate. Stamens inserted in corolla tube; filaments 5.5–8.5 mm long; anthers 2–3.5 mm long, ellipsoid. Style short; stigma with triangular lobes. Capsules 1.5–1.8 cm; ovoid-ellipsoid; gynophores up to 4cm, slender. Seeds ellipsoid, 2–2.5 mm long, dark brown; seed coats with simple pits.

**Flowering and Fruiting:** July–October.

**Distribution:** India (Sikkim, Arunachal Pradesh), Bhutan, Nepal, China (SW Qinghai, E Xizang) (Ho & Liu 2001).

**Specimen examined:** 1013(GBP), 10.viii.2017, Nagula Lake, Tawang District, Arunachal Pradesh, India, 27.647°N, 91.861°E, 4,000m, coll. Lod Yama & KS. Kanwal (Images 2 & 3).

**Habitat and Ecology:** The plants were found growing in some isolated pockets in Nagula Lake area of western Arunachal Pradesh in alpine meadows and gravel slope at 4,000m altitude. It is facing threats from livestock mainly from trampling by yaks and horses, unregulated tourism and developmental activities which result in habitat destruction and fragmentation in the area. In future, the species may face further threat from climate change due to very limited population size and restricted distribution in the Himalayan region. Therefore, conservation action should be taken for this rare and endemic species before it becomes extinct in this region. Extensive grazing by yaks along with the consequent human intrusion for plant exhibited more pronounced habitat destruction and made the plant status crucial for immediate management intervention. Proper updated information regarding the species is



Image 1. *Gentiana urnula* Harry Sm.: A—plants in natural habitat | B—flowers. © K.S. Kanwal.

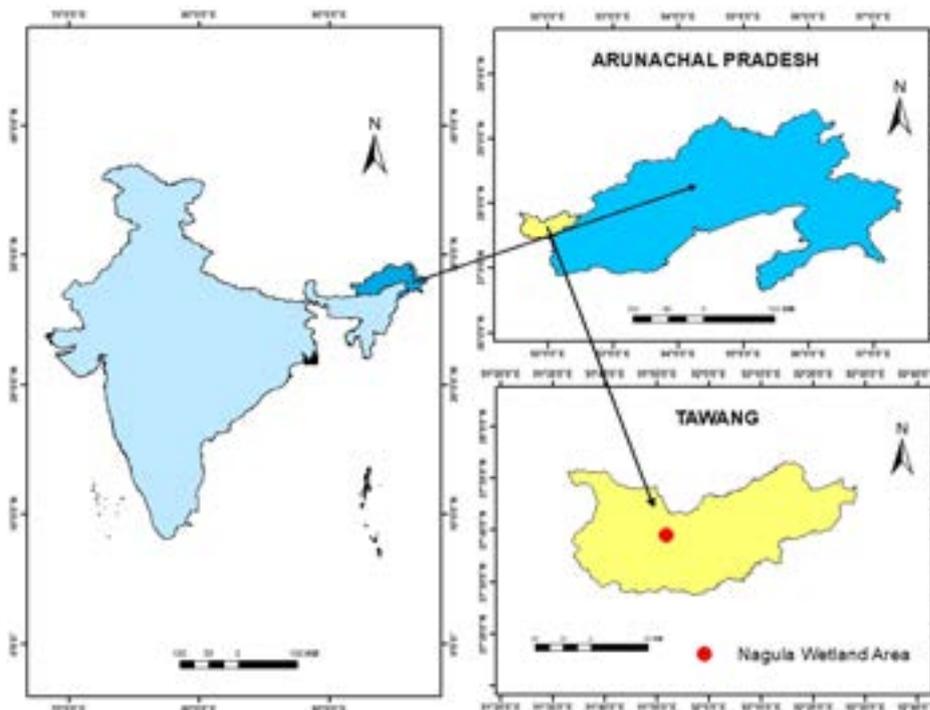


Image 2. Distribution of *Gentiana urnula* Harry Sm. near Nagula wetland, Tawang District.

lacking in India or neighbouring countries, especially with regard to ecological and habitat information, thereby creating huge lacuna in the knowledge base. Qualitative and quantitative inventory of the species is urgently needed for evolving a long term conservation plan of the species. In addition to this, in situ and ex situ conservation measures, awareness through educational programmes, and community participation should also be required for the conservation of *Gentiana urnula* in the region.

