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Journal of Threatened Taxa

10.11609/jott.2022.14.5.20951-21126
www.threatenedtaxa.org

26 May 2022 (Online & Print)
14(5): 20951–21126
ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

Open Access

ISSN 0974-7907 (Online); ISSN 0974-7893 (Print)

Publisher
Wildlife Information Liaison Development Society
www.wild.zooreach.org

Host
Zoo Outreach Organization
www.zooreach.org

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Cover: Dorsal view of Mantis Shrimp *Cloridina ichneumon* (Fabricius, 1798) & *Gonodactylellus demanii* (Henderson, 1893). © Fisheries Research Station, Junagadh Agricultural University, Sikka.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 20951–20963

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7835.14.5.20951-20963

#7835 | Received 16 January 2022 | Final received 13 April 2022 | Finally accepted 27 April 2022

OPEN ACCESS

COMMUNICATION

Drought may severely reduce the ability of wild Asian Elephants *Elephas maximus* (Mammalia: Proboscidea: Elephantidae) to resist opportunistic infections

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Abstract: The present study was conducted to assess the microbial quality of water in forest waterholes in different seasons and its possible impact on wild animals, at Bandipur and Nagarahole Tiger Reserve forests in the state of Karnataka, India, during the year 2012 which evidenced drought, and the year 2014 which witnessed normal rainfall in these forests. The forests recorded the death of 39 wild elephants during April and May of 2012. One ailing elephant was confirmed to have high fever, diarrhoea, leucocytosis, and symptoms of colic. Water samples collected from major waterholes during the peak drought showed higher numbers of coliforms and several species of opportunistic bacteria including species of *Vibrio* and *Campylobacter.* In the year 2014–15, with normal rainfall, the death of less than 10 wild elephants was documented during April to May, 2015. We collected water samples from 20 major waterholes every month from June 2014 to May 2015 and assessed the water quality. We found that the microbial water quality improved in rainy season (June–September), started deterioration in winter (October–January) and became poor in summer (February–May). Though, the water during the summer of 2014–15 was equally of poor microbial quality as seen during peaks of droughts, the elephant deaths were relatively lower, signifying the role of normal rainfall in forests which provides the availability of fodder and water, which determines the general body condition and ability to resist opportunistic infections. We discuss the measures suggested and implemented from this study and their utilities at ground level.

Keywords: *Campylobacter*, Coliforms, forest waterholes, microbial quality, rainfall, *Vibrio*, water, wildlife.

Editor: Heidi Riddle, Riddle's Elephant and Wildlife Sanctuary, Arkansas, USA. **Date of publication:** 26 May 2022 (online & print)

Citation: Chandranaik, B.M., V. Patil, D. Rathnamma, G.S. Mamatha, K.S. Umashankar, D.N. Nagaraju & S.M. Byregowda (2022). Drought may severely reduce the ability of wild Asian Elephants *Elephas maximus* (Mammalia: Proboscidea: Elephantidae) to resist opportunistic infections. *Journal of Threatened Taxa* 14(5): 20951–20963. https://doi.org/10.11609/jott.7835.14.5.20951-20963

Funding: This study was funded by Govt. of India's Centrally Sponsored Scheme-Project Elephant-2013–14 vide Govt. of Karnataka order No. PCCF(WL)/B1/CR-01/2013-14 dated 31.10.2013 for the research project 'Monitoring of quality of water at major waterholes in Bandipur and Nagarahole forest area'.

Competing interests: The authors declare no competing interests.

Author details & **Author contributions:** See end of this article.

Acknowledgements: The authors are thankful to the Ministry of Environment and Forest, Government of India, and to the Forest Department of Government of Karnataka for funding the research work. Authors are also thankful to the unconditional support of all forest officers, forest guards, jeep drivers of Bandipur and Nagarahole tiger reserves, who had to travel in the wild forest under difficult climatic conditions at different stages of this study spanning over three years.

INTRODUCTION

Concern about climate change has intensified interest in understanding how climatic variability affects animal life. Despite such effects being potentially most dramatic in long-lived, slow reproducing, large terrestrial mammals, little is known of the effects of climatic variation on survival in such species. A series of complex climatic changes affecting the equatorial pacific region causes reversal of wind patterns in the Pacific Ocean and leads to consecutive droughts in Australia and Asia (Wenju et al. 2014; Chris 2015).

Water is essential for living. Wild animals depend on rainwater that accumulates in waterholes in forests. The rainwater that accumulates in waterholes remains throughout the year and is prone to microbial contaminations arising out of various sources, of which faecal contaminations from humans and wild animals are most important (Obi et al. 2002). Unpredictable chronic droughts lead to acute shortage of drinking water, forcing wild animals either to depend on limited water available in waterholes or they get no water at all (Durham et al. 2008). It has been extensively studied and reported that the microbial diarrhoeal diseases are a major public health problem from ingestion of water contaminated with human and /or animal faeces (Seas et al. 2000; Cabral 2010). However, no studies have been undertaken to correlate wild animal mortality with droughts and water quality in the wild.

The present study attempts to assess possible factors contributing to the deterioration of microbial quality of water during different seasons of a year, and its possible impact on wild animals. We used elephant mortality as evidence to compare the water quality and its impact during chronic droughts, in comparison to seasons of normal rainfall (Figure 2). Though microbial quality is not the only reason for animal deaths, this study analyses how the microbial quality of water in waterholes in forests could predispose elephants to mortality during extended droughts. This study was carried out in the Bandipur Tiger Reserve (also known as Bandipur National Park) and Nagarahole Tiger Reserve (also known as Nagarahole/Rajiv Gandhi National Park) forests in the state of Karnataka, India, during 2012 which witnessed severe drought in these forests, and in the year 2014–15 which had normal rainfall. The study is of significance since recurrent droughts could be a common feature in times to come, owing to severely disrupted global weather patterns and we need to know its impact on wildlife.

MATERIALS AND METHODS

Study area

The Bandipur Tiger Reserve with an area of 874.20 km^2 and the Nagarahole Tiger Reserve with an area of 643 km^2, are important components of the 5,500 km^2 'Nilgiri Biosphere Reserve' which is one of the largest conservation areas in the world (Chandranaik et al. 2016, 2017) (Figure 1). The forests are a large chunk of dry deciduous forest which receives heavy pre-monsoon showers in late May. The south-west monsoon starts by mid-June and lasts until September. These two forests are one of the richest wildlife areas in India, being noted for their assemblage of seven large ungulate species—Muntjac *Muntiacus muntjak*, Chital *Axis axis*, Sambar *Rusa unicolor*, Chousingha *Tetracerus quadricornis*, Gaur *Bos gaurus*, Wild Pig *Sus scrofa cristatus*, & Asian Elephant *Elephas maximus* and three major carnivores—Tiger *Panthera tigris*, Leopard *Panthera pardus*, & Dhole *Cuon alpinus*. The forest supports a high ratio of predator and prey species. As per the 2012 elephant census, Bandipur forest has a population of 1,697 elephants and Nagarahole forest has 1,320 elephants, constituting 27.9 % and 21.8 % of the total 6,072 elephants in Karnataka state, respectively (Varma & Sukumar 2012).

Ten major waterholes each in Bandipur forest and Nagarahole forest were selected for the purpose of monitoring the quality of water during this study period. Ten major waterholes selected in Bandipur forest included; Moolapurakere (Range: Bandipur), Kharapurakere (Range: Kundkere), Tavarekattekere (Range: Bandipur), Hirikere (Range: G.S. Betta), Natkalkere (Range: Maddur), Madrakatte (Range: Moolehole), Nataraja Kolachi (Range: A.M. Gudi), Hidgalpanchi (Range: Muliyur), Chikkamauthige Kolachi (Range: N. Begur), and South Kere (Range: Omkar)

Ten major waterholes selected in Nagarahole forest for the purpose of monitoring the quality of water included; Kambapurakere (Range: Anechoukur), Maralakandakere (Range: Anechoukur), Kallahalla (Range: Kallhalla), Doddahallakere (Range: Nagarahole), Marappanakere (Range: Nagarahloe), Bisilawadikere (Range: Antharasanthe), Bidirukattekere (Range: Veeranahosahalli), Rajegowdanakatte (Range: Veeranahosahalli), Holerahundikere (Range: Metikuppe), and Seegurukere (Range: D.B. Kuppe).

For the purpose of this study we have considered the months from June to September as rainy season; October to January as winter season, and February to May as summer season.

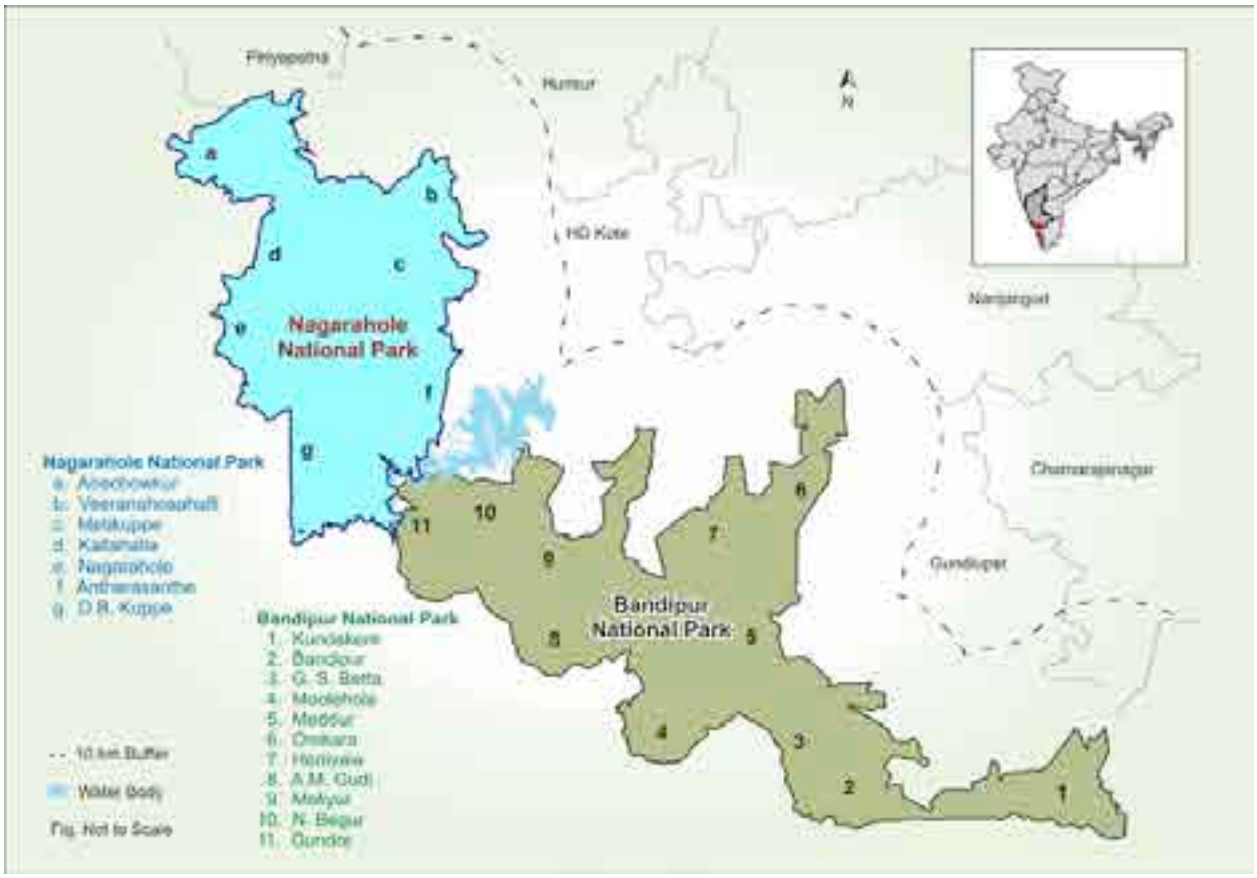


Figure 1. Map of the study areas.

Sample Collections

i) During droughts of 2012

Clinical samples from ailing and dead elephants.

Thirty-nine wild elephants died during the months of April–May, 2012. An ailing elephant was examined on the banks of the dried-up Kabini River in Bandipur forest and blood samples were collected for laboratory examination. The elephant was treated symptomatically with fluids and antibiotics but the animal did not survive. Post-mortem examination was conducted on the fresh elephant carcass.

In most other cases of elephant deaths it was very difficult to get fresh carcasses for post-mortem examination and hence, alternatively, bone marrow samples from femur bones were aseptically collected from 12 near putrefied elephant carcasses in Bandipur and Nagarahole forests during April–May, 2012.

Water samples

Water samples from the waterholes were aseptically collected during April–May, 2012, as per the procedure described previously (Obi et al. 2002) and transported on ice to the Institute of Animal Health and Veterinary Biologicals, Bengaluru, India, for microbiological and parasitological investigations.

ii) During normal rain fall year of 2014–15

Water samples were collected from each of the above 20 major waterholes every month, starting from June 2014 (beginning of rainy season) to May 2015 (end of the summer season) for microbial and parasitological analysis. Samples were collected as described previously and transported to laboratory under cold chain conditions.

Microbiological Analysis

(i) Water samples: Microbiological analysis of water samples were performed as described previously (Standard Methods 1998; Nevodo & Cloete 1999; Obi et al. 2002; Quinn et al. 2011). Briefly, for heterotrophic bacteria, the spread-plate method was done on nutrient agar and plates were incubated at 37 °C for 48 hours. The total Coli forms and *E. coli* counts were enumerated using USFDA and WHO approved petri-films procured from 3M Company, USA, as per previously described methods (Jordano et al. 1995). All the bacteria that were

isolated under the present study were confirmed by specific biochemical tests as prescribed previously (Obi et al. 2002; Quinn et al. 2011).

(ii) Organ samples: Blood samples collected from an ailing elephant, organ samples collected at post-mortem, and bone marrow samples collected from putrefied carcasses were subject to microbiological culture as per previously described procedures (Quinn et al. 2011; Chandranaik et al. 2015, 2016).

Parasitological quality assessment

Presence of parasitic worms and/or their eggs in water samples was done by floatation and sedimentation techniques as previously described by Soulsby (1982).

Polymerase Chain reaction

For DNA extraction, five milliliters of nutrient broth inoculated with bacteria from a single colony of the isolate, and culture was incubated overnight with shaking. Bacterial cells were harvested by centrifugation at 1,000 X g for 15 min. Genomic DNA was extracted from the disrupted cells using DNA extraction kit procured from Amnion Biotech Pvt. Ltd. Bengaluru, Karnataka, India, following the protocols provided by the manufacturer. The previously described primers and the protocols were used for PCR confirmation of *Escherichia, Compylobacter*, and *Vibrio* species (Hollond et al. 2000; Soren & Katharina 2005; Cheryl et al. 2007).

RESULTS

Drought of 2012

Out of 20 major waterholes in forest area under the study, nine had dried up by April–May of 2012 (3b,c in Image 1). The other eleven waterholes under the study had very little water, which appeared muddy, greenish, with heaps of dried-up as well as fresh elephant dung (Supplementary Image 1). It is important to note that these two forests received less than the normal rainfall in the year 2011 (Figure 2), and the drought of 2012 was an extended period of dry spell (Figure 2). Water samples collected in all the waterholes had high microbial contamination with an average total coli form counts of 6.7×10^5 cfu/ml, mean total *E. coli* counts of 9.2×10^3 cfu/ml (Table 1). On petri films, the coli formed red colonies and *E. coli* formed blue colonies (Supplementary). The water samples collected from the 11 waterholes which contained water at the time of collection during April–May, 2012, yielded growth of *Vibrio cholerae*, *V. parahaemolyticus*, and species of *Salmonella, Klebsiella, Shigella, Staphylococcus, Streptococcus, Bacillus*, and *Campylobacter* (Supplementary Image 3). These bacterial isolates were confirmed by biochemical tests, viz., Indole, citrate, catalase, nitrate, urease, oxidase, methyl red, voges-prausker, ornithine-decarboxylase, nitrate reduction, lysine decarboxylase and arginine hydrolase test. *Escherichia, Vibrio*, and *Campylobacter* were additionally confirmed by polymerase chain reaction.

The water samples contained eggs of gastrointestinal parasites of Strongyles, Amphistomes and Fasciola flukes (A, B, C in Supplementary Image 4). During the drought, the forests witnessed recurrent massive forest fires (Supplementary Image 5) destroying minimally available fodder to larger mammals, and killing several smaller wild animals which could not escape the raging forest fire.

The ailing elephant that was examined on the banks of Kabini River at the peak of drought conditions had a high fever of 104 ^{o}F. Blood samples revealed elevated liver enzyme SGOT at 219 IU/µl (Normal value: 5–55 IU/µl), total leukocyte counts at 18,000/µl (Normal value: less than 12, 000/µl) (Miller & Fowler 2012). The ailing elephant finally succumbed to acute colic symptoms. Post mortem revealed lesions of severe enteritis, empty bowels, heavy worm loads (Image 2), and hepatitis. Out of 12 bone marrow samples collected from elephant carcasses in late decomposition, nine yielded growth of mixed cultures of *E. coli, Salmonella* sp., *Shigella* sp., and *Klebsiella* sp. The study recorded death of 39 elephants during April–May, 2012.

Normal rainfall year of 2014–15

During this study, it was observed that the quantity of water in waterholes started increasing from June through the rainy season in August and reached the maximum levels by November. The water level started depleting from December and reached minimum levels by April to mid-May (1a,b,c in Image 1). Total coli form counts and *E. coli* counts were lowest during rainy season which gradually increased during late winter and the counts reached highest number during summer months (Table 1). Water samples collected during the months of June, July, August, September, October, November, and December yielded growth of *Escherichia, Aeromonas, Psuedomonas, Staphylococcus, Salmonella, Streptococcus, Bacillus, Klebsiella*, and *Shigella* bacterial species. Water samples collected during January, March, April, and May in addition to the above bacterial species yielded growth of *Vibrio cholerae*, *V. haemolyticus*, and species of *Campylobacter* (Table 2)

Table 1. Bacterial counts observed during different seasons in major waterholes of Bandipur and Nagarahole Tiger Reserve forests.

	During normal rainfall year of 2014–15			During the drought year 2012
Parameter	**Rainy season**	**Winter season**	**Summer season**	**April and May, 2012**
Coli form count	Mean: 2.4 x 10^2 S.D: 2.1 x 10^2	Mean: 1.8 x 10^3 S.D: 2.45 x 10^2	Mean: 4.3 x 10^5 S.D: 3.2 x 10^5	Mean: 6.7 x 10^5 S.D: 4.2 x 10^5
E.coli count	Mean: 3.7 x 10^2 SD: 3.1 x 10^1	Mean: 2.7 x 10^2 SD: 2.7 x 10^2	Mean: 6.2 x10^3 SD: 4.2 x 10^3	Mean: 9.2 x 10^3 SD: 5.1 x 10^3

Image 1. Water levels in waterholes: 1a, 2a, 3a—Completely filled up waterholes in rainy season | 1b, 2b, 3b—Waterholes in winter | 1c, 2c, 3c—Dried up waterholes in summer. © Authors.

Water samples collected during all the months (June 2014–May 2015) revealed the presence of eggs of Fasciola, Amphistomes, Strongyles, Taenia and Coccidian oocysts (Table 2). The study found that the habit of wild animals to defecate while consuming water (as observed in several instances during this study while collecting water samples) had possibly resulted in an abundance of faecal droppings in the water holes, especially at the fringes of the waterholes where they stand and drink water (D, E, F, G in Supplementary Image 4). Abundant numbers of different types of snails which act as intermediate hosts for trematode flukes (Fasciola and Amphistomes) were observed near the waterholes (H, I, in Supplementary Image 4). The monthly average rainfall data in the study area during 2011, 2012, and 2014 is depicted in Figure 2. Forests witnessed the death of less than 10 elephants in April–May, 2015.

DISCUSSION

Bandipur and Nagarahole Tiger Reserves witnessed an extended drought during 2012. Most of the findings that are described in this study are the first time reports in elephants; hence, we have discussed our results in comparisons with available reports in domestic animals

and humans.

Drought of 2012

The major waterholes had either completely dried up or were left with little water which was highly contaminated. There was an acute shortage of fodder to elephants as the green vegetation had dried-up in the forest. Also, the dried-up grass, shrubs, and trees had been destroyed by recurrent forest fires. These factors lead the elephants to chronic starvation and dehydration; gradually contributing to poor nutrition, poor body condition, and consequent immunosuppression.

In the absence of any other water sources, elephants had to drink the contaminated water available in the waterholes, which were the source of heavy loads of different types of opportunistic pathogens especially the coli forms. Under natural conditions when the elephants are healthy with good nutrition and immunity, they can withstand most opportunistic pathogens including coliforms and the gastrointestinal parasitic infestations (Quinn et al. 2011; Miller & Fowler 2012). However, under severe drought conditions, the immune compromised wild animals are susceptible to opportunistic and/or acute bacterial infections/septicemia (Quinn et al. 2011; Chandranaik et al. 2015) which cause high fever, hepatitis, pancreatitis, acute enteritis, dehydration, and other systemic disorders. Hepatitis, pancreatitis, and enteritis are highly painful conditions which cause colic and struggling, as observed in most of the elephant deaths in the present investigation.

Potential pathogenic and/or opportunistic bacterial species of *Escherichia, Vibrio, Aeromonas, Shigella, Klebsiella, Salmonella, Bacillus, Pseudomonas*, and *Campylobacter* were isolated from all the water sources studied during drought. The presence of these bacteria in water sources is in agreement with previous reports (Cabral 2010). These enteric bacteria have been reported to act as the causative agents of various diseases and their complications such as diarrhoea/dysentery, septicaemia, dehydration, hypovolaemic shock, acidosis, and haemo-concentration (Ongunsanya et al. 1994; Seas et al. 2000; Cabral 2010).

Vibrio cholerae can grow at 40°C with pH 9–10. The growth is stimulated by the presence of sodium chloride which is available as a result of rapid evaporation of water in waterholes due to heat of the summer. There are more than 200 serovars of *V. cholera,* characterized based on the structure of the lipopolysaccharide. Only two serovarieties named O1 and O139 are involved in causing true cholera. However, other serovarieties can cause gastroenteritis, but not cholera. The severity of

Table 2. Bacterial isolates and parasitic eggs/cysts recovered from the water samples collected during this study.

Escherichia spp.	
Vibrio spp.	
Salmonella spp.	
Klebsiella spp.	Fasciola
Campylobacter spp.	Amphistomes
Pseudomonas spp.	Strongyles
Streptococcus spp.	Taenia
Staphylococcus spp.	Coccidia
Shigella spp.	
Bacillus spp.	
Aeromonas spp.	

Image 2. Post mortem lesions in elephants died during drought: A—Lesions of severe enteritis | B, C—Heavy helminth worm load.

the disease depends on several factors, and importantly on the individual's immunity and the inoculums (Sack et al. 2004; Todar 2009). *Vibrio parahaemolyticus* is a well-documented causal agent of acute food-borne gastroenteritis (Sack et al. 2004; Quinn et al. 2011).

The principal habitat of *Salmonella* is the intestinal tract of humans and animals including wild animals. Food-borne Salmonella gastroenteritis is frequently caused by ubiquitous *Salmonella* serovars (Quinn et al. 2011). *Shigella* is typically an inhabitant of the intestinal tract of humans and other primates. It is primarily spread by fecal-contaminated drinking water causing bacillary dysentery (Kapperud et al. 1995; Farque et al. 2002; Tetteh & Beuchat 2003).

E. coli strains have been grouped into several groups of which enterotoxigenic, enterohemorrhagic and enteroinvasive (Cabral 2010; Quinn et al. 2011) serotypes are of significant importance and can be transmitted through contaminated water. Disease caused by *E. coli* follows ingestion of contaminated food or water and is characterized by acute abdominal pain, profuse watery diarrhoea lasting for several days that often leads to dehydration. Outbreaks involving consumption of drinking water contaminated with human sewage or cattle feces have been documented in human dwellings. An increasing number of outbreaks are associated with the consumption of fruits and vegetables (e.g., sprouts, lettuce) contaminated with feces from domestic or wild animals at some stage of growth. EHEC has also been isolated from water bodies (ponds, streams), wells and water troughs, and has been found to survive for months in manure and water-trough sediments (Scheutz & Strockbine 2005).

Possible sources of contamination of the water bodies in forests include animal faeces or introduction of micro-organisms by birds and insects (Paul et 1995; Nevodo & Cloete 1999; Obi et al. 2002; Cabral 2010). Higher bacterial levels could also be due to heightened ecological activities (Strockbine & Maurelli 2005). The habits of wild animals to defecate and urinate in the waterholes as they drink water could be important sources of faecal contamination with coli forms, the parasitic eggs and other opportunistic pathogens isolated during this study. The flow of water into waterholes from adjacent (surrounding) villages with human habitations where open defecation is practiced by their populace could also be another significant source of coli forms and parasitic eggs/cysts noticed in the waterholes. It should, however, be noted that the presence of faecal coli forms in the water sources may not be definitive for a faecal origin of the bacteria (Paul et al. 1995). Investigators have reported the presence of faecal coli forms in tropical environments in the absence of any source of fecal contamination (Hardina & Fujioka 1991; Palupi et al. 1995; Hazen 1998; Fernandez et al. 2000).

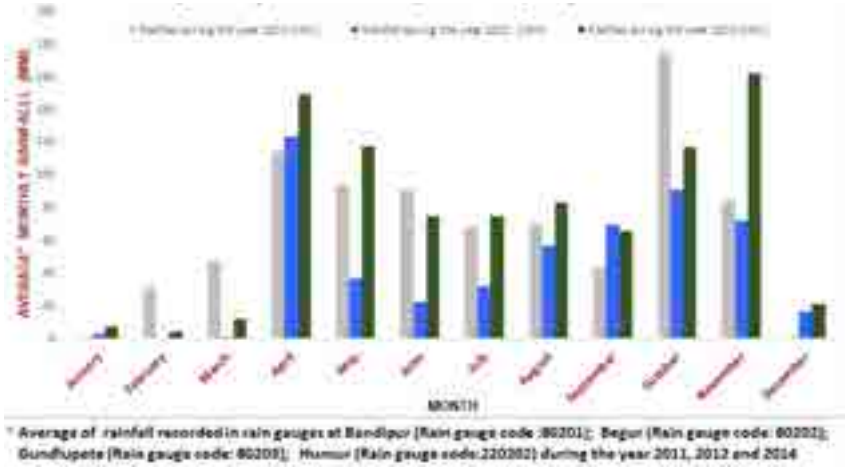
Snails act as intermediate hosts for *Fasciola* and *Amphistome* trematodes (Soulsby 1982), the presence of abundant snails of different species on the shores of waterholes could be a prominent reason for detection of fluke eggs in water samples.

These two forest areas had received lower than normal rains in the year 2011, and the situation worsened in 2012 leading to severe drought conditions (Figure 2). Possibly, as a consequence of all these factors, 39 elephants died during April–May, 2012 in these two forest areas. Most of the elephants had died with symptoms of colic as observed by severe struggling of the animals before death. The blood picture of leucocytosis indicated bacterial infection, and increased liver enzymes indicated toxic changes. The post-mortem examination revealed lesions of severe inflammation of intestines and septicaemic changes in an elephant that was examined on the banks of Kabini River during the peak of drought in 2012. Further, the bone marrow samples of the elephants that died during droughts yielded growth of *E. coli* and other coli forms and these opportunistic pathogens have been reported to be aetiologies for severe enteritis and septicaemia in immunosuppressed animals (Quinn et al. 2011). The post-mortem also revealed the presence of heavy loads of parasites in the gastro-intestinal tract, which correlates with the current findings of parasitic eggs in water samples.

Normal rainfall of 2014–15

After good pre-monsoon and monsoon rains, all the waterholes were full to their brim by November. The quantity of water gradually decreased from the month of December, reached the lowest in the summer months of March, April, and May. Even when the rainfall is normal, the water in waterholes continue to be the source of opportunistic pathogens and various species of gastrointestinal parasites as evidenced by growth of coliforms and presence of eggs /ova in water.

During 2014–15, the forests received normal rainfall but the bacterial counts were very high during the summer season (March–April, 2015) which was almost similar to the counts recorded during the drought conditions of 2012. However, the death of less than ten elephants was noted in April–May, 2015. The normal monsoon rains of 2014–15 had possibly resulted in



* Average of rainfall recorded in rain gauges at Bandipur (Rain gauge code :80201); Begur (Rain gauge code: 80202); Gundlupete (Rain gauge code: 80203); Hunsur (Rain gauge code:220302) during the year 2011, 2012 and 2014

Figure 2. Average monthly rainfall in the study area during the years 2011, 2012, and 2014.

Image 3. Measures suggested from the findings of this study and their implementation: A—Construction of smaller artificial water tanks and fill them with water tankers | B—Removing obstructing bigger shrubs surrounding major waterholes before every rainy season so that more and more water gets accumulated in waterholes | C—Installation of solar powered pumping bore wells near major waterholes at feasible locations in the forest.

sufficient availability of fodder for animals keeping them in good body condition and relatively better immunity, which possibly gave them the ability to resist infections caused by opportunistic pathogens present in the water they consume.

The study records that rainfall directly controls the availability of feed and water in forests; and availability of feed and water determines the general body condition of wild animals and their ability to resist infections. During droughts there is an acute shortage of feed and water leading to poor body condition with total immunosuppression; possibly making them susceptible for opportunistic pathogens present in water they consume leading to colic, diarrhoea, dehydration, septicemia, and death.

El Nino events are a prominent feature of climate variability with global climatic impacts, severely disrupted global weather patterns, affecting ecosystems agriculture, tropical cyclones, drought, bushfires, floods, and other extreme weather events worldwide. Here we present evidence of such changing climate on the survivability of wildlife. Increasing temperatures, combined with changes in rainfall and humidity, may have significant impacts on wildlife, domestic animals, and human health. When combined with expanding human population, these changes could increase demand on limited water resources, leading to more habitat destruction, and provide yet more opportunities for infectious diseases (Hofmeister et al. 2012) and the elimination of wildlife species (McLean 2016). Droughts of the future are likely to be more frequent, severe, and longer lasting than they have been in recent decades (Toby 2020). Through this present study we have attempted to give a glimpse of the future of wildlife in events such as drastic climatic changes.

MANAGEMENT IMPLICATIONS

Measures suggested from the findings of this study and impact of their implementation

1. The study found that the growth of heavy shrubs in and around major waterholes had prevented the flow of water into waterholes. It was suggested to take measures to clear theses shrubs before every rainy season so that more water accumulates in waterholes.

2. In absence of water in major waterholes during drought conditions, it was suggested to take measures to provide water in a few major waterholes through water tankers.

3. To help smaller animals in the forest it was suggested to construct small artificial water tanks and fill them with water.

4. It was suggested to install solar powered pumping bore wells at feasible locations in the forest.

All the suggested measures have been implemented (Image 3) at most major waterholes by the Government of Karnataka, possibly helping many wildlife species during summer and drought situations at Bandipur and Nagarahole Tiger Reserves in recent years.

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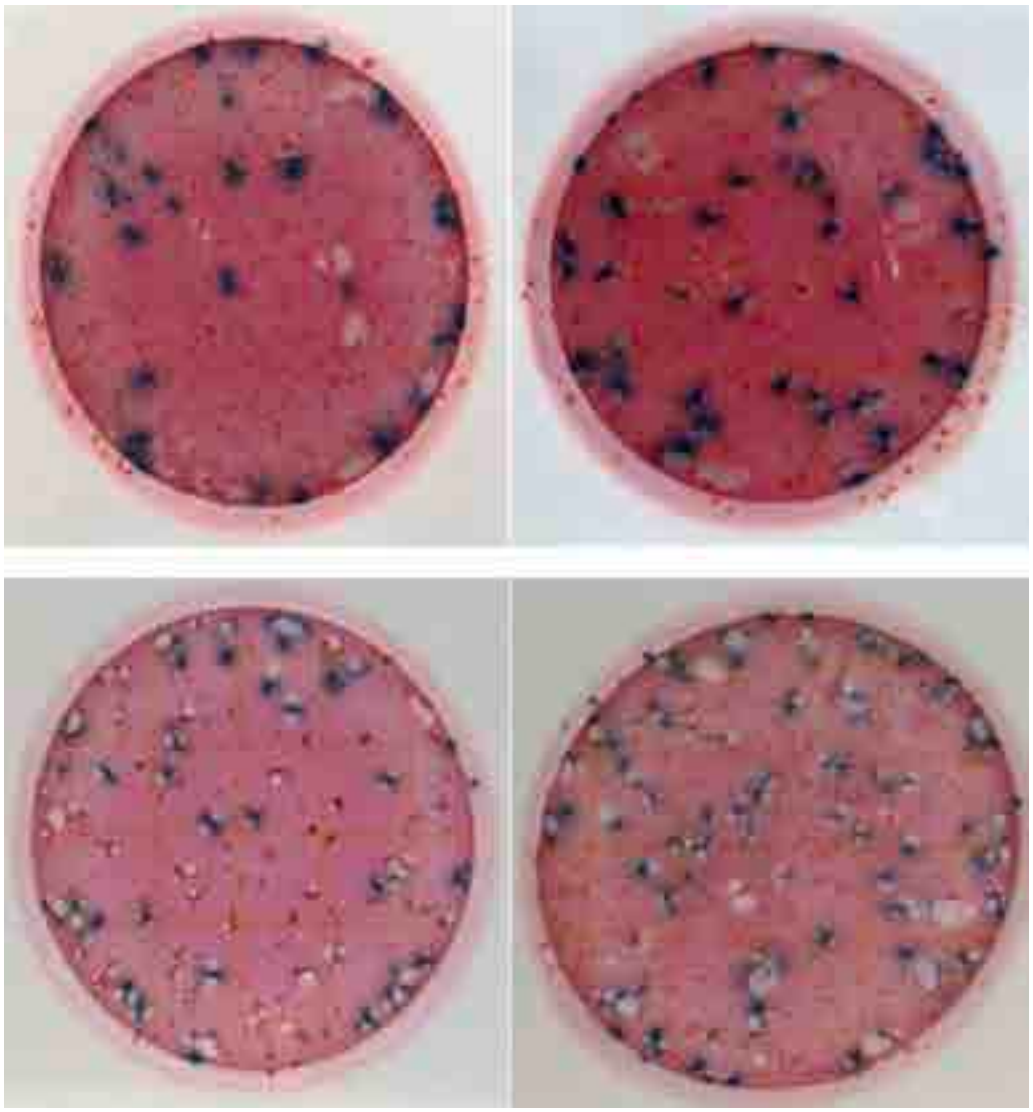
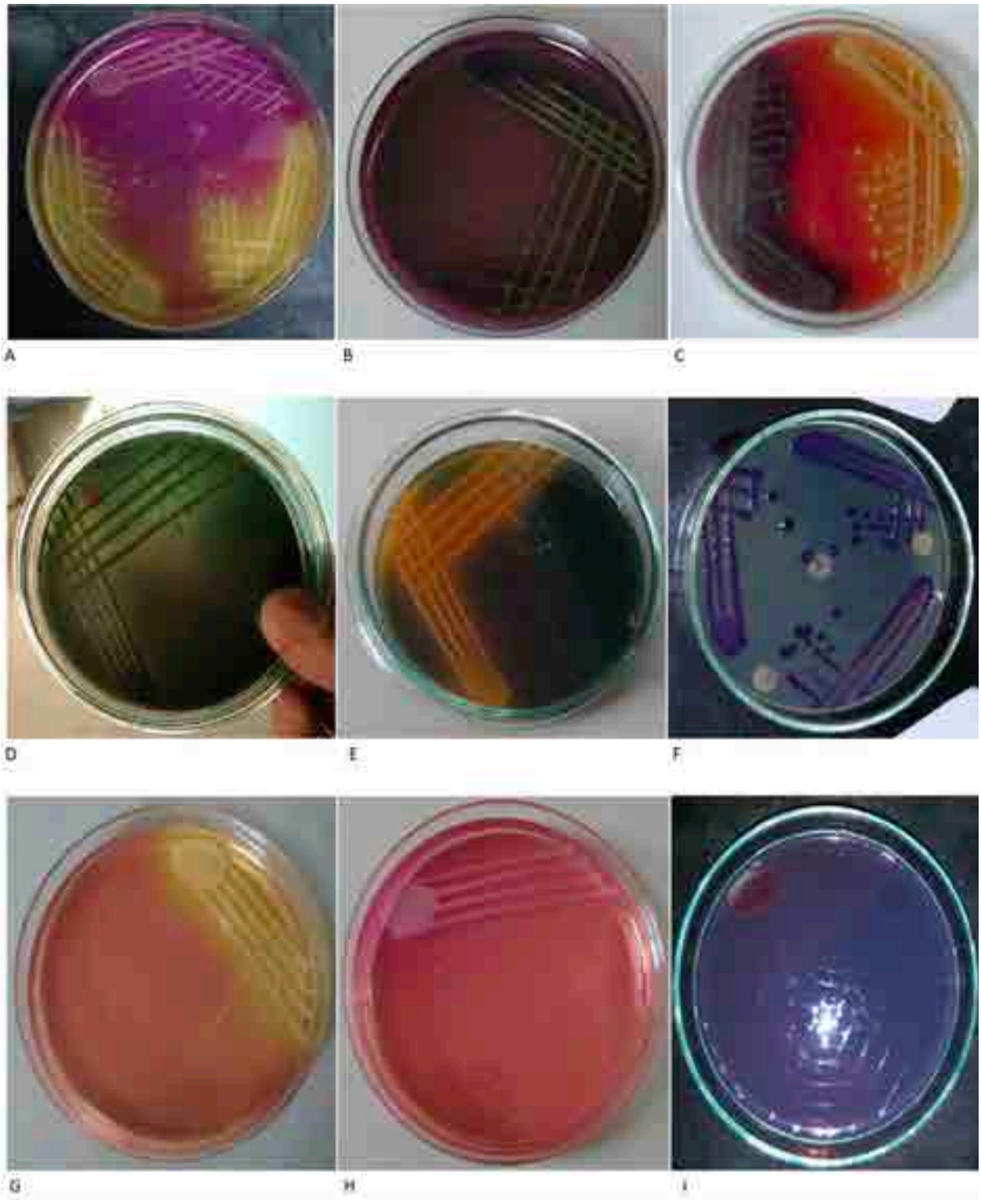
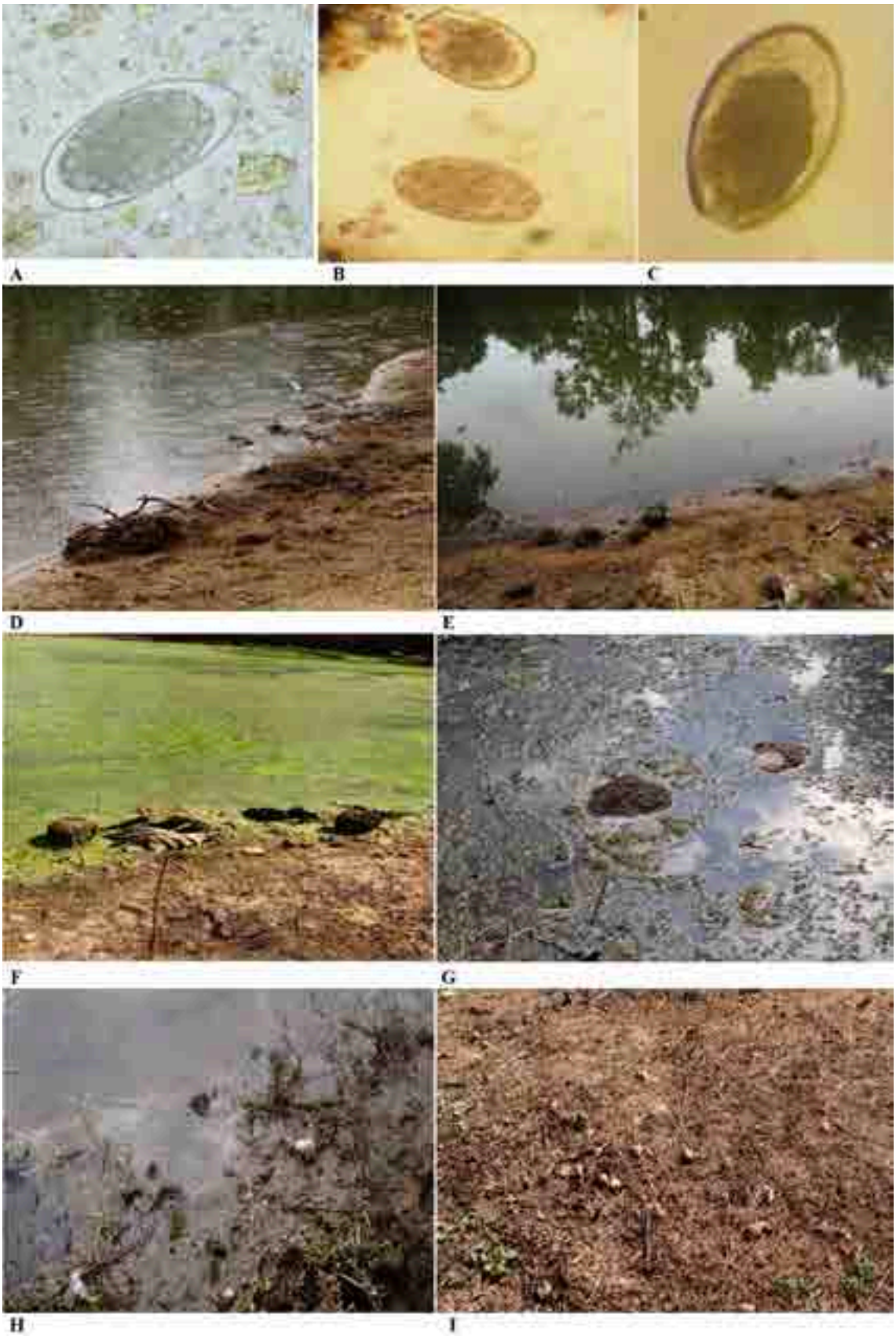
Supplementary Image 1. The water in waterholes during drought appeared greenish with heaps of elephant dung at periphery.