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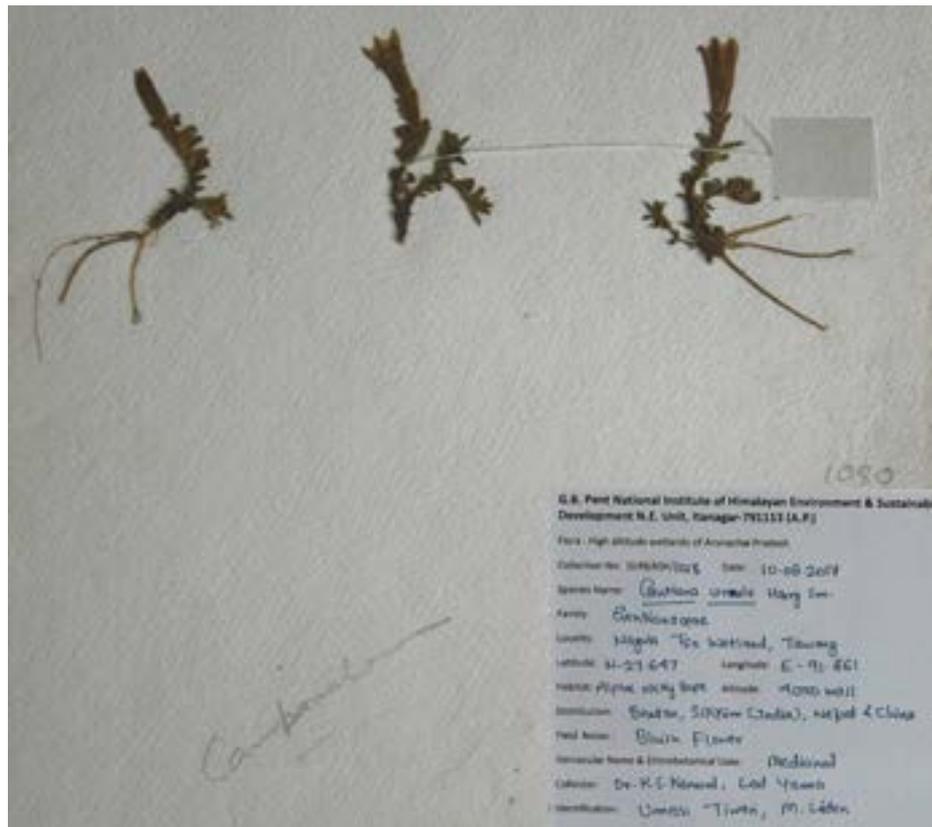


Image 3. Herbarium image of *Gentiana urnula* (GBP) [1013].

*aperta* (Gentianaceae) - a new record to India from Ladakh Himalaya. *Journal of Threatened Taxa* 10(9): 12286–12289. <https://doi.org/10.11609/jott.4233.10.9.12286-12289>

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**CAREX PHACOTA, SPRENG. (CYPERACEAE):  
A NEW RECORD FOR THE CENTRAL  
WESTERN GHATS OF KARNATAKA, INDIA**

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The genus *Carex* L. of the family Cyperaceae is represented by 1,800–2,000 species. It has a cosmopolitan distribution at high altitudes of the temperate and tropical regions (Mabberley 2008). Hazra & Verma (1996) reported 62 species of *Carex* from Sikkim and the Darjeeling Himalaya. During a survey of grasses in Kudremuk National Park, Karnataka (13.169°E & 75.281°N) a species of *Carex* was collected and confirmed as *Carex phacota* by using the floristic literature. The genus *Carex* is characterized by a perennial rhizome, paniced spike, unisexual flowers rarely dioceses, glumes numerous, persistent and imbricate around the rachilla (Images 2 & 3). Unisexual spikelets with one or two male florets at the tip and remaining female florets in the inflorescence, shortly beaked utricles are the key characters of *Carex phacota* (Gamble, 1928). They usually grown on wetlands, stream sides or lakes; hence they are commonly called lakeshore sedge. It is distributed in southern and eastern Asia, Bhutan, Nepal, China, Indonesia, Japan, Malaysia, Myanmar, Sri Lanka, northern Thailand, and Vietnam (Chowdary 2016). In India it was first reported by Gamble

in 1928 at Nilgiri Hills, Tamil Nadu; later from Assam, Chhattisgarh, Kerala, Meghalaya, Sikkim, West Bengal and now Karnataka also. The herbarium is deposited in Central National Herbarium, Botanical survey of India, Howrah and also Herbaria, Department of Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka (Image 4).

Flowering and Fruiting: November–March

Habitat and Ecology: Wet soil of grasslands, ditches, banks of streams and roadsides ditches at an altitude of up to 1,400m of shola (Image 1).

Specimens examined: KUABYLKS21, 21.i.2017, Gogudda, Kudremuk National Park, Chikkamagalore District, Karnataka, coll. H.U. Abhijit.

***Carex phacota* Spreng.,**

F.B.I. vi.708. Syst. Veg. 3: 826. 1826; Hook.f., Fl. Brit. India 6: 708. 1894; Gamble, Fl. Pres. Madras 1686(1169). 1931; Manilal, Fl. Silent Valley 338. 1988; Karthik. et al., Fl. Ind. Enum. Monocot. 40. 1989; Vajr., Fl. Palghat Dist. 542. 1990; Rejani, Cyperaceae. Kerala 101. 1991; C.D.K. Cook Aquat. Wetl. Pl. Ind. 99. 1995; Sasidh. et al., Bot. Stud. Med. Pl. Kerala 39. 1996; Sasidh., Fl. Shenduruny WLS 363. 1997; Swarup. et al., Shola For. Kerala 78. 1998; Sasidh., Fl. Periyar Tiger Reserve 484. 1998; Sasidh., Fl. Parambikulam WS 372. 2002; Mohanan & Sivad., Fl. Agasthyamala 772. 2002.

An erect, perennial herb with tufted culms 50–60 cm long, leafy mainly at the base. Leaves are slightly yellowish-brown in color size about 40x0.5 cm. Utricles



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Image 1. Habitat of *Carex phacota*, Spreng.



Image 2. Habit



Image 3. Inflorescence

not or very shortly beaked. Female glumes 2mm long with hispid awn, oblong or ovate, apex deeply emarginated, sides pale brown, midrib and awn green. Bracts long, upper filiform; spikelet's unisexual, one terminal male, rest female with some times a few male flowers at the tip,



Image 4. Herbarium sheet of *Carex phacota*, Spreng.

linear- cylindric, 1–4.6 mm long, solitary, pedicelled, often drooping; stigmas 2; utricles biconvex, ovate or trapezoid, acute, hardly beaked, usually very shortly stipitate, brown with pale angles, faces covered with white papillae which turn chocolate or purplish-brown on drying (Gamble 1928; Prasad 2002)

Flowering and Fruiting: November–March

Habitat: Growing in swampy places or streams of Shola.

Specimens examined: KUABYLKS21, Gogudda, Kudremuk National Park, Chikkamagalore District, Karnataka, coll. H.U. Abhijit.

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## COMPENDIUM OF TRADED INDIAN MEDICINAL PLANTS

Reviewed by A. Rajasekaran

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Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. The traditional practitioners sustainably utilized the medicinal plants for various formulations with high degree of accuracy. However, the resurgence of global interest in herbal based health care has brought about a shift in preparation of herbal health care products on industrial scale involving high volume trade of many medicinal plants. In this context, there is a need for clear understanding on the sources of species under trade and their scale of demand and supply. The book titled “Compendium of Traded Indian Medicinal Plants” by K. Ravikumar, S. Noorunnisa Begum, D.K. Ved, J. R. Bhatt and G. S. Goraya, (2018) is fascinating and concise, though comprehensive information on the traded Indian medicinal plants.

Herbal raw drugs are generally traded using local trade name or vernacular names (but the use of trade / vernacular names to identify) plant taxa traded in herbal medicine markets is unreliable as they vary considerably from place to place and even between traders within the same market. However, there can be only one valid botanical name for a plant species and the quality of herbal formulations prepared as per the guidelines of classical texts, is highly dependent on the correct identification of the plant species being traded. This book has appropriately followed the latest ‘The International Code of Nomenclature for Algae, Fungi and Plants’ (ICN) 2012 to facilitate correct identification of the traded Indian medicinal plants. Further, basionyms and important synonyms are provided for many medicinal plants so as to include their popularly known botanical names. Another issue which affect the quality of traded medicinal plants is unauthorized substitutes and unknown adulterants. To address this issue, the compendium under review followed a scientific approach by providing modern taxonomic descriptions which equates the descriptions of plants in the classical texts along with 736 colour photographs covering various plants in trade and their official parts to help in

correct botanical identity.