Supplementary Image 2. Colonies of coli forms and *E. coli* on the petri films.



Supplementary Image 3. Growth of opportunistic pathogens from water samples: A—colonies of Aeromonas (yellow) and Pseudomonas (white) on Aeromonas-Pseudomonas agar | B—*E. coli* on EMB agar | C—Salmonella *E. coli* on XLD agar | D—Vibrio parahaemolyticus on TCBS agar | E—Vibrio cholera on TCBS agar | F—Klebsiella species on Klebsiella agar | G—Staphylococcus aureus on MSA agar | H—Staphylococcus intermedius on MSA agar | I—Streptococcus species on KF Streptococcal agar.



Supplementary Image 4. Eggs of gastro intestinal parasites in water samples: A—Strongyle egg | B—Amphistome egg | C:—Fasciola egg | D, E, F, G—Dung in the water in the waterholes | E, F—Different species of snails observed in and around the waterholes.

Supplementary Image 5. The forest witnessed massive forest fires during the drought.

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Author contributions: BMC conceptualised the study, obtained the funding, designed the experiments, collected samples, conducted experiments, analysed and interpreted the data; VP collected the samples and conducted bacteriological analysis on the water samples; DR conducted bacteriological analysis on the water samples and interpreted the data; GSM conducted parasitological examination on the water samples and interpreted the data; KSU and DNN treated ailing animals and collected samples for this study; SMB contributed in analysis and data interpretations.

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 20964–20969

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7758.14.5.20964-20969

#7758 | Received 22 November 2021 | Final received 21 February 2022 | Finally accepted 09 May 2022

OPEN ACCESS

COMMUNICATION

Cases of fatal electrocution of the endangered Javan Gibbons (Mammalia: Primates: Hylobatidae) by power lines

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Abstract: Human-made structures are often deadly to wildlife. Power lines from electric poles can cause serious injury and even death of wildlife via electrocution, especially of arboreal primate species that can easily access power lines. Here, we reported three cases of wild 'Endangered' Javan Gibbons *Hylobates moloch* electrocuted by power lines along a road between a tea plantation and a village adjacent to Gunung Halimun-Salak National Park area, West Java, Indonesia. In all cases, the adult male Javan Gibbons jumped and grabbed a power line hanging at the edge of the forests and immediately died. Our case reports highlight that power lines can have a critically adverse effect on the survival of wild animals, particularly on highly arboreal species such as Javan Gibbons. We argue the need for mitigation plans (e.g., cable insulation) for power lines in areas at risk. Such areas should be determined based on wildlife habitat monitoring and the study of ranging behaviors, focusing on areas with high risks of electrocution.

Keywords: Anthropogenic mortality, arboreal primates, human-made structures, *Hylobates moloch,* power lines.

Indonesian abstract: Infrastruktur buat an manusia seringkali mematikan bagi satwa liar. Sengatan listrik dari tiang listrik dapat menyebabkan cedera serius dan bahkan kematian bagi satwa liar, terutama spesies primata arboreal yang dapat dengan mudah meraih jaringan listrik. Kami melaporkan tiga kasus owa jawa (*Hylobates moloch*) liar yang tersengat listrik oleh jaringan listrik yang melewati daerah perbatasan antara perkebunan teh dan Taman Nasional Gunung Halimun-Salak, Jawa Barat, Indonesia. Pada ketiga kasus tersebut, owa jawa jantan dewasa melompat dan menangkap jaringan listrik yang tergantung di tepi hutan, dan langsung mati. Laporan kasus kami ini membuktikan bahwa aliran listrik dapat memiliki dampak negatif, tidak hanya di daerah perkotaan tetapi juga di alam liar, terutama pada spesies arboreal seperti owa jawa. Oleh karena itu, diperlukan rencana mitigasi (misalnya menambahkan isolator pada kabel) untuk jaringan listrik pada areal yang berisiko mencelakai satwa liar. Areal tersebut perlu dikenali berdasarkan pemantauan habitat satwa liar dan pengamatan daerah jelajah, dengan fokus pada areal dengan risiko tinggi untuk tersengat listrik.

Editor: H.N. Kumara, Sálim Ali Centre for Ornithology and Natural History, Coimbatore, India. **Date of publication:** 26 May 2022 (online & print)

Citation: Yi, Y., S. Ham, R. Oktaviani, M.C. Dewi, M. Nur, A. Mardiastuti & J.C. Choe (2022). Cases of fatal electrocution of the endangered Javan Gibbons (Mammalia: Primates: Hylobatidae) by power lines. *Journal of Threatened Taxa* 14(5): 20964–20969. https://doi.org/10.11609/jott.7758.14.5.20964-20969

Funding: The present research has been supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2021R1A6A3A03039709) to YY and Amore Pacific Academic and Cultural Foundation (AACF). YY is supported by the Foreign Youth Talent Program (QN2021014010L) from the Ministry of Science and Technology and Research Grants (2021K310C) from Department of Human Resources and Social Security of Jiangsu Province of the People's Republic of China.

Competing interests: The authors declare no competing interests.

Author details & **Author contributions:** See end of this article.

Acknowledgements: This project was conducted in collaboration with the IPB University and Ewha Womans University. We thank the Indonesian Ministry of Research and Technology (RISTEK), the Indonesian Ministry of Environment and Forestry (KLHK), and the Gunung Halimun-Salak National Park (GHSNP) for the research permissions. We thank Amael Borzee for helpful comments on the manuscript.

INTRODUCTION

Human-made structures often disconnect and fragment the habitat of wildlife animals, negatively impacting their survival and potentially leading species to local extirpation (Rudolphi et al. 2014; Warner et al. 2021). Electric poles, a power line element, are one of the human-made facilities that can be deadly to various animal species. A power line can cause immediate death by electrocution or serious injuries when touched simultaneously with another non-electric current object or a wire with a different voltage (Kumar & Kumar 2015; Schulze et al. 2016). Although bird electrocutions are well documented and studied (Loss et al. 2014; Pérez-García et al. 2017), arboreal mammal species such as primates are also especially vulnerable given their high accessibility to the power lines (Moore et al. 2014; Kumar & Kumar 2015; Katsis et al. 2018).

Considering electrocutions of primates, several long-term studies reported that 32 to 40% of them eventually died after an electric shock from a power line (*Macaca mulatta*: Kumar & Kumar 2015; *Alouatta guariba clamitans*: Lokschin et al. 2007; *Semnopithecus vetulus vetulus*: Roscoe et al. 2013). The most extended study from 1998 to 2019 in Kenya found 73% of the death is due to electrocution in four monkey species (Cunneyworth & Slade 2021). In addition, electrocution was the reason for 36% of deaths observed in Hanuman langurs in Jodhpur, India (*Semnopothecus entellus entellus*; Ram et al. 2015) and 16% of four primate species in Kenya (*Colobus angolensis palliates*, *Cercopithecus mitis albogularis*, *Chlorocebus pygerythrus hilgerti*, & *Papio cynocephalus cynocephalus*: Cunneyworth et al. 2021). These results suggest that electrocution contributes highly to mortality in primates, especially in urban areas where humans and wildlife co-exist.

Java is the most heavily populated island of Indonesia with more than 140 million people while also hosting various wildlife habitats. Due to the continued deforestation in Java, less than 10% of forests remain, and the remnant is also heavily fragmented (Nijman 2004, 2013). Gunung Halimun-Salak National Park (GHSNP) has the largest remaining forest blocks in Java and it is the host to high biodiversity (MacKinnon & MacKinnon 1986). These areas can provide ecological services not only to wildlife but also to humans. However, throughout the year, with the increasing growth rate of the human population, infrastructure like roads and power lines are traversing areas close to the conservation area and could cause negative effect on wildlife.

The Javan Gibbon *Hylobates moloch* is an 'Endangered' primate and the main threats are deforestation and hunting for pet trade (Nijman 2020). While around 4,000 Javan Gibbons are left in the wild, GHSNP holds between 900 and 1,221, the largest remaining wild population (Nijman 2004; Supriatna 2006). Similar to other gibbon species, Javan Gibbons are highly arboreal, spending most of their time in the canopies (Cheyne 2011). As a result of their movement through brachiation, gibbons are at high risk of electrocution when electric poles are installed in their habitats. Several electrocution of gibbons have been reported in the media (The Wildlife Trade Monitoring Group 2020; The Straits Times 2019; Zon Pann Pwint 2019), but rarely in the scientific literature (Talukdar et al. 2018). In this study, we report and describe three cases of electrocuted wild Javan Gibbons in 2011, 2015, and 2021 at the edge of the forest close to GHSNP.

METHODS

Study site

The Javan Gibbon is one of the three key species of GHSNP, along with Javan Leopards *Panthera pardus melas* and Javan Hawks *Nisaetus bartelsi*. In addition to Javan Gibbons, the endemic Javan Lutung *Trachypithecus mauritius* and the endangered Javan Surili *Presbytis comata* are also found in the National Park. While GHSNP supports one of the largest Javan Gibbon populations, their habitats are still fragmented due to deforestation and human facilities (Smith et al. 2017). The field site in Citalahab is also located on the edge of primary forests and is surrounded by tea plantations and rice paddies (Yi et al. 2020).

Study subject

The Javan Gibbon Research & Conservation Project (JGRCP) began in 2007 in Citalahab area in GHSNP (-6.739167 S, 106.530000 E), with a focus on behavior and ecology of wild Javan Gibbons (Kim et al. 2012; Ham et al. 2017; Oktaviani et al. 2018; Yi et al. 2020; Jang et al. 2021). The home ranges of the gibbon groups that are regularly followed by the research team are located along the tea plantation, separated by a one-lane dirt road. We expect a similar habitat shared with human facilities for other gibbon groups in GHSNP. Given the long-term research going on in the area, local people are aware of Javan Gibbons and therefore direct threats towards the gibbons are relatively low compared to other habitats.

Data collection

The observations of electrocuted Javan Gibbons were

Image 1. The location of three electrocuted gibbons and the Javan Gibbon Research and Conservation Project field station on the map.

opportunistic, temporarily matching within the frame of the long-term research project. We collected the date, location and context of the electrocution events.

RESULTS

We observed three cases of electrocution of Javan Gibbons on power lines over the 15 years of long-term research along the road and the tea plantation adjacent to GHSNP (Image 1).

Case 1

22 February 2011: We found a dead gibbon holding a power line at Malani area (-6.710944, 106.512500). The individual had died a few days prior (pers. com. Cikaniki Research Station) and it was collected before 25 February 2011. The GHSNP staff witnessed the electrocution at the edge between forest and tea plantation. The gibbon jumped and held on to the power line unexpectedly because of a falling tree behind and was electrocuted. The dead gibbon was stuck to the power line until the GHSNP staff requested the national electricity company to turn the power off a few days later (Image 2). There was not much flesh left on the body and its head was missing after falling to the ground probably due to the electrocution and decomposition. Because of the bad condition, the GHSNP staff buried the gibbon body directly and we could not obtain further information. We suspect the dead gibbon was an adult male from the body size.

Case 2

23 March 2015: A local resident found an electrocuted gibbon close to Malani area (-6.727778, 106.493889), and suspected that human disturbance made the gibbon to jump onto the power line. The electrocuted gibbon's face and left arm which held the power line were damaged. No other gibbons were observed around at the time of electrocution. For necropsy, the body was delivered to LIPI (Indonesian Institute of Sciences) in Bogor. The individual was an adult male, with a body length of 530 mm and a weight of 5.5 kg (Image 3). Later, the specimen was deposited at the GHSNP headquarter in Kabandungan area as education material.

Case 3

13 December 2021: A local resident found an electrocuted gibbon close to Ciwalen (-6.705809, 106.521170). The right side, especially the arm and flank, of the dead gibbon were seriously damaged probably

Image 2. A Javan Gibbon *Hylobates moloch* electrocuted on a power line around Malani area (-6.710944, 106.512500) close to Gunung Halimun Salak National Park (22 February 2011). © Soojung Ham.

when touching the power lines. The residents buried the dead body close to the road. From the pictures taken by the resident, we assume the dead gibbon was an adult male.

Power line installed in the region

The voltage running through the power line is above 20 kv for main, 20 kv for medium, 220 v for low voltage. The number of wires is: three phases and three wires for medium, three phases and four wires for low voltage. All cables are horizontally arranged. Therefore, the gibbons likely held medium voltage (20 kv) cables. There is an insulator in the medium cable, however, the insulation strength is only up to 6 kv, and holding it for more than a few seconds will break the insulator and lead to death.

DISCUSSION

In this study, we described three cases of electrocution of Endangered Javan Gibbons on electric power lines along with their natural habitat. These observations highlight the effect of power lines on highly arboreal species such as Javan Gibbons. Given that these data are obtained opportunistically, there may be more actual number of electrocution cases in more remote and uninhabited areas. At the study site, some of the power lines pass along the road and inside forest patches. As a result, its presence can be a great threat to the wildlife in the forest, mainly for birds and arboreal mammals. Thus, we suspect similar but unreported cases of Javan Gibbon, or other wildlife injury or death, caused by electrocution in the study area.

While arboreal species have relatively low chances to be hit by vehicles (roadkill), power lines are easily reachable for them. Among primates in general, mortality resulting from electrocution seems to be higher in arboreal than in terrestrial species (Al-Razi et al. 2019; Cunneyworth & Slade 2021). While previous reports on primate electrocution are mostly from urban areas (Printes 1999; Goulart et al. 2010; Corrêa et al. 2018; Pereira et al. 2020), our cases indicate that power lines can cause fatality in forested areas as well. Moreover, along with the cases reported in this study, three more electrocuted gibbons were found outside the protected areas in the last four years: two cases from Gunung Gelap Garut, Cisewu between January and February 2022 (-7.370986, 107.497615; pers. com. Sigit

Image 3. A male adult Javan Gibbon *Hylobates moloch* electrocuted on a power line around Malani area (-6.727778, 106.493889) close to Gunung Halimun Salak National Park (23 March 2015). Pictures of the whole body (left), the face (top right), and the injury on the left arm due to the electrocution (bottom right). The body was collected by Muhammad Nur. © Mia Clarissa Dewi.

Ibrahim, The Aspinall Foundation, Indonesia) and one case in 2018 from Lengkong, South Sukabumi (-7.127500, 106.687222). This suggests that the endangered Javan Gibbons suffers from power line mediated electrocution both in urban and forested areas.

People living in the study site were previously isolated because there was no electricity and mobile signal before electric poles were set up in 2011–2012. Despite the benefits of offering electricity to the local community, the cases reported in the study highlight that the increase in human facilities, especially in the areas surrounding protected areas, has negative effects on wildlife. A recent study of population viability analysis revealed that Javan Gibbons might go extinct within the next 100 years if existing threats such as hunting and deforestation remain the same (Smith et al. 2017). Electrocution caused by power lines may hasten this trend. Therefore, mapping the priority areas to apply mitigation measures will be an urgent step to decrease the threats. Furthermore, a typical conservation approach to preserve Javan Gibbon populations, the distribution of power lines should be considered a relevant determinant of possible mortality risk.

With the cases reported in our study, we conclude that the installation of human infrastructures such as electric poles needs to take into account conservation management practices. First, power line installations should practically avoid the habitats of Javan Gibbons according to the mitigation hierarchy, and should follow the application of wildlife standards (Kiesecker et al. 2010). Then, mitigation efforts such as insulation or burial of electric cables, artificial canopy bridges will help lower the mortality of the species (Janss & Ferrer 1999; Katsis et al. 2018). In Sri Lanka, the electrocution cases of the Toque Macaques *Macaca sinica* significantly dropped from 18 to zero after the installation of shields on electric poles (Dittus 2020). Given the cost of mitigation efforts, it is critically important to understand habitat use and the ranging behaviors of species as well as to investigate and prioritize mitigation in high-risk areas, for instance where power lines pass along the forests. For example, mitigating actions are spatially prioritized based on the systematical analyses of spatial occurrence of electrocution in five primate species between 1998 and 2016 (Katsis et al. 2015). Wildlife Javan Gibbon electrocution mortality in areas close to GHSNP must be promptly and appropriately addressed through monitoring and conservation management practices.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 20970–20987

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7762.14.5.20970-20987

#7762 | Received 28 November 2021 | Final received 09 April 2022 | Finally accepted 01 May 2022

OPEN ACCESS

COMMUNICATION

Nesting habits of the Baya Weaver *Ploceus philippinus* (Linnaeus, 1766) in the agricultural landscape of Tindivanam, Tamil Nadu, India

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Abstract: This paper pertains to the nesting habits of *Ploceus philippinus* (Linnaeus, 1766) with specific reference to the agricultural landscape of Tindivanam Taluk, Villupuram District, Tamil Nadu during the breeding period between April and October 2021. A total of 11,386 nests (wad stage-840, ring stage-478, helmet stage-3,980, egg-chamber closed stage-2,865, completed nests-2,028, abnormal nests-938, and damaged nests-257) and 12,600 birds were observed on 832 nest-supporting plants. Nest-supporting plants belonged to 27 species, 26 genera, and 17 families. The three principal nest-supporting palm species—*Borassus flabellifer*, *Cocos nucifera*, and *Phoenix sylvestris*—represented 85.21% of the total nest-supporting plants. The number of nests (including all the stages) per colony varied from 1 to 109 and 70.16% nests were oriented towards the east. Abnormal nests constituted 8.24% of the recorded nests with 17 variations and 90.12% helmet stage nests contained plastering of clay on the inner walls. Nest predation by House Crow, Large-billed Crow, Asian Koel, Black Drongo, & Rufous Treepie and killing of adult Baya Weaver by Shikra were recorded.

Keywords: Abnormal nests, associate birds, clay deposits, foraging behaviours, nest colonies, nest predation, Villupurum District.

Editor: H. Byju, Coimbatore, Tamil Nadu, India.

Date of publication: 26 May 2022 (online & print)

Citation: Pandian, M. (2022). Nesting habits of the Baya Weaver *Ploceus philippinus* (Linnaeus, 1766) in the agricultural landscape of Tindivanam, Tamil Nadu, India. *Journal of Threatened Taxa* 14(5): 20970–20987. https://doi.org/10.11609/jott.7762.14.5.20970-20987

Funding: None.

Competing interests: The author declares no competing interests.

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Acknowledgements: I thank D. Balaji, K. Sriram (Villupuram) for help in data collection and photography, S. Suresh, (Madras University), Minnal A. Giridharan and Narasingapuram T. Selvapandian for help with data analysis and Cuddalore N.Eswari for preparation of study area map.

INTRODUCTION

Globally, India is among the 10 top countries with highest bird species richness harbouring about 1,332 species (Lepage 2016; Praveen et al. 2020). The family *Ploceidae* includes 15 genera and 118 species (Oiveros et al. 2019). The Afro-Asian region has 64 species of weavers, the genus *Ploceus* spp. (Dickinson & Christids 2014), of which four species occur in India (Baya Weaver *Ploceus philippinus*, Black-throated Weaver *Ploceus benghalensis*, Streaked Weaver *Ploceus manyar*, and Finn's Weaver *Ploceus megarhynchus)* (Ali & Ripley 1987). The IUCN Red List of Threatened Species has classified Baya Weaver as 'Least Concern' species (BirdLife International 2016). Baya Weaver is a social, gregarious, and polygamous bird, occurring throughout the Indian subcontinent (Ali & Ambedkar 1956) and also in Java and Sumatra (Wood 1926). In India, the Baya Weaver breeds between June and November (Rasmussen & Anderton 2005). Baya Weavers prefer *Cocos nucifera* along the western coast of the Indian peninsula, *Borassus flabellifer* along the eastern coast, and *Vachellia nilotica* in the arid north-west (Sharma 1989). Males usually build partial nests and complete them only after courting females (Ali et al. 1956). Several authors have stated that nests almost invariably hang exposed towards an easterly direction so as to be the least affected by battering winds of the south-west monsoon (Ali 1931; Ambedkar 1964; Davis 1971; Quader 2003; Borges et al. 2012; Pandian & Ahimas 2018; Pandian 2021a). Nests are built as colonies and the sizes of nest colonies have been found to vary (Sharma 1989; Borkar & Komarpant 2003; Pandian 2018, 2021a).

The behaviour of Baya Weaver constructing different types of abnormal nests were reported by Ali et al. (1956), Ambedkar (1958, 1980), Sharma (1985, 1988, 1995), Borkar & Komarpant (2003), and Pandian (2018). Plastering of mud/clay on the inner walls of helmet stage nests is prevalent among Baya Weaver *P.* philippinus (Crook 1962). Baya Weavers strictly followed mixed communal roosting and foraging (Zahavi 1971; Gadgil 1972; Ward & Zahavi 1973; Gadgil & Ali 1975; Pandian 2020). The occurrence of nest predation by avian predators and fall of nests due to abiotic factors like monsoon rains and battering winds, also by rival male birds and various anthropogenic factors were reported by Ali (1931) and Pandian (2021a,b).

In this paper, I document the quantitative analysis of nests, birds, nest-supporting plants, roosting and foraging behaviours of Baya Weaver with specific reference to the agricultural landscape of Tindivanam Taluk, Villupuram District, Tamil Nadu. The following objectives were kept in mind in the study: (i) Nest tree use pattern and its microhabitat (power cables, roads, and human dwellings, water bodies), (ii) Features of nest building including sources of nesting materials, stages of nest development, orientation, plastering of clay on inner walls, and abnormalities, (iii) Roosting and foraging behaviours including preference of crops, and (iv) Interactions with other bird species and threats faced.

MATERIALS AND METHODS

Study area

The present study was carried out in 115 villages (Appendix-I) in Tindivanam Taluk (12.236N—79.649E), Villupuram District, spread over 80 km^2. The human population of the district is c. 500,000 (2011 Census). Agriculture is the primary occupation of the people here. The major crops of the area are Paddy *Oryza sativa*, Jowar *Sorghum bicolor*, Pearl Millet *Pennisetum glaucum*, Finger Millet *Eleusine coracana*, Foxtail Millet *Setaria italica*, Sugarcane *Saccharum officinarum* (Poaceae), Green Gram *Vigna radiata*, Groundnut *Arachis hypogaea* (Fabaceae), and Cassava *Manihot esculenta* (Euphorbiaceae). Small-scale cultivation of ornamental flower, vegetable, fruit, and monoculture of *Cauariana equisetifolia* (Equisetaceae) also occurs. The maximum and minimum temperatures of the district are 36°C and 20°C, respectively. The average annual rainfall of the district is 1,060 mm (www.viluppuram.nic.in) (Figure 1).

Methods

With help of two field assistants, I identified 115 villages in Tindivanam taluk having a history of habitations of Baya Weavers. These villages were surveyed daily between 0545–1200 h and 1500–1830 h when the birds were active between the first week of April and the second week of October 2021. The heights of the nest-supporting trees were measured using Silva Clinometer while GBH (Girth at breast height) and distances between the nesting trees and power cables, road, human dwellings, various type of crop fields were measured using a 100 m measuring tape. The canopy width was obtained by cross method (Blozan 2006) by measuring the edge of the canopy shadow on the ground. The distances between nest-supporting plants and the above-listed factors were grouped under 01–50 m, 51–100 m, 101–150 m, 151–200 m, and >200 m or

Figure 1. Study area map: a—India map showing Tamil Nadu and marked Tindivanam taluk as a white dot | b—Tindivanam taluk map showing villages (yellow color) containing nesting habitats of Baya Weaver.

01–100 m, 101–200 m, 201–300 m. The locations of the inventoried 832 nest-supporting plants were determined using a standard GPS (Garmin Etrex 20x). The total number of nests observed on one nest-supporting plant was considered one nest colony. Using Super Zenith 20 x 50 field binoculars, the number of nests in the colonies, their developmental stages, abnormalities, damaged nests, clay deposits on inner walls of helmet stage nests, and number of birds were enumerated. The orientations of the nests were determined using a 'Compass App' in a smart phone iPhone (Model A1530). Every nest-supporting plant was observed uninterruptedly for 60 min and the maximum number of birds perched at one time on the nest-supporting plants during the observation period was determined as the number of birds per plant. The fallen nests spread over on the ground under the nest-supporting plants were enumerated. Roosting and foraging behaviours of flocks, preferred plants for foraging were observed for 20 days (10–29 July 2021) from 0545 to 1830 h, nest predation by avian predators and interactions with other birds were observed using binoculars. Utmost care was taken not to disturb the nests or birds, maintaining a minimum distance of c. 30 m during observations. No live nests, eggs, chicks, or adult birds were disturbed. Nikon P1000 digital camera was used for photography and videography.

Data analysis

One-way Analysis of Variance (ANOVA) was applied to test the differences among the total number of nests and total number of birds observed on the nest-supporting plant species such as *Borassus flabellifer*, *Phoenix sylvestris*, *Cocos nucifera*, *Prosopis juliflora*, *Morinda tinctoria*, *Casuarina equisetifolia*, *Phyllanthus reticulatus,* and others by using Statistical Package for Social Sciences. Those nest-supporting plant species (n = 19) which represented more than 10 individuals per species were taken as separate variables and the plant species which represented less than 10 individuals were grouped as 'others' for analysis. Test of significance was assessed at p = 0.05. The correlation between variables such as GBH (cm), heights (m) and canopy sizes (m) of nest-supporting plants and the number of nests enumerated on them was calculated using Pearson's Correlation Coefficient test. Collected data were tabulated, analysed and shown as graphical representations.

RESULTS

Baya Weavers and their plant preference to build nests

A total of 832 nest-supporting plants belonging to 27 species, 26 genera, and 17 families bearing nests of Baya Weaver were observed in 115 villages in Tindivanam Taluk. Among the 17 families, three families such as Arecaceae, Musaceae, and Poaceae are monocotyledons. Family Fabaceae represented a maximum of seven species, followed by Arecaceae representing three species, Moraceae and Phyllanthaceae are representing two species each and other 13 families representing one species each. A total of 12,600 adult birds were counted on those 832 nest-supporting plants. Maximum 73.69% birds (n = 9,285) were observed on *Borassus flabellifer* trees, followed by 11.38% birds (n = 1,434) on *Cocos nucifera*, 8.94% birds (n = 1,127) on *Phoenix sylvestris*, and the remaining 5.99% birds (n = 754) were enumerated on 24 other nest-supporting plant species (Table 1).

Table 1. Details of nest-supporting plants, number of birds, nests, various developmental stages of nests and nest orientation (as on 2nd week of October 2021) in the study area.

	Name of the plant	Total no. of nest-supporting plants	Life-form	Developmental stages of nests					Abnormal nests	Damaged nests	Total no. of nests	Orientation of nests				Total no. of birds
				Wad	Ring	Helmet	Egg- chamber closed stage	Completed nests				East	West	North	South	
1	*Borassus flabellifer*	490	Tree	519	309	2683	2246	1518	807	222	8304	5586	963	1298	457	9285
2	*Phoenix sylvestris* (Arecaceae)	118	Tree	163	63	509	175	112	49	12	1083	899	72	100	12	1127
3	*Cocos nucifera* (Arecaceae)	101	Tree	56	44	499	353	262	51	12	1277	919	124	171	63	1434
4	*Prosopis juliflora* (Fabaceae)	28	Shrub	32	14	85	16	31	6	2	186	148	29	3	6	194
5	*Morinda tinctoria* (Rubiaceae)	16	Tree	4	4	24	7	18	7	0	64	60	0	0	4	66
6	*Casuarina equisetifolia* (Casuarinaceae)	10	Tree	24	16	62	0	0	0	0	102	100	0	2	0	104
7	*Phyllanthus reticulatus* (Phyllanthaceae)	10	Shrub	7	4	8	4	6	0	2	31	27	0	4	0	32
8	*Vachellia nilotica* (Fabaceae)	10	Tree	2	4	22	11	0	2	0	41	19	1	21	0	53
9	*Azadirachta indica* (Meliaceae)	9	Tree	5	1	10	6	14	3	0	39	38	0	0	1	50
10	*Flueggea leucopyrus* (Phyllanthaceae)	8	Shrub	6	2	13	11	21	1	4	58	28	0	16	14	59
11	*Ficus benghalensis* (Fabaceae)	6	Tree	8	11	37	18	25	11	3	113	79	13	15	6	87
12	*Lantana camara* (Verbanaceae)	4	Shrub	1	2	3	3	2	1	0	12	12	0	0	0	14
13	*Pithecellobium dulce* (Fabaceae)	3	Tree	1	0	9	0	0	0	0	10	9	0	1	0	12
14	*Senna siamea* (Fabaceae)	3	Tree	1	0	3	3	1	0	0	8	8	0	0	0	8
15	*Chromolaena odorata* (Asteraceae)	2	Shrub	1	1	1	0	0	0	0	3	3	0	0	0	3
16	*Ficus religiosa* (Moraceae)	2	Tree	2	0	0	4	2	0	0	8	8	0	0	0	14
17	*Leucaena leucocephela* (Fabaceae)	2	Tree	0	1	7	6	7	0	0	21	21	0	0	0	30
18	*Albizia lebbeck* (Fabaceae)	1	Tree	1	0	0	2	9	0	0	12	12	0	0	0	12
19	*Cortaderia selloana* (Poaceae)	1	Herb	1	0	0	0	0	0	0	1	1	0	0	0	1
20	*Passiflora foetida* (Passifloraceae)	1	Climber	0	1	0	0	0	0	0	1	1	0	0	0	1
21	*Tamarindus indica* (Tamarindus indicus)	1	Tree	1	0	2	0	0	0	0	3	3	0	0	0	3
22	*Eheretia pubescens* (Boraginaceae)	1	Tree	1	0	0	0	0	0	0	1	1	0	0	0	1
23	*Ziziphus oenopolia* (Ramnaceae)	1	Tree	1	0	0	0	0	0	0	1	1	0	0	0	1
24	*Cocculus carolinus* (Menispermaceae)	1	Climber	1	0	0	0	0	0	0	1	1	0	0	0	1
25	*Solanum trilobatum* (Solanaceae)	1	Climber	1	0	0	0	0	0	0	1	1	0	0	0	1
26	*Musa paradisiaca* (Musaceae)	1	Herb	0	0	1	0	0	0	0	1	1	0	0	0	1
27	*Moringa oleifera* (Moringaceae)	1	Tree	1	1	2	0	0	0	0	4	3	0	1	0	6
	Total	832		840	478	3980	2865	2028	938	257	11386	7989	1202	1632	563	12600

Pandian

Preference of birds to primary nest-supporting trees to build nests

Among 27 species, the three primary nest-supporting plant species were palms (Arecaceae), (*B. flabellifer* 58.9%, n = 490; *P. sylvestris* 14.18%, n = 118; and *C. nucifera* 12.14%, n = 101), which represented 85.21% (n = 709) of the total nest-supporting plants (Table 1). Among 490 *B. flabellifer* trees, 55.10% were male trees (n = 270) bearing 58.72% nests (n = 4,876) and other 44.9% were females trees (n = 220) bearing 41.28% nests (n = 3,428). One rare instance of Baya Weaver constructing a nest on *Musa paradisiaca* using a torn leaf lamina was recorded. In another instance, a nest was found attached to the rachilla of inflorescence of *C. nucifera* as against the usual practice of birds constructing nests from tip of leaflets (Image 1).

ANOVA test reveals that significant differences existed between the type of nest-supporting plant species and the number of nests (F-value = 7.691, p <0.001) and birds (F-value = 7.269, p <0.001) at 5% (p <0.05) level of significance. Analysis also revealed that there existed significant differences among the three primary nest-supporting plant species and the number of nests (F-value = 11.155, p <0.001) and number of birds (F- value = 10.589, p <0.001) at 5% (p <0.05) level of significance. Positive correlation was observed (r = 0.231) between the number of nests and GBH and tree height of nest-supporting plants but negative correlation (r = -0.043) existed between the number of nests and canopy sizes of nest-supporting plants.

Preference of type of lands

The study on the preference of Baya Weaver towards the type of lands revealed that 89.30% nest-supporting plants (n = 743) which bore 90.81% nests (n = 10,340) occurred in cultivated lands; 7.33% nest-supporting plants (n = 61) bearing 2.86% nests (n = 326) occurred near water bodies; 2.16% plants (n = 18) with 4.72% nests (n = 537) occurred in fallow lands; and 1.20% plants (n = 10) with 1.61% nests (n = 183) occurred in residential areas (Figure 2).

Preference of Baya Weaver to build nests close to grain crops

The study revealed that 65.6% of nest-supporting plants bearing 65.67% of nests enumerated were situated in crop lands where cereal grain crops were under cultivation, such as paddy, pearl millet, finger millet, sorghum, and foxtail millet. Apart from this, 12.5% of the nest-supporting plants were within 500 m of such crops, while another 21.9% plants were at a distance of 500–1,000 m from cereal grain crops. This shows overwhelming preference for crop lands or their vicinity as choice of nesting colonies (Table 2).

Preference of Baya Weaver in building nests on plants occurring close to power cables, roads and human dwellings

The study also tested the relationship between proximity of overhead transmission power cables, roads, human dwellings, and selection of nest-supporting plants by populations of Baya Weaver. The study revealed that maximum nest-supporting plants, nests and birds occurred within 50 m distance from power cables (Figure 3). The study also revealed that maximum nest-supporting plants, nests, and birds occurred within 100 m distance from the adjacent roads (Figure 4). Similarly, maximum nest-supporting plants, and birds occurred within 100 m distance from human dwellings (Figure 5).

Hedges under nest-supporting trees

Study on the type of vegetation covered around the stems of nest-supporting plants revealed that 81.97% nest-supporting plants (n = 682) lacked any bushes/ shrubs around the stems/trunks, whereas dense shrubs were growing around the bases of stems of 18.03% nest-supporting plants (n = 150). The shrubs around the stems were indentified as *P. juliflora*, *L. camara*, *A. indica*, *S. trilobatum*, *S. xanthocarpum*, *C. carolinus*, and *F. leucopyrus*. These plants were found thickly covering the basal parts of stems of nest-supporting plants/ trees and probably prevented humans or monkeys from accessing the plants/trees.

Source of nest materials

The study on the source of nest materials revealed that Baya Weavers had plucked fibres from three plant species, such as leaves of Sugarcane, Narrow Leaf Cattail *Typha angustifolia*, and leaflets of Indian Date Palm.

Various stages of nests

The enumerated 11,386 nests were under various developmental stages, viz., wad stage-7.38% (n = 840), ring stage-4.20% (n = 478), helmet stage-34.96% (n = 3,980), egg-chamber closed stage-25.16% (n = 2,865), complete nests-17.81% (n = 2,028), abnormal nests-8.24% (n = 938), and damaged nests-2.26% (n = 257). The study revealed that each nest-supporting plant bore an average of 13.68 nests (Figure 6).

Orientation of nests

The study revealed that, 70.16% nests (n = 7,989)

Image 1. Pictures showing various nest-supporting plants bearing nests: a—Male bird with breeding plumage | b—Female bird carrying prey | c—Nest colony on *Borassus flabellifer* | d—Nest colony on *Cocos nucifera* | e—Nest colony on *Phoenix sylvestris* | f—Solitary nest on *Moringa olifera* | g—Solitary nest on *Musa paradisiaca* | h—Solitary nest on *Morinda tinctoria*. © M. Pandian.

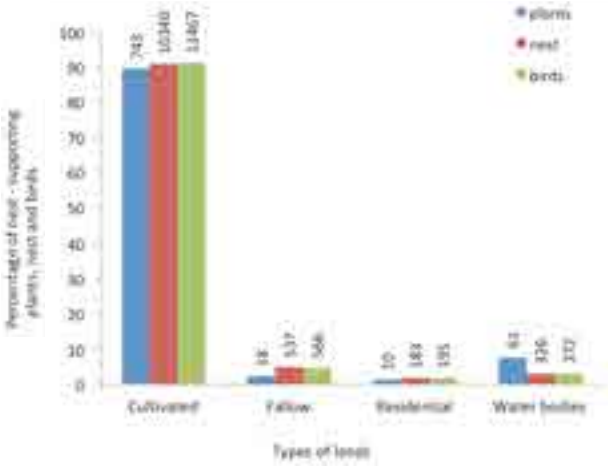

Figure 2. Preference of Baya Weavers' in selection of nest-supporting plants close to types of lands.

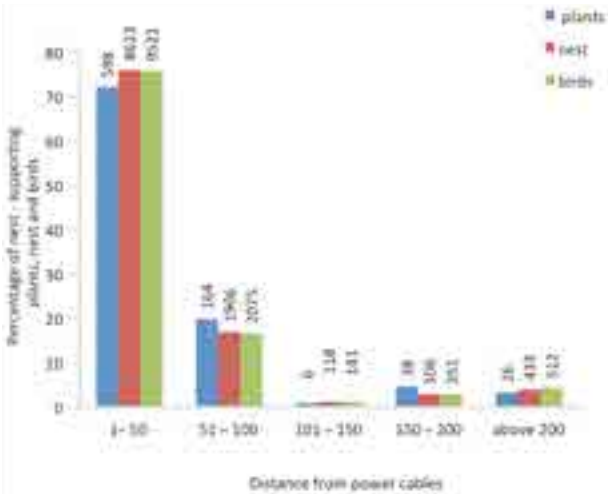

Figure 3. Relationship between the distance of nest-supporting plants and nearest overhead power transmission cables.

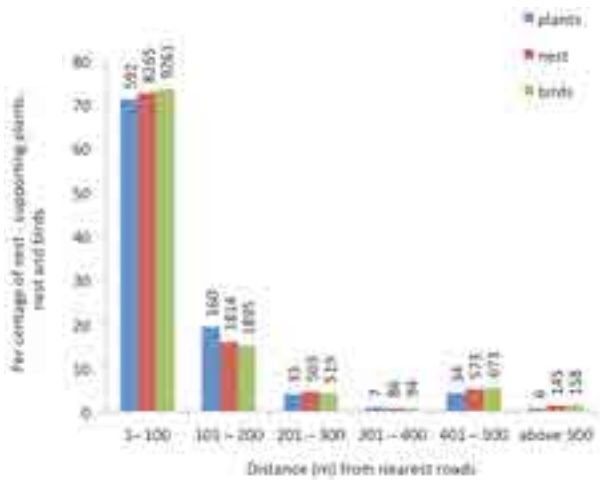

Figure 4. Relationship between the distance of nest-supporting plants and nearest roads.

were oriented towards the east, facing the rising sun, followed by 10.55% (n = 1,202) nests oriented towards the west, 14.33% (n = 1,632) nests facing north, and only 0.49% of nests (n = 563) facing south. Out of 89 solitary nests, 87 nests were found facing an east orientation and one nest each was found facing north and south orientations. Of the total nests (n = 7,989) facing towards the east, 69.92% nests (n = 5,586) were found on *B. flabellifer*, 11.5% nests (n = 919) on *C. nucifera*, 11.25% nests (n = 899) on *P. sylvestris*, 1.85% nests (n = 148) on *P. juliflora*, 1.25% nests (n = 100) on *C. equisetifolia* and 4.23% nests (n = 337) were found on the remaining 20 nest-supporting plant species.

Nest colonies

The number of nests (including all the stages) in each nest colony varied: 78.13% of nest-supporting plants (n = 650) bore nests between 01–20, whereas 13.46% of

nest-supporting plants (n = 112) bore 21–40 nests, 5.5% plants (n = 47) bore 41–60 nests, 2.20% plants (n = 20) bore 61–80 nests, 0.24% plants (n = 2) bore 81–100 nests, and one plant (0.12%) bore above 100 nests, i.e., 109 nests. A maximum of 109 nests in a colony were observed on a single *B. flabellifer* tree in Vengadur Village (12.228°N, 79.566°E). The study revealed 89 nest colonies contained solitary nests.

Nests overhanging water bodies

The study revealed that 2.86% nests (n = 326) including 140 abnormal nests on 61 nest-supporting plants were overhanging water bodies, i.e., irrigation wells, river, lakes, ponds, and sewage stagnant water occurring in 20 villages. A total of 372 individuals of Baya Weavers (2.95%) were observed on those 61 nest-supporting plants. Those nest-supporting plants (n = 61) belonging to 12 species, such as *B. flabellifer, V. nilotica, P. juliflora*, *L. camara*, *A. lebbeck, A. indica, F. benghalensis, F. religiosa, F. leucopyrus*, *P. reticulatus*, *M.tinctoria* and *S. siamea* were found growing on the edges of water bodies. Among 326 nests, 244 nests attached to 46 nest-supporting plants were found in irrigation wells. The remaining 82 nests were attached to 14 nest-supporting plants were observed on the edges of lakes, ponds, river, and sewage stagnant water. The number of nests per colony was found to be varied. A maximum of 28 nests was counted on one *F. benghalensis* tree, followed by 25 nests on one *B. flabellifer* tree, and 15 nests on one *A. indica*. Solitary nests were observed on 15 nest-supporting plants. The study revealed that an average

Table 2. Relationships between the type of crops and selection of nest-supporting plants by Baya Weaver.

	Name of the crops/ groves	No. of plants bearing nests	Total no. of nests	Total no. of birds
1	Cereal grain crops	546	7477	8236
2	Sugarcane	119	1641	1807
3	Pulses & oil seeds	47	767	852
4	Fallow lands	37	381	355
5	Casuarina groves	44	568	719
6	Residential area	10	173	191
7	Flower crops	7	106	130
8	Other groves	22	273	310
	Total	832	11386	12600

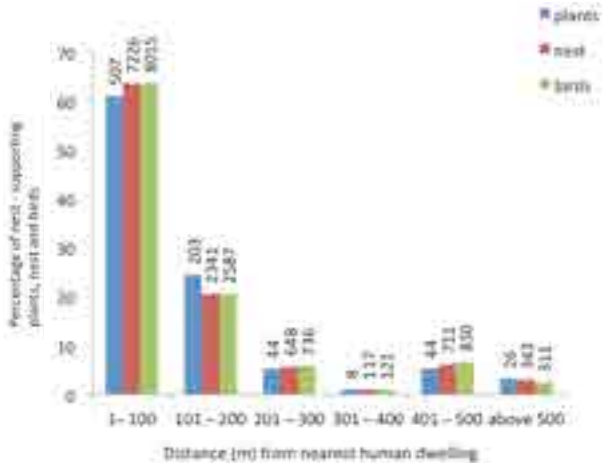

Figure 5. Relationship between the distance of nest-supporting plants and nearest human dwellings.

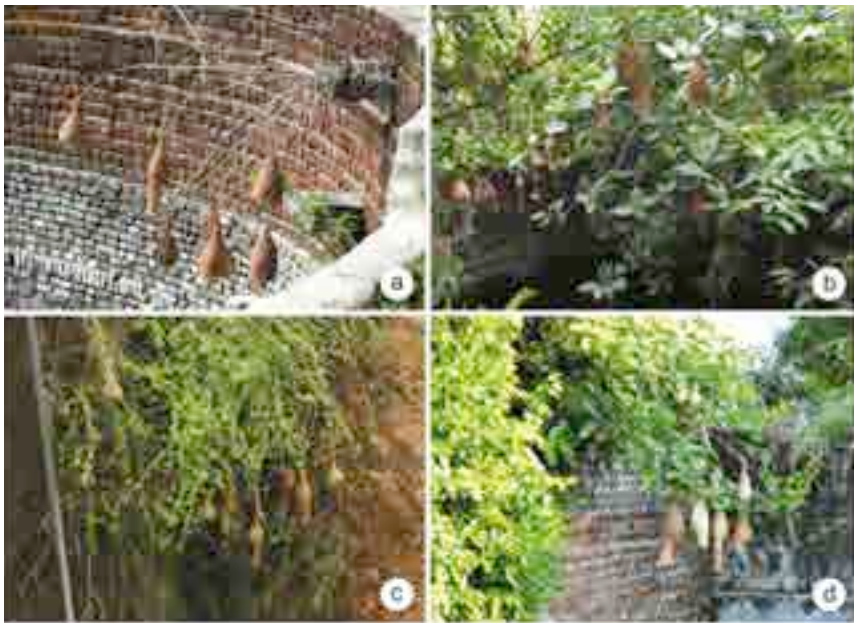

Image 2. Pictures showing nests overhanging water bodies: a—Birds built nests on dried *L. camara* twigs tied in the well | b—*A. indica* and *F. benghalensis* bearing nests | c—Overhanging nests attached to *P. reticulatus* |d—Overhanging nests attached to *F. benghalensis*. © M. Pandian.

of 5.34 nests per nest-supporting plant was observed. In one instance, a landholder in Periyathatchur village (12.115°N, 79.523°E) had cleared all the bushes for the safety of the well and had tied a bunch of dried *L. camara* twigs in the well during the fourth week of April 2021 to facilitate Baya Weavers to build nests and accordingly the birds built seven nests on those dried twigs during May–June 2021. In Kambur village (12.303°N, 79.771°E), one nest colony containing eight nests was submerged in a well due to the rising of the water level (Image 2).

Abnormal nests

Abnormal nests constituted 8.24% (n = 938) of the recorded nests and 17 different types of variations in nests were noticed: 86.03% (n = 807) abnormal nests were found on *B. flabellifer* trees (n = 188), 5.43% (n = 51) abnormal nests on *C. nucifera (*n = 24), and 5.22% (n = 49) on *P. sylvestris* (n = 19), and the remaining 31 abnormal nests were found on 10 other nest-supporting plant species. Out of 938 abnormal nests, 56.07 (n = 526) abnormal nests were found on male *B. flabellifer* trees whereas 29.95% (n = 281) abnormal nests were found on female *B. flabellifer* trees. Seventeen different types of abnormal nests were recorded: 28.99% (n = 272) abnormal nests belonged to multi-stalked type, 26.65% (n = 250) were 1+1/2 storeyed type, 25.79% (n = 242) were 1+1 storeyed, and 4.69% (n = 44) were mixed abnormal types. The remaining 13.86% (n = 130) abnormal nests belonged to other 13 types of abnormal nests. A solitary nest abnormally having two egg-chambers attached to a common stalk and another helmet stage nest containing three openings were noticed. Each nest-supporting plant bore an average of 3.76% abnormal nests (Table 3; Image 3).

Deposit of clay in the nests

The males had plastered the inner walls of helmet stage nests with wet clay immediately after the completion of construction of helmet stage nests and before selection of such nests by females. Out of a total of 11,386 nests, 3,980 nests (35.24%) were found in the helmet stage. Observation of the inner walls of those helmet stage nests through binoculars and digital camera revealed that 90.12% helmet stage nests (n = 3,587) contained plastering of clay on the inner walls. The remaining 9.88% helmet stage nests (n = 393) had no such smearing of clay on their inner walls. It was not possible to view and study the nature of clay deposits in the completed nests through binoculars, as the nest chambers were found closed. Continuous observations revealed no incidents of males taking readily available

Table 3. Details of nest-supporting plants bearing various types of abnormal nests of Baya Weavers in the study area (as on 2nd week of October 2021).

	Name of the nest-supporting plant	Total no. of plant	Total no. of nest	Abnormal	Multi-stalked nest	1+½ Storeys	1+1 Storeys	Mixed abnormal nest	Chain storeyed nest	½+½ Storeys	Wide stalked nest	½+1 Storeys	Buttressed nest	Fused nest	Bell-jar shaped	Fused Branching nest	Helmet with three openings	Meshed nest	Free branching nest	Symmetrical nest	Double egg chamber
1	*Borassus flabellifer*-female	113	3682	526	169	137	138	21	12	9	11	11	4	6	2	3	0	1	1	0	1
2	*Borassus flabellifer*-male	75	2272	281	81	71	77	14	10	8	4	1	3	4	5	1	1	0	0	1	0
3	*Cocos nucifera*	24	776	51	7	13	15	3	0	5	2	0	4	0	0	1	1	0	0	0	0
4	*Phoenix sylvestris*	19	452	49	12	20	6	0	4	2	2	2	0	0	0	0	0	1	0	0	0
5	*Morinda tinctoria*	5	43	7	1	0	4	0	0	0	0	0	2	0	0	0	0	0	0	0	0
6	*Prosopis juliflora*	5	73	6	1	3	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
7	*Vachellia nilotica*	2	11	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	*Azadirachta indica*	1	15	3	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
9	*Ficus benghalensis*	3	90	11	0	3	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
10	*Flueggea leucopyrus*	1	10	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	*Lantana camara*	1	7	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	**249**	**7431**	**938**	**272**	**250**	**242**	**44**	**26**	**25**	**20**	**14**	**13**	**11**	**9**	**5**	**2**	**2**	**1**	**1**	**1**

wet clay from paddy fields. Between 0600 and 0800 h daily, all the males swarmed to the adjacent wet fallow lands (200 to 700 m distance from nest-supporting plants) and scooped the bulk of wet clay through their beaks in many trips and carried it to helmet stage nests. It was not possible to ascertain whether the birds added clay on the inner walls after closing of the egg-chamber and construction of the entrance tube. No females were seen on wet soil surfaces, scooping clay or carrying it to the nests (Image 5a,b).

Communal roosting and foraging

The study on 20 flocks engaged in roosting and foraging revealed that the individuals of Baya Weaver always moved as flocks, the flock size ranging 40–75 birds. All the flocks flew in close formations by performing complicated manoeuvers and moved out of roosting sites such as sugarcane crops and *P. juliflora* bushes between 0600 and 0630 h daily for foraging. Baya Weavers strictly followed communal roosting and foraging. They foraged mainly on cereal grain crops but occasional foraging on other crops/grasses was also observed. Out of twenty flocks studied, 13 flocks were found foraging on paddy crops. During foraging the flocks used nearby overhead power transmission cables as transit roosting sites. After foraging, the flocks split and returned to their nesting colonies in various directions. Then nest construction activities, roosting and preening continued on the nest-supporting plants, and adjacent roosting sites. Again they moved as small flocks for foraging between 1030 and d 1130 h and afterwards some birds returned to their nesting trees and the remaining roosted on adjacent sugarcane crops and *Prosopis juliflora* trees for day roost. Third foraging trips were observed in the evening period between c. 1600 and 1740 h. After evening forage, some birds returned to their nesting trees and others moved to adjacent sugarcane and *P. juliflora* trees for night roosts. The foraging continued for a short span of time, i.e., 20 to 50 min and the flocks moved frequently from one site to another on the foraging crops. Apart from grain crops, the birds also consumed unripe seeds of *S. indicum*, *C. annuum*, *L. camara*, and grasses such as *S. pallide-fusca* & *P. geminatum* (Image 4). The foraging flocks contained individuals of other bird species, such as Tricolored Munia *Lonchura malacca*, Scaly-breasted Munia *Lonchura punctulata*, and White-rumped Munia *Lonchura striata* (Table 4).

No individual of Baya Weaver was found night roosting on the nesting trees during the entire study period. After evening forage, all the birds used to flee from the nest colonies and roost on the shrubs/

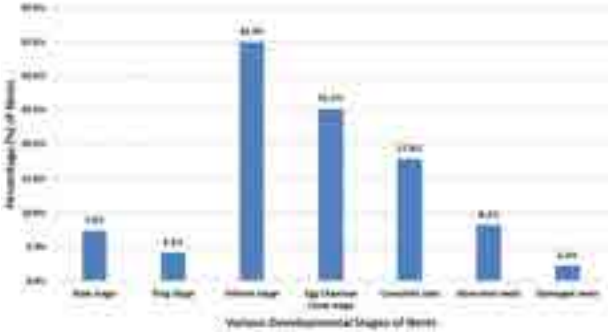

Figure 6. Number of various developmental stages and damaged nests of Baya Weaver enumerated in the study area.

sugarcane crops and return to their nest colonies the next morning. Continuous monitoring on nest colonies revealed that some females entering their nests during the evening hours did not come out and it was presumed that those females might have been incubating their eggs or nestlings.

Threats

A total of 257 nests were found torn and dangling from the nest-supporting plants, of which 86.38% of damaged nests (n = 222) were found attached to fronds of *B. flabellifer* trees, 4.67% damaged nests each (n = 12) were found on *C. nucifera* and P. sylvestris, respectively, and the remaining 4.28 % damaged nests (n = 11) occurred on other nest-supporting plants, such as *P. reticulatus*, *P. juliflora*, *F. benghalensis*, and *F. leucopyrus*. Among 257 nests, 47 nests had circular openings opposite egg-chambers (Image 5c,d,e).

The survey revealed that apart from 11,386 nests enumerated, a total of 1,050 nests in various stages (helmet stage-45.80%, n = 481; egg-chamber closed stage-21.90%, n = 230; complete nests-30%, n = 315; and abnormal nest-2.28%, n = 24) had fallen from 163 nest-supporting plants and were found scattered on the ground. During the study period, 25 eggs and 18 dead chicks were found in the fallen nests. Among 1,050 fallen nests, 72.66% nests (n = 763) were found under 113 *B. flabellifer* trees, whereas 16% fallen nests (n = 168) were under 25 *P. sylvestris* trees, 9.71% fallen nests (n = 102) were under 21 *C. nucifera* trees, 1.05% fallen nests (n = 11) under two *P. reticulatus*, 0.48% fallen nests (n = 5) under solitary *C.equisetifolia* tree, and a solitary nest (0.10%) was found under one *V. nilotica* tree (Image 5f,g,h).

Threats

The study revealed that the farmers have the

Table 4. Details of flocks containing individuals of Baya Weaver foraging on various crops in the study area.

	Name of the plants	Common name	Family	No. of forages observed	No. of birds observed
1	*Oryza sativa*	Paddy	Poaceae	13	60–75
2	*Sorghum bicolor*	Jowar	Poaceae	1	60
3	*Pennisetum glaucum*	Pearl Millet	Poaceae	3	60
4	*Eleusine coracana*	Finger Millet	Poaceae	1	60
5	*Setaria italica*	Foxtail Millet	Poaceae	1	60
6	*Setaria pallide-fusca*	Pigeon Grass	Poaceae		
7	*Paspalidium geminatum*	Egyptian Panic Grass	Poaceae		
8	*Sesamum indicum*	Sesame	Pedaliaceae	1	40
9	*Capsicum annuum*	Chilli	Solanaceae		
10	*Lantana camara*	West Indian Lantana	Verbenaceae		

Table. 5. Details of nest predations and interactions between Baya Weaver and other bird species.

	Name of the birds observed	No. of sighting of the birds	No. of damaged nests of Baya Weavers	No. of adult Baya Weavers killed
1	House Crow	21	2	0
2	Large-billed Crow	14	1	0
3	Shikra	12	0	2
4	Rufous Treepie	39	6	0
5	Black Kite	11	0	0
6	Black Drongo	85	7	0
7	Asian Koel	6	1	0
8	White-rumped Munia	11	0	0
9	Common Myna	72	0	0
10	Indian Roller	4	0	0
	Total	275	17	2

practice of clearing bushes around irrigation wells every year for their safety. When it involved destruction of nest-supporting plants, it would cause lack/scarcity of nesting substrata for the birds. Burning of herbs/shrubs under nest-supporting trees before commencement of cultivation every year resulting in smoke and fire drove away birds. In Kilvailamur villages, the land holders pruned the leaves of *C. nucifera* trees to avoid nesting of Baya Weavers with the intention of protecting cereal grain crops and 34 nests were found attached to the pruned leaves. It was observed that farmers of Rettanai had plucked the nests from trees using a hook tied to bamboo sticks to avoid possible damage to grain crops by Baya Weavers. In Kambur Village, a nest colony containing eight nests had been submerged in the irrigation well due to the rising of the water level after monsoon rains and the birds had to abandon the site (Table 5).

There were opportunistic sightings of 10 species of other birds, such as House Crow *Corvus splendens*, Large-billed Crow *Corvus macrorynchus*, Shikra *Accipiter badius*, Rufous Treepie *Dendricitta vagabunda*, Black Kite *Milvus migrans*, Black Drongo *Dicrurus macrocercus*, Asian Koel *Eudynamys scolopaceus*, White-rumped Munia *Lonchura striata*, Common Myna *Acridotheres tristis*, and Indian Roller *Coracias benghalensis* on the nest colonies. Seventeen incidents of nest damages by House Crow, Large-billed Crow, Rufous Treepie, Black Drongo, and Asian Koel were observed during the study, whereas no antagonistic relationships existed between Baya Weavers and Common Myna and Indian Roller. Rufous Treepie had plucked fibres and made a circular opening on the anterior side of egg-chamber and inserted their heads (Image 5a). In seven instances, individuals of Black Drongo had plucked fibres from nests and caused damage to the nests. Seven nests (helmet-1 & complete nests-6) of Baya Weaver were occupied by White-rumped Munia and no antagonistic relationship was observed between these two species. It was not possible to ascertain whether the individuals of White-rumped Munia occupied abandoned nests or by usurping the nests from resident Baya Weavers. No incident of either damage to nests or killing of adult birds by Black Kites was noticed, but Baya Weavers were seen to be frightened and fleeing from the nesting colonies when a Black Kite landed on nesting trees (Table 5; Image 6).

DISCUSSION

Baya Weavers and their preference of plants to build nests

Baya Weavers used *B. flabellifer* trees extensively for construction of nests in the eastern parts of peninsular India (Sharma 1989). Davis (1974) indicated that 60% of nests occurred on both *B. flabellifer* and *C. nucifera.* In the present study, I found that Baya Weavers preferred *B. flabellifer* (58.9%; n = 490), since 72.93% of nests (n = 8,304) occurred on them. It was also observed that Baya Weavers preferred more male *B. flabellifer* trees (55.10%; n = 270) than female trees (44.9%; n = 220) for construction of nests. The probable reasons for preferring male trees might be due to less human disturbance faced by male trees as compared to female trees. However the exact causes for such a preference will require further investigation. In one instance, a male bird constructed a nest by plaiting a knot encircling the stems of *Cocculus carolinus*, *Prosopis juliflora*, and rachis of *Phoenix sylvestris.* In another case the nest was found attached to the tip of stems of *Prosopis juliflora* and *S. trilobatum*.

Ambedkar (1969) had stated that Baya Weavers of different regions preferred different plant species for construction of nests. He also recorded six species in Tamil Nadu, viz., *B. flabellifer*, *P. sylvestris*, *C. nucifra*, *P. dulce*, *T. indica*, and *Acacia* spp. Birds used 25 plant species as nesting substrata in Uttar Pradesh (Mathew 1972) and 17 plant species in Arakkonam taluk of Tamil Nadu (Pandian 2021a). In the present study, 27 plant species have been recorded including the six species as recorded by Ambedkar (1969).

Preference of Baya Weavers in building nests on plants occurring close to power cables, roads and human dwelling

As a social bird, Baya Weavers generally prefer to live near agricultural areas with significant human activity. For example, Ali (2009) found that the Weaver populations used electricity lines as fetching sites for collection of food and nesting materials. Ninety-three percent of nest-supporting plants occurred in close proximity to power cables, 64% nest-supporting plants near roads, and 86% nest-supporting plants near human dwellings were reported in Villupuram district (Pandian & Ahimas 2018). In the present study, the maximum nest-supporting plants occurred close to power cables that passed through crop fields and they were used as fetching and roosting sites while foraging, collection of nesting materials and feeding broods. The birds selected apparently nest-supporting plants that occurred in close proximity to roads with busy vehicular traffic and human dwellings close to cultivated lands hence, this matches with the findings of Ali (2009) and (Pandian & Ahimas 2018).

Source of nest materials

The nest materials used by Baya Weavers were found to vary according to the locality. In India, the birds used leaf fibres of *C. nucifera* and *P. sylvestris* except in the north (Dewar 1909). Baya Weavers used fibres from grass and palm fronds to construct nests in the Northern Province of Sri Lanka, India, Africa, and Seychelles (Wood 1926; Crook 1962), leaves of *Phoenix* sp., coarse grass and paddy in Kolaba district, Maharashtra (Ali 1931), and *Phoenix* sp., paddy, millets, coconut, and lemon grass in Cuddapah district of Andhra Pradesh (Mathew 1972). The present findings of birds using fibres of *P. sylvestris* for construction of nests partly matches with the observations of Dewar (1909), Wood (1926), Ali (1931), Crook (1962), Mathew (1972), and Davis (1974). Apart from *P. sylvestris,* the birds used leaves of *S. officinarum* and *T. angustifolia* as nest materials in the study area.

Orientation of nests

Nests of Baya Weavers were found hanging in an easterly direction to protect the nests from winds of the south-west monsoon in the Northern Province of Ceylon (Wood 1926). Many authors have commented on the occurrence of more nests on the eastern side (windward) of the plants as protection from strong monsoon winds (Ali 1931; Ambedkar 1964; Davis 1971; Quader 2003). The nests of the White-browed Sparrow (*Plocepasser mahali)* constructed on the windward side of trees suffered more damage than those on leeward side (Ferguson & Siegfried 1989). It was reported that 40.4% nest colonies in Rajasthan (Sharma 1990), 87% nests in Chorao Island, Goa (Borges et al. 2012), 88.6% of nests in Tindivanam taluk (Pandian & Ahimas 2018), and 80.86% of nests in Arakkonam taluk, Tamil Nadu (Pandian 2021a) were oriented towards the east probably to protect their nests from the battering south-west monsoon winds. In the present study also, 70.16% nests were found hanging towards the east, hence it matches with the findings of Wood (1926), Ali (1931), Ambedkar (1964), Davis (1971), Quader (2003), Borges et al. (2012), Pandian & Ahimas (2018), and Pandian (2021a). Sharma (1990) observed all solitary nests faced other than the eastern side in Rajasthan whereas in the present study, 97.7% solitary nests (n = 87) were found facing the eastern side, hence it contradicts the observations of Sharma (1990).

Image 3. Pictures showing abnormal nests: a—Multi-stalked nest | b—Fused branching nest | c—Buttressed nest | d—Chain-storeyed nest | e—Bell-jar shaped nest | f—A bistoreyed nest with both the alive storeys | g—Wide stalked nest | h—1+1 storeys nest | i—Nest with two egg-chambers attached to a common stalk | j—1+1/2 type nest | k—Fused branching nest | l—Helmet stage nest with three openings. © M. Pandian.

Image 4. Pictures of Baya Weavers showing forage on various seeds. A — Baya Weavers transit roost on Sugarcane crop before forage |— b A flock containing Baya Weaver with associate birds foraging on paddy crop| c— A female bird gleans grains of Pearl millet| d— A female bird gleans paddy grains| e— A male bird forages on fruits of Lantana camara| f— A male bird forages on foxtail millet crop| g— Birds foraging unripe fruits of Sesame crop, and |h— Birds forage on Chilli fruits. © M. Pandian.

Nesting colonies

Baya Weaver is a colony-nester and the number of nests in each colony has been reported to be varied: 1–250 nests in Rajasthan (Sharma 1989), 5–24 nests in South Goa (Borkar & Komarpant 2003), 1–93 nests in Villupuram district (Pandian 2018), and 1–61 nests in Vellore district, Tamil Nadu (Pandian 2021a). In the present study also, the number of nests per colony was found between 1–109.

A total of 27 solitary nests were recorded on *A. Arabica* trees in Satna district of Madhya Pradesh (Pandey 1991), 22 solitary nests in Arakkonam taluk (Pandian 2021a) and now I recorded 89 solitary nests in the study area. Nest colonies with small numbers of nests tend to be more likely to be abandoned than large and established ones, as Baya Weavers are of a more shifting nature (Ali et al. 1956). The present enumeration of less than 20 nests in 78.13% of nest colonies (including solitary nests

on 89 nest-supporting plants) indicates that the present nest colonies are found weak, not well-established as stated by Ali (1931).

Nests overhanging water bodies

Many authors have reported the occurrence of nests of Baya Weaver hanging over water bodies (Ali 1931; Ambedkar 1964; Collias & Collias 1964; Crook 1964; Davis 1974; Khan 1799; Subramanya 1982; Sharma 1987). Nests on plants hanging over water bodies in South Goa were reported by Borkar & Komarpant (2003), in Parbati hill, Poona by Crook (1960), in Nanded region, Maharashtra by Achegawe *et al*. (2016), and in Assam, by Yashmita-Ulman *et al*. 2017. In Tamil Nadu, 3.2% of nests in Tindivanam taluk (Pandian 2018) and 4.38 % nests in Arakkonam taluk, Tamil Nadu (Pandian 2021a) were found hanging over water bodies. During the present study, 2.86% nests (n = 140) were found hanging over irrigation wells, canals and ponds, as reported in many other studies (Khan 1799; Ali 1931; Ambedkar 1964; Collias & Collias 1964; Crook 1964; Davis 1974; Subramanya 1982; Sharma 1987; Borkar & Komarpant 2003; Pandian 2018, 2021a). The reason for birds selecting nest-supporting plants close to water bodies is attributed to the safety of the nests and broods from terrestrial predators (Davis 1974). Sharma (1987) recorded four nest-supporting plants, namely *Calotropis procera*, *Cordia gharaf* (= *Cordia sinensis*), *Adhatoda vasica*, and *Cynodon dactylon,* bearing nests found hanging over wells and water bodies in Rajasthan. Pandian (2021a) had recorded eight nest-supporting plant species bearing nests, namely *V. nilotica*, *P. juliflora*, *B. flabellifer*, *P. sylvestris*, *C. nucifera*, *P. reticulatus*, *F. religiosa*, and *Ziziphus oenoplia* growing on the edges of water bodies in Arakkonam Taluk, Tamil Nadu. But in the present study, 12 plant species bearing nests which were not recorded by Sharma (1987) in Rajasthan were observed. It indicates that the preference of nest-supporting plants by Baya Weavers near water bodies is found to vary in different geographic regions.

Abnormal nests

Abnormal nesting behaviour of Baya Weaver was reported by Ali et al. (1956) and Ambedkar (1958, 1980) in Pune, Maharashtra, and Sharma (1985, 1988, 1995) in Rajasthan. Borkar & Komarpant (2003) listed 13 distinct types of anomalous nests in South Goa. In Tamil Nadu, 15 types of abnormal nests in Tindivanam Taluk and eight types of abnormal nests in Arakkonam taluk were reported (Pandian 2018, 2021a). Now 17 types of abnormal nests were recorded in the study area, hence it matches with the observations of the above said authors.

Abnormal nesting behaviour also occurs in other species of the genus *Ploceus.* For example, Southern-masked Weaver *P. velatus* constructs one of the most abnormal nests among the Weaver birds in South Africa, Angola, Zambia and Mozambique (www.weavers.adu.org). Black-throated Weaver *P. benghalensis* builds an abnormal entrance tube of more than a metre length (Mishra 2004) and Spectacled Weaver *P. ocularis* constructs an abnormal entrance tube with a two-metre length in southern Africa (Maclean 1985). African Weaver *P. cucullatus* constructs an abnormal nest with supernumerary antechamber or bottomless or canopy type nests with variations in the entrance tubes (Collias & Collias 1962; Crook 1963). Intraspecific variations in the length of entrance tubes are found in the nests of Streaked Weaver (*P. manyar*) and Sakalava Weaver (*P. sakalava*). The Streaked Weaver constructs a nest with a short entrance tube in reeds in India, but with a long entrance tube in trees in Java (Delacour 1947) and the Sakalava Weaver constructs a nest with a short entrance tube in the arid habitats and a long entrance tube in the other habitats in Madagascar. Hence, like other species of *Ploceus*, Baya Weavers are also found to have constructed abnormal nests with 17 variations in the study area.

Deposition of clay

It was found that plastering of clay by males started when the nest construction was in the helmet stage, as also reported in other studies (Dewar 1909; Ali 1931; Borkar & Komarpant 2003). According to Davis (1973), wet mud smudging in nests takes place prior to pairing with females. The behavior of deposition of mud on the inner walls of nests is also prevalent among the other species of *Ploceus*, *viz*., Black-breasted Weaver *P. benghalensis* and Streaked Weaver *P. manyar* (Crook 1962). Wood (1926) suggested that plastering of clay helps to stabilize the nest in strong winds and also speculated that it might have been the habit of some ancestors of Baya Weaver, which built nests entirely or partly made of mud. Crook (1963) and Davis (1973) opined that mud plaster gives reinforcement to the fibres when the female conducts violent examination prior to her selection of nests. Ali (1931) and Sharma (1996) stated that intricate ethology is behind this peculiar habit of plastering and hence it requires further research. In this study, 90.12% helmet stage nests (n = 3,587) contained clay deposits on the inner nest walls and the exact reasons for plastering of clay needs further

Image 5. Pictures showing damaged and fallen nests: a—A male scoops clay | b—Helmet stage nest with plastering of clay | c—Partly torn nest | d—Dangling damaged nests | e—A circular opening opposite to egg-chamber | f—A fallen nest containing damaged eggs | g—Fallen nests | h—Fallen nest containing dead chick. © M. Pandian.

Image 6. Baya Weavers and their interactions with other bird species: a—Shikra chasing Baya Weaver on *Borassus flabellifer* tree | b—House Crows chasing nest colony | c—Pruned nest-bearing leaves of *Cocos nucifera* | d—Black Drongo damaging a nest | e—Rufous Treepie perching on power cable adjacent to nest colony | f—White-rumped Munia occupied a comlete nest of Baya Weaver. © M. Pandian.

study as stated by Ali (1931) and Sharma (1996).

Communal roosting and foraging

The mixed communal roosting consisting of different bird species serves as a centre for the exchange of information regarding the locations of food sources and warning signals about the approach of predators (Zahavi 1971; Gadgil 1972; Ward & Zahavi 1973; Gadgil & Ali 1975). Pandian (2020) had observed communal foraging and roosting of Baya Weaver in Ranipet district, Tamil Nadu. In the present study, flocks containing individuals of Baya Weaver, Tricolored Munia, Scaly-breasted Munia, and White-rumped Munia moved collectively without any competition over sharing of food and roosting sites. The behaviour of mixed roosting of four different species might have shared information on sources of cereal grain crops and protection from predators as stated by Gadgil (1972), Zahavi (1971), Ward & Zahavi (1973), Gadgil & Ali (1975). The food of the adult Baya Weaver comprises of cereal grains, grasses, weeds, flower nectar, and insects (Ali & Ripley 1987), paddy and weed seeds (Mukherjee & Saha 1974), paddy grains followed by bajra and sorghum (Ali et al. 1978). In the present study, the birds preferred cereal grain crops mainly paddy, pearl millet, finger millet and foxtail millet, grasses and a weed *L. camara* as observed by Ali & Ripley (1987) and Ali et al. (1978). Additionally Baya Weavers foraging on seeds of sesame and chilli crops were observed in the current study.

Threats

The males made openings on the nests from the outside directly into the egg-chamber to feed the chicks (Wood 1926). Borges et al. (2002) observed eight nests with a hole near the egg-chamber in Goa. Ali et al. (1956) felt that most circular holes bored opposite the egg-chamber recorded in nests in Pune, Maharashtra, could have been caused by predators. Rufous Treepie made a circular opening near the egg-chamber and predated eggs/chicks (Pandian 2021a). In the present study, a total of 257 damaged nests were found attached to the nest-supporting plants, of which 47 nests had circular holes near the egg chambers confirming that individuals of Rufous Treepie made circular holes on six nests corroborating the findings of Ali (1931) and Pandian (2021a). Another 11 nests were damaged by House Crows, Large-billed Crows, Black Drongos, and Asian Koels. The reasons for damages in the remaining 240 nests were not possible to ascertain during the present study.

Many complete nests were blown down due to recurring spells of bad weather during June–August in the Bombay area and the males cutting down the nest of rival cocks was common when the owner had gone to fetch nesting materials in Poona City (Ali et al. 1956). The males usually had the habit of cutting down their own nests, including those rejected by females and complete nests after broods have departed (Collias & Collias 1959, 1962). An instance of male Baya Weaver cutting down a complete nest occupied by White-rumped Munia was recorded in Villupuram district (Pandian 2021b). In the present study, a total of 1,050 nests had fallen down from the nest colonies. A total of 25 eggs and 18 dead chicks were found spread near fallen nests. The occurrence of such a great number of fallen nests may have been due to various biotic and abiotic factors as suggested by Ali et al. (1957), Collias & Collias (1959, 1962), and Pandian (2021b) and it needs further study.

House Crows and Large-billed Crows were the major predators of nests, eggs and broods (Ali 1956). Nest predation by Rufous Treepie was reported in Arakkonam taluk, Tamil Nadu (Pandian 2021a). Agitated behaviour of birds when Crow Pheasants *Centropus sinensis* appeared in close proximity of nesting trees and a Shikra making an unsuccessful stoop on a nest colony was observed in Kolaba district, Maharashtra (Ali 1931). In the present study, individuals of Baya Weavers had exhibited an agitated behaviour when House Crows and Large-billed Crows landed on nesting trees and two incidents of predation on adult male birds by Shikra and 17 incidents of nest damages by avian predators, such as House Crow, Large-billed Crow, Asian Koel, Black Drongo, and Rufous Treepie were observed as stated by Ali (1931), Ali (1956), and Pandian (2021a) hence, these predators posed a threat to the populations of Baya Weaver in the study area.

CONCLUSION

This is a systematic quantitative study on the preference of Baya Weaver towards various nest-supporting plants as nesting substrata, stages of nests, abnormal nests and probable threats to the nests on such nesting plants in the study area. The survey revealed that out of 27 plant species, Baya Weavers preferred three primary nest-supporting palm species, such as *B. flabellifer*, *C. nucifera*, and *P. sylvestris* for nesting. These three palms are an integral part of rural areas and they are also associated with rural cottage industries. The birds preferred nests on plants close to power cables, roads and human dwellings. Maximum nest-supporting plants occurred in cereal grain crop land. Probably the

nests are located on the eastern side of trees to protect them from the strong south-west monsoon winds. High variations of nests (17 types of abnormal nests) were reported. The birds strictly followed mixed communal roosting and foraging. Nest predation by avian predators was also found. Increasing urbanization by conversion of cultivated lands into residential areas, industrialization, widening of roads along with indiscriminate felling of these principal nest-supporting plants that are vital for Baya Weaver is a conservation issue in this landscape. Increasing practice of monoculture of *Casuarina*, sugarcane, vegetables, and flower crops, declining areas of cultivation of cereals and millets cause shortage of food grains to adult birds. Destruction of nests due to various anthropogenic factors and abiotic factors (monsoon winds and rains) may also affect the breeding of the Baya Weaver. The survey is limited to one taluk, but this is part of a larger geographical area that has a potential for high nesting population of the Baya Weaver which, however, faces threats from the changing rural landscape. Therefore, a conservation program focused on Baya Weaver could be taken up in the area, primarily through protection of nests and birds, keeping a check on anthropogenic threats, along with a sensitization program for local farmers towards conservation.

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Appendix I. List of villages having nesting habitats of Baya Weaver in Tindivanam taluk, Villupuram district.