The book aimed at documenting medicinal plants traded in the country, including vernacular names, description of the part in trade, trade information, taxonomic descriptions, habitat, distribution in India and the world and medicinal uses of 178 species that are in high volume trade (> 100 MT/Year) whereas for each low volume trade plants (776) species details such as accepted botanical names, widely used synonyms, trade names, parts traded, medical systems (viz. Ayurveda, Siddha, Unani, Sowa-rigpa, Homeopathy,



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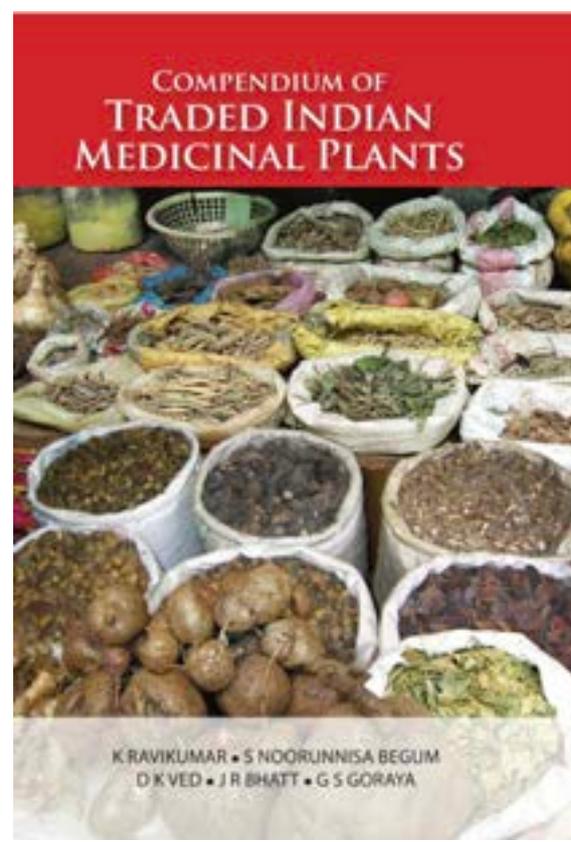
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Folk), brief botanical description, distribution and habitat are given. In essence, the compendium is well organised with excellent scheme of presentation with the details of species name, family, medical system, trade and vernacular names, plant parts in trade with distribution map including the medicinal uses portraying brief account of raw drugs traded with their known substitutes and adulterants along with a short plant profile.

In India, most of the medicinal plant materials are being harvested from the wild so it is very important to clearly establish the specific regions from where the medicinal plants are being sourced. Such information in the form of distribution maps are provided for 159 high volume traded species in the compendium which will be highly useful for formulating necessary conservation and management measures for these medicinal plant sources.

Most of the data for this compendium originated from the field work carried out during 2002-2017 under a study of demand and supply of medicinal plants in India by the Foundation for Revitalization of Local

Health Traditions (FRLHT), a Centre of Excellence on Medicinal Plants and Traditional Knowledge under the Ministry of Environment, Forest and Climate Change (MoEFCC), Government of India. The research efforts made by various individuals and institutes who have contributed in finalization of this book resulted in the successful and timely completion of this Compendium. The authors of this compendium and other individuals who have contributed towards remarkable and valuable photographs for this Compendium deserve much appreciation.

The work presented in this book will be most advantageous for students, researchers as well as academic staff researching plants for medicinal purposes in India and indeed the rest of the world. It will be useful for wide range of stakeholders including herbal pharmacies, exporters and importers of medicinal plants, managers of the forest resources and regulatory authorities. This book is an important contribution and is useful for maximizing the realization of the potential of traded medicinal plants found in India.



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**Observations on the ex situ management of the Sumatran Rhinoceros *Dicerorhinus sumatrensis* (Mammalia: Perissodactyla: Rhinocerotidae): present status and desiderata for conservation**

– Francesco Nardelli, Pp. 14927–14941

#### Communications

**Revisiting genetic structure of Wild Buffaloes *Bubalus arnee* Kerr, 1792 (Mammalia: Artiodactyla: Bovidae) in Koshi Tappu Wildlife Reserve, Nepal: an assessment for translocation programs**

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