	Name of the village		Name of the village		Name of the village
1	Mambakkam	41	Muppuri	81	Then Kalavay
2	Sembakkam	42	Pandamangalam	82	Annamputtur
3	Mel Siviri	43	Kenippattu	83	Kovadi
4	Konalur	44	Kodima	84	Manur
5	Attippakkam	45	Manthagapattu	85	Roshanai
6	Neduntondi	46	Alagraman	86	Ural
7	Vellimedupettai	47	Soli Sokkunam	87	Karuvapakkam
8	Vada Siruvalur	48	Kutterippattu	88	Vairapuram
9	Taniyal	49	Chinna Nerkunam	89	Tengapakkam
10	Puliyanur	50	Kizhavaliyamur	90	Evallur
11	Ilamangalam	51	V. Nallalam	91	Purangarai
12	Akkur	52	Se. Kotamangalam	92	Konerikuppam
13	Vilukkam	53	Nedi	93	Saram
14	Tivanur	54	V. Panchalam	94	Kil Gudalur
15	Salai	55	Sendiyambakkam	95	Vithalapuram
16	Kollar	56	Mozhiyanur	96	Kattalai
17	Kattusiviri	57	Periathachur	97	Nolambur
18	Pampundi	58	Perani	98	Ayyanavaram
19	Peramandur	59	Palapattu	99	Eppakkam
20	Pattanam	60	Chittani	100	Kuttikulattur
21	Pelakuppam	61	Elay	101	Kambur
22	Tindivanam	62	Andipalayam	102	Vada Kalavay
23	Bootheri	63	Pombur	103	Avanippur
24	Singanur	64	Ganapathipattu	104	Sendamangalam
25	Then Pasiyar	65	Anganikuppam	105	Kil Mannur
26	Vempundi	66	Athikuppam	106	Andappattu
27	Muttiyur	67	Vidur	107	Kil Serur
28	Peramandur	68	Padirippuliyur	108	Kil Buderi
29	Goplalapuram	69	Ten Alappakkam	109	Senalur
30	Mel Peradikuppam	70	Kuralur	110	Vandarampundi
31	Vengandur	71	Chendur	111	Naramagani
32	Kongarampet	72	Velangambadi	112	Kil Nemali
33	Nanalmedu	73	Siruvai	113	Kunnapakkam
34	Narerikuppam	74	Veliyanur	114	Mandaperumbakkam
35	Rattanai	75	Kallakulattur	115	Mettunatham
36	Annankulathumedu	76	Nallamur		
37	Maroor	77	Kannigapuram		
38	Thavalapattu	78	Kil Idaiyalam		
39	Then Puthur	79	Vairampattu		
40	Peramapttu	80	Avanampattu		

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 20988–21001

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.6705.14.5.20988-21001

#6705 | Received 13 September 2020 | Final received 05 December 2020 | Finally accepted 21 April 2022

OPEN ACCESS

COMMUNICATION

A checklist of avifauna from different habitats of semi-arid landscape in western parts (Mandsaur and Ratlam districts) of Madhya Pradesh, India

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Abstract: We prepared a checklist of avian species recorded from two western districts (Mandsaur and Ratlam) of Madhya Pradesh, situated in a semi-arid region with comments on their habitat preference, feeding habits, migratory, and conservation status; 133 bird species belonging to 47 families were recorded during the present study. About 30% of the species were migratory in status. In terms of habitat fidelity, 74 avian species were found only in a single habitat. Habitat-wise avian richness varied widely. Among five habitats identified during the present study, wetland supported the highest number (69) of avian species of which 58 species were exclusively recorded from this habitat. Eight foraging guilds were identified among which omnivores were dominant. Six species of globally threatened and seven species of near-threatened species were recorded during the present study. The presence of significant numbers of winter migrants and globally threatened species indicated the importance, both ecologically and biologically, of the semi-arid landscape for breeding and migratory birds. Therefore, this work will provide baseline information to conservationists for the development of conservation and management policies for the two districts.

Keywords: Avian diversity, central India, conservation, feeding habits, habitat fidelity, migratory, semi-arid landscape, threatened species, wetlands, winter migrants.

Editor: H. Byju, Coimbatore, Tamil Nadu, India. **Date of publication:** 26 May 2022 (online & print)

Citation: Bhattacharjee, K. & S. Adhikari (2022). A checklist of avifauna from different habitats of semi-arid landscape in western parts (Mandsaur and Ratlam districts) of Madhya Pradesh, India. *Journal of Threatened Taxa* 14(5): 20988–21001. https://doi.org/10.11609/jott.6705.14.5.20988-21001

Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: Both of the authors were involved in field survey, documentation, literature review and manuscript preparation, editing and finalizing the manuscript.

Acknowledgements: We are thankful to anonymous reviewers for their critical review and valuable suggestions.

INTRODUCTION

Birds are one of the most widely distributed and abundant vertebrate taxa, living in diverse habitat types across different ecosystems. In a particular ecosystem, the avian community plays a variety of functional roles and provides different important ecosystem services (Sekercioglu 2006, 2012; Whelan et al. 2008). Change in vegetation composition affects the habitat quality for birds in terms of food, nesting site, which in turn affect the species richness, abundance, and distribution (Western & Grimsdell 1979). Birds generally colonize an area that is suitable in terms of resources for their survival (Veech et al. 2011). Many species of birds show fidelity towards a particular habitat (Chatterjee et al. 2013) so, alteration or degradation of that particular habitat leads to a declining population (Khan et al. 2019). Therefore, they act as an important bioindicator of habitat quality, environmental degradation, pollution, and ecosystem health (Gregory et al. 2003; Zhang & Ma 2011). Due to these essential ecological functions, birds have always been extensively used for conservation and environmental impact assessment studies (Knegtering et al. 2005).

Semi-arid regions are climatic zones that are intermediate between humid and arid (desert) climates. This zone is characterized by scanty precipitation and an aridity index between 0.20–0.50. Generally, the semi-arid region supports scrubby and grassland vegetation along this region has agricultural potential (with proper irrigation and management practices). Globally, about 65% of the area of total dry land in the world comes under arid and semi-arid regions (FAO 1993). In India, the semi-arid biogeographic zone is situated in transition between desert (arid) and other zones like the Himalaya, Western Ghats, Deccan Peninsula, and Gangetic plains (Rodgers & Panwar 1988). This region accounts for 16.60% of the total geographic area of the country covering the states of Rajasthan, Gujarat, Madhya Pradesh, Punjab, Haryana, Uttar Pradesh, and Maharashtra. Two-hunderd-and-seventeen endemic bird areas (EBA) with a large number of endemic bird species, identified by Birdlife International, are located within the arid and semi-arid regions of the World (Stattersfield et al. 2005). Avian populations in semi-arid regions are declining rapidly due to several factors such as unplanned development activities, climate change, and urbanization. Therefore, proper documentation of avian species, identification of their habitats, and breeding grounds is essential for proper conservation and management of avian communities and their habitat (Khan et al. 2019).

Avifaunal study in central India started before independence with the earliest work by King in the year 1911 who enlisted around 155 resident bird species from Saugar and Damoh (Chandra & Singh 2004). Later several other studies in this field were conducted by authors like Baker (1930a,b); D'Abreu (1931); Hewetson (1939), and Ali (1939, 1940). Grimmett & Inskipp (2003) listed 469 species of birds but due to lack of a comprehensive study, Chandra & Singh (2004) undertook a literature survey and reported around 488 taxa from Madhya Pradesh. In western Madhya Pradesh, 139 avian species were reported from the Gandhisagar reservoir (Vyas & Singh 2004) while Dange & Kumar (2013) listed 94 species of birds from the Ratlam district.

Preparing a checklist of taxa is the first and foremost task to acquire knowledge of biodiversity in a particular geographic area. This checklist acts as a basis for further in-depth studies, viz., systematics, taxonomy, distribution, evaluation, and conservation (Núnez-Zapata et al. 2016). Keeping this in mind the main aim of this study was to observe, record, and prepare a checklist of avian species from two western districts of Madhya Pradesh, situated in the semi-arid region with comments on their habitat preference, feeding habit, migratory and conservation status.

MATERIALS AND METHODS

Study area

Mandsaur (Site I) and Ratlam (Site II), two western districts of Madhya Pradesh located in the Malwa region covers an area of 5,521 and 4,861 km^2, respectively (Figure 1). The climatic conditions of these two areas are generally dry except during the monsoon season when it receives rainfall from the southwest monsoon. This area is generally classified under a semi-arid biogeographical zone (Rodgers & Panwar 1988). The average annual rainfall is 786 and 937 mm, respectively for the sites. Summer temperature ranges 38–44 °C while the winter temperature ranges 4–8 °C (Dange & Kumar 2013; NIC 2020).

We classified our study area into five habitat types (Image 1) based on their vegetation type, land use, and land cover which includes:

Wetland (WL): Both natural and man-made wetlands are present in the study area that are home to a wide variety of bird species that includes wintering and resident waterfowl, waders, raptors, etc.

Grassland (GL): Dominated by grass species of the

Figure 1. Map of the study sites (Site I: Mandsaur and Site II: Ratlam) at semi-arid landscape of western Madhya Pradesh.

genus *Bothriochloa*, *Themeda*, and *Dichanthium* provide shelter to different raptors like Montagu's Harrier, Short-toed Snake Eagle, various passerines, and others.

Open scrub jungle (OS): Dominated by *Acacia* and *Balanities.* Bird species inhabiting this area include Shikra, Common Kestrel, dove, bushchat, bunting, and starling.

Agricultural land (AL): Several crops like Soybean *Glycine max*, Wheat *Triticum aestivum*, Gram *Cicer arietinum*, Mustard *Brassica*, and Maize *Zea mays* are cultivated in this area.

Dry deciduous forest (DF): Most of the forests covers in these two districts are mainly Tropical dry deciduous forests which are dominated by Teak *Tectona grandis* and *Butea* sp. mixed with other species like Saja *Terminalia tomentosa*, Sal *Shorea robusta*, Bija *Pterocarpus marsupium*, Lendia *Lagerstroemia parviflora*, Haldu *Adina cardifolia*, Dhaora *Anogeissus latifolia*, Salai *Boswellia serrata*, Amla *Emblica officinalis*, Amaltas *Cassia fistula*, and Gamhar *Gmelina arborea* (Singh 2014).

Field visits were carried out in the morning (0600–1100 h) and in the afternoon (1500–1900 h), when birds were found to be most active during December 2015 and February 2016. Various survey methods like line transect (3 in each habitat, about 900–1,150 m long track) and point transect (5 in each habitat) that were randomly placed, along with opportunistic sightings were used to record various bird species of the region (Bibby et al. 2000; Sutherland 2006). Olympus 10×50 DPSI binoculars and Canon PowerShot sx500 IS camera were used for observation and photographs were taken whenever it was possible. Identification and categorization of avian species according to their migratory status either resident (R), winter visitor (WV), or passage visitor (PM) was done using field guides (Kazmierczak & van Perlo 2000; Grimmett et al. 2011). Bird species included in different IUCN Red List categories (IUCN 2020) and Schedule under the Wildlife (Protection) Act, 1972 (BNHS 2002) were also taken into account while preparing the checklist. Based on the frequency of observation, following categorizations were made: Common (C): frequently observed in the study area (encountered during sampling in more than 60% cases); Uncommon (UC): spotted on multiple occasions but not as frequently as in case of common (encountered during sampling in more than 30% but less than 60% cases); Rare (R): not frequently encountered in the entire study period (encountered during sampling in less than 30% cases). Feeding habits (guilds) of birds were recorded as per observation and following published literature (Ali & Ripley 1987).

RESULTS AND DISCUSSION

One-hundred-and-thirty-three bird species belonging to 47 families and 13 orders were recorded during the

study period of which 123 were recorded from Site I and 112 were recorded from Site II (Table 1). The highest number of bird species were recorded from Accipitridae and Anatidae family (11 spp. each) followed by Turdinae (8 spp.) and Ardeidae (7 spp.). The checklist of birds of the two districts is represented in Table 1 while the number of bird species and their families is graphically represented in Figure 2. According to the observed frequency, 37 bird species (27.82 %) were common, 79 species (59.40 %) were uncommon, and 17 species (12.78 %) were rare. Availability of diverse resources, habitat heterogeneity, and different anthropogenic factors influences the avian diversity of the studied sites. Avian species were categorized according to their migratory status (either resident, winter visitor, or passage visitor). Ninety-three species were resident, 39 species were winter visitors and only a single species was passage visitor (Rosy Starling).

Habitat-wise avian richness varied widely during the present study. Wetland (WL) harboured a maximum number (69) of avian species, followed by agricultural land (AL, 56 spp.) and open scrub jungle (OS, 50 spp.). These high numbers might indicate the ability of certain bird species to occupy diverse habitat types. However, in grassland (GL) and dry deciduous forest (DF) habitats, avian richness was quite low; 33 and 24 species were recorded from GL and DF habitats respectively (Figure 3). Wetlands were an important habitat that sustains a substantial number of waterbirds and wetland-associated birds (Kumar et al. 2005). However, most of the small water bodies were temporary that eventually dried up in summer. During winter, water was pumped out for irrigation by farmers of nearby agricultural fields but still, the wetlands harboured rich avifauna. Agricultural fields were also an important habitat that sustains the rich diversity of avifauna in different landscapes (Hossain & Aditya 2014; Swamy et al. 2015; Kumar & Sahu 2020). The present study also revealed the importance of wetland and agricultural fields as avian habitats.

In the present study, we recorded that 74 avian species (i.e., 55.64 % of total recorded spp.) were found exclusively in a single habitat (Figure 4). Among this, highest number of species (58 spp.) were exclusively recorded from WL habitat. Similar findings were also recorded by Chatterjee et al. (2013) while working in sub-Himalayan forest patches. Waterbirds were specialists in resource utilization (utilizing feeding habitat and foraging technique selection) therefore they show strong fidelity towards wetlands (Chatterjee et al. 2020). However, in other habitats, the number of species that were solely found in that habitat was much less. In DF, AL, and OS the number of species was seven, six, and three, respectively. In GL no such species were recorded

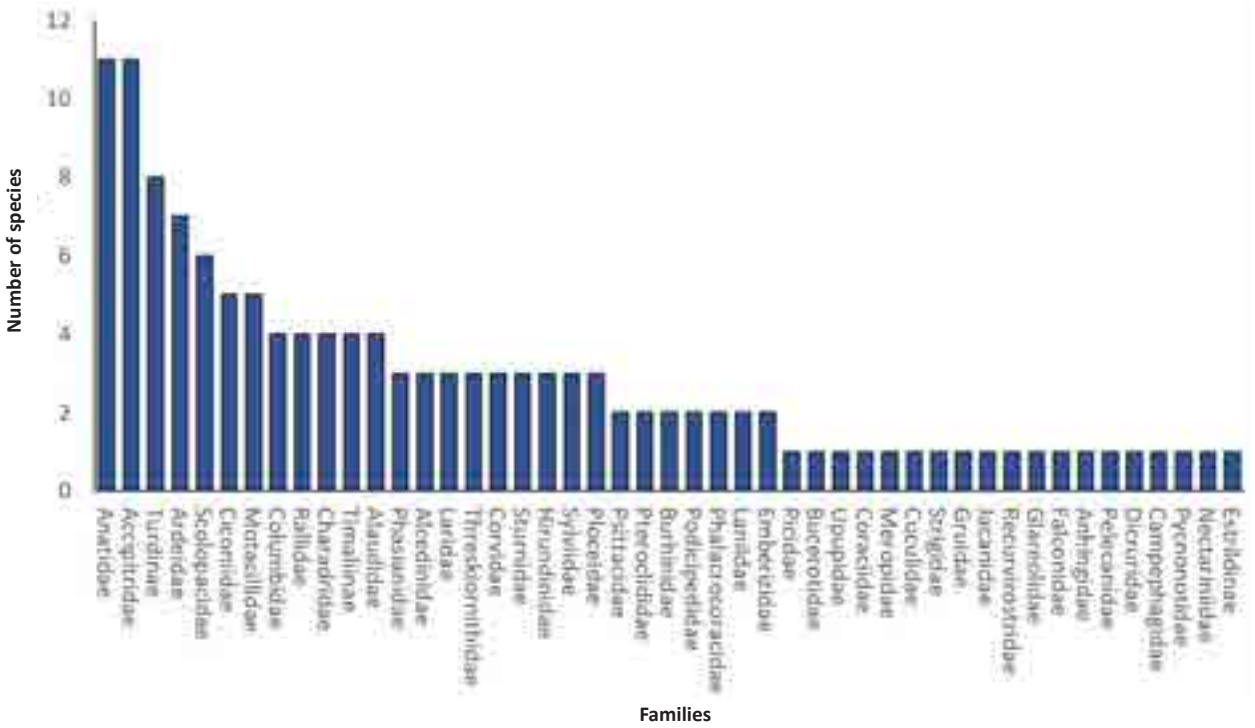

Figure 2. Family wise distribution of bird species recorded during present study.

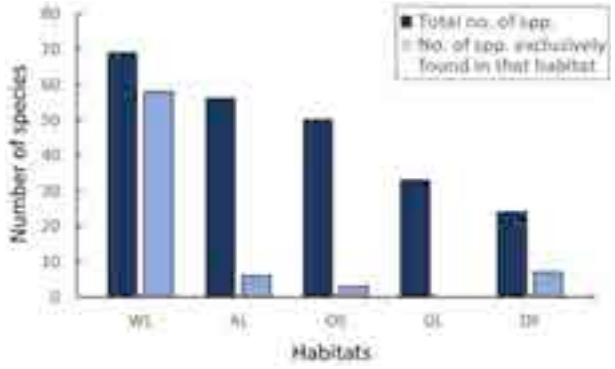

Figure 3. Distribution of bird species in different habitats (total number of species and species exclusively found in that habitat), viz., wetland (WL), grassland (GL), open scrub jungle (OS), agricultural land (AL) and dry deciduous forest (DF).

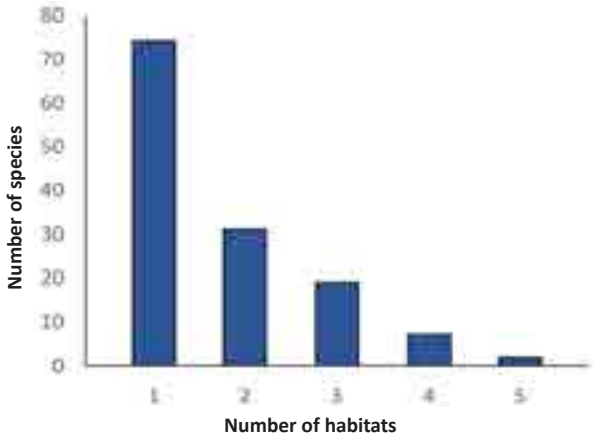

Figure 4. Habitat fidelity of bird species at semi-arid landscape of western Madhya Pradesh. Number of bird species in a single habitat, and consequently 2–5 studied habitats are given in the graph.

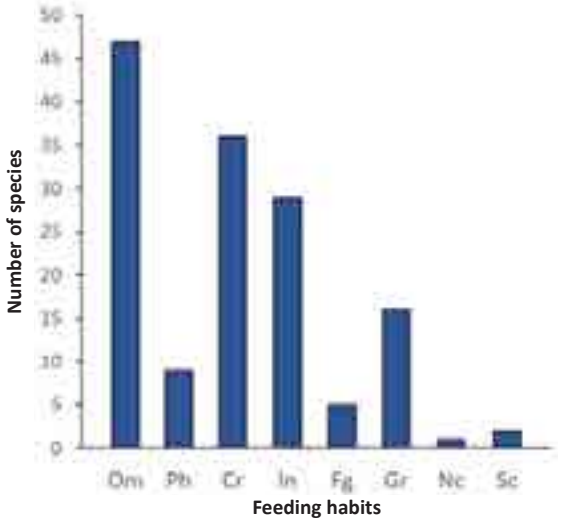

Figure 5. Distribution of bird species of different foraging habits (guilds) i.e., omnivorous (Om), phytophagous (Ph), carnivorous (Cr), insectivorous (In), frugivorous (Fg), granivorous (Gr), nectarivores (Nc) and scavengers (Sc) recorded during present study.

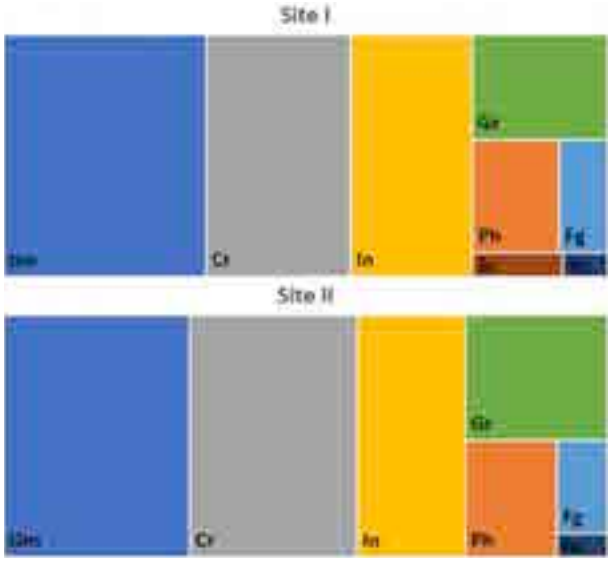

Figure 6. Site wise distribution of bird species belonging to different foraging habits (guilds).

(Figure 3). The rest of the species are found in more than one habitat. 31 species (23.31 %) were found in two habitats, 19 species (14.29 %) in three habitats, seven species (5.26 %) in four habitats, and only two species (1.50 %) were recorded from all five habitats (Figure 4).

A total of eight feeding habits (guilds) were recorded including omnivorous (Om), phytophagous (Ph), carnivorous (Cr), insectivorous (In), frugivorous (Fg), granivorous (Gr), nectarivores (Nc), and scavengers (Sc). Few species had more than one feeding habit and species feeding on diverse food items belonging to different trophic levels were designated as Om. Om birds were most abundant (47 spp.) followed by Cr (36 spp.), In (29 spp.), Gr (16 spp.), Ph (9 spp.), and Fg (5 spp.). Foraging guilds dominated by Om birds were also recorded by several workers like Yashmita-Ulman & Singh (2021), Noreen & Sultan (2022). The increase in the abundance of Om birds is possibly due to a change in food resources as a result of urbanization (Marzluff & Rodewald 2008). Two species, recorded during the present study were scavengers (Sc) viz., *Neophron percnopterus* (Egyptian Vulture), and *Gyps bengalensis* (White-rumped Vulture). Scavengers play an important role in the ecosystem by feeding on carcasses and/or human garbage (Moleón et al. 2014) and are much susceptible to anthropogenic effects (Ogada et al. 2011). Once vultures were abundantly distributed in this region as mentioned by the local inhabitants in and around

Table 1. Checklist of avifauna recorded from semi-arid landscape of western Madhya Pradesh (Site I: Mandsaur and Site II: Ratlam).

Scientific name	Common name	Migratory status	IUCN Red List category	WPA schedule	Site I	Site II	Frequency of observation	Habitat (s)	Feeding habit
Order: Galliformes									
Family: Phasianidae									
Pavo cristatus	Indian Peafowl	R	LC	I	+	+	UC	AL, OS	Om
Francolinus pondicerianus	Grey Francolin	R	LC	IV	+	+	C	GL, OS	Om
Francolinus pictus	Painted Francolin	R	LC	IV	+	+	UC	GL, OS	Om
Order: Anseriformes									
Family: Anatidae									
Anser indicus	Bar-headed Goose	WV	LC	IV	+	+	UC	WL	Ph
Anas crecca	Common Teal	WV	LC	IV	+	+	UC	WL	Ph
Nettapus coromandelianus	Cotton Pygmy-Goose	R	LC	IV	+	+	UC	WL	Ph/Cr
Tadorna ferruginea	Ruddy Shelduck	WV	LC	IV	+	+	UC	WL	Om
Aythya fuligula	Tufted Duck	WV	LC	IV	+	+	UC	WL	Ph/Cr
Anas acuta	Northern Pintail	WV	LC	IV	+	+	UC	WL	Ph/Cr
Anas clypeata	Northern Shoveler	WV	LC	IV	+	+	UC	WL	Cr
Anas poecilorhyncha	Indian Spot-billed Duck	R	LC	IV	+	+	UC	WL	Ph/Cr
Anser anser	Greylag Goose	WV	LC	IV	+	+	R	WL	Ph
Netta rufina	Red-crested Pochard	WV	LC	IV	+	+	UC	WL	Ph/Cr
Aythya ferina	Common Pochard	WV	VU	IV	+	+	UC	WL	Ph/Cr
Order: Piciformes									
Family: Picidae									
Dendrocopos mahrattensis	Yellow-crowned Woodpecker	R	LC	IV	-	+	UC	OS, DF	In
Order: Bucerotiformes									
Family: Bucerotidae									
Ocyceros birostris	Indian Grey Hornbill	R	LC	I	+	+	UC	DF	Om
Order: Upupiformes									
Family: Upupidae									
Upupa epops	Common Hoopoe	R	LC	NI	+	+	UC	AL	In
Order: Coraciiformes									
Family: Coraciidae									
Coracias benghalensis	Indian Roller	R	LC	IV	+	+	C	OS, AL	Om
Family: Alcedinidae									
Ceryle rudis	Pied Kingfisher	R	LC	IV	+	+	UC	WL	Cr
Halcyon smyrnensis	White-throated Kingfisher	R	LC	IV	+	+	C	WL	Om
Alcedo atthis	Common Kingfisher	R	LC	IV	+	+	C	WL	Cr
Family: Meropidae									
Merops orientalis	Green Bee-eater	R	LC	NI	+	+	C	OS, AL	In
Order: Cuculiformes									
Family: Cuculidae									
Eudynamys scolopaceus	Asian Koel	R	LC	IV	+	+	C	DF	Om
Order: Psittaciformes									
Family: Psittacidae									
Psittacula cyanocephala	Plum-headed Parakeet	R	LC	IV	+	+	C	AL, DF	Fg
Psittacula krameri	Rose-ringed Parakeet	R	LC	IV	+	+	C	AL, DF	Fg

Scientific name	Common name	Migratory status	IUCN Red List category	WPA schedule	Site I	Site II	Frequency of observation	Habitat (s)	Feeding habit
Order: Strigiformes									
Family: Strigidae									
Athene brama	Spotted Owlet	R	LC	IV	+	-	UC	OS, AL, DF	Cr
Order: Columbiformes									
Family: Columbidae									
Columba livia	Common Pigeon	R	LC	IV	+	+	C	AL, DF, OS, WL, GL	Gr
Streptopelia decaocto	Eurasian Collared-Dove	R	LC	IV	+	+	C	AL, DF, OS, WL, GL	Gr
Spilopelia senegalensis	Laughing Dove	R	LC	IV	+	+	C	OS, AL, DF	Gr
Spilopelia chinensis	Spotted Dove	R	LC	IV	+	+	C	AL, DF, OS, GL	Gr
Order: Gruiformes									
Family: Gruidae									
Antigone antigone	Sarus Crane	R	VU	IV	+	+	R	AL	Om
Family: Rallidae									
Gallinula chloropus	Common Moorhen	R	LC	IV	+	+	UC	WL	Om
Porphyrio porphyrio	Grey-headed Swamphen	R	LC	IV	-	+	UC	WL	Om
Amaurornis phoenicurus	White-breasted Waterhen	R	LC	IV	+	+	UC	WL	Om
Fulica atra	Eurasian Coot	R	LC	IV	+	+	C	WL	Om
Order: Ciconiiformes									
Family: Pteroclididae									
Pterocles exustus	Chestnut-bellied Sandgrouse	R	LC	IV	+	+	UC	AL	Gr
Pterocles indicus	Painted Sandgrouse	R	LC	IV	-	+	UC	AL, OS	Gr
Family: Scolopacidae									
Gallinago gallinago	Common Snipe	WV	LC	IV	+	+	UC	WL	Cr
Limosa limosa	Black-tailed Godwit	WV	NT	IV	+	+	UC	WL	Cr
Tringa totanus	Common Redshank	WV	LC	IV	+	+	UC	WL	Cr
Actitis hypoleucos	Common Sandpiper	WV	LC	IV	+	+	C	WL	Cr
Tringa stagnatilis	Marsh Sandpiper	WV	LC	IV	-	+	R	WL	Cr
Calidris pugnax	Ruff	WV	LC	IV	+	-	R	WL	Om
Family: Jacanidae									
Metopidius indicus	Bronze-winged Jacana	R	LC	IV	+	+	UC	WL	Om
Family: Burhinidae									
Burhinus oedicnemus	Eurasian Thick-knee	R	LC	IV	-	+	UC	WL	Cr
Esacus recurvirostris	Great Thick-knee	R	NT	IV	-	+	R	WL	Cr
Family: Charadridae									
Charadrius dubius	Little Ringed Plover	R	LC	IV	+	+	UC	WL	Cr
Charadrius alexandrinus	Kentish Plover	WV	LC	IV	+	+	UC	WL	Cr
Vanellus malabaricus	Yellow-wattled Lapwing	R	LC	IV	+	+	UC	WL, AL	Cr
Vanellus indicus	Red-wattled Lapwing	R	LC	IV	+	+	C	WL, AL	Cr
Family: Recurvirostridae									
Himantopus himantopus	Black-winged Stilt	WV	LC	IV	+	+	UC	WL	Cr
Family: Glareolidae									
Cursorius coromandelicus	Indian Courser	R	LC	NI	+	-	R	AL	In

Scientific name	Common name	Migratory status	IUCN Red List category	WPA schedule	Site I	Site II	Frequency of observation	Habitat (s)	Feeding habit
Family: Laridae									
Larus ridibundus	Black-headed Gull	WV	LC	IV	+	+	UC	WL	Om
Sterna aurantia	River Tern	WV	NT	IV	+	+	UC	WL	Om
Chlidonias hybrida	Whiskered Tern	WV	LC	IV	+	-	R	WL	Om
Family: Falconidae									
Falco tinnunculus	Common Kestrel	WV	LC	IV	+	+	C	OS, DF, GL, AL	Om
Family: Accipitridae									
Neophron percnopterus	Egyptian Vulture	R	EN	IV	+	-	R	OS, DF	Sc
Gyps bengalensis	White-rumped Vulture	R	CR	IV	+	-	R	DF	Sc
Milvus migrans	Black Kite	R	LC	I	+	+	C	AL, OS	Om
Elanus caeruleus	Black-winged Kite	R	LC	I	+	+	C	AL, OS	Om
Spilornis cheela	Crested Serpent Eagle	R	LC	I	-	+	UC	DF	Cr
Circaetus gallicus	Short-toed Snake Eagle	R	LC	I	+	+	C	AL, GL, DF, OS	Cr
Circus pygargus	Montagu's Harrier	WV	LC	I	+	+	UC	OS, AL, WL, GL	Om
Accipiter badius	Shikra	R	LC	I	+	+	UC	AL, GL, DF, OS	Cr
Pernis ptilorhynchus	Oriental Honey Buzzard	R	LC	I	+	+	UC	DF	In
Butastur teesa	White-eyed Buzzard	R	LC	I	+	-	UC	AL, GL, OS	Om
Pandion haliaetus	Osprey	WV	LC	I	+	+	UC	WL	Cr
Family: Podicipedidae									
Tachybaptus ruficollis	Little Grebe	R	LC	IV	+	+	UC	WL	Om
Podiceps cristatus	Great-crested Grebe	WV	LC	IV	+	+	R	WL	Om
Family: Anhingidae									
Anhinga melanogaster	Oriental Darter	WV	NT	IV	+	+	R	WL	Cr
Family: Phalacrocoracidae									
Microcarbo niger	Little Cormorant	R	LC	IV	+	+	C	WL	Cr
Phalacrocorax carbo	Great Cormorant	R	LC	IV	+	+	UC	WL	Cr
Family: Ardeidae									
Ardea cinerea	Grey Heron	R	LC	IV	+	+	UC	WL	Cr
Egretta garzetta	Little Egret	R	LC	IV	+	+	UC	WL	Om
Bubulcus ibis	Cattle Egret	R	LC	IV	+	+	C	WL, AL	Om
Ardea alba	Great Egret	R	LC	IV	+	+	UC	WL	Cr
Ardea intermedia	Intermediate Egret	WV	LC	IV	+	+	UC	WL	Cr
Ardeola grayii	Indian Pond Heron	R	LC	IV	+	+	C	WL	Om
Nycticorax nycticorax	Black-crowned Night Heron	R	LC	IV	+	-	UC	WL	Om
Family: Threskiornithidae									
Threskiornis melanocephalus	Black-headed Ibis	R	NT	IV	+	+	UC	WL	Om
Plegadis falcinellus	Glossy Ibis	R	LC	IV	+	-	UC	WL	Om
Platalea leucorodia	Eurasian Spoonbill	R	LC	IV	+	+	UC	WL	Om
Family: Pelecanidae									
Pelecanus philippensis	Spot-billed Pelican	R	NT	IV	+	-	R	WL	Cr
Family: Ciconiidae									
Anastomus oscitans	Asian Openbill	R	LC	IV	+	+	UC	WL	Om
Ciconia nigra	Black Stork	WV	LC	IV	+	+	UC	WL	Cr

Scientific name	Common name	Migratory status	IUCN Red List category	WPA schedule	Site I	Site II	Frequency of observation	Habitat (s)	Feeding habit
Mycteria leucocephala	Painted Stork	R	NT	IV	+	+	UC	WL	Cr
Ciconia episcopus	Woolly-necked Stork	R	VU	IV	+	+	UC	WL	Cr
Ciconia Ciconia	White Stork	WV	LC	IV	+	-	R	WL	Om
Order: Passeriformes									
Family: Laniidae									
Lanius vittatus	Bay-backed Shrike	R	LC	NI	+	+	C	OS	In
Lanius meridionalis	Southern Grey Shrike	R	VU	NI	+	+	UC	OS, AL	Om
Family: Dicruridae									
Dicrurus macrocercus	Black Drongo	R	LC	IV	+	+	C	GL, AL, OS	Om
Family: Corvidae									
Dendrocitta vagabunda	Rufous Treepie	R	LC	IV	+	+	UC	DF	Om
Corvus macrorhynchos	Indian Jungle Crow	R	LC	IV	+	+	C	OS, GL, WL	Om
Corvus splendens	House Crow	R	LC	V	+	+	C	AL, OS, WL, GL	Om
Family: Sturnidae									
Acridotheres tristis	Common Myna	R	LC	IV	+	+	C	AL, OS, GL	Om
Pastor roseus	Rosy Starling	PM	LC	IV	+	+	UC	AL, OS, GL	Om
Gracupica contra	Asian Pied Starling	R	LC	IV	+	-	UC	AL, OS, GL	Om
Family: Hirundinidae									
Ptyonoprogne concolor	Dusky Crag Martin	R	LC	NI	+	-	UC	AL	In
Hirundo rustica	Barn Swallow	WV	LC	NI	+	+	UC	WL, AL	In
Hirundo smithii	Wire-tailed Swallow	R	LC	NI	+	+	UC	WL, AL	In
Family: Campephagidae									
Pericrocotus ethologus	Long-tailed Minivet	R	LC	IV	+	-	R	DF	In/Fg
Family: Pycnonotidae									
Pycnonotus cafer	Red-vented Bulbul	R	LC	IV	+	+	C	OS, AL, DF, GL	Fg
Family: Timaliinae									
Chrysomma sinense	Yellow-eyed Babbler	R	LC	IV	+	-	UC	OS, AL	Om
Turdoides striata	Jungle Babbler	R	LC	IV	+	-	UC	OS, DF	Om
Argya malcolmi	Large Grey Babbler	R	LC	IV	+	+	C	AL, OS, GL	Om
Argya caudata	Common Babbler	R	LC	IV	+	+	C	AL, OS, GL	Om
Family: Sylviidae									
Prinia socialis	Ashy Prinia	R	LC	IV	+	+	C	AL, OS, GL	In
Prinia inornata	Plain Prinia	R	LC	IV	+	+	C	AL, OS, GL	In
Orthotomus sutorius	Common Tailorbird	R	LC	IV	+	+	C	AL, OS, GL, DF	In
Family: Alaudidae									
Eremopterix griseus	Ashy-crowned Sparrow Lark	R	LC	IV	+	+	C	AL, OS, GL	Gr/In
Galerida cristata	Crested Lark	R	LC	IV	-	+	UC	AL	Gr/In
Mirafra erythroptera	Indian Bushlark	R	LC	IV	+	+	UC	OS	Gr/In
Ammomanes phoenicurus	Rufous-tailed Lark	R	LC	IV	+	+	UC	AL, GL	Gr/In
Family: Nectariniidae									
Cinnyris asiaticus	Purple Sunbird	R	LC	IV	+	+	UC	OS, DF, AL	Nc
Family: Ploceidae									
Ploceus philippinus	Baya Weaver	R	LC	IV	+	-	UC	OS	Gr

Scientific name	Common name	Migratory status	IUCN Red List category	WPA schedule	Site I	Site II	Frequency of observation	Habitat (s)	Feeding habit
Passer domesticus	House Sparrow	R	LC	IV	+	+	C	AL, GL	Gr
Gymnoris xanthocollis	Chestnut-tailed Petronia	R	LC	IV	-	+	R	AL, OS	Gr
Family: Estrildinae									
Euodice malabarica	Indian Silverbill	R	LC	IV	+	+	UC	AL, GL, OS	Gr
Family: Turdinae									
Monticola solitarius	Blue Rock Thrush	WV	LC	IV	+	-	UC	OS, AL	In
Saxicoloides fulicatus	Indian Robin	R	LC	IV	+	+	C	AL, OS	In/Fg
Copsychus saularis	Oriental Magpie Robin	R	LC	IV	+	+	UC	AL, OS, DF	In
Phoenicurus ochruros	Black Redstart	WV	LC	IV	+	+	UC	OS, GL	In
Saxicola torquata	Common Stonechat	WV	LC	IV	+	+	C	AL, GL, OS	In
Saxicola caprata	Pied Bushchat	WV	LC	IV	+	+	UC	AL, GL, OS	In
Oenanthe isabellina	Isabelline Wheatear	WV	LC	IV	+	-	R	AL, GL	In
Cyanecula svecica	Bluethroat	WV	LC	IV	+	+	UC	WL, GL	In
Family: Motacillidae									
Motacilla alba	White Wagtail	WV	LC	IV	+	+	UC	WL	In
Motacilla maderaspatensis	White-browed Wagtail	R	LC	IV	+	+	UC	WL	Om
Motacilla citreola	Citrine Wagtail	WV	LC	IV	+	-	UC	WL	In
Motacilla cinerea	Grey Wagtail	WV	LC	IV	+	+	UC	WL	In
Anthus rufulus	Paddyfield Pipit	R	LC	IV	+	-	UC	GL, AL	In
Family: Emberizidae									
Emberiza melanocephala	Black-headed Bunting	WV	LC	IV	+	+	R	AL, OS	Gr
Melophus lathami	Crested Bunting	R	LC	IV	+	+	UC	OS, GL	Gr

Migratory status: R—Resident | WV—Winter visitor | PM—Passage migrant; IUCN Category: CR—Critically Endangered | EN—Endangered | LC—Least Concern | NT—Near Threatened | VU—Vulnerable; WPA Schedule: NI—bird species that are not included either in Schedule-I, Schedule-IV or Schedule-V of the Wildlife (Protection) Act, 1972; Frequency of observation: R—Rare | C—Common | UC—Uncommon; Habitat type: WL—Wetland | GL—Grassland | OS—Open scrub jungle | AL—Agricultural land | DF—Dry deciduous forest; Feeding habit: Om—omnivorous | Ph—phytophagous | Cr—carnivorous | In—insectivorous | Fg—frugivorous | Gr—granivorous | Nc—nectarivores | Sc—scavenger.

the study site, but during our study period, they were recorded only from Site I and the encounter rate was rare (less than 5 individuals in the entire study area) for both the species. The decline in the number of vultures may be attributed to the use of diclofenac drugs, habitat destruction, food scarcity, deforestation, and other reasons like power line collisions & natural disasters (Jha et al. 2020). *Cinnyris asiaticus* Purple Sunbird was the only Nc species observed during the present study (Figure 5). Site-wise composition of avian species based on feeding habit was represented in Figure 6.

During our present study, we recorded more than 400 individuals of *Anser indicus* Bar-headed Goose (Image 2A) from a partially dried-up wetland. This species is assessed as Least Concern (LC) according to the IUCN Red List. Though the population trend appears to be declining (BirdLife International 2020b), based on the IUCN Red List of threatened species (version 2020-1), Critically Endangered species like *Gyps bengalensis* (White-rumped Vulture, Image 2B), Endangered species like *Neophron percnopterus* (Egyptian Vulture, Image 2C), Vulnerable species like *Aythya ferina* (Common Pochard), *Antigone antigone* (Sarus Crane), *Lanius meridionalis* (Southern Grey Shrike, Image 2D) and *Ciconia episcopus* (Woolly-necked Stork, Image 2E) were recorded during the present study. Near Threatened bird species like *Threskiornis melanocephalus* (Black-headed Ibis, Image 2F), *Limosa limosa* (Black-tailed Godwit, Image 2G), *Esacus recurvirostris* (Great Thick-knee, Image 2H), *Anhinga melanogaster* (Oriental Darter), *Mycteria leucocephala* (Painted Stork, Image 2I), *Sterna aurantia* (River Tern, Image 2J) and *Pelecanus philippensis* (Spot-billed Pelican, Image 2K) were also observed in the study area. Besides 11 avian species listed in Schedule I under the Wildlife (Protection) Act, 1972 were recorded during the present study. The majority of the globally threatened species were recorded exclusively from wetlands. During wintering seasons these water bodies

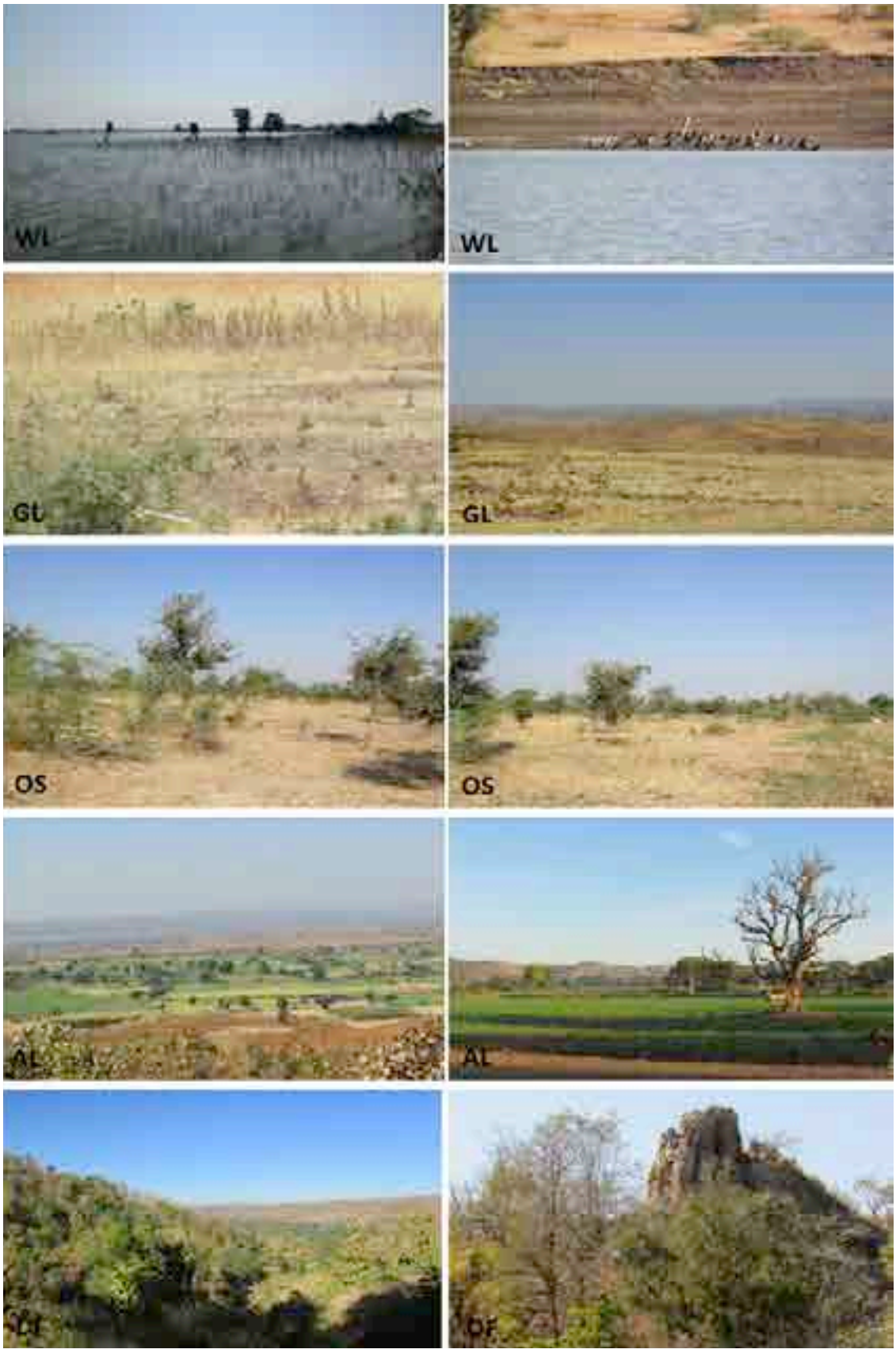

Image 1. Photographs of different habits i.e., wetland (WL), grassland (GL), open scrub jungle (OS), agricultural land (AL) and dry deciduous forest (DF), identified for present study at semi-arid landscape of western Madhya Pradesh.

Image 2. Some species recorded during present study: A—Bar-headed Goose *Anser indicus* | B—White-rumped Vulture *Gyps bengalensis* | C—Egyptian Vulture *Neophron percnopterus* | D—Southern Grey Shrike *Lanius meridionalis* | E—Woolly-necked Stork *Ciconia episcopus* | F—Black-headed Ibis *Threskiornis melanocephalus* | G—Black-tailed Godwit *Limosa limosa* | H—Great Thick-knee *Esacus recurvirostris* | I—Painted Stork *Mycteria leucocephala* | J—River Tern *Sterna aurantia* | K—Spot-billed Pelican *Pelecanus philippensis*.

were used for irrigation of agricultural fields. This water usage accelerates the drying of small wetlands. To protect the waterbird diversity in this region proper management policies should be taken. It is necessary to adopt alternative irrigation schemes for farmers to conserve the wetland habitats.

This semi-arid landscape had immense importance in the avian study. There were two Important Bird and Biodiversity Areas (IBAs) situated within the present study area viz, Gandhisagar Reservoir (IN-MP-06) and Sailana Kharmor Sanctuary (IN-MP-15). This region especially, Sailana Kharmor Sanctuary is a native breeding ground

of endangered Lesser Florican *Sypheotides indicus* and many other species (Rahmani et al. 2016). This region of the Indian subcontinent serves as a terminus for avian species who migrate following the Central Asian/ South Asian Flyway. These birds breed in the northern part of Russia (Siberia) in the east to as far west as Europe covering parts of China, central and western Asia. *Anser indicus*, the world's highest-altitude migrant follow this route directly over the Himalaya (BirdLife International 2020a) and congregate in different wetlands of sub-Himalayan regions. The presence of a large non-breeding wintering population of *Anser indicus*, never reported earlier within the study site indicated the importance of conservation of wetland habitats of this region.

The present study area is situated within Biome 11 (i.e., Indo-Malayan tropical dry zone) and harboures many biome-restricted birds. BirdLife International identified 59 such species from this biome. We recorded several biome-restricted species which included *Gyps bengalensis* (White rumped vulture), *Butastur teesa* (White-eyed Buzzard), *Pavo cristatus* (Indian Peafowl), *Vanellus malabaricus* (Yellow-wattled Lapwing), *Psittacula cyanocephala* (Plum-headed Parakeet), *Ocyceros birostris* (Indian Grey Hornbill), *Eremopterix griseus* (Ashy-crowned Sparrow-lark), *Saxicoloides fulicata* (Indian Robin), *Turdoides malcolmi* (Large Grey Babbler), *Turdoides striatus* (Jungle Babbler), *Prinia socialis* (Ashy Prinia) (Parveen & Ilyas 2019).

The presence of significant numbers of winter migrants, globally threatened species, biome–restricted species amply supported that this semi-arid landscape was both ecologically and biologically significant for breeding and migratory birds. However, presently avian populations in semi-arid regions are declining rapidly due to unplanned developmental activities, urbanization, and climate change (Khan et al. 2019). Therefore, it is need of the hour to develop different conservation strategies like raising awareness among local residents, community participation, long term monitoring, and research, conservation of unique avian habitats, and restoration of degraded habitats. Implementation of proper management policies both at the central and state government levels with the involvement of different stakeholders is necessary to protect the avian species and their habitats outside the protected areas.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21002–21009

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.6692.14.5.21002-21009

#6692 | Received 09 September 2020 | Final received 13 April 2022 | Finally accepted 27 April 2022

OPEN ACCESS

COMMUNICATION

Post-release growth of captive-reared Gharial *Gavialis gangeticus* (Gmelin, 1789) (Reptilia: Crocodilia: Gavialidae) in Chitwan National Park, Nepal

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Abstract: Supplementation of wild populations of the Critically Endangered Gharial *Gavailis gangeticus* with individuals reared in captivity is a widely used conservation management tool in Nepal and India, although its efficacy is uncertain. Measuring post-release growth in Gharial can provide valuable information on acclimation of captive-reared Gharial to the wild and provide growth rates to inform population recovery models. We studied post-release growth of Gharial reared in the Gharial Conservation Breeding Centre, Nepal, following their release into the Chitwan National Park. We used recapture data from known individuals to determine growth and change of mass for 26 Gharial recaptured 0.5–10 years after release. We found that Gharial recaptured two or more years post-release had increased in mass and length despite being over six years old at release, however there was a triangular relationship between time since release and growth: some Gharial had grown very slowly, whilst others had grown much faster. All Gharial recaptured less than two years since release had lost mass and had negligible growth in total length. This data show that there is considerable variation in post-release growth rates, which will lead to some individuals being very old before they reach a potentially mature size class, with unknown implications for reproduction. This variation is important for predicting or modelling recovery in populations where the release of Gharial from captivity is a management tool. Our results also suggest the two years after release are an acclimation phase—when Gharial lose mass and do not grow—which should be considered by release strategies in order to give Gharial the best chance of survival after release.

Keywords: Conservation, Crocodylia, growth rate, head starting, rear and release.

Abbreviations: CNP—Chitwan National Park | GCBC—Gharial Conservation and Breeding Centre | OLS—Ordinary least squares regression | SVL—Snout-vent length | TL—Total length.

Editor: Raju Vyas, Vadodara, Gujarat, India. **Date of publication:** 26 May 2022 (online & print)

Citation: Khadka, B.B., A. Bashyal & P. Griffith (2022). Post-release growth of captive-reared Gharial *Gavialis gangeticus* (Gmelin, 1789) (Reptilia: Crocodilia: Gavialidae) in Chitwan National Park, Nepal. *Journal of Threatened Taxa* 14(5): 21002–21009. https://doi.org/10.11609/jott.6692.14.5.21002-21009

Funding: Chitwan National Park and Gharial Conservation Breeding Centre. Recaptures were supported by the National Geographic Society, the UK Trust for Nature Conservation in Nepal and the NERC Oxford Environmental Research Doctoral Training Partnership (NE/L002612/1).

Competing interests: The authors declare no competing interests.

Author details: Bed Bahadur Khadka has wide experience in wetland and freshwater ecology and has been studying Gharials for the last 18 years. Presently, he works as consultant to the Gharial Conservation Breeding Centre. Ashish Bashyal is a co-founder of Biodiversity Conservancy Nepal—a non-profit dedicated to wildlife conservation in Nepal. He has been studying genetics and ecology of crocodilians including Gharials since 2009. Phoebe Griffith is a conservation scientist, specializing in crocodilians. She is currently a DPhil candidate at the Zoological Society London and University of Oxford, and research fellow at Himalayan Nature, studying the ecology and conservation of gharial crocodilians in Nepal.

Author contributions: BK conceptualized and designed the study. BK and PG collected data, and AB and PG performed data analysis. All authors interpreted the results and prepared the manuscript.

Acknowledgements: We thank the Department of National Parks and Wildlife Conservation, Chitwan National Park, and chief conservation officer Narayan Rupakheti. Thank you to all the staff at the Gharial Conservation Breeding Centre, nest watchers Kale and Suka Maya Bote, and the late Som Lal Bote for their vital contributions in the field. We thank the ZSL Nepal Office, Himalayan Nature and NTNC. Thanks also to Ram Kumar Aryal, Dr Baburam Lamichhane, and Santosh Bhattarai at NTNC BCC.

INTRODUCTION

The Critically Endangered Gharial *Gavialis gangeticus* crocodile was once common in the Narayani River of southern Nepal, as well as its tributaries including the Kali Gandaki and Rapti, with hundreds of Gharial found in the lower Narayani prior to the construction of the dam on the Indo-Nepal border (Maskey 1989). However, by the 1970s the population had crashed, and in response the Government of Nepal instigated the Gharial Conservation Breeding Centre (GCBC), based at Kasara, Chitwan National Park (CNP) in 1978 (Maskey 1989). At the GCBC, Gharial eggs laid in captivity or collected from the wild are reared in captivity until they reach a size of 1.5–2 m, usually at an age of 5–7 years, when they are released into rivers within the species' range in Nepal (Khadka 2012). The goal of the GCBC is to reinforce Nepal's Gharial populations, with a major focus on Chitwan (Khadka 2010; Acharya et al. 2017).

Before 2004, the vast majority of Gharial were released in the Narayani River (375 from 1981–2003). However, the population of Gharial did not recover (Maskey & Percival 1998; Ballouard et al. 2010), and the release programme was shifted predominantly to the Rapti in 2004, with all Gharial released in the Rapti since 2008. To date (April 2022), 972 Gharial have been released in the Rapti, with 788 of these released from 2006 onwards (Bed Bahadur Khadka, pers. comm. April 2022). It was estimated that captive-released Gharial had survival rates of only 7% in the Narayani (Maskey & Percival 1998), however the rear-and-release programme has seen greater success since the shift to releasing the Gharial individuals in Rapti. The overall Gharial population in Chitwan is estimated to have increased from 39 in 2005, to a minimum count of over 200 in 2022 (Acharya et al. 2017; Khadka 2022). However, even if the entirety of this increase is attributed to the head-start programme, this still accounts for only about 30% of released the Gharials, which suggests that mortality and/or loss from the system remains high. Ballouard et al. (2010) suggested the first two years after release was a time of particularly low survival (~20% survival) for released Gharials.

There is currently less data on growth rates of Gharial reared in captivity followed by release into the wild. Work on captive and released crocodilians suggests that released animals may not thrive, especially immediately after release (Blake & Loveridge 1975; Singh 1978). Growth rate data for Gharial at different stages post-release into a natural system such as the Rapti River will be very informative to understand acclimation of captive-reared Gharial to wild conditions, provide growth rates to inform population recovery models, and also indicate at which stage there is likely to be high mortality as limited growth and negative or limited mass changes may indicate difficulties in adapting to conditions post-release.

The goal of this study was to investigate post-release growth rates in recaptured Gharials, in order to:

1. inform GCBC release strategy, by providing a better understanding of the post-release response of Gharial to their new environment in terms of change in mass and growth,
2. inform predictions of population recovery, by providing a better understanding of the variation in Gharial growth rates and the time taken for Gharial to reach a potentially reproductive size class.

MATERIALS AND METHODS

This study evaluated growth rates in length and mass following release of Gharials raised in captivity into the wild. We used recapture data from known individuals to determine growth and change of mass for 26 Gharials released from the GCBC, 0.5–10 years after release.

This study took place on the Rapti River and its tributaries, the Dhugre Khola and the Budhi Rapti, in and around Chitwan National Park (CNP), southern Nepal (Image 1). The Rapti is a tributary of the larger Narayani River, and Gharials freely move between the two rivers. The Rapti River forms the northern boundary of the CNP, whilst the Dhugre Khola and Budhi Rapti fall within community forest outside the northern park boundary. Our team was made up of staff from the GCBC and catchers from the indigenous fisherfolk communities. We captured the Gharials in daytime using either a throw net deployed from a dugout canoe, or via gill nets drifted along basking sand banks, with one end attached to a float and the other held by a person upstream. Once basking Gharial was located, long gill nets were set up under the water adjacent to the bank. The Gharials were captured by flushing them into water, after which they became entangled when they dived into the net, or were captured by traditional throw nets cast from the canoes offshore. In clear, shallow water Gharials were located underwater, and captured using throw nets. Following entanglement, we used hessian sacks to blindfold the Gharial whilst still in the water, then removed captures to the nearest shore, where the Gharials were restrained on ladders to minimise risks during measurements.

For all captured individuals, total length (TL; distance

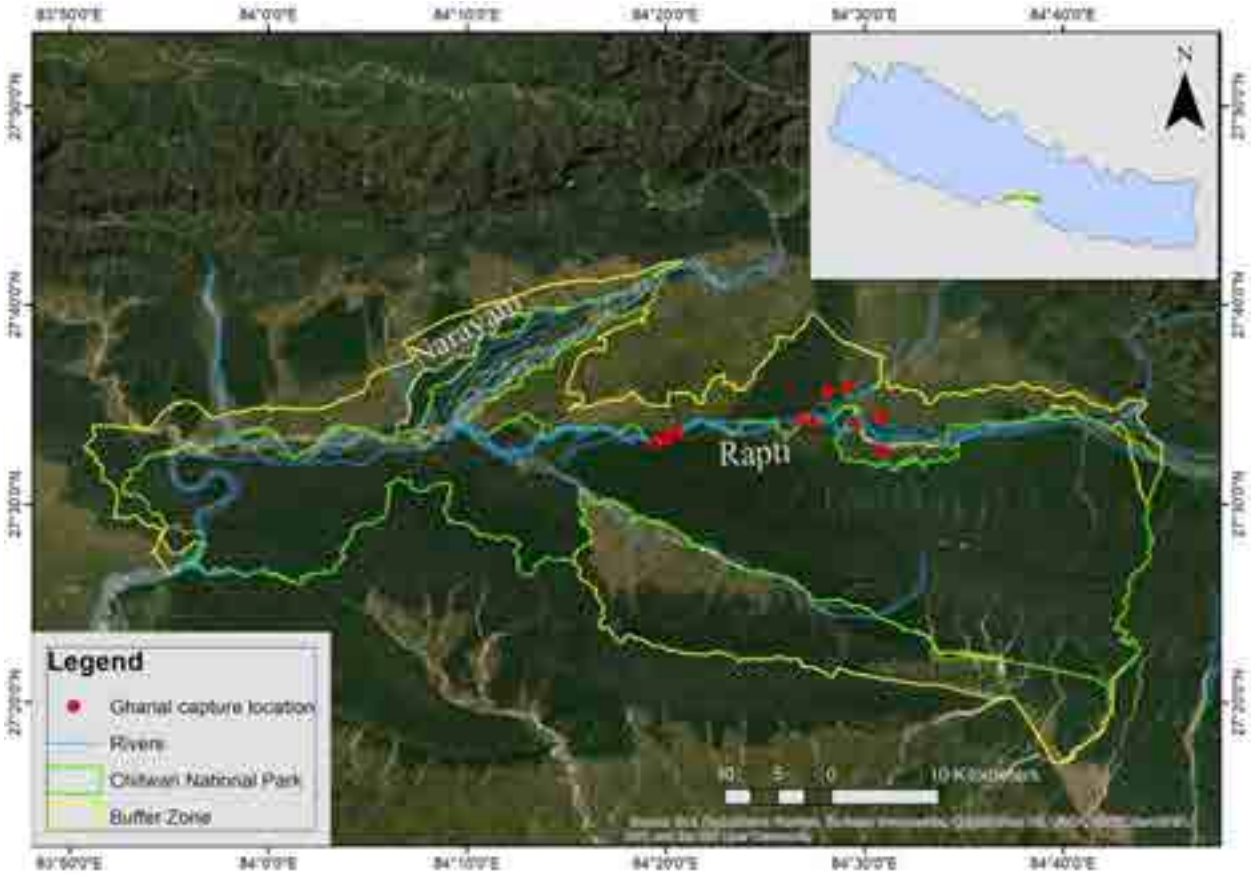

Image 1. Map of the Study Site in Chitwan National Park. The major rivers (Rapti and Narayani) are labelled, and locations of Gharial capture are indicated. Inset shows position of Chitwan National Park in Nepal.

from anterior tip of the snout to the posterior tip of the tail) was measured to the nearest 0.5 cm, and mass was measured to the nearest 0.5 kg. If captured Gharial had clipped tail scutes, we matched position of clipped scutes to the catalogue of previously marked Gharial maintained at the GCBC. Sex was determined at recapture by physical examination of the outer genitals and were designated male, female or indeterminate. Indeterminate individuals had intermediate sized genital organs that could not confidently be designated as either a clitoris or penis. After morphometric and sex measurements were made, Gharial were released back into the river at their capture location. The total process from capture to release took less than one hour. Gharial were recaptured from 2005–2019 as part of the GCBC programme for detangling Gharial from fishing nets (n = 7), and from 2018–2019 as part of an ongoing telemetry study (n = 19). Size classes were designated as adult (>300 cm TL), sub-adult (200–299 cm TL) and juvenile (100–199 cm TL).

To determine growth rates, morphometric values (TL, mass) were compared to the same values recorded at the time of release, (measured using the same protocol described above for recaptured Gharial) which are kept on record in the GCBC database. Morphometric measurements of all Gharial released from the GCBC are taken and recorded on the day of release as part of standard practice at the centre.

A linear regression was carried out to establish whether time since release significantly predicted growth (in TL and mass). A Breusch-Pagan test showed there was heterogenous variance in both the mass and TL linear regression models. Therefore, a quantile regression was used to model empirical relationships between time since release and mass or TL. To identify which quantile regression predictions fell outside of the confidence intervals of the ordinary least squares regression, we used a stepwise approach identify quantile regressions at 5% intervals from the 5–95 % quantile.

Total length was used for all analyses rather than snout-vent length (SVL), as TL and SVL in this study were highly correlated both at time of release ($r = 0.99$, $p < 0.001$, $n = 26$) and for recaptured Gharials ($r = 0.99$, p

<0.001, n = 18). We corrected for missing values (TL or mass) for some individuals by using a scaling relationship that predicted the missing values based on the data we had for recaptured Gharial for which complete measurements were available. The relationship was: mass = $1.3602*TL^{3.7175}$ (R^2 = 0.9824, n = 18). A scaling relationship of this form has been shown to be appropriate for crocodilians (Grigg & Kirshner 2015). These computed values are designated with asterisks in Table 2. We only used this method for Gharials in good condition for which we had a minimum mass estimate (>80 kg, the maximum of our equipment). For four Gharials we did not have a mass measure or minimum mass estimate, and these individuals had poor body condition. Therefore, the mass of these Gharials was excluded from the analysis.

Growth rates for Gharials released for two years or less (mass n = 8; TL n = 10) were calculated by taking the mean change in mass or TL and dividing it by time since release (in years) to give as estimated per-year change. We used a paired t-test to determine whether per-year change in mass and TL for these Gharials differed from the change in mass and TL predicted by the ordinary least squares regression. One Gharial was excluded from all mass analyses as it was recaptured for welfare reasons due to extreme emaciation following a long-term entanglement in a gill-net. Analysis was conducted in R (R Core Team 2013), with the package 'quantreg' (Koenker 2020) used for quantile regressions. Figures were produced using the package ggplot2 (Wickham 2016).

RESULTS

The 26 Gharials included in this study were recaptured 0.5–10 years after release. Gharial recaptured less than two years post-release had generally lost mass and grown negligibly. Gharial released over two years earlier had all increased in length and mass, but the relationship was triangular, with some Gharial growing very slowly, and others much faster. We collected morphometric measurement from a total of 28 Gharials, however two were excluded from this study as they had not been previously scute clipped.

Time since release significantly predicted post-release change in mass (B = 10.18±2.80, t = 7.86, p <0.01) and accounted for 74% (adjusted R^2) of variability in mass change, according to the ordinary least squares linear regression (OLS). However, the relationship was triangular in shape: all Gharials released within less than two years had lost or maintained mass, whereas Gharials released for after more than two years split into individuals that had grown considerably, and those that had grown very little (Figure 1). The quantile regressions showed that at the lowest and highest quantiles, the quantile coefficients fall outside the confidence intervals of the OLS coefficient. At the 5%, 10% and 15% quantiles the coefficient was lower than that of the OLS (slow growth), and at the 90% and 95% quantiles the coefficient was higher than that of the OLS (fast growth; Table 1, Figure 1a).

A very similar pattern was seen in post-release TL growth. Time since release (predictor variable) significantly predicted the response variable of growth in total length (B = 17.94±3.93, t = 9.42, p <0.01) and accounted for 78% (adjusted R^2) of variability in total length growth. This relationship was also triangular, with the quantile regression (see Figure 1b and Table 1) showing that at the 15% and 20% quantiles the coefficient was lower than that of the OLS (slow growth), and at the 90% and 95% quantiles the coefficient was higher than that of the OLS (fast growth).

This variation in post-release growth of both mass and TL can be seen clearly in the data (Table 2), for example two Gharials released 5.67 and 5.75 years before capture showed a difference in mass change of 30.5 kg and TL change of 40 cm, when at their release in the same year their difference in mass was just 2.5 kg and in TL was just 3 cm. As a consequence, Gharials from the GCBC will reach a size of 300 cm (thought to be adult size) at very different ages: one slow-growing Gharial is only 247 cm at 15.5 years old, whilst another is 306 cm at 9.92 years old.

There was no correlation between age and either TL or mass at release: older Gharials in our sample were

Table 1. Value of regression coefficient (estimated change in growth (TL or mass) per year post-release) for differing quantiles that fall outside of the confidence intervals of the ordinary least squares (OLS) model, with OLS regression as reference.

	Value of Regression Coefficient	
Quantile	Mass	Total Length
5%	5.93	
10%	5.93	
15%	5.46	13.57
20%		12.91
90%	13.01	22.01
95%	13.01	24.27
OLS Estimate	10.18±2.80	17.94±3.93

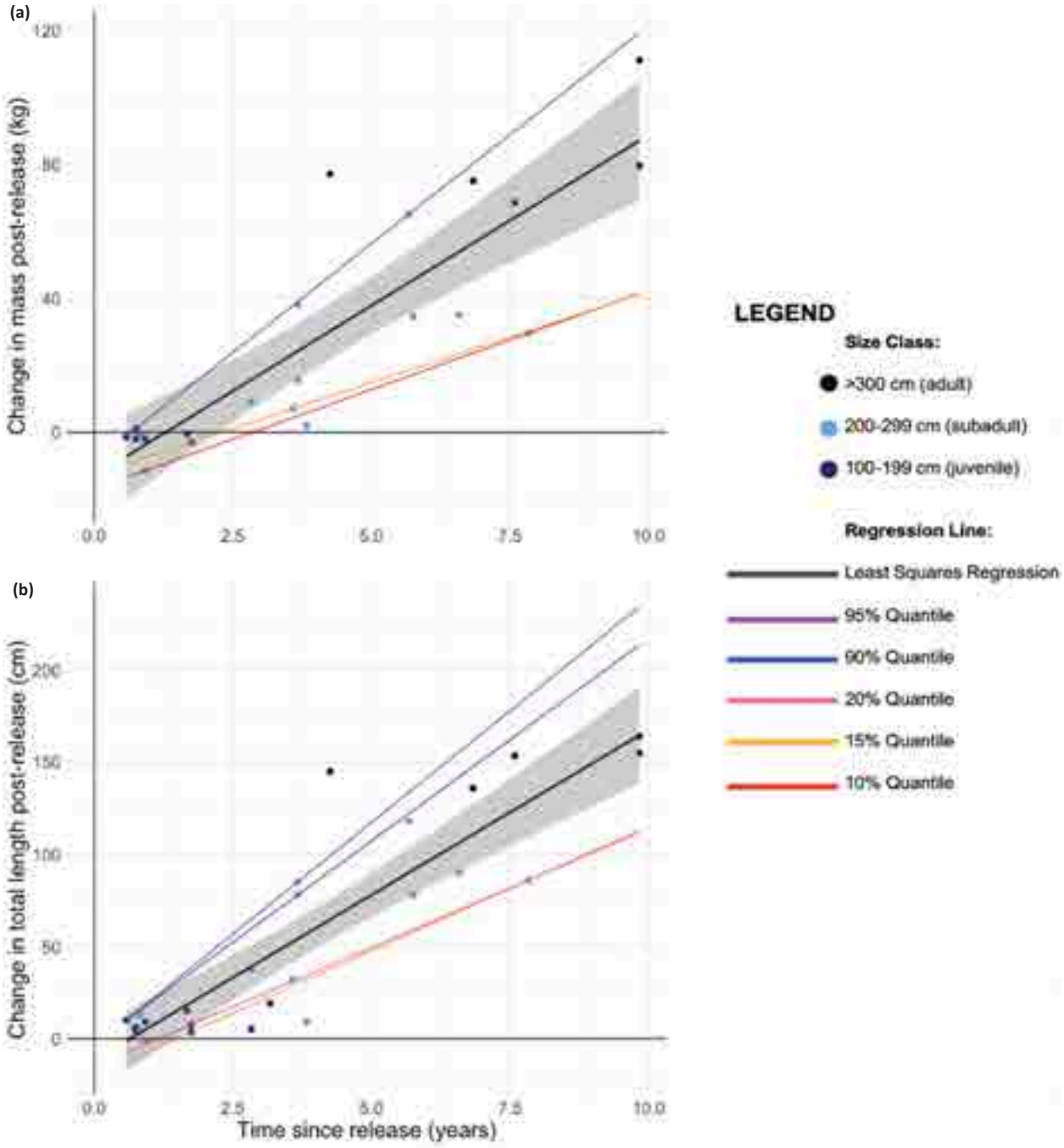

Figure 1. Change in (a) mass, n = 22 or (b) change in total length post-release, n = 26 for Gharial since release from captivity. Predictions are illustrated with the ordinary least squares (OLS) regression showing the mean growth trajectory, the 95% and 90% quantile regressions illustrate the estimated fast growth trajectory, with the 10%, 15%, and 25% quantile regressions illustrating the slow growth trajectory. The grey shaded area illustrates the confidence intervals of the OLS regression. Each data point is an individual Gharial colourised to show size-class at recapture.

no longer or heavier than younger Gharial upon release from captivity.

Mass change was positively correlated with time since release (Pearson's r = 0.86, n = 21, p = 0.01). However, all except one of the Gharials released less than two years ago had lost weight after release. The paired t-test estimated the mean change in mass for the two years following release was between -6 and +0.4 kg per year (t(7) = 2.36, p = 0.05, n = 8), considerably less than the OLS prediction of 10.18±2.80kg increase in mass per year.

Total length was positively correlated with time

Table 2. Measurements taken at both release and recapture of 26 captive-reared Gharial released into the Rapti River. Sex is stated as male (M), female (F) and indeterminate (I). Gharial for which the relationship mass = 1.3602*TL3.7175 was used to calculate mass (n = 4) or length at release (n = 1) are marked as so*. Four Gharial do not have a mass value and mass couldn't be estimated due to poor body condition.

Release				Recapture					
Release Date	Age (years)	Total Length (cm)	Mass (kg)	Sex	Capture Date	Age (years)	Age Difference Post-Release	Total Length (cm)	Mass (kg)
11-xi-2004		197		M	31-viii-2005	0.83	0.83	210	
02-ii-2013	5.67	172	16.5	M	25-viii-2013	6.25	0.58	182	15
02-ii-2010	5.67	162	10.5	F	06-x-2013	9.34	3.67	247	26
02-ii-2010	5.67	161	10	F	14-v-2014	9.92	4.25	306	**87***
05-iii-2014	7.75	173	12.5	F	09-v-2017	10.92	3.17	192	
07-ii-2017	6.67	205	29	F	08-i-2018	7.58	0.92	203	17.5
10-iii-2018	5.75	173	13	F	26-xi-2018	6.5	0.75	179	11
09-iii-2018	5.75	170	12	F	27-xi-2018	6.5	0.75	174.5	13
14-ii-2018	7.67	200	22	F	27-xi-2018	8.5	0.83	204	21
02-ii-2012	10.67	177	20	F	27-xi-2018	17.5	6.83	313	**95***
09-ii-2016	5.67	181	15	I	28-xi-2018	8.5	2.83	218.5	24
08-iii-2018	6.75	181	15.5	F	28-ii-2019	7.67	0.92	190	13.5
10-iii-2018	5.75	170	13.5	F	11-xi-2019	7.42	1.67	185	13
05-iv-2016	5.83	184	18	F	11-xi-2019	9.42	3.58	216	25
05-iii-2014	8.75	182	17.5	F	16-xi-2019	14.5	5.75	260	52
10-iii-2018	5.75	176	13	M	17-xi-2019	7.5	1.75	179.5	10
05-iv-2016	5.83	192	18	I	18-xi-2019	9.5	3.67	270	56
24-iii-2014	6.83	179	15	F	18-xi-2019	12.5	5.67	297	80
20-iv-2013	5.92	184	19	M	19-xi-2019	12.5	6.58	274	54
19-iv-2012	6.92	151	8.5	F	19-xi-2019	14.5	7.58	304.5	77
02-ii-2012	7.67	161	11.5	F	20-xi-2019	15.5	7.83	247	41
02-ii-2010	5.67	171	11	F	25-xi-2019	15.5	9.83	335	**122***
02-ii-2010	5.67	150	6.5	F	26-xi-2019	15.5	9.83	305	**86***
07-ii-2017	5.67	176	12.5	F	27-xi-2019	8.5	2.83	181	
09-iii-2018	5.75	181	14.5	F	09-xii-2019	7.5	1.75	188.5	
10-ii-2016	5.67	**200***	18	F	09-xii-2019	9.5	3.83	209	20

since release (Pearson's r = 0.91, n = 26, p = 0.01). All Gharials released less than years ago had shown only slight growth in TL, the paired t-test estimated the mean increase in TL for the two years following release was between 3.47 and 11.42 cm per year (t(10) = 2.26, p = 0.05, n = 10), less than the OLS prediction of 17.94±3.93 increase in TL per year, indicating that all Gharial grow slower (if at all) in the two years post-release.

DISCUSSION

Previous work (Singh 2018) found that TL growth in Gharial drops very suddenly around the 6th or 7th year, however we found that Gharials released from the GCBC continue growing post-release, despite their age (5.67–10.67 years old at release). This suggest there are factors limiting growth in captivity before release. The lack of correlation between age and TL or mass at the point of release suggests individuals are small for their age at release, especially the older Gharials. Singh (2018) found that when the TL growth rate dropped at the 6th or 7th year, the Gharial in his study had attained a near-adult

length, and most facilities have found fast growth rates for captive Gharials, with them reaching over 200 cm in 3–4 years (Singh 1978, 2018; De Vos 1982). Growth rates at the GCBC in Nepal are slower, with Gharial reaching sizes of ~150 cm within four years of hatching (Khadka & Bashyal 2019). Gharials are therefore already 5+ years old when they are released from the GCBC, but a long way off mature size (300 cm for females, 400 cm for males). After release in suitable riverine habitat, the Gharials in this study resumed varying rates of growth, with some individuals reaching adult size at the time of recapture. The impact of this delayed maturity on the head started Gharial is unknown. The similar values for TL growth post-release of adult-sized Gharial at recapture, regardless of time since release, suggests that growth in length slows once Gharials reach adult size, likely indicating a shift in energy allocation from somatic growth to reproduction (Czarnołe'ski & Kozłowski 1998).

We found a large amount of variation in the growth rates of Gharials that had been released longer than two years. This variation is substantial – in the 5% quantile, mass change is estimated at a 5.93kg increase per year, whereas at the 95% quantile mass change estimate is as double this – at 13.01 kg per year. Most Gharials followed either a 'fast growth' or 'slow growth' trajectory. The underlying cause of this variation is not known, but it suggests there are key factors impacting post-release growth that we have not yet been measured. These differing growth rates will lead to some individuals reaching maturity much later than others – slow growing individuals could be close to 20 years old before reaching an adult size, which could have implications for reproduction. The differing lengths of time taken for Gharials to be recruited into the potentially reproductively active adult size class is also important for predicting population recovery, and should be incorporated into population models for Gharial management in Chitwan. Slow growing individuals will also spend a longer time in the smaller size classes, when they appear to be more vulnerable to threats such as net entanglement. Substantial variation in growth rates between individuals have also been found in captive studies of Gharials (Singh 1978; Khadka & Bashyal 2019), but the reasons underlying this variation are unclear.

Our results showed that Gharial lose mass in their first year or two after release, and gain mass after a 1–2 year acclimation phase, especially once they reach >300 cm. Gharial also appear to only increase TL very slowly in this acclimation phase. Singh (2018) also reported that Gharial growth rate will slow following a 'shift', such as to a new habitat or pen, for at least a year. This was suspected to be due to the shock of a shift to a new habitat, with time required to enable crocodilians to adjust and resume normal feeding.

One potential cause of the loss in mass and reduced growth rate is the new environment (Blake & Loveridge 1975; Singh 2018). This shift may lead to a difficulty or time-lag in shifting from eating dead fish to hunting live prey, increased activity related to adapting to riverine flow, and the need to find a suitable habitat to settle in and avoid new threats such as predators and entanglement in illegal fishing gear. The direct impact of these challenges, may cause chronic stress for the Gharials, and stress is thought to be a major cause of high mortality rates in reintroductions (Teixeira et al. 2007). Studies on crocodilians in captivity show a strong negative relationship between levels of corticosterone (stress hormone) and increase in body mass (Elsey et al. 1990; Morici et al. 1997; Turton et al. 1997), suggesting that crocodilians that lose weight are likely to also be physiologically stressed. Physiologically stressed crocodilians show elevated mortality rates (Morici et al. 1997), which could contribute to high mortality in the immediate two-years post-release that has been recorded by Ballouard et al. (2010). Gharials are currently released with a 'soft-release' approach: they are placed in in-situ grass enclosures at the river to acclimate to flow, and after some time break out themselves. The post-release loss of mass in Gharials from the GCBC suggests that this soft release programme could be further supported by supplemental feeding in the in situ release enclosure, ensuring Gharials do not deplete their resources during this period.

Another potential cause of this acclimation phase is that this is the lag-time required to overcome the impacts of chronic stress in captivity. High stress in captive crocodilians has been documented and is known to effect growth (Elsey et al. 1990), and can have a number of causes, including high stocking density, limited availability of a sufficient thermogradient, and fear due to high visitor numbers, or an inability to seek cover (Huchzermeyer 2003). Research into stress of Gharials at GCBC under different housing and husbandry conditions could help inform the programme.

It is also possible that Gharials have an elevated mass in the GCBC compared to wild Gharials of the same TL, due to the captive feeding regime and conditions, as this elevated mass of captive crocodilians is seen in many captive settings (Blake & Loveridge 1975; Elsey et al. 1992). Initial post-release declines may reflect a shift to a more 'natural' mass of Gharial. However, since the

Gharials were recaptured in this study less than two years post-release also showed poor conditions (thin body and tail) compared to observed Gharials of the same size in either captive collections or wild populations regardless of TL, we suspect that losses in mass reflected a decline of Gharial post-release to condition below the natural 'wild' state. Gharials recaptured after more than two years post release had more convex bodies and tails, suggesting a healthier condition.

The pre-monsoon and monsoon seasons are thought to be the best season for Gharials to hunt fish, due to murky water caused by high sediment load in the river. These seasons are also the time at which Gharials increase the most in both length and mass in captivity, due to high temperatures (Singh 2018; Khadka & Bashyal 2019). Warmer temperatures also lead to higher body temperatures in crocodilians, and these are therefore the seasons with the highest energetic costs (Lang 1987). The release of Gharials pre-monsoon, when energetic costs are high but they are attempting to catch live prey for the first time, could lead to the observed loss of mass. This may be compounded with high levels of corticosterone which is known to depress crocodilian growth regardless of resources (Elsey et al. 1990; Morici et al. 1997). This may lead to a 'missed' season of growth for released Gharials immediately after release, and they may enter their first winter without sufficient reserves. Release of Gharials in the post-monsoon or early winter, may enable them to adapt to the habitat earlier, and maximise opportunity for growth when the warmer season starts.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21010–21018

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7666.14.5.21010-21018

#7666 | Received 19 September 2021 | Final received 18 April 2022 | Finally accepted 13 May 2022

OPEN ACCESS

COMMUNICATION

Occurrence patterns of herpetofauna in different habitat types of western Terai Arc Landscape, India

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Abstract: The Terai Arc Landscape (TAL) is an important region of biodiversity in India. Situated in the foothills of the Himalaya, it is spread across India and Nepal. We describe the herpetofauna of the western part of TAL encompassing Ramnagar Forest Division, which falls in Uttarakhand state of India. We primarily used visual encounter survey method for sampling. A total of 47 species of herpetofauna belonging to three orders, 17 families and 36 genera were recorded from 10 habitat types (6 terrestrial and 4 aquatic). Highest species richness (n=32) was recorded from the human settlement and least (n=4) species richness was reported from pond habitat. In this paper, the diversity of amphibians and reptiles in each habitat type is discussed.

Keywords: Amphibians, biodiversity, ecoregion, habitat type, Himalaya, Ramnagar Forest Division, reptiles, Uttarakhand, visual encounter survey.

Editor: S.R. Ganesh, Chennai Snake Park, Chennai, India.

Date of publication: 26 May 2022 (online & print)

Citation: Mehra, G.S., N. Mohanty & S.K. Dutta (2022). Occurrence patterns of herpetofauna in different habitat types of western Terai Arc Landscape, India. *Journal of Threatened Taxa* 14(5): 21010–21018. https://doi.org/10.11609/jott.7666.14.5.21010-21018

Funding: The Corbett Foundation- Corbett, Nainital (Uttarakhand), 244715, India.

Competing interests: The authors declare no competing interests.

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Acknowledgements: We thank the Head P.G. Department of Zoology, MSCBD University, Baripada, Odisha, for providing research facilities and The Corbett Foundation (TCF) for providing financial support to the study. We also thank the Forest Department of Uttarakhand, especially D.F.O., Ms. Neha Verma (IFS) for her kind help during the study. Sincere thanks to the staff of Ramnagar Forest Division for cooperation and field staff of TCF for the assistance during field surveys. We are extremely thankful to our Field Assistant, Mr. Kuber Mehra for his help and guidance in the field. A cordial thanks to Mr. H.S. Dev and Mr. Mohan Joshi for their assistance. Last but not least a big thanks to Mr. Prakash Mehta for helping in map work.

INTRODUCTION

Terai Arc Landscape (TAL) is situated in the foothills of the Himalaya spread across India and Nepal, and is listed among 200 important ecoregions of the globe (Olson & Dinerstein 1998, 2002). The total area of TAL is 49,500 km^2 out of which 30,000 km^2 falls in India and 19,500 km^2 in Nepal (Semwal 2005). TAL harbors various habitat types such as Sal forest, Sal mixed forest, mixed forest, grassland, riverbed, swamp forest, moist riverine forest, dry riverine forest, scrubland, rivers, barren land, and wetlands (Jhala et al. 2015). Ramnagar Forest Division (RFD) is situated in the western part of Indian TAL with an area of approx. 593 km$^{2.}$ RFD is a region with rich biodiversity, and shares its western boundary with Corbett Tiger Reserve (CTR). RFD serves as a corridor in TAL from CTR to Nandhaur Wildlife Sanctuary (both in Uttarakhand), which is contiguous to Shuklaphanta National Park of Nepal (Poudyal & Chaudhary 2019).

Habitat is the place where a species survives and thrives (Odum 1971), while 'habitat type' refers to the kind of vegetation of an area (Hall et al. 1997). Intervention by humans may modify habitat types such that these areas differ from original vegetation types.

Amphibians and reptiles are collectively called herpetofauna, and they can be found in various habitat types and are adapted for various modes of living (Bowo et al. 2018). Although amphibians and reptiles provide various ecological services (Aguilar 2013) most of the time herpetofauna are not given proper consideration in decision making for forest management (de Maynadier & Hunter 1995). Some species of herpetofauna are habitat generalist and utilize various habitats, while some are habitat specialist species which reside in a specific habitat only. Habitat loss in general is destructive to the whole biodiversity but is the most severe threat for herpetofauna (Gibbons et al. 2000). Specialist species which are restricted to less number of habitats are more prone to extinction than generalist species living in multiple habitat types (Segura et al. 2007).

Although this part of western TAL is a well-known destination for wildlife enthusiasts and the information about other vertebrates such as tigers, elephants, and avifauna are available, the status of herpetofauna is still unknown. Hence this study was undertaken with the objectives to determine the diversity of herpetofauna species and their distribution in various habitat types of RFD.

METHODS

Study area

RamnagarForest Division (RFD) falls in the Nainital district of Uttarakhand state, on the latitudes 29.552–20.503 °N and longitudes 79.079–79.544 °E(Image 1) with an altitudinal range of 300–700 m. Annual temperature range is 5–40 °C, and is the lowest in January and the highest in June. The average annual rainfall is around 2,000 mm, which occurs mainly during monsoons with some showers during the winters. In this study, sampling was done in 10 different habitat types, out of which six were terrestrial and four were aquatic. These habitat types were selected on the basis of vegetation, ecology and terrain, to avoid resampling in similar habitat type in different location (Table 1).

All 10 habitat types vary in locations and vegetation (Table 1, Image 1). The terrestrial habitat types surveyed in the study were boulder region (BR), grassland (GL), scrubland (SL), mixed forest (MF), Sal forest (SF), and human settlement (HS) (Image 2). The aquatic habitat types surveyed were pond (PN), monsoon river (MR), perennial river (PR), and marshland (ML) (Image 3).

Sampling methods

Sampling was primarily carried out by area constrained visual encounter survey (VES) method (Crump & Scott 1994; Sutherland 2006). A total of 118 surveys were done in all 10 habitat types starting from September 2016 to February 2018. Totally, 12 surveys per habitat type were done by two or three persons. A total of 720 man-hours were spent on the sampling. The locations of all habitat types were at least 10 km away from each other. Photographs were taken for identification and no specimen was collected during the study. Data was also gathered by using other methods such as opportunistic observation (Behangana 2014), road kill survey (Langenet el. 2009), night searches (Bennett 1999), and rescue and release program. Species identification was made in the field, with the help of field guides, identification keys (Daniel 2002; Vasudevan & Sondhi 2010) and some recent taxonomic works (Lajmi et al. 2016; Bisht et al. 2021; Ganesh et al. 2021; Gowande et al. 2021; Amarasinghe et al. 2022; Bandara et al. 2022).

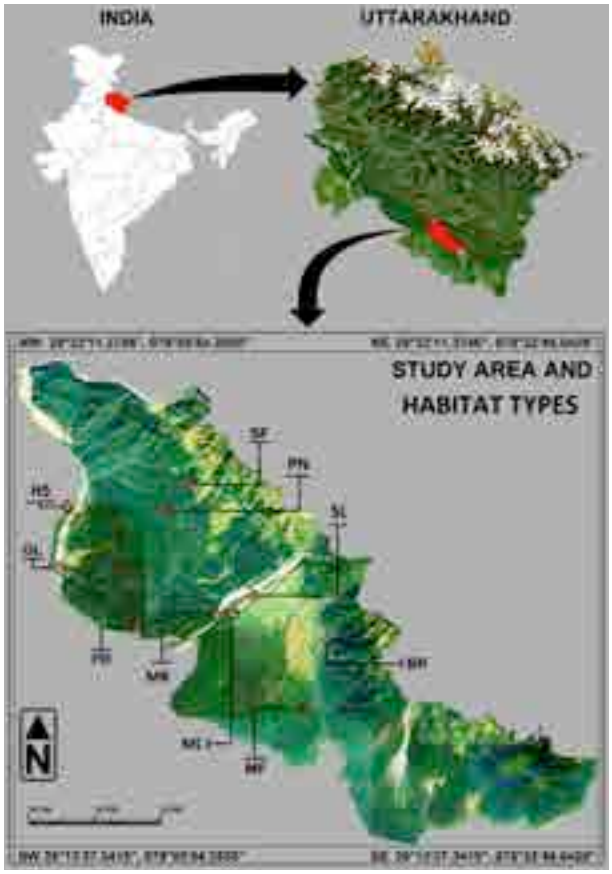

Image 1. Map showing the locations of different habitat types in Ramnagar Forest Division.

RESULTS

A total of 47 species were recorded from RFD. We recorded 10 anurans, 13 lizards, 20 snakes and four species of turtles (Table 2). The occurrence patterns of herpetofauna species in different habitat types of RFD is presented in Table 3.

Terrestrial habitat types

Boulder Region (BR) (Image 2A)—A total of six species of herpetofauna out of 47 (13%) were encountered in BR. Thisincludes five species of lizards and one species of snake – *Amphiesma stolatum.* No species of Testudinata or anurans were found.

Grassland (GL) (Image 2B)—A total of seven species out of 47 species of herpetofauna were encountered in this habitat type. Presence of four species of anurans, two species of lizards—*Calotes vultuosus* & *Eutropis carinata,* one species of snake – *Amphiesma stolatum*, was reported, but no species of Testudinata was found. In total, around 15% of species of herpetofauna were encountered in GL.

Scrubland (SL) (Image 2C)—A total of seven species of herpetofauna, were recorded from this habitat type. Four species of anurans and three species of lizards were found, but no species of snake or Testudinata were encountered. Around 15% of species of herpetofauna were encountered in SL.

Mixed forest (MF) (Image 2D)—A total ofnine species of herpetofauna species were reported from this habitat type. One anuran – *Sphearotheca breviceps*, four lizards, two snakes – *Trimerusurus septentrionalis* & *Sibynophys sagittarius*, and two turtles – *Melanochelys trijuga* & *Melanochelys tricarinata* were recorded; 19% of the total species were encountered in MF.

Sal forest (SF) (Image 2E)—A total of 12 species of herpetofauna were encountered in SF. One species of anuran –*Sphearotheca breviceps*, three species of lizards, seven species of snakes, and one species of tortoise–*Indotestudo elongata* were recorded; 25% of species of herpetofauna were encountered in SF.

Human settlement (HS) (Image 2F)—A total of 32 species out of 47 (68.08%) were reported from HS. Among these, seven species of anurans out of total 10species, eight species of lizards out of total 13 species, 15 species of snakes out of total 20 species, and two species of Testudinata out of a total four, were recorded in HS. Two species of anurans – *Duttaphrynus melanostictus* & *Duttaphrynus stomaticus*, and two species of lizards – *Hemidactylus flaviviridis* & *Hemidactylus kushmorensis*, and eight species of snakes were encountered only in HS (Table 2).

Aquatic habitat types

Pond (PN) (Image 3A)—Four species of herpetofauna were found in this habitat type. Three species of anurans and one species of lizard – *Varanus bengalensis* were observed, but no species of snakes or Testudinata were encountered. Only 9% of total species of herpetofauna were encountered in PN.

Monsoon river (MR) (Image 3B)—A total of six species of herpetofauna, were recorded from MR. Four species of anurans, one species of lizard – *Calotes vultuosus*, one species of snake – *Fowlea piscator*, were found, but no species of Testudinata was encountered; 13% of species of herpetofauna were encountered in MR.

Perennial river (PR) (Image 3C)—A total of nine species of the total herpetofauna were reported from this habitat type. From this habitat type three species of anurans, four species of lizards, and two species of Testudinata – *Indotestudo elongata* & *Lissemys punctata*, were recorded. However, no species of

Table 1. Description of the habitat types in Ramnagar Forest Division.

Habitat types	Geographic location	Description of habitat type
Boulder Region (BR)	29.367N, 79.339E	This site is a rocky terrain occupied with huge boulders and very less vegetation. Vegetation consist of *Senegalia catechu* trees, shrubs of *Lantana camara* and *Adhatoda vasica*.
Grassland (GL)	29.411N, 79.135E	This site is located nearby Kosi river and surrounded by scrubland. Major grass species on the site are *Cynodon dactylon, Sorghum halepense*,and *Eleisine indica*.
Scrubland (SL)	29.394N, 79.279E	It is *Lantana camara* dominated bushland, along with other shrub species like *Ziziphus mauritiana, Murraya koenigii* and *Acacia himalayana*.
Mixed forest (MF)	29.3185N, 79.325	This is a forest with two-layered canopy and variety of plant species. Among these primary canopy consist of trees like, *Trevia nudiflora, Syzygium cumini, Mallotus philippensis*, and *Ficus benghalensis.* While the secondary canopy consists of shrubs such as *Adhatoda vasica, Glycosmis pentaphylla*, and *Murraya koenigii*.
Sal forest (SF)	29.468N, 79.233E	This site is a Sal- *Shorea robusta* dominated area, along with Sal associated tree species like *Mallotus philippensis, Lagerstroemia parviflora* and *Clerodendrum viscosum.*
Human settlement (HS)	29.452N, 79.143E	This site is located in Dhikuli village. Surveys were done around houses, drains, lawns, gardens, and courtyards.
Pond (PN)	29.450N, 79.215E	A man-made waterhole near *Tectona grandis* forest, which remains filled with water throughout the year.
Monsoon river (MR)	29.380N, 79.254	This site is a monsoon river with a broad river bed occupied by sand and pebbles, remains dry beyond monsoons and floods during the rainy season.
Perennial river (PR)	29.372N, 79.193E	This site is on an ever-flowing river, with the narrow river bed. River bed is occupied by sand, rocks and boulders with scanty vegetation on the banks.
Marshland (ML)	29.384N, 79.266E	A marshy area which remains water-logged for around eight months of the year. Major vegetation found are *Bacopa monnieri, Amaranthus viridis, Senna tora* and *Equisetum diffusum.*

snake was found; 19% of species of herpetofauna were encountered in PR.

Marshland (ML) (Image 3D)—Seven species of herpetofauna were recorded. Five species of anurans, one lizard –*Calotes vultuosus,* and one species of snake – *Ptyas mucosus*, were recorded, but no Testudinata was observed; 15% of species were encountered in ML.

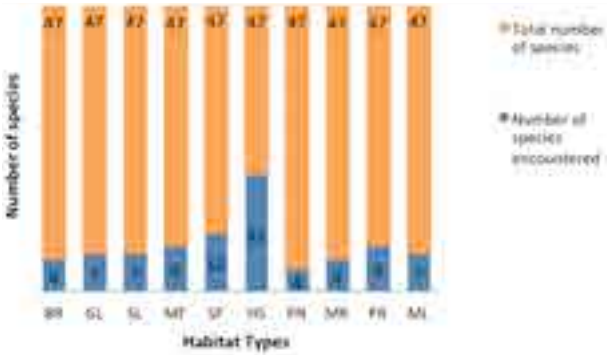

Figure 1. Habitat types in Ramnagar Forest Division with number of species encountered in them. BR—Boulder region | GL—Grassland | SL—Scrubland | MF—Mixed forest | SF—Sal forest | HS—Human settlement | PN—Pond | MR—Monsoon river | PR—Perennial river | ML—Marshland.

DISCUSSION

The maximum number of species of herpetofauna was recorded from the human settlement. Of 47 species of herpetofauna, 32 were encountered in human settlements while only four were observed in the pond. The higher number of species in human settlement might be due to the availability of a wider variety of microhabitats such as drains, lawns, leaf litter, kitchen gardens, and front & backyards. Night bulbs present around human settlement might also attract more insects, which could lure amphibians and reptiles for easy prey. In southern India, a similar result was observed in the Kalpakkam area (12.551°N & 80.168°E) where reptilian diversity was found high in human-dominated regions (Ramesh et al. 2013). Herpetofauna diversity was also found higher in human habitation in Sri Lanka (Karunarathna et al. 2008). In another study in Sri Lanka, the home gardens were found to be the second most diverse habitat for herpetofauna, after forest habitat, in a tea plantation ecosystem (Kottawa-Arachchi et al. 2014).

In RFD no anuran or testudine species were encountered in the Boulder region. Karunarathna et al. (2008) also found fewer herpetofauna species in the boulder habitat type, and considered it as a xeric habitat for herpetofauna. In Kalpakkam area of southern India, the highest number of herpetofauna species were reported from scrubland (Ramesh et al. 2013). However, we did not find the same pattern of herpetofauna diversity in this habitat type in RFD. In contrast, we found only 15% species diversity in scrubland, with comparison to human settlement which had the highest diversity (68.08%) among all 10 habitat types studied.

Sal forest which is the dominant habitat type in the

Table 2. Occurrence patternsof herpetofauna in the various habitat types of Ramnagar Forest Division.

	Species	Common names	Terrestrial habitat types	Aquatic habitat types
Anurans				
1.	*Euphlyctis cyanophlyctis*	Indian Skipper Frog	GL, PR, SL	ML, PN, MR, PR
2.	*Minervarya* sp.	Paddy Field Frog	HS, GL, PR, SL	ML, PN, MR, PR
3.	*Hoplobatrachus tigerinus*	Indian Bull Frog	HS, GL, PR, SL	ML, PN, MR, PR
4.	*Hoplobatrachus crassus*	Jerdon's Bull Frog		ML, MR
5.	*Sphaerotheca breviceps*	Indian Burrowing Frog	HS, SF, MF, SL	ML
6.	*Duttaphrynus melanostictus*	Common Indian Toad	HS	
7.	*Duttaphrynus stomaticus*	Marbled Toad	HS	
8.	*Uperodon systoma*	Indian Balloon Frog	GL	
9.	*Microhyla nilphamariensis*	Nilphamari Narrow-mouthed Frog	HS	
10.	*Polypedates maculatus*	Indian Tree Frog	HS	
Lizards				
11.	*Varanus bengalensis*	Indian Monitor Lizard	HS, SF, BR, SL	PN
12.	*Calotes vultuosus*	Bengal Garden Lizard	HS, GL, PR, BR, SL	MR, PR
13.	*Laudakia tuberculata*	Himalayan Rock Lizard	HS	PR
14.	*Asymblepharus himalayanus*	Himalayan Rock Skink		PR
15.	*Eutropis carinata*	Keeled Grass Skink	HS, GL, SF, PR, MF, BR, SL	PR
16.	*Eutropis* cf. *macularia*	Bronze Grass Skink	MF	
17.	*Eutropis trivittata*	Striped Grass Skink	MF	
18.	*Riopa punctata*	Dotted Grass Skink	HS, SF, MF	
19.	*Riopa albopunctata*	White-spotted Supple Skink	BR	
20.	*Cyrtodactylus fasciolatus*	Bent Toed Gecko	HS	
21.	*Hemidactylus kushmorensis*	Kusmore's House Gecko	HS	
22.	*Hemidactylus flaviviridis*	Northern House Gecko	HS	
23.	*Hemidactylus leschenaultii*	Leschenault's House Gecko	BR	
Snakes				
24.	*Ophiophagus hannah*	King Cobra	HS, SF	
25.	*Naja naja*	Indian Cobra	HS	
26.	*Bungarus caeruleus*	Common Krait	HS	
27.	*Daboia russelii*	Russell's Viper	HS	
28.	*Trimerusurus septentrionalis*	Himalayan White-lipped Pit Viper	MF	
29.	*Python bivittatus*	Burmese Python	HS, SF	
30.	*Dendralephis tristis*	Bronze Back Tree Snake	HS, SF	
31.	*Ptyas mucosa*	Indian Rat Snake	HS	ML
32.	*Oligodon russelius*	Russell's Kukri	HS	
33.	*Boiga trigonata*	Common Cat Snake	HS, SF	
34.	*Boiga forsteni*	Forsten's Cat Snake	SF	
35.	*Coelognathus helena*	Common Trinket Snake	HS, SF	
36.	*Coelognathus radiata*	Copper-headed Trinket	SF	
37.	*Lycodon aulicus*	Common Wolf Snake	HS	
38.	*Lycodon jara*	Twin-spotted Wolf Snake	HS	
39.	*Sibynophis sagittarius*	Contor's Black-headed Snake	MF	
40.	*Fowlea piscator*	Checkered Keelback		MR
41.	*Amphiesma stolatum*	Striped Keelback	HS, GL, BR	
42.	*Indotyphlops braminus*	Brahminy Blind Snake	HS	
43.	*Argyrophis diardii*	Indochinese Blind Snake	HS	
Testudines				
44.	*Melanochelys tricarinata*	Tricarinate Hill Turtle	MF	
45.	*Melanochelys trijuga*	Indian Black Turtle	MF	
46.	*Lissemys punctata*	Indian Flap Shell Turtle	HS,	PR
47.	*Indotestudo elongata*	Elongate Tortoise	HS, SF	PR

BR—Boulder region | GL—Grassland | SL—Scrubland | MF—Mixed forest | SF—Sal forest | HS—Human settlement | PN—Pond | MR—Monsoon river | PR—Perennial river | ML—Marshland.

Image 2. Terrestrial habitat types in Ramnagar Forest Division: A—Boulder region| B—Grassland | C—Scrubland | D—Mixed forest | E—Sal forest | F—Human settlement. © Gajendra Singh Mehra.

Table 3. Presence of herpetofauna species in various habitat types of Ramnagar Forest Division.

Habitat types	Species of anurans	Species of lizards	Species of snakes	Species of Testudinata
BR	0	5	1	0
GL	4	2	1	0
SL	4	3	0	0
MF	1	4	2	2
SF	1	3	7	1
HS	7	8	15	2
PN	3	1	0	0
MR	4	1	1	0
PR	3	4	0	2
ML	5	1	1	0

BR—Boulder region | GL—Grassland | SL—Scrubland | MF—Mixed forest | SF—Sal forest | HS—Human settlement | PN—Pond | MR—Monsoon river | PR—Perennial river | ML—Marshland.

TAL region, was found to be the second most diverse region for herpetofauna diversity in this study. The least herpetofauna diversity was found in the pond habitat, possibly because it is a stagnant water body, hence only species preferring lentic water might live here.

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Image 3. Aquatic habitat types in RamnagarForest Division: A—Pond | B—Monsoon river | C—Perennial river | D—Marshland. © Gajendra Singh Mehra.

Image 4. A—*Duttaphrynus stomaticus* | B—*Minervarya* sp. | C—*Calotes vultuosus* | D—*Laudakia tuberculata* | E—*Amphiesma stolatum* | F—*Bungarus caeruleus* | G—*Indotestudo elongata* | H—*Lissemys punctata*. © Gajendra Singh Mehra.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21019–21024

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7583.14.5.21019-21024

#7583 | Received 31 July 2021 | Final received 05 May 2022 | Finally accepted 09 May 2022

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Ichthyo-parasitological studies in northeastern India

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Abstract: Fish constitutes an essential component of the diet for most of the people of northeastern India. It provides nutrition and employment opportunities for most of the population of the region. Still, fish diseases due to helminth parasites pose a severe threat to fish health and the fishery industry. Helminths are worm-like parasites affecting the fishes and thus reduce their food value. Fishes are mostly infected with four groups of helminths, viz., Trematoda, Cestoda, Nematoda, and Acanthocephala. The article reviews the investigation and research on the trend of helminth parasites in the freshwater fishes of northeastern India through the study of available literature. For the present study, secondary data was collected from published research articles, journals, reports, and books on this major issue and compiled together. Google Scholar is the leading search engine used to search for scholarly literature in this area broadly. The study revealed that helminth parasites are extensively distributed in the freshwater fishes of different regions of northeastern India and are primarily found in the intestine of the fishes. Females are found to be highly infested than males. Also, the seasonal influence was observed on the occurrence of parasites. Several workers have conducted considerable works in Assam, Meghalaya, Arunachal Pradesh, Manipur, Mizoram, and Tripura. But to date, there is no published record on the occurrence of helminth parasites in the fishes of Sikkim and Nagaland. Therefore, it is imperative to conduct further research on the current topic that could help the scientific community and pisciculturists understand the biodiversity of parasites in different host fishes for proper aquaculture management.

Keywords: Acanthocephalans, aquaculture, cestodes, freshwater fish, helminths, nematodes, parasites, trematodes.

Editor: Mandar Paingankar, Government Science College Gadchiroli, Maharashtra, India. **Date of publication:** 26 May 2022 (online & print)

Citation: Hazarika, A.K. & B. Bordoloi (2022). Ichthyo-parasitological studies in northeastern India. *Journal of Threatened Taxa* 14(5): 21019–21024. https://doi.org/10.11609/jott.7583.14.5.21019-21024

Funding: Our research is not funded by any agency or organization.

Competing interests: The authors declare no competing interests.

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Author contributions: Both authors have contributed equally throughout the survey work, manuscript preparation and editing of the manuscript.

Acknowledgements: The authors are thankful to the Department of Zoology, and all the laboratoty mates of Ecology laboratory, Cotton University. The authors would like to acknowledge Professor Bhabesh Chandra Goswami, Hon'ble Vice Chancellor, Cotton University for inspiring us to write research papers. Also, the authors would like to thank all the reviewers and subject editors for their valuable suggestions to improve the manuscript.

INTRODUCTION

Global fish production has been increasing steadily over the past five decades. It reached an all-time high production of 73.8 million tonnes in 2014. Additionally, the fisheries sector of India has witnessed a several-fold increase in fish production from 0.75 million tonnes in 1950–51 to 10.07 million tonnes in 2015–16 (Debnath et al. 2019). Fish is one of the most important and easily digestible protein-rich food items of humankind worldwide. The northeastern region of India comprises the eight states—Assam, Arunachal Pradesh, Manipur, Mizoram, Tripura, Sikkim, Meghalaya, and Nagaland—covers an area of about 262,179 km^2 which is almost eight percent of that of India and lies between 25.5736° N, and 93.2473° E. This region has been gifted with vast aquatic resources (Barman et al. 2012) comprising floodplain wetlands, locally known as beels, swamps, ponds, and paddy fields. Therefore, aquaculture has been increasing rapidly in this region (Munilkumar & Nandeesha 2007). Moreover, the northeastern part of India enjoys a favourable climate for aquaculture with annual rainfall exceeding 2,000 mm. Additionally, more than 60% of the area is covered by forest, where the soil is primarily acidic with pH ranging from 4.5–5.0 (Munilkumar & Nandeesha 2007). Northeastern India has been blessed with a wide variety of ichthyofauna and is considered one of the hotspots of freshwater fish diversity globally. Out of the nearly 806 species of freshwater fishes in India, the northeastern region represents 267 species belonging to 114 genera and 38 families and 10 orders, which constitutes approximately one-third of the Indian freshwater fishes (Jyrwa et al. 2016). Fish includes a significant diet component for most people of northeastern India (Debnath et al. 2019). The demand for fish is high as more than 90% of the population are fish-eaters (Munilkumar & Nandeesha 2007). Moreover, fish provide nutrition and employment opportunities for most people. However, fish disease due to helminth parasites poses a severe threat to fish health and the fishery industry directly or indirectly. The disease causes poor growth, poor quality, and the low market price of the products, thus affecting the livelihood and living standards of the people due to loss of income and employment. Fish diseases led to a loss of about 15% of production in China (Debnath et al. 2019). About 30,000 helminth species have been estimated as parasites of fishes, most known to be of serious threat to their hosts. The word 'helminth' is a general term meaning 'worm'. They are worm-like parasites that constitute a significant group of pathogens, causing infection and diseases of fish both in freshwater and marine environments (Jyrwa et al. 2016). Fish serve as hosts for a wide variety of taxonomically diverse parasites (Barber et al. 2020). They are mostly infected with four groups of helminths, viz., trematodes, cestodes, nematodes, and acanthocephalans. As estimated by the World Health Organisation (WHO), the number of people currently infected with fish-borne trematodes alone exceeds 18 million, and many more are at risk. People who eat raw, lightly smoked, lightly salted, dried, and pickled fish are the most at risk (Jyrwa et al. 2016). The occurrence of fish disease due to helminthic infestations has become a major constraint in aquaculture. It affects the usual health conditions of fish, thus causing fish mortality and resulting in significant economic loss to fisheries. Therefore, successful fish parasitological research is essential in various fishery development programs as fish yield improvement can mainly be achieved from healthy fish stock (Chandra 2006). Various workers and researchers have studied the occurrence and distribution of helminth parasitic fauna in the freshwater fishes of northeast India. Also, several workers have described newer species of parasites from different fish hosts and made further advancements in this field. With the importance of aquaculture as a source of livelihood for most people in northeastern India and economic loss due to fish diseases, this review aims to summarize the helminth parasitological studies in fishes conducted in northeastern India and give suggestions for future research directions for further research in this field for proper aquaculture development.

NATURE OF STUDIES CONDUCTED IN THE STATES OF NORTHEASTERN INDIA

Assam

Assam is bountiful in aquatic resources, thus supporting the fisheries sector as a potential economic activity. The state covers 78,438 km^2 with two major river systems, viz., the Brahmaputra and the Barak River, with their tributaries (Chakravarty et al. 2017). In the context of Assam, there are sufficient pieces of evidence on the occurrence of helminth parasites in freshwater fishes. Several workers and researchers have conducted studies in various aspects of fish pathology. In the Cachar district, the intensity of cestode parasites of *Monopterus cuchia* was studied, which showed fourteen infected specimens out of 30 samples examined, and most of the infection was restricted to the intestine of the hosts. A considerable number of helminth parasites

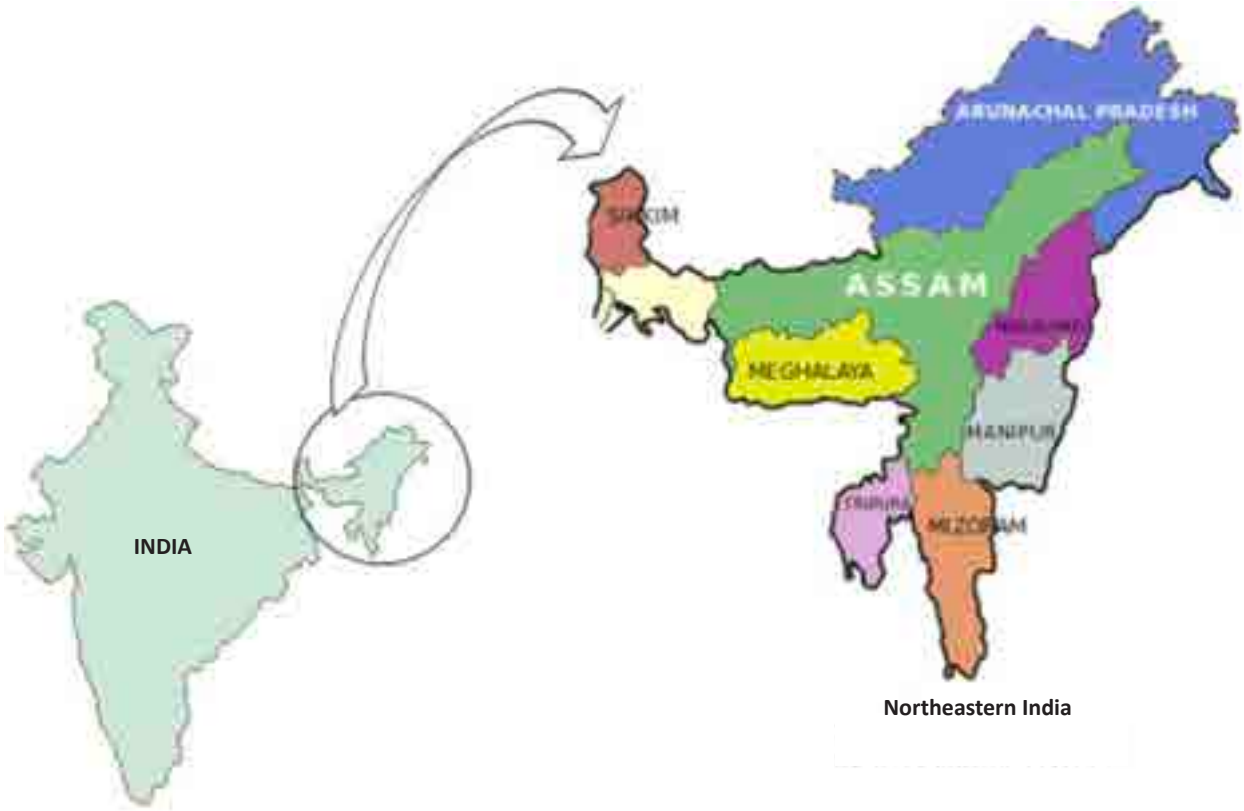

Figure 1. Map of northeastern India showing its eight different states. (Map source: https://images.app.goo.gl/TV3RFq1YNAFQ4yee9)

were also reported from freshwater fishes of Goalpara revealing seasonal variation in the prevalence, mean intensity, and abundance of parasitic infestation of fishes. The fishes of the Sone Beel of Karimganj district were investigated for the occurrence of parasites. The Sone Beel contains about 42 species of fishes belonging to 27 genera, 16 families, and six orders. The common fishes found in this lake are *Channa* sp., *Puntius* sp., *Trichogaster* sp., *Amblypharyngodon mola*, *Channa* sp., and *Mystus* sp. A total of 238 helminth parasites were recorded from this wetland (Beel), out of which 16 were nematodes, 132 were cestodes, and 22 were trematodes, and 168 of acanthocephalans, respectively. The highest infection was observed in *Channa striata* and the lowest infection in *Macrognathus aral* (Ngasepam & Kar 2014). The diversity of helminth infection in the fishes of the Jatinga River was recorded from where 14 different species of parasite groups belonging to nematodes, acanthocephalan, and cestodes were found. Maximum infection was observed in *Mastacebelus armatus* & *Mystus cavasius* and lowest in *Tenualosa ilisha*, *Channa punctata*, & *Sperata aor* (Singh et al. 2015). Three freshwater fishes, viz., *Notopterus notopterus*, *Channa punctata*, and *Heteropneustes fossilis* from Dolu Lake, Silchar was found to be infested with 358 helminth parasites out of which 270 were nematode, six were cestodes, 50 were trematodes, and 32 were acanthocephalan. The degree of infestation was higher in *Notopterus notopterus* and lower in *Heteropneustes fossilis*. The degree of infection in females was higher in all three fish species .Also, different trends were observed in the occurrence of parasites in different sex and length groups of host fishes during different seasons of the study period. The morphological features of a digenetic trematode *Isoparochis hypselobagri* recovered from three freshwater bottom-dwelling fishes, viz., *Mastacembelus armatus, Notopters notopterus,* and *Wallago attu* of Kolong river are described (Tamuli et al. 2017).

Meghalaya

Meghalaya, a hilly state in the north-eastern region of India, with its numerous hill streams and rivers, is enriched with variety of ichthyofauna (Jyrwa et al. 2016). The platyhelminth spectrum of some edible fishes of Meghalaya was documented where several fishes were examined belonging to *Labeo, Cyprinus carpio, Cirrhinus reba*, *C. mrigala*, *Danio* sp., *Puntius sarana*, *Garra* sp.

(Cypriniformes), *Channa* sp. (Channiformes) and *Clarias batrachus*, *C. gariepinus*, *Heteropneustes fossilis*, *Rita rita*, *Monopterus cuchia*, *Ompak* sp., *Bagarius bagarius,* and *Mystus tengara* (Siluriformes). Four new species of the genus *Lytocestus* were reported from the edible catfishes *Clarias batrachus* and *Heteropneustes fossilis* in Assam and Meghalaya (Tandon et al. 2005). A study on the helminth parasite spectrum of freshwater food fishes in Meghalaya was conducted for which a total of 1,674 host fishes were examined belonging to 17 genera and 26 species, including *Labeo* sp., *Cirrhinus* sp., *Cyprinus* sp., *Puntius* sp., *Neolissocheilus* sp., *Botio* sp., Garra sp., and *Catla* sp. (Cypriniformes), *Channa* sp. (Channiformes), *Mystus* sp., and *Clarias* sp., *Heteropneustes fossilis, Rita rita, Chaca chaca, and Bagarius bagarius* (Siluriformes), *Monopterus cuchia* (Symbranchiformes), and *Anguilla Anguilla* (Anguilliformes). The helminth parasites recovered from the various host species in the study area comprised of two monogeneans, eight trematodes, 12 cestode, six nematode, and one acanthocephalan species (Jyrwa et al. 2016).

Tripura

Tripura has rich fish diversity and fishery resources in the form of rivers, streams, ponds, lakes and mini barrages and paddy fields. Freshwater aquaculture is promising source of economy of the state (Singh et al. 2009). A study conducted on the seasonal incidence of parasitic helminth infestation in *Clarias batrachus* of Tripura reported 606 fishes to be infected with parasites out of 868 host fishes examined. Of the recovered parasites, one was trematode, i.e., *Astiotrema reniferum*, seven were cestodes, *Lytocestus indicus L.birmanicus*, *L. Longicollis*, *L. attenuates*, *L. filiformes*, *L. clariae*, *Djombangia penetrans*, and one larval nematode species of the genus *Anisakis* (Koiri & Roy 2016).

Manipur

Manipur covers 22,327 km^2 and has four major river basins with rich fish diversity (Vishwanath et al. 2007). Documentation on the intensity of nematode infections in the fishes of Ultra Lake revealed 121 fishes heavily infected with nematodes out of the 183 fishes examined. The study indicated maximum infection in *Anabas testudineus* by *Camallanus anabantis* (Geetarani et al. 2010). There is evidence of the occurrence of trematode parasites in the fishes of Awangsoi Lake. A total of five species of trematodes were collected in the study, namely, *Clinostomum complanatum, Allocreadium handia, Allocreadium fasciatusi, Astiotrema reniferum* and *Genarcopsis goppo* from the fish species *Channa punctatus, Clarias batrachus, Channa striatus, Channa orientalis, Anabas testudineus,* and *Heteropneustes fossilis*. The abundantly found parasite is *Astiotrema reniferum. Anabas testudineus* was found to be mostly infected with the parasites (Puinyabati et al. 2010). A good number of helminths have been reported from Awangsoi fishery including four nematodes *Procamallanus saccobranchi, Camallanus anabantis, Paraquimperia manipurensis* and one juvenile stage belonging to genus *Syphacia,* five trematodes *Allocreadium handiai, A. faciatusi, Astiotrema reniferum, Clinostomum complanatum* and *Genarcopsis goppo*, three cestodes *Djombangia penetrans, Capingentoides Singhi, Lytocestus bishnupurensis* and two acanthocephala namely *Pallisentis ophiocephali* and *Acanthocephalus* sp. (Puinyabati et al. 2013). Seasonal variation in the prevalence, intensity, and abundance of infection of nematode parasite *Camallanus anabantis* was revealed in the fish *Anabas testudineus* of Loktak Lake; 335 fishes were infected with the nematode parasites out of 460 fishes examined having maximum prevalence and intensity of infection in summer (Ranibala et al. 2013). Nine species of nematodes were recovered from the fishes of Oinam Lake. They are *Camallanus anabantis, Procamallanus saccobranchi, Paraquimperia manipurensis, Paragendria* sp. juvenile stage of *Syphacia, Haplonema, Spinitectus, Philometra and Parascarophis* sp. (Sangeeta et al. 2011).

Mizoram

In the recent years, Mizoram has witnessed a positive growth in aquaculture. An attempt to document the distribution and diversity of helminth infection in Freshwater Garfish *Xenentodon cancila* was made where a total of 40 specimens of host fishes were examined that reported only two taxonomic groups of helminth parasites namely trematodes and Acanthocephala. No cestodes and nematodes were recorded from the hosts in their study .

Arunachal Pradesh

Arunachal Pradesh is a network of watercourses has significant fish habitats (Nath & Dey 2000). The investigation of parasites in different catfishes of River Siang revealed maximum cestode infection in all the samples of fish species. The highest parasite burden was observed in the intestine of the fishes. Also, host fishes of the intermediate length group were mostly infected than the smaller length groups (Das et al. 2014). *Dactylogyrus barnae*, a platyhelminth (Monogenoidea), was found infecting the gills of *Barilius barna* (Cyprinidae) captured

Table 1. The most common parasite groups in the fishes of the states of northeastern India.

State	Fish species	Most common parasite group
Assam	*Monopterus cuchia*	Cestode
	Anabas testudineus	Nematode
	Colisa fasciata	Nematode
	Trichogaster lalius	Trematode
	Trichogaster fasciatus	Monogenea
	Channa punctata	Trematode
	Channa striata	Acanthocephala
	Notopterus chitala	Nematode
	Macrognathus aral	Cestode
	Ompok bimaculatus	Cestode
	Wallago attu	Cestode
	Clarias batrachus	Cestode
	Heteropneustes fossilis	Cestode
	Notopterus notopterus	Nematode
Meghalaya	*Clarias batrachus*	Cestodes
	Heteropneustes fossilis	
	Labeo sp.	
	Cirrhinus sp.	
	Cyprinus sp.	
	Puntius sp.	
	Neolissocheilus sp.	
	Botio sp.	
	Garra sp.	
	Catla sp.	
	Channa sp.	
Arunachal Pradesh	*Barilius barna*	Monogenea (*Dactylogyrus barnae*)
Manipur	*Anabas testudineus*	Nematode, Trematode
	Channa punctatus	Trematodes
	Channa striatus	
	Channa orientalis	
	Heteropneustes fossilis	
	Clarias batrachus	
Mizoram	*Xenentodon cancila*	Trematode, Acanthocephala
Tripura	*Clarias batrachus*	Cestode

from the local rivers of Arunachal Pradesh. *Barilius barna* is one of the commonly exported indigenous species of ornamental fish of northeastern India (Wangchu et al. 2017).

Sikkim and Nagaland

To date, there is no published record on the occurrence of helminth parasites in the fishes of Sikkim and Nagaland.

CONCLUSION

Helminth parasites are extensively distributed in the freshwater fishes of different regions of northeastern India. Fish diseases due to helminth parasites pose a serious threat to fish health and the fishery industry. Parasitic infestation affects the physiology of the fishes, thus reducing their food value. It has been observed that most of the parasites were recovered from the intestine of the host fishes, and females were found to be mostly infested than the males. It also provides information on the variation in the prevalence, intensity, and abundance of infection seasonally. Cestode and trematode infections are comparatively higher in the fishes than nematodes and acanthocephalans. Among the studies conducted it has been observed that cestodes are the most common parasites of the fishes especially in the catfishes, however, acanthocephalans are less common. But insufficient information of most of the parasites greatly handicaps the efforts at their positive control. Therefore, it is necessary to access the potential impact of helminth parasites in fishes to recognize the fish diseases so that essential control measures can be taken up to interrupt the steps of parasitic transmission from one host to another. Emphasis should be given to increase protein production and the rapid growth of fish. Moreover, further studies in the present study could help the scientific community and pisciculturists understand the biodiversity of parasites in different host fishes for proper aquaculture management.

Future perspectives

There is a wealth of evidences on the occurrence of helminth parasites in the freshwater fishes of northeastern India except for two states from where there is no published record on the occurrence of parasites. However, no planned investigations have been carried out to incur the loss in fish production due to parasites. Therefore, it is imperative to conduct a further ichthyo-parasitological investigation to assess the nature of parasitism and its effect on fishes. Also, preventive and therapeutic measures appropriate for farms should be taken for proper aquaculture management.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21025–21031

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7203.14.5.21025-21031

#7203 | Received 19 February 2021 | Final received 31 March 2022 | Finally accepted 05 April 2022

OPEN ACCESS

COMMUNICATION

Serosurvey of viral pathogens in free-ranging dog populations in the high altitude Trans-Himalayan region

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Abstract: Dogs, as reservoir hosts, have been implicated in the decline of carnivore populations across the globe. We conducted a serosurvey of free-ranging dog populations to assess the population level exposure rates to three viral pathogens, canine parvovirus (CPV), canine distemper virus (CDV) and canine adenovirus (CAV) in a Trans-Himalayan landscape in India that is home to the endangered Snow Leopard. A total of 97 dogs were sampled across six villages as a part of a surgical sterilization campaign during the study period. Samples were tested for IgG antibodies using a table top ELISA kit. Exposure rates to the three viral pathogens in the dog populations was high; 100% for CPV, 54% for CDV and 66% for CAV, with high positive immunoglobulin titer values for CAV and CPV, and low to moderate values for CDV. Overall conservation efforts for native carnivores need to address the role of free-ranging domestic dogs in disease transmission.

Keywords: *Canis lupus familiaris*, canine distemper, canine parvovirus, canine adenovirus, commensal, epidemiology.

Editor: Bahar S. Baviskar, Wild-CER, Nagpur, India.

Date of publication: 26 May 2022 (online & print)

Citation: Home, C., A. Bijoor, Y.V. Bhatnagar & A.T. Vanak (2022). Serosurvey of viral pathogens in free-ranging dog populations in the high altitude Trans-Himalayan region. *Journal of Threatened Taxa* 14(5): 21025–21031. https://doi.org/10.11609/jott.7203.14.5.21025-21031

Funding: International Foundation for Science (IFS) Grant to CH (D-5325/1) and National Research Foundation of South Africa (Grant Number 103659) to ATV.

Competing interests: The authors declare no competing interests.

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Author contributions: Study conception and design: CH and ATV; Funding: CH raised funds for research; Data collection in field: CH and AB; Logistic support for data collection: AB and YVB; Analysis and interpretation of result: CH and ATV; Manuscript preparation: CH with inputs from AB, YVB and ATV. All authors reviewed the results and approved the final version of the manuscript.

Acknowledgements: This study was financially supported by the International Foundation for Science grant (D/5325-1) to CH, and in part by the National Research Foundation of South Africa (Grant Number 103659) to ATV. AB thanks the Himachal Pradesh Forest Department and Leonard X Bosack and Bette M Kruger Charitable Foundation for providing the financial support for the sterilization camps. We thank Tanzin Thinley, Kalzang Gurmet, Kesang Chunit, Tanzin Thuktan, Rinchen Tobge, Dorje Chudim, Tanzin Sherup and Takpa Tanzin for their support and help in data collection; the Animal Husbandry Department, Kaza, Dharamsala Animal Rescue (DAR) and Tibet Charitable Trust who were involved in the conducting the sterilization camps; Dr. Rohit Guleria (AH), Dr. Anuradha (AH), Dr. Takpa Tanzin (DAR), and Dr. Nyzil Massey (DAR) who led the sterilization camps; the Pradhans of Kaza and Rangrik Panchayat who helped mobilize the people for the sterilization program; Dr. Maria Thaker for providing laboratory space for analysis of serum samples; and Dr. Aniruddha Belsare for providing useful comments on the manuscript.

INTRODUCTION

Infectious diseases in wildlife are an important conservation concern, particularly with increasing evidence of human-induced escalations in incidence rates (Daszak et al. 2001). Changes such as habitat loss, biodiversity loss, homogenization of ecosystems, and a rapidly changing domestic-wildlife interface have been associated with increased disease risks in wild species (Funk et al. 2001; Van De Bildt et al. 2002; Morgan et al. 2006; Pongsiri et al. 2009; Murray & Daszak 2013). Domestic animals in particular are reservoirs as well as vectors for pathogens such as distemper virus, *Trypanosoma*, *Echinococcus,* and *Brucella*. A majority of the pathogens of domestic animals are multi-host pathogens (Cleaveland et al. 2001). Many pathogens of conservation concern are transmitted by populations of free-ranging domestic animals, such as cats and dogs. Domestic dogs have been implicated in carnivore population declines across the globe (Funk et al. 2001; Knobel et al. 2005; Acosta-Jamett et al. 2011). Pathogens such as canine parvovirus (CPV) and canine distemper virus (CDV) have been reported to cause disease in several wild carnivores (Truyen et al. 1998; Cleaveland et al. 2000; Fiorello et al. 2007; Acosta-Jamett et al. 2015), resulting in death (Seimon et al. 2013; Belsare et al. 2014) and subsequent severe population declines (Osterhaus et al. 1997; Randall et al. 2006). Viral pathogens such as CDV are highly immunizing and require a large host population for persistence (Acosta-Jamett et al. 2010; Almberg et al. 2010). Large dog populations facilitate the transmission and maintenance of such pathogens within the ecosystem.

The global population of domestic dogs is close to a billion, and they are ubiquitous in most terrestrial landscapes (Gompper 2014). Dogs have been globally known to threaten 188 species, with greatest impacts in global biodiversity hotspots (Doherty et al. 2017). With a population of ~60 million (Gompper 2014), domestic dogs have emerged as an important conservation problem for native wildlife in India (Home et al. 2018). With the exception of a few studies (Hiby et al. 2011; Belsare & Gompper 2013;Tiwari et al. 2018), there is a significant lack of information on dog demography in both urban and rural areas, considering that they co-occur with several other native carnivores in human-dominated landscapes (Vanak & Gompper 2010; Vanak et al. 2014). High sero-prevalence of antibodies for CPV, CDV and canine hepatitis (CAV) has been documented for rural dog populations in Central India (Belsare & Gompper 2013). The study not only detected pathogens in an endemic canid, Indian Fox *Vulpes bengalensis* but also reported high mortality in foxes putatively infected with CDV (Belsare et al. 2014). The sero-prevalence of antibodies against these three viral pathogens was also high among domestic dogs sampled around Ranthambore National Park (Sidhu et al. 2019) and Kanha Tiger Reserve (Chaudhary et al. 2016). The recent deaths of Asiatic Lions *Panthera leo persica* in Gujarat, putatively due to CDV infection <https://in.reuters.com/article/india-wildlife-lions/more-asiatic-lions-in-india-test-positive-for-virus-after-23-deaths-idINKCN1MK22A>, highlights the importance of gathering long term epidemiological surveillance data for these pathogens in both wild and domestic carnivores.

Irrespective of human population densities, high dog densities facilitate a favorable environment for pathogen spillover, which can put native species at risk (Vanak & Gompper 2009). The Trans-Himalayas in India support amongst the lowest density of humans in the subcontinent, but domestic dogs in this region pose a threat to humans and wildlife. Domestic dogs contribute to a majority of the livestock losses in this landscape (Suryawanshi et al. 2013; Home et al. 2017) and interact with wildlife as predators and competitors (Pal 2013; Ghoshal et al. 2016; Home et al. 2017). Such interactions can result in pathogen spillover into native carnivores, in particular, the globally threatened Snow Leopard, an apex predator of the high altitude Himalayan landscapes.

There is a general dearth of information on the disease ecology of free-ranging dogs in the Himalaya. We undertook a serosurvey of free-ranging dog populations in the upper Spiti landscape in the Indian Trans-Himalaya to assess sero-positivity levels to three viral pathogens, CPV, CDV, and CAV. An understanding of the population level exposure to these viruses is important to evaluate the risks posed by dogs to wild carnivores, and to help in planning mitigation programs in the landscape.

MATERIALS AND METHODS

Study area

The study was conducted in the Upper Spiti Landscape (32–32.700 ^{0}N & 77.617–78.500 ^{0}E), in Lahaul and Spiti district, a high altitude region in the state of Himachal Pradesh, India (Figure 1). This landscape has the lowest densities of humans and is comprised mainly of agro-pastoralists. The area supports endangered wild herbivores and large predators such as Snow Leopard *Panthera uncia*, and Tibetan Wolf *Canis lupus chanco*, medium to small-sized predators (Red Fox

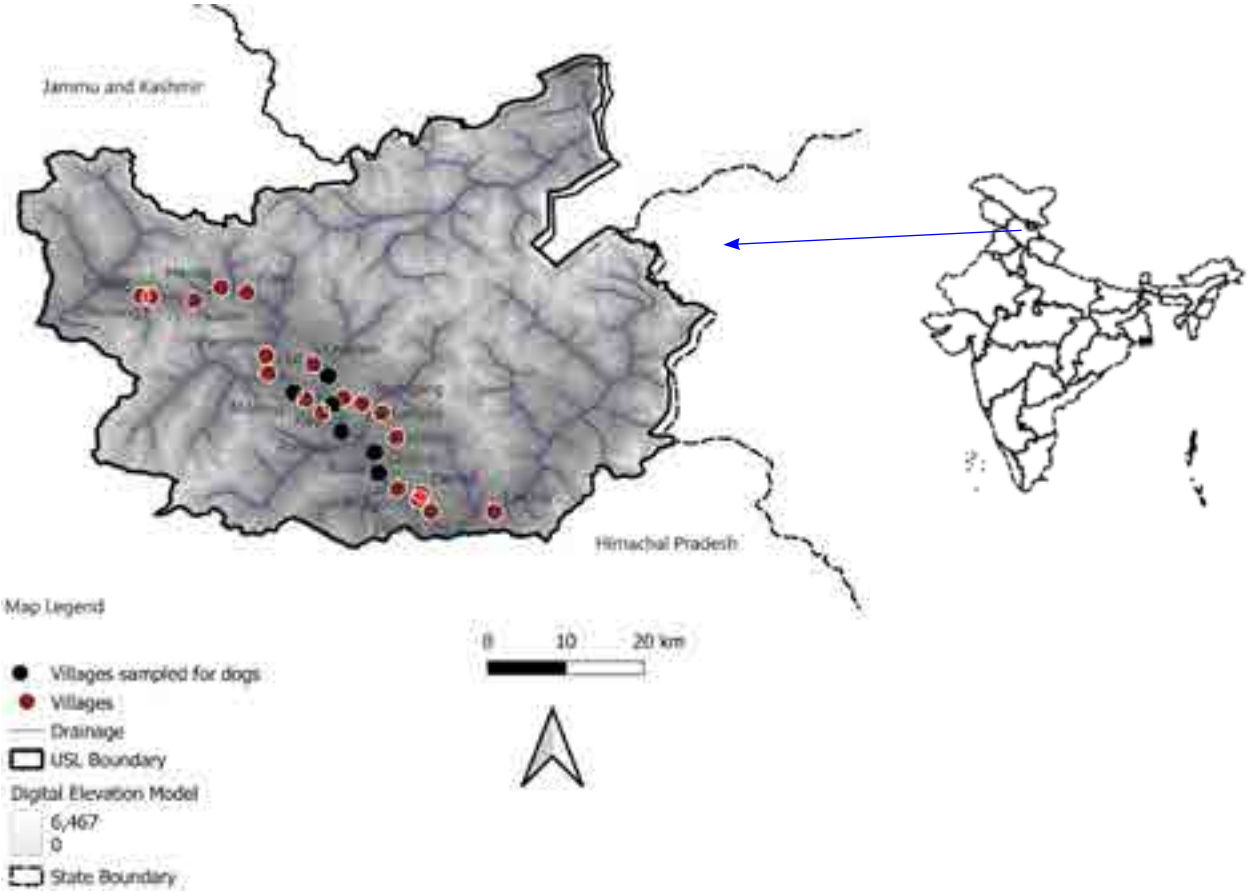

Figure 1. Map of the upper Spiti landcape. The villages sampled for dogs as a part of the sterilization camp are are marked in black circles.

Vulpes vulpes and other mustelid species) (Anonymous 2011). Domestic dogs are ubiquitous in the landscape. The last two decades have seen a rapid increase in the tourism infrastructure of the Spiti valley, particularly the township of Kaza and the largest village Rangrik. As a consequence of the increased availability of garbage and other resources, the domestic dog population in the study area has increased rapidly (Home et al. 2017, 2018). As dogs have spread to neighboring upland villages, there has been a concomitant increase in negative interactions with wildlife (Pal 2013; Hennelly et al. 2015; Ghoshal et al. 2016) as well as a severe problem of livestock depredation by free-ranging dogs (Home et al. 2017; Home 2018).

Capture and handling

We collected blood samples from dogs that were opportunistically captured during two surgical sterilization camps conducted in six villages in the study area in October 2013 and June–July 2014. The township of Kaza, which had the highest number of dogs, was sampled in both years. Dogs associated with households were brought on leash while free-ranging dogs were baited and captured using nets. Dogs were immobilized for sterilization using a combination of xylazine hydrochloride-ketamine hydrochloride, and blood was collected by venipucture of the cephalic vein. Blood was stored in vacutainers (BD vacutainer, 5 ml) with clot activating factor. The serum was decanted in 2 ml vials (Tarsons) and stored in a -20 ˚C freezer in Kaza that was hired on monthly basis till the samples were transported. The samples were transported on ice and stored in a -20˚C freezer in Bengaluru prior to analysis.

Ethics approval for this study was granted by Ashoka Trust for Research in Ecology and the Environment Animal Ethics committee (AAEC-IRB/ACA/0015/CH/2009X). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. The participation of owners who brought their dogs to the sterilization camps was voluntary.

Sero-prevalence of antibodies

For serological assessments, commercially available dot-ELISA immunoassay kits (ImmunoComb ®, Biogal

Galed Laboratories, Israel) were used. These kits have previously been validated for antibody assays in domestic dogs (Waner et al. 1996, 1998). The ImmunoComb ® kits were used to determine the IgG antibody titres against canine distemper virus (CDV), canine parvovirus (CPV) and canine adenovirus (CAV), for the dogs sampled in the villages. The antibody kits are based on solid phase immunoassay technology and their concentrations in the serum are measured on a qualitative scale. The kits provide a colour-coded scale (CombScale) which scores each sample on a scale of 0–6 "S" units, where S3 corresponds to titers at which the virus is neutralized. The information provided by the manufacturer mentions that values of S3 and above suggest that the animal has protective levels of antibodies to CDV, CPV, and CAV while values below S3 but above S1 suggests that the individual has been exposed to the pathogen and has seroconverted (https://www.biogal.com/wp-content/uploads/2019/09/PI-CVV-31_03_2016-4.pdf.) As a measure of prevalence exposure, we used descriptive statistics for summarizing the information and calculated the percentage of sampled animals with detectable levels of IgG against the pathogen (> S1) stratified by age and sex.

RESULTS

We sampled a total of 97 dogs (52 males: 45 females) across six villages (Kaza; N= 38, Rangrik; N= 33, Quiling; N= 8, Kibber; N= 5, Kee; N= 10, and Morang; N= 3) (Bijoor 2016), which included 73 adults, 19 juveniles, and five pups. Nearly 73% of the samples were collected from the township, Kaza and the largest village, Rangrik, which account for 74% of the dog population in the study area (Home et al. 2017). None of the dogs had any prior vaccination as this was the first attempt for a dog sterilization program in the region.

The assay detected antibodies against CPV in all the samples collected across all the villages, while antibodies against CDV were detected in 54% (52/97; 95% CI 43 – 63%; Sterne's exact method) of the samples and against CAV in 66% (64/97; 95% CI 56 – 75%; Sterne's exact method) of the samples (Reiczigel et al. 2019) (Table 1). We observed low to medium values (Inadequate immunity) for CDV for a majority of the sampled population (Table 2).

DISCUSSION

We documented high exposure rates to the three pathogens for the free-ranging dog populations in the upper Spiti landscape. Antibody response to CPV was detected in all the dogs sampled indicating that it is circulating widely in the population. Domestic dogs in the study area have never been vaccinated, and thus the antibody titers observed can be reliably interpreted as evidence of pathogen exposure (Belsare & Gompper 2013). Given the widespread distribution of dogs in this landscape, and their frequent interactions with carnivores, our study provides evidence that dogs can pose a serious disease risk to endangered species.

All the three pathogens; CDV, CPV, and CAV are contagious viruses that infect canids as well as other carnivores, demonstrating their ability to cross species barriers. In the case of CPV, its ability to persist in the environment for months is of particular concern, since direct contact is not required for transmission (Castanheira et al. 2014). For CDV, the primary mode of infection is inhalation requiring direct contact with infected animals (Beineke et al. 2015). While CDV may or may not be fatal for dogs, they are usually fatal for the wild counterparts (Cleaveland et al. 2000; Van De

Table 1. Pathogen exposure in sampled dog populations in six villages in the upper Spiti landscape. Numbers are dogs sampled, with percentages in parenthesis.

	Canine Parvovirus	Canine Distemper		Canine Adenovirus (ICH)	
	Detected (% in parenthesis) (N= 97)	Detected (% in parenthesis) (N= 52)	Not detected (% in parenthesis) (N= 45)	Detected (% in parenthesis) (N= 64)	Not detected (% in parenthesis) (N= 33)
Adult male	39 (40)	23 (44)	16 (36)	30 (47)	9 (27)
Adult female	34 (35)	23 (4)	11 (24)	27 (42)	7 (21)
Juvenile male	(10) 10	1 (2)	9 (20)	1 (2)	9 (27)
Juvenile female	(9) 9	3 (6)	6 (13)	3 (5)	6 (18)
Pup male	3 (3)	2 (4)	1 (2)	1 (2)	2 (6)
Pup female	2 (2)	0 (0)	2 (4)	2 (3)	0 (0)

Table 2. Seropositivity scores of CPV, CDV and CAV for dog samples across six villages.

Villages	CPV					CDV					CAV				
	0	1–2	4<5	5–6	>6	0	1–2	4<5	5–6	>6	0	1–2	4<5	5–6	>6
Kaza (N= 38)	0	2	0	24	12	19	13	6	0	0	11	4	1	7	15
Kee (N= 10)	0	0	0	10	0	8	2	0	0	0	7	1	2	0	0
Kibber (N= 5)	0	0	1	2	2	2	3	0	0	0	0	0	0	2	3
Morang (N= 3)	0	0	0	3	0	3	0	0	0	0	1	0	0	1	1
Quiling (N= 8)	0	0	0	5	3	4	4	0	0	0	3	3	0	2	0
Rangrik (N=33)	0	3	3	23	4	9	16	8	0	0	11	7	3	7	5

0—Not detected | 1–2—Low or inadequate immunity | 4<5—Positive | 5–6—High Positive | >6—Very High Positive. Total number of dogs sampled—97.

Bildt et al. 2002; Belsare et al. 2014). In a recent study in the Nepal Himalaya, CDV antibodies were not only detected in a large proportion of the dog population, but the virus was also found to be circulating in the population, as revealed through molecular analysis (Ng et al. 2019). Canine Adenovirus (CAV) can also survive in the environment for long periods and is transmitted through both excreta as well as secretions (Balboni et al. 2013). While variation in seropositivity levels across villages may be difficult to interpret due to a lower sample size in two villages (Morang & Kibber), positive values for CDV seroprevalence levels were reported only in the two largest villages Kaza and Rangrik (See Table 2). A proportion of dogs had sero-positivity scores that were lower than the "control" threshold. This could be interpreted either as non-detection (https://www.biogal.com/wp-content/uploads/2019/09/PI-CVV-31_03_2016-4.pdf), or evidence of waning immunity. Vaccination for CDV provides long term protection from CDV reinfection (Belsare & Gompper 2013). As mentioned earlier, none of the dogs were vaccinated for any of these pathogens. While it is difficult to interpret why CDV titres were low for dogs in Kaza and Rangrik, there could be possibility of future reinfections due to lower immunity. Although most adult dogs may survive a CDV reinfection, the impacts will be fatal for wild carnivores. However, this requires a better understanding for free-ranging dogs and its implications for transmission.

There is a considerable degree of movement of dogs from these high dog population clusters (Kaza & Rangrik) to other villages (Home et al. 2017) within the landscape, which could potentially facilitate pathogen circulation and persistence. Long-term research work on snow leopards in the study area has detected domestic dog movements in areas used by snow leopards <http://snowleopardblog.com/camera-reveals-dog-pack-attacking-snow-leopard/>. There is therefore a potential for pathogen spillover for the endangered snow leopards. Domestic dogs have also been observed to frequently interact with Red Foxes (Ghoshal et al. 2016) and a single occurrence of mating with the Tibetan Wolf has been observed (Hennelly et al. 2015). Since domestic dogs co-occur and interact with native carnivores in the landscape, high pathogen exposure rates in dogs could potentially pose a pathogen spill-over risk for these species especially in the context of human-mediated environmental change (Daszak et al. 2001; Foley et al. 2005; Brearley et al. 2013).

A model-based approach to understanding the spillover of infectious pathogens show that the proportion of free-ranging dogs in a population has a strong influence on CDV infections in dogs as well as spill-over events (Belsare & Gompper 2015). The adult dog population in the study area was estimated to be ~570 dogs (Home et al. 2017), and the total population of dogs including juveniles and pups could be estimated to about ~1,500 for the upper Spiti landscape. Considering that almost all dogs are free-ranging, and a proportion of the dog population is also wide ranging, moving across villages (Home et al. 2017) to areas used by Snow Leopard, Wolf, and Red Fox there is a strong potential for these pathogens to be transmitted to wild carnivores with potentially fatal consequences.

Our study has important implications for disease surveillance and monitoring for both domestic and wild carnivores within the landscape. Since the maintenance of infectious pathogens is determined by the host population, managing dog populations and restricting free-ranging movement would be imperative to prevent spillover. Conservation efforts for native carnivores should concurrently address the role of free-ranging domestic dogs in disease transmission.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21032–21042

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7471.14.5.21032-21042

#7471 | Received 26 May 2021 | Finally accepted 28 April 2022

OPEN ACCESS

COMMUNICATION

Diversity and distribution of mantis shrimps (Arthropoda: Crustacea: Stomatopoda) in the Gulf of Kachchh, Gujarat, India

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Abstract: Diversity and distribution study of Stomatopoda has been carried out in selected locations of Gulf of Kachchh from 2014 to 2020. Four species belonging to four genera in two families were recorded from Gulf of Kachchh, Gujarat. *Carinosquilla multicarinata* (White, 1848) was recorded for the first time from the west coast of India. *Cloridina ichneumon* (Fabricius, 1798) was recorded for the first time from Gujarat coast. *Gonodactylellus demanii* (Henderson, 1893) was reported after 50 years from Gulf of Kachchh, Gujarat, and *Gonodactylus smithii* Pocock, 1893 is a commonly occurring species in the intertidal zone of the Gulf of Kachchh. Species are described and illustrated with key characters and distributional status in Gulf of Kachchh. An annotated checklist of nine species of Stomatopoda occurring in Gujarat is presented.

Keywords: Annotated checklist, *Carinosquilla multicarinata, Cloridina ichneumon, Gonodactylellus demanii, Gonodactylus smithii,* intertidal zone, new records, west coast India.

Gujarati: કચ્છના અખાતમાં પસંદગીના સ્થળોએ વર્ષ ૨૦૧૪ થી ૨૦૨૦ દરમ્યાન સ્ટોમેટોપોડાની જૈવિક વિવિધતા અને વિતરણનો અભ્યાસ હાથ ધરવામાં આવેલ. ગુજરાતના કચ્છના અખાતમાંથી બે કુળની ચાર જાતિની ચાર પ્રજાતિ નોંધવામાં આવેલ છે. ***Carinosquilla multicarinata*** **(White, 1848) પ્રજાતિ ભારતના પશ્ચિમ કિનારેથી પ્રથમ વખત નોંધાયેલ છે, જ્યારે *Cloridina ichneumon* (Fabricius, 1798) પ્રજાતિ ગુજરાતના દરિયા કિનારેથી પ્રથમ વખત નોંધાયેલ છે. *Gonodactylellus demanii* (Henderson, 1893) પ્રજાતિ ૫૦ વર્ષ બાદ ફરીથી ગુજરાતના કચ્છના અખાતમાંથી નોંધાયેલ છે અને *Gonodactylus smithii* Pocock, 1893 કચ્છના અખાતના ભરતીઓટના વિસ્તારમાં સહજ રીતે જોવા મળતી પ્રજાતિ છે. કચ્છના અખાતમાં નોંધાયેલ પ્રજાતિઓ સચિત્ર, તેના વર્ણન, મુખ્ય લાક્ષણીકતાઓ અને વિતરણની સ્થિતિ સાથે આપવામાં આવેલ છે. ગુજરાતમાં જોવા મળતી સ્ટોમેટોપોડાની નવ પ્રજાતિઓની વિસ્તૃત યાદી સાથે આપવામાં આવેલ છે.**

Editor: Anonymity requested. **Date of publication:** 26 May 2022 (online & print)

Citation: Vadher, P., H. Kardani & I. Beleem (2022). Diversity and distribution of mantis shrimps (Arthropoda: Crustacea: Stomatopoda) in the Gulf of Kachchh, Gujarat, India. *Journal of Threatened Taxa* 14(5): 21032–21042. https://doi.org/10.11609/jott.7471.14.5.21032-21042

Funding: Junagadh Agricultural University, Junagadh.

Competing interests: The authors declare no competing interests.

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Acknowledgements: The authors are thankful to Junagadh Agricultural University, Junagadh for providing necessary facilities and encouragement. The authors would like to acknowledge officials of Marine National Park & Sanctuary for providing support and necessary permission to visit area during the field visit, the authors are also thankful to the Technical Staff Fisheries Research Station, Junagadh Agricultural University, Sikka for assisting data collection in the field. Authors are also grateful to Prof. Shane T. Ahyong (Principal research scientist & manager, Marine Invertebrates Australian Museum Sydney, Australia) and Dr. M. Kathirvel (Retd. principal scientist, Central Institute of Brackishwater Aquaculture, Chennai, Tamil Nadu, India) for valuable suggestion in identification of Stomatopoda species.

INTRODUCTION

Stomatopoda Latreille, 1817 are commonly known as Mantis Shrimp, which are among the most aggressive predators with the most complex behaviour (Ahyong & Harling 2000; Ahyong 2001, 2012; van der Wal et al. 2017). They have unique raptorial appendages for hunting their prey. Prey is captured by 'spearing' or 'smashing', based on dactyl position (extended or folded) during the strike (Ahyong & Harling 2000; Ahyong 2001, 2012; Schram et al. 2013). They are cosmopolitan in distribution in tropical and subtropical coastal waters, found in a wide range of habitats including continental shelf, slope and intertidally, down to the depth of about 1,500 m; a few species are also found in cool temperate habitat of sub-Antarctic waters (Ahyong 2012; Schram et al. 2013; van der Wal et al. 2017). Worldwide, a total of 486 stomatopod species belonging to 119 genera in 17 families are known (WoRMS 2021) and more than 330 species are reported from the Indo-West Pacific region (Ahyong 2012).

Fabricius (1798) was the first to study Stomatopoda from India. Some of the notable studies on stomatopods with their distribution and biology from Indian waters include Wood-Mason (1875, 1895), Wood-Mason & Alcock (1891), Henderson (1893), Thurston (1895), Kemp (1911, 1913, 1915), Kemp & Chopra (1921), Gravely (1927), Chopra (1934), Alikunhi (1952), Chhapgar & Sane (1967, 1968), Manning (1967), Shanbhogue (1969, 1986), Dutt & Ravindranath (1975), Ghosh (1984, 1987, 1991, 1995, 1998), Rao et al. (1989), Lyla et al. (1997), Holthuis (2000), Ramakrishna et al. (2003), Venkataraman et al. (2004), Pillai & Thirumilu (2008), Kathirvel (2008) Gopalakrishnan et al. (2012), Divipala & Thirumilu (2013), Ahyong (2016), Kumaralingam & Raghunathan (2016), Sen et al. (2016), Ahyong & Kumar (2018), and Niveditha et al. (2019).

Kathirvel (2008) compiled a checklist of 66 species belonging to 23 genera in eight families of Stomatopoda in Indian waters. Most recently, Trivedi et al. (2020) compiled a comprehensive checklist of Indian stomatopods consisting of 72 species belonging to 35 genera in 10 families.

Stomatopoda of the Gujarat coast are limited to a few studies of Shanbhogue (1969, 1986), Murthy et al. (2015), Vachhrajani (2015), Zynudheen et al. (2004), and Trivedi et al. (2020). From Gujarat, seven species belonging to six genera in two families of Stomatopoda are recorded (Trivedi et al. 2020). In addition to the list, we added two more records to the Gujarat mantis shrimp fauna. Hence a total of nine species belonging to eight genera in two families have been recorded until now (Table 1).

MATERIALS AND METHODS

The Gulf of Kachchh is situated on the west coast of India and comprises of 42 islands and reefs that provide shelter for corals, mangroves, seaweed, seagrass, and associated faunas. The intertidal area of Gulf of Kachchh is rocky, sandy, muddy and salt marshes. Seven locations were selected for the study, i.e., Kalubhar Island, Narara Reef, Dantiyo Reef, Sikka Reef, Goose Reef, Dedeka & Mundeka Island, and Pirotan Island. Four species of stomatopods were collected from the Gulf of Kachchh during the study period. The collected specimens were preserved in 95% ethyl alcohol. All specimens were deposited in the museum of the Fisheries Research Station, Junagadh Agricultural University, Sikka, Gujarat, India with accession numbers FRSACS-01 to FRSACS-04. Taxonomic identification was done using Henderson (1893), Pocock (1893), Kemp (1913), Kemp & Chopra (1921), Manning (1967), Ahyong (2001, 2016) and personal communication with experts. Comprehensive checklist of Gujarat water is prepared based on present field work and past published literatures (Table 1 & 2). Diagnostic characters, habitat, and distribution of each species is given. The size of specimens is given as the total length (TL) measured from the tip of the rostrum to the tip of the sub median spines of the telson; the carapace length (CL) excludes the rostrum. Synonymy of all species treated in this study follows that of WoRMS (2021). The present paper follows the standard classification of Ahyong (2001).

RESULTS AND DISCUSSION

Four species of Stomatopoda belonging to four genera and two families were recorded from Gulf of Kachchh, Gujarat, India. *Carinosquilla multicarinata* (White, 1848) and *Cloridina ichneumon* (Fabricius, 1798) were recorded for the first time from Gujarat coast. *Gonodactylellus demanii* (Henderson, 1893) reported 50 years ago from Gulf of Kachchh was observed again and *Gonodactylus smithii* Pocock, 1893 is a commonly occurring species in the Gulf of Kachchh. A checklist of stomatopod species of Gujarat with their occurrence sites are shown (Table 1). All the species are described and illustrated, and their world distribution summarized.

Table 1. Checklist of Stomatopoda recorded in Gujarat coast.

	Species	References (Gujarat coast)
Family: Gonodactylidae Giesbrecht, 1910		
1	*Gonodactylellus demanii* (Henderson, 1893)	Gujarat (Shanbhogue, 1986; Trivedi et al. 2020; present study)
2	*Gonodactylus chiragra* (Fabricius, 1781)	Gujarat (Shanbhogue, 1986; Trivedi et al. 2020)
3	*Gonodactylus smithii* Pocock, 1893	Gujarat (Trivedi et al. 2020; present study)
Family: Squillidae Latreille, 1802		
4	*Carinosquilla multicarinata* (White, 1848)	present study
5	*Clorida bombayensis* (Chhapgar & Sane, 1967)	Gujarat (Vachhrajani, 2015; Trivedi et al. 2020)
6	*Cloridina ichneumon* (Fabricius, 1798)	present study
7	*Erugosquilla hesperia* (Manning, 1968)	Gujarat (Trivedi et al. 2020)
8	*Harpiosquilla harpax* (de Haan, 1844)	Gujarat (Trivedi et al. 2020)
9	*Miyakella nepa* (Latreille in Latreille, Le Peletier, Serville & Guérin, 1828)	Gujarat (Zynudheen et al. 2004; Murthy et al. 2015; Vachhrajani, 2015; Trivedi et al. 2020)

Table 2. Distribution of Stomatopoda recorded in selected location of the Gulf of Kachchh.

	Species	KI	NR	DR	SR	GR	D&MI	PI
Family: Gonodactylidae Giesbrecht, 1910								
Genus: *Gonodactylellus* Manning, 1995								
1	*Gonodactylellus demanii* (Henderson, 1893)	-	-	-	+	+	+	-
Genus: *Gonodactylus* Berthold, 1827								
2	*Gonodactylus smithii* Pocock, 1893	+	+	+	+	+	+	+
Family: Squillidae Latreille, 1802								
Genus: *Carinosquilla* Manning, 1968								
3	*Carinosquilla multicarinata* (White, 1848)	-	-	+	+	-	-	-
Genus: *Cloridina* Manning, 1995								
4	*Cloridina ichneumon* (Fabricius, 1798)	-	-	+	+	+	-	+

KI—Kalubhar Island | NR—Narara Reef | DR—Dantiyo Reef | SR—Sikka Reef | GR—Goose Reef | D&MI—Dedeka & Mundeka Islands | PI—Pirotan Island.

TAXONOMY

Order: Stomatopoda Latreille, 1817
Family: Gonodactylidae Giesbrecht, 1910
Genus: Gonodactylellus Manning, 1995

***Gonodactylellus demanii* (Henderson, 1893)**
(Image 1a–f)

Gonodactylus demani Fishelson, 1971: 119, 128 [type locality: Red Sea]

Gonodactylus demanii Henderson, 1893: 455, pl. 11, fig. 23–24 [type locality: India]

Gonodactylus hendersoni Manning, 1967: 4, fig. 1–2 [type locality: Myanmar]

Material examined: 01 Male (1) TL= 35 mm, CL= 11 mm; Obs. by Piyush Vadher (Goose Reef), FRSACS-01

Description:

Carapace smooth, slightly narrower anteriorly, rostrum with sharp median spine; lateral margin divergent anteriorly, anterolateral angles rounded. Eyes large, cornea 1/4th length of stalk. Mandibular palp composed of three segments.

Raptorial claw folded beneath carapace; propodus dilated at distal end, deeply channeled for reception of dactylus. Dactylus inflated basally, slender distally, minutely serrated on inner margin upward to apex. Thoracic somites 6–7, lateral processes narrowed with rounded anterior and posterior margin. Thoracic somite 8 lateral process bluntly rounded. Abdominal segments 1–5 smooth, with low marginal carina; somites 1–4 rounded posterolaterally, somite 5 bluntly rectangular. Abdominal somite 6 slightly convex dorsally, dorsally with six longitudinal carinae terminating in small spine.

Telson broader than long; mid-dorsally with three longitudinal ridges, the intermediate marginal teeth

Image 1. ***Gonodactylellus demanii*** **(Henderson, 1893), Male, TL 35 mm, Goose Reef, FRSACS-01: a—dorsal view | b—ventral view | c—telson | d—uropod | e—carapace | f—raptorial claw. © Fisheries Research Station, Junagadh Agricultural University, Sikka.**

well-developed, lateral teeth small, quite distinct, two rounded tubercles at posterior end. Median and submedian with keel-shaped spinules on dorsal surface. Uropod with a short dorsal spine over first segment of exopod.

Colour: Entire body of the animal greenish with black dots scattered on some thoracic and abdominal somites. Preserved species in spirit or formalin yellowish-brown with a speckling of black chromatophores, which tend to form a transverse band in the posterior third region of the carapace.

Habitat: *Gonodactylellus demanii* is commonly found in tide pools of lower-inter tidal zone.

Distribution: Gulf of Aden, Mozambique, Pakistan, Persian Gulf, Somalia (Cappola & Manning 1995); Myanmar (Manning 1967); Red Sea (Fishelson 1971; Cappola & Manning 1995).

India: Gujarat (Shanbhogue 1969, 1986); Maharashtra (Kemp 1913; Chhapgar & Sane 1968); Tamil Nadu (Henderson 1893; Thurston 1895; Kemp 1913; Kemp & Chopra 1921; Gravely 1927; Manning 1967; Shanbhogue 1969, 1986). *Gonodactylellus demanii* (Henderson, 1893) was recorded by Shanbhogue (1969) from the Gulf of Kachchh, Gujarat, India. Presently, it is known from Sikka Reef, Goose Reef, and Dedeka & Mundeka Islands of Gulf of Kachchh (Table 2).

Remarks: *Gonodactylellus demanii* resembles *G. chiragra* (Fabricius 1781) in shape and structures but distinguished in dorsal process of ophthalmic somite minute or inconspicuous whereas conspicuous in *G. chiragra*. Telson of both the species has swollen ridges but median of *G. demanii* has more strongly convex than *G. chiragra*. Telson ridges ending with spines possess "V" shaped furrow in *G. demanii* whereas "V" shaped furrow absent in *G. chiragra* (Kemp 1913). This species is rare in the Gulf of Kachchh. *G. osheai* Ahyong, 2012 is identical to *G. demanii* in having a small cluster of setae on inner proximal margin of uropod endopod but *G. osheai* differentiated in having its anterior margin of the rostral plate sloping posteriorly, the anterolateral corners of the rostral plate distinctly rounded whereas *G. demanii* anterior margins of the rostral plate are concave and the anterolateral corners are angular to sharp (Ahyong 2012). In addition to dissimilarity, intermediate teeth of the telson distinctly longer than half the length of the submedian teeth in *G. osheai* whereas, the intermediate teeth of the telson are shorter than half the length of the submedian teeth in *G. demanii* (Ahyong 2012). This species is rare in Gulf of Kachchh.

Genus: Gonodactylus Berthold, 1827

***Gonodactylus smithii* Pocock, 1893**

(Image 2a–f)

Gonodactylus smithii Pocock, 1893: 475, pl. 20B [type locality: Arafura Sea]

Gonodactylus arabica Ghosh, 1991: 201, 205, fig. 2 [type locality: India]

Gonodactylus chiragra var. anancyrus Borradaile, 1900: 395, 397, 401 [type locality: New Caledonia, Papua New Guinea]

Gonodactylus chiragra var. intermedia de Man, 1929: 2, 25, pl. 3, fig. 9 [type locality: Myanmar]

Gonodactylus minikoiensis Ghosh, 1991: 201, 202, fig. 1 [type locality: India]

Material examined: 02 Male (1) TL= 70 mm, CL= 19 mm; (2) TL= 65 mm, CL= 14 mm; Obs. by Piyush Vadher (Goose Reef); 01 Female (1) TL= 64 mm, CL= 15 mm; Obs. by Hitesh Kardani (Sikka Reef), FRSACS-02.

Description

Carapace smooth, slightly narrower in front, rostrum slightly broader than long, rostral plate's margin concave anteriorly, apical spine on rostrum longer than base; lateral margin divergent anteriorly, anterolateral angles convex or rounded.

Ocular scales broad, flattened. Eyes large, cornea 1/4th longer than stalk. The mandibular palp present; composed of three segments. Raptorial claw folded underside of the carapace; propodus dilated at distal end. Dactylus inflated basally, slender dactylus possess rows of microscopic spinules on its inner margin upward to the apex.

Thoracic somites 6–7 lateral processes narrowed with rounded anterior and posterior margin. Thoracic somite 8 possesses blunt rounded lobe. Abdominal segments 1–5 smooth. Abdominal segment 6 with sharp carinae, posteriorly ending with six sharp acute spines.

Telson anchor shaped, broader than long with distinct median carina, sub median and lateral carina. Intermediate and sub–mediate denticles with minute sharp movable spine. Outer margin of uropod exopod distal segment with 10–13 uneven movable spines.

Habitat: *Gonodactylus smithii* Pocock, 1893 is commonly found under the crevices of dead corals.

Colour: Entire body deep green with light green patches; propodus of the raptorial limb reddish purple at the anterior end; dactylus light purple.

Distribution: Arafura Sea (Pocock 1893); Myanmar (Man 1929); New Caledonia (Borradaile 1900; Ahyong 2001); Papua New Guinea (Borradaile 1900); Western Indian Ocean to Vietnam (Ahyong 2001).

Image 2. *Gonodactylus smithii* Pocock, 1893, Male, TL 70 mm, Goose Reef, FRSACS-02: a—dorsal view | b—ventral view | c—telson | d—uropod | e—carapace | f—raptorial claw. © Fisheries Research Station, Junagadh Agricultural University, Sikka.

India: Gujarat (Trivedi et al. 2020); Lakshadweep Islands (Shanbhogue 1969, 1986; Rao et al. 1989; Ghosh 1991; Venkataraman et al. 2004; Sen et al. 2016); Andaman & Nicobar Islands (Niveditha et al. 2019). The species was found in all locations sampled in the Gulf of Kachchh (Table 2). *Gonodactylus smithii* Pocock, 1893 has cosmopolitan distribution in Gulf of Kachchh.

Remarks: This species resembles *Gonodactylus chiragra* (Fabricius, 1781) and *Gonodactylus platysoma* Wood–Mason, 1895 in shape and structure but are immediately distinguished by the acute anterolateral angles of rostrum, generally more slender median carinae of telson and merus of claw with crimson coloured blotch on inner margin (Ghosh 1991). This species is common throughout Gulf of Kachchh.

Family: Squillidae Latreille, 1802

Genus: Carinosquilla Manning, 1968

***Carinosquilla multicarinata* (White, 1848)**

(Image 3a–f)

Squilla multicarinata White, 1849: 144, pl. 6, fig. 1 [type locality: Philippines]

Material examined: 01 Male (1) TL= 73 mm, CL= 17 mm; Obs. by Hitesh Kardani (Sikka Reef), FRSACS-03

Description

Rostral plate as long as broad; carapace with longitudinal rows of sharp carina. Eye stalk lack carinae. Ocular scales without bifurcation, whole. Medial carina distinct, anteriorly bifurcated, opening posterior to dorsal pit. Raptorial claw oblong, merus outer region with longitudinal raw of carina, carpus dorsal margin with rows of small teeth; dactylus with five uneven sharp teeth. Mandibular palp present. Maxilliped 1–4 with epipod. TS5 dorsal carinae transverse, except medially. TS6–8 and AS1–6 dorsal carinae subparallel, most or all posteriorly armed above intermediate carinae.

Thoracic somite 5 lateral process possesses a slender spine anterolaterally on anterior lobe; posterior lobe small, broad, rounded laterally. Thoracic somite 6 lateral process anterior lobe quadrate shaped, rounded apex, posterior lobe broad rounded. Thoracic somite 7 lateral process anterior lobe short, blunt; posterior lobe larger than anterior lobe, broad, rounded. Thoracic somite 8 triangular anterolateral margin, apex acute; sternal keel triangular. All abdominal somites with equals carinae spines; submedian 1–6; intermediate 1–6, lateral 1–6, marginal 1–5. Telson as long as broad; prelateral lobe with sharp tooth at the apex longer than margin of lateral tooth, dorsal surface with numerous longitudinal carinae structure, denticles submedian 3, intermediate 8–9, lateral 1. Uropodal protopod inner margin possess eight slender spines, endopods articuled with ventral tuberculation process. Uropodal exopodal segments possess nine movable spines on outer margin. Endopods carinate distally.

Habitat: *Carinosquilla multicarinata* (White, 1848) is found in the crevices of rocks in the Gulf of Kachchh.

Distribution: Japan to Vietnam, Malaysia, northeastern Indian Ocean, Singapore, Thailand (Ahyong 2016); Philippines (White 1849; Ahyong 2016).

India: Tamil Nadu (Kemp 1913; Shanbhogue 1969, 1986; Lyla et al. 1997; Gopalakrishnan et al. 2012; Divipala & Thirumilu 2013; Ahyong & Kumar 2018); West Bengal (Ghosh 1995, 1998). *C. multicarinata* (White, 1848) reported for the first time from the west coast of India. It is here reported from Dantiyo Reef and Sikka Reef of Gulf of Kachchh (Table 2).

Remarks: Descriptive characters of the present specimen agree well with the detailed descriptions of Kemp (1913), Ahyong & Moosa (2004), and Ahyong et al. (2008). *C. multicarinata* resembles *Carinosquilla carita* Ahyong, 2001 but immediately distinguished in a presence of a mandibular pulp whereas absent in *C. carita* and *C. carita* possess more slender anterior lobe on thoracic somite 6 and a blunt spiniform apex on the prelateral lobe where as a sharp in *C. multicarinata* (Ahyong 2001).

Genus: Cloridina Manning, 1995

***Cloridina ichneumon* (Fabricius, 1798)**

(Image 4a–f)

Squilla ichneumon Fabricius, 1798: 416 [type locality: India]

Squilla microphthalma H.M. Edwards, 1837: 523 [type locality: India]

Material examined: 01 Male (1) TL= 53 mm, CL= 15 mm; Obs. by Piyush Vadher (Sikka Reef), FRSACS-04

Description

Dorsal surface of the carapace and abdomen smooth, carapace lacks median or lateral carinae. Rostrum triangular, as long as wide, rounded apically. Cornea wider two times in the total length of eye. Eyes pear shaped, as longs as 2/3rd which reaches to the basal segment of antennular peduncle. Medial and submedial carina absent on first five abdominal segments. Raptorial claw oblong, merus unarmed, carpus dorsal margin with rows of small teeth; dactylus with five uneven, evenly spaced sharp teeth. Mandibular palp present.

Thoracic somite 5 lateral process possesses a sharp spine. Thoracic somites 6–8 lateral processes smooth

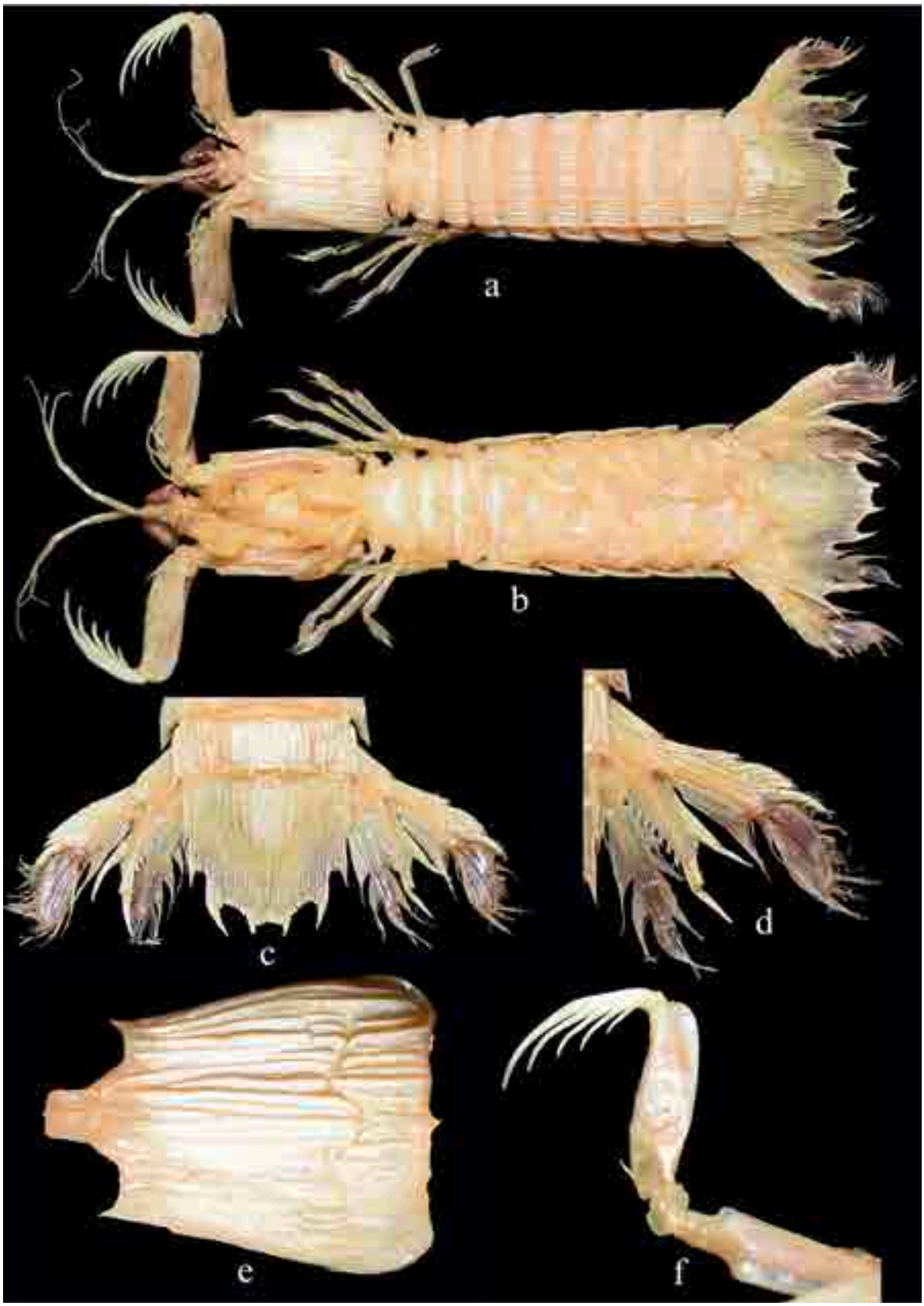

Image 3. ***Carinosquilla multicarinata*** **(White, 1848), Male, TL 73 mm, Sikka Reef, FRSACS-03: a—dorsal view | b—ventral view | c—telson | d—uropod | e—carapace | f—raptorial claw. © Fisheries Research Station, Junagadh Agricultural University, Sikka.**

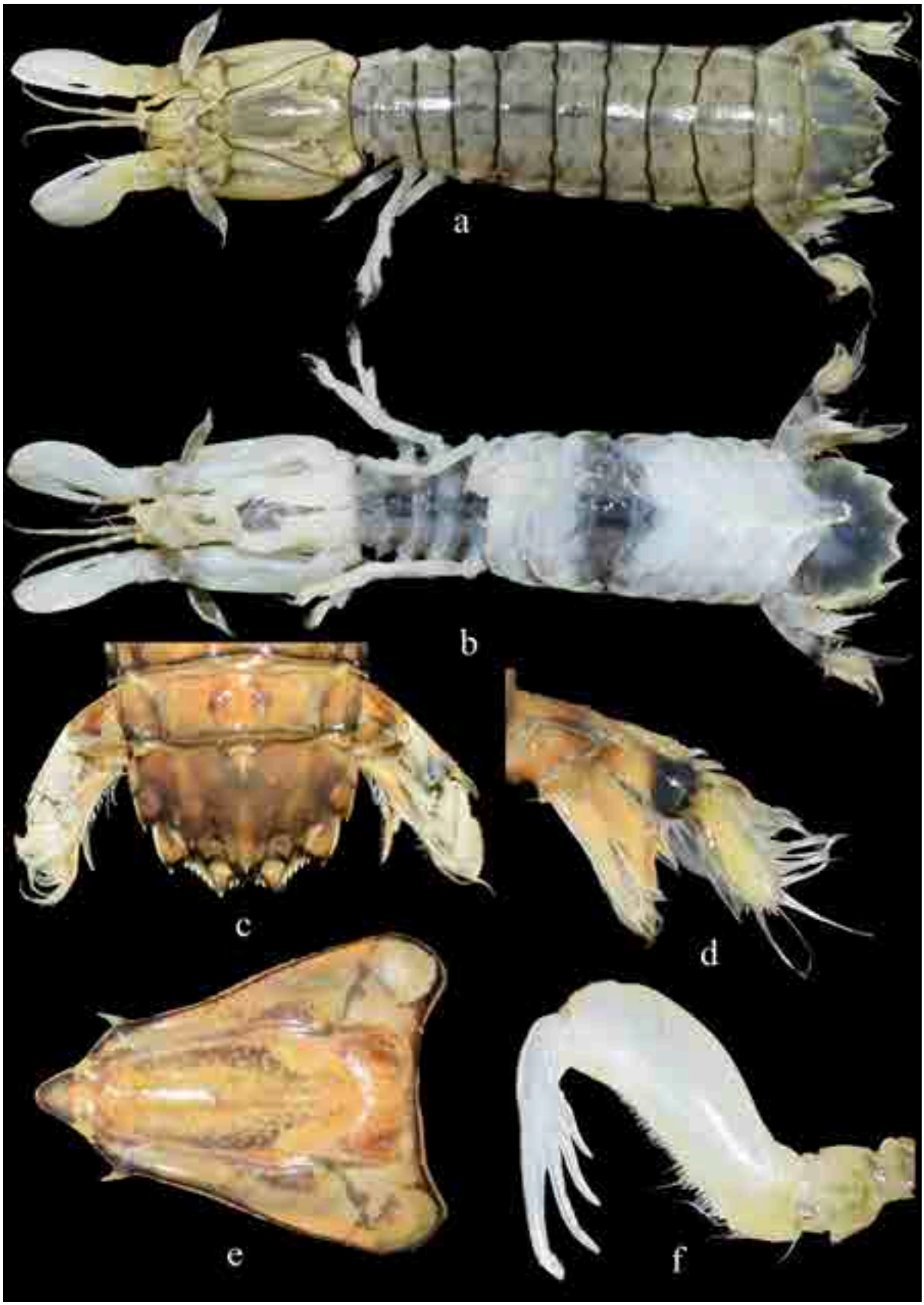

Image 4. ***Cloridina ichneumon*** **(Fabricius, 1798), Male, TL 53 mm, Sikka Reef, FRSACS-04: a—dorsal view | b—ventral view | c—telson | d—uropod | e—carapace | f—raptorial claw. © Fisheries Research Station, Junagadh Agricultural University, Sikka.**

convex lobed. Abdominal somites 1–6 smooth, unarmed. Telson as long as wide, median carinae distinct, dorsal surface with uneven small tooth, marginal carina with distinct sharp teeth at posterior margin, lateral and submedian carinae possess minute sharp teeth at distal margin. Uropod comprises a row of seven small spines on inner margin.

Habitat: *Cloridina ichneumon* (Fabricius, 1798) commonly found in sandy zone of lower intertidal zone.

Colour: Specimens observed after about 2-month preservation in formalin show general body colour pale white: posterior margins of carapace, exposed thoracic and first five abdominal somites bear black colour, in male these bands are more prominent, characteristic patch of black colour present on distal part of proximal segment of uropodal exopod.

Distribution: Eastern Africa, Gulf of Thailand, Singapore, South China Sea (Ahyong 2016).

India: Maharashtra (Kemp 1913; Chhapgar & Sane 1968; Shanbhogue 1986; Holthuis 2000; Ahyong 2016); Tamil Nadu (Kemp 1913; Kemp & Chopra 1921; Shanbhogue 1986). *C. ichneumon* (Fabricius, 1798) reported first time from Gujarat coast. It is here reported from Dantiyo Reef, Sikka Reef, Goose Reef, and Pirotan Island of Gulf of Kachchh (Table 2).

Remarks: Descriptive characters and diagnosis of the present specimen well agreed with the detailed descriptions by Kemp (1913), Kemp & Chopra (1921), and Shanbhogue (1986).

C. ichneumon resembles *Cloridina stephensoni* Ahyong, 2001 but distinguished in having as long as or longer than broad rostral plate and the apices of the dorsal processes formed to acute spines whereas in *C. stephensoni* possess shorter rostral plate, unarmed apices of dorsal processes of the antennular somite (Ahyong 2001).

CONCLUSION

In the Gulf of Kachchh, Mantis Shrimp possess high diversity in Goose Reef and Sikka Reef compared to the other reef of the Gulf of Kachchh (Table 2). They are mostly found in the crevices of rocks, under dead corals and inside the holes of water pools. *Gonodactylellus demanii* (Henderson, 1893), *Carinosquilla multicarinata* (White, 1848), and *Cloridina ichneumon* (Fabricius, 1798) are rare and sparsely distributed in the lower intertidal zone of the Gulf of Kachchh whereas *Gonodactylus smithii* Pocock, 1893 is very common in the intertidal zone. *G. smithii* Pocock, 1893 is found in the crevices of pools exposed at low tide. The reproductive season for mantis shrimp in the region seems to be during the monsoon given the abundance of larvae seen during the monsoon in the field. Juveniles were found in winter season.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21043–21054

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.5490.14.5.21043-21054

#5490 | Received 23 August 2019 | Final received 16 January 2021 | Finally accepted 07 April 2022

OPEN ACCESS

COMMUNICATION

Bionomics study of *Mansonia* (Diptera: Culicidae) in a filariasis-endemic area of Sedang Village, Banyuasin Regency, South Sumatra, Indonesia

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Abstract: An investigation of bionomic study of *Mansonia* species was successfully conducted in Sedang village which is one of the filariasis-endemic areas in Indonesia. The study was carried out for 14 months from April 2017 to May 2018. In order to trap the local mosquitoes in the study area, indoor and outdoor human landing collection method was adopted. During the study, 7,908 mosquitoes were collected which consisted of 13 genera and 40 species of mosquitoes. Moreover, *Mansonia uniformis, M. annulifera*, and *M. indiana* were found to be the most abundant, dominant, and high frequency mosquitoes. The filariasis vector analysis through polymerase chain reaction test confirmed that only *Mansonia annulifera* positively detected as the filariasis vector. Furthermore, the longevity calculation showed that 81% of all the collected *Mansonia* spp. had already oviposited their eggs which indicates that the studied area possesses high possibilities of filariasis transmission.

Keywords: Filariasis diseases, filariasis vector analysis, *Mansonia* spp., mosquitoes, tropical diseases, vector transimission.

Editor: Anonymity requested

Date of publication: 26 May 2022 (online & print)

Citation: Pratiwi, R., C. Anwar, A. Ghiffari & A. Huda (2022). Bionomics study of *Mansonia* (Diptera: Culicidae) in a filariasis-endemic area of Sedang Village, Banyuasin Regency, South Sumatra, Indonesia. *Journal of Threatened Taxa* 14(5): 21043–21054. https://doi.org/10.11609/jott.5490.14.5.21043-21054

Funding: There is no funding declared in the present study.

Competing interests: The authors declare no competing interests.

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Author contributions: RP—conceptualization, research design, field supervisor, writing. CA—research supervisor, evaluation, data interpretation. AG—data interpretation, data analysis. AH—writing, data visualization, writing, data analysis.

Acknowledgements: Authors acknowledge the Government of Banyuasin District for supporting the present research.

INTRODUCTION

Filariasis is a zoonotic disease caused by the infestation with microfilaria, which is found in tropical areas, such as Indonesia. This disease has caught the attention of world researchers and policy-makers alike, especially in tropical and subtropical countries because it has infected more than 120 million people in 72 countries and more than 90% of filarial infections are infected by *Wuchereria bancrofti* and *Brugia malayi* which are transmitted by *Culex* and *Mansonia* mosquitoes (cdc.gov). Several studies have reported that *Brugia malayi* has dominantly caused the transmission of filarial infection in many Asian countries like China, South Korea, Japan, India, Myanmar, Indonesia, Malaysia, the Philippines, Thailand, and Bonaire Islands (Kanjanavas et al. 2009; Tan et al. 2011; Saeed et al. 2015).

To be more specific in Indonesia, Banyuasin is a regency in the South Sumatra province of Indonesia, which has been designated as an area where filariasis is endemic (Ministry Health of Republic of Indonesia 2016). In 2014, there were 142 cases of 173 provincial cases of chronic filariasis in Indonesia, in which Banyuasin has a high rate of endemicity, with an average microfilarial rate of 2.02%. Geographically, Banyuasin is a lowland filled with swamps, coastlines, rice paddies, and plantation fields, which makes it an ideal mosquito breeding ground. However, based on our knowledge, there is no record of comprehensive bionomics study in Banyuasin. Thus, this study will contribute to eradicating filariasis, mainly by vector control and case management (Saeed et al. 2015).

Herein, the present study aims to determine the bionomic study of *Mansonia* species in Banyuasin including their diversity, abundance, dominance, and preference. The main reason for choosing this area was based on the high filariasis cases reported in provincial case of filariasis in Indonesia. It is supported by the fact that the studied area still has high microfilaria rate which is 0.93% after conducting Mass Drug Administration (MDA) programmes by the Ministry of Health, Republic of Indonesia for the last three year (2013–2016) to eliminate the filariasis in this area. Therefore, the results are expected to provide evidence base and references to strategize a further prevention action to reduce the number of filariasis cases in Banyuasin regency, South Sumatera, Indonesia. The results could also become the reference and information baseline about the diversity and behavior of *Mansonia* spp. in Indonesia which enhance knowledge in Entomology.

MATERIALS AND METHODS

Study Area

The research was focused in Sedang Village which is located in Suak Tapeh District in Banyuasin Regency, South Sumatera-Indonesia. The research area had the coordinate of 2.853S and 104.579E with the altitude of 10 m. The studied area has a tropical weather with an average temperature of 26–28 °C and humidity ranging 89–92 %. The study area is dominated by high water bodies such as swamplands, ponds which has water plantations (Department Health of Banyuasin District, 2016). Image 1 presents the landscape of studied area taken using a drone.

Mosquito collection

All the obtained mosquitoes in this study were collected once a month for 14 months started from April 2017 to May 2018. Human landing collection (HLC) method was followed for both indoor and outdoor for 24 hours from 18.00 until 17.00 in the next day by six teams which consist of 12 volunteers. The six teams were divided into two teams (three team each condition) to collect the biting mosquitoes indoor and outdoor condition. The mosquitoes attached and rested to humans or wall shelters (rested only) were collected using aspirator for 40 minutes/hour and 10 minutes/hour, respectively. For consideration, all the research activities had been approved by the ethics team of Sriwijaya University (Ethical Access Certificate No. 522 / kepkrsmhfkunsri / 2016).

Image 1. The studied area in Sedang village. The light green ground was dominated by swamps and rice field, and the dark green refers to plantation. © Author.

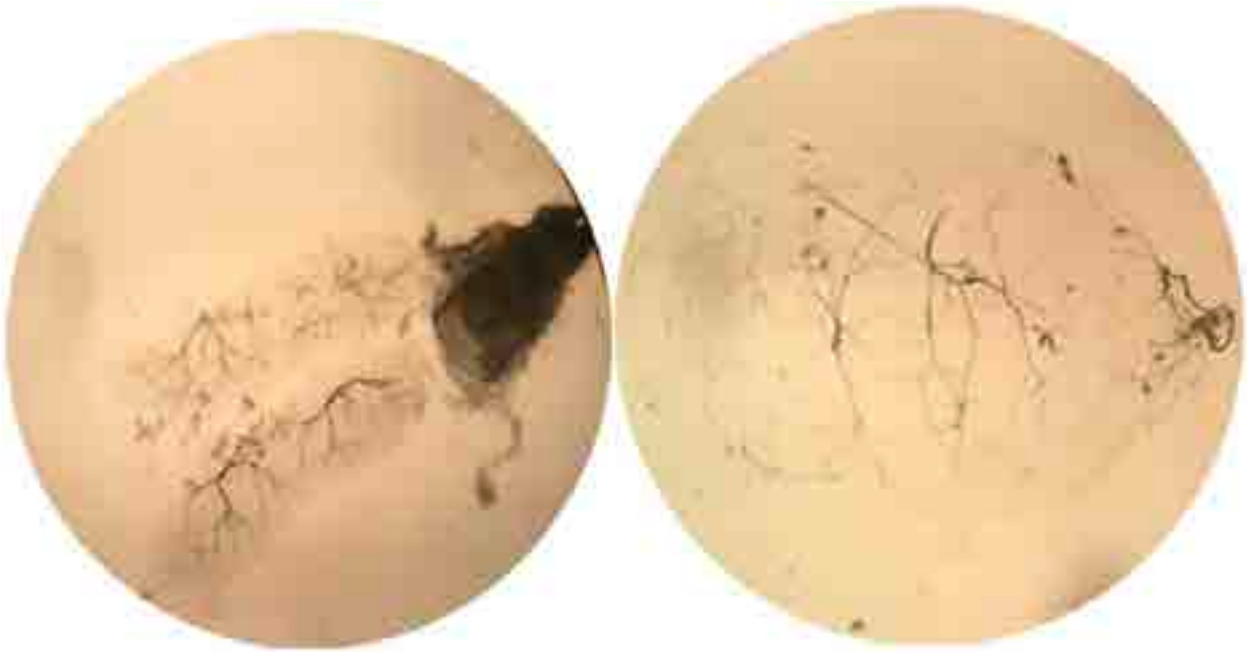

Image 2. Dilatation uterus.

Mosquito identification

All the collected-mosquitoes were further identified using the Rampa & Wharton identification book and carefully counted (Wharton 1978; Rattanarithikul 2005). In this study, only female mosquitoes were collected as biting mosquitoes since the male mosquitoes did not bite the volunteers in human landing method (Shirai et al. 2002). The females of *Mansonia* were then dissected to determine parity using dilatation methods and identify the ovarian as parous or nulliparous (Image 2).

Data analysis

All the collected mosquitoes in biting and resting positions were summarized and divided into several categories including diversity, abundance, frequency, dominance, man-hours density, man-biting rate, and resting rate. The detailed calculation to determine each category was shown in several formulas below.

Abundance= (Total number of collected mosquitoes per species / Total number of collected mosquitoes) x 100 % (1)

Frequency= Total number of collected mosquitoes per species / Total collecting hours (2)

Dominance= Frequency x Abundance (3)

Man-hours density= Total number of collected mosquitoes per species / Total collection hours per day x number of day x number of collector x duration of collection (minutes) (4)

Man-biting rate= Total number of collected biting mosquitoes / Total number of collector x number of collection hours (5)

Resting rate= (Total number of collected resting mosquitoes per species / Total number of collected resting mosquitoes) x 100% (6)

Longevity (P)= ^{A}VB

where A= Physiological age of collected mosquitoes (gonotrophic cycle); B= Proportion of porous from several dissected mosquitoes; P= Daily life opportunities. Estimated age of mosquito population= 1 / -log e^{P} (7)

Biomolecular examination

The molecular examination was carried out to determine the filariasis vector through DNA isolation from the heads of the *Mansonia* spp. Twenty-five mg sample kept in 1.5 ml microtube was smashed with pastel and added with 180 µl buffer ATL and 20 µl proteinase K. After vortex, the sample was incubated at 56 °C until lysed. Then 200 µl buffer AL and 200 µl ethanol (96–100 %) was added and vortexed, respectively, followed by the spinning period with Dneasy Mini Spin (Blood & Kit 2006). *Brugia malayi* specific primers were forward (5'-GCGCATAAATTCATCAGC-3') and reverse (5'-GCGCAAAACTTAATTACAAAAGC-3') amplified using thermal PCR (Haryuningtyas & Subekti 2008). The PCR temperature and the master mixes were according to Goodman et al. (2003). The amplicon was later electrophoresed at 80 volts for 40 minutes. The gel was 2% agarose TAE with ethidium bromide and read after

Table 1. The diversity of mosquitoes in Sedang Village, Banyuasin Regency, South Sumatera-Indonesia collected in the period of April 2017 to May 2018.

	Species	Number of collected mosquitoes	%
1	*Mansonia uniformis*	1,835	23.20
2	*Mansonia annulifera*	1,585	20.06
3	*Mansonia indiana*	985	12.50
4	*Mansonia bonneae*	30	0.40
5	*Mansonia annulata*	9	0.11
6	*Mansonia dives*	4	0.05
7	*Culex gelidus*	795	10.06
8	*Culex quinquifasciatus*	629	7.96
9	*Culex tritaeniorhynchus*	211	2.67
10	*Culex vishnui*	124	1.56
11	*Culex sitiens*	46	0.58
12	*Culex fuscocephalus*	23	0.29
13	*Culex hutchinsoni*	10	0.12
14	*Culex bitaeniorhyncus*	1	0.01
15	*Culex pseudosinensi*	1	0.01
16	*Culex nigropunctatus*	1	0.01
17	*Culex infula*	1	0.01
18	*Culex sinensis*	1	0.01
19	*Aedes aegypti*	339	4.29
20	*Aedes albopictus*	55	0.70
21	*Aedes butleri*	30	0.40
22	*Aedes pulchriventer*	11	0.14
23	*Aedes albolineatus*	6	0.07
24	*Aedes* sp.	5	0.06
25	*Aedes lineatopennis*	3	0.04
26	*Aedes anandelei*	1	0.01
27	*Aedes poicilius*	1	0.01
28	*Anopheles nigerrimus*	12	0.15
29	*Anopheles separatus*	7	0.09
30	*Anopheles barbirostris*	1	0.01
31	*Coquillettidia crassipes*	5	0.06
32	*Coquilettidia nigrosignata*	3	0.03
33	*Topomyia* sp.	542	6.90
34	*Armigeres subalbatus*	340	4.30
35	*Tripteroides* sp.	235	2.97
36	*Mimomyia* sp.	5	0.06
37	*Malaya jacobsoni*	5	0.06
38	*Uranataenia* sp.	2	0.02
39	*Hodgesia* sp.	1	0.01
40	*Uratonia longinistis*	1	
	Total	**7,901**	**100.00**

the DNA ladder addition under ultraviolet light using Gel doc. The PCR results positively result in a band at 326 bp as *Brugia malayi*.

RESULT AND DISCUSSION

Mosquito collections

Table 1 showed the diversity and total number of collected mosquitoes. During the research period, there were 7,908 mosquitoes collected which consisted of 13 genera including *Mansonia, Culex, Aedes, Anopheles, Coquilettidia, Topomyia, Armigeres, Triptoides, Miomyia, Malaya, Uranataenia, Hodgesia*, and *Urotonia*. From the 13 genera, all the obtained mosquitoes were analyzed and divided into 40 species. The most dominant diversity was from the genera *Culex* which has 12 species, followed by *Aedes* (9 species), *Mansonia* (6 species), *Anopheles* (3 species), *Coquilettidia* (2 species), and 1 species from *Armigeres, Triptoides, Malaya, Urotonia, Uranataenia, Topomyia, Coquilettidia, Hodgesia, Topomyia*, & *Miomyia*. In case of number, *Mansonia* spp. was found as the highest collected mosquitoes where 4,448 *Mansonia* spp. (56.30%) had successfully collected and identified during the research period. On the other hand, *Culex* spp. had the highest species diversity and was the second most abundant of collected mosquitoes which consists of 1,843 mosquitoes (23.33%).

The result was similar with the work conducted by Rohani (2013) who had reported the bionomic study in Malaysia and reported six genera of mosquitoes collected which were *Aedes, Anopheles, Armigeres, Culex, Coquilettidia*, and *Mansonia* which consist of only 27 species. However, the study reported that *Culex* spp. was the highest collected mosquito followed by *Anopheles, Armigeres*, *Mansonia*, *Aedes*, and *Coquilettidia*. As comparison in Indonesia, Sugiarto (2017) also reported the bionomic study in North Borneo Island, and found that *Anopheles* mosquitoes as the most collected mosquitoes during the research period. We can infer that there is a difference in the diversity and abundance of mosquito species in each studied area due to variations in geographical characteristics, climate, and the availability of breeding grounds and resting places (Rohani et al. 2013; Sugiarto et al. 2017). Moreover, this finding can contribute as the information about a fingerprint of specific species located in Sedang village of Banyuasin district, South Sumatera-Indonesia.

In order to see the potential of filariasis transmission vector, the further study was focused on *Mansonia* spp. as the most collected mosquitoes during the present

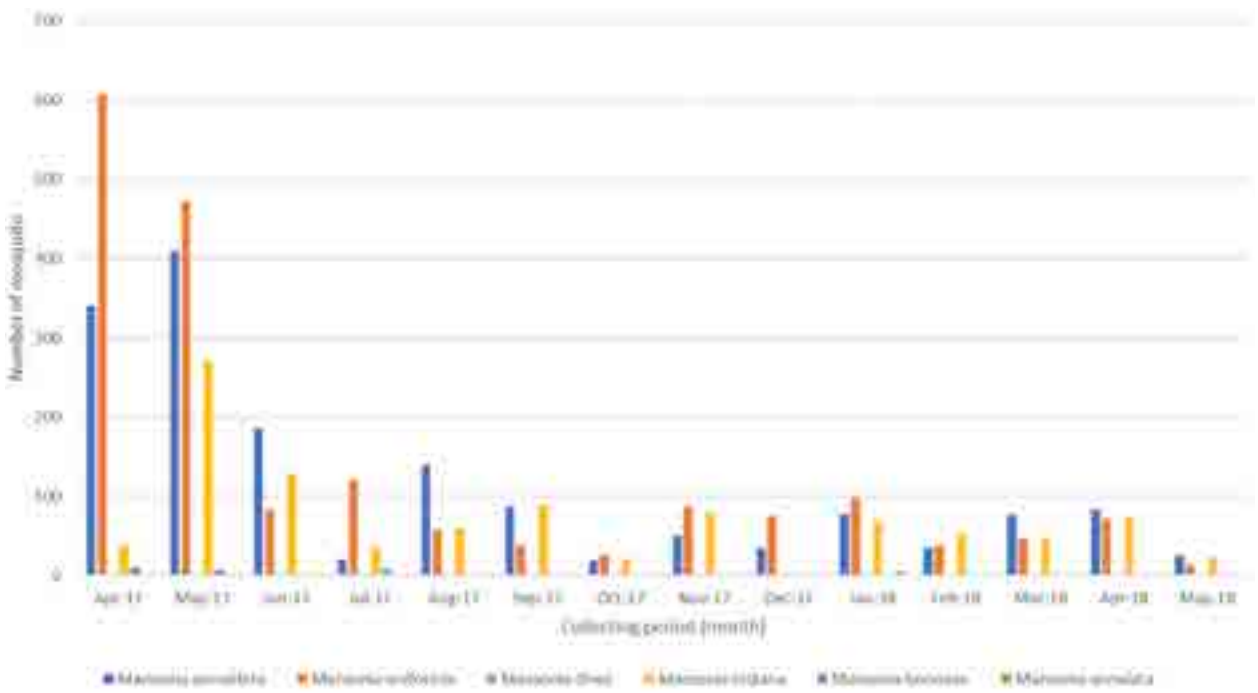

Figure 1. Seasonal distribution of *Mansonia* species.

study. It was because of the report of *Mansonia* spp. as the vector of filariasis compared to the other genera (Kumar et al. 1992).

The species identification showed six species of *Mansonia* collected in this study including *M. uniformis, M. annulifera, M. indiana, M. bonneae, M. annulata,* and *M. dives*. *M. uniformis* was found as the most abundant species (41.25%) followed by *M. annulifera* (35.63%), and *M. indiana* (22.14%). The detailed number and percentage of collected *Mansonia* spp. as a function of its species can be seen in Table 2.

To be more specific in seasonal distribution during the study period, *M. uniformis,* and *M. annulifera* were found as the most predominant mosquito species as they were present in almost all months, while *M. indiana* as the third highest number collected mosquito was not present in December 2017. The other *Mansonia* species have relatively low dominance by occurring only in the specific months. For example, *M. bonneae* was collected only in April 2017, May 2017, July 2017, January 2018, and March 2018; *M. annulata* in May 2017, September 2017, October 2017, January 2018, and February 2018; and *M. dives* was only detected in June 2017 and December 2017. Moreover, the period between April 2017 and June 2017 were found as the highest occurrence period of *Mansonia* species. It is because the air temperature and relative humidity were 27–28 °C and 90%, respectively which is the most suitable period for mosquitoes to breed than other seasons.

Table 2. The diversity of Mansonia spp in Sedang Village, Banyuasin Regency, South Sumatera, Indonesia.

Species	Number of collected mosquitoes	Percentage (%)
M. uniformis	1,835	41.25
M. annulifera	1,585	35.63
M. indiana	985	22.14
M. bonneae	30	0.7
M. annulata	9	0.2
M. dives	4	0.08
Totally	4448	100

Frequency, abundance, and dominance of *Mansonia* mosquito

The analysis result for the frequency, abundance, and dominance of mosquitoes biting outdoor and biting indoor is presented in Table 3. *M. uniformis, M. Annulifera*, and *M. indiana* become the top number of abundance and dominance compared to other species. Correlating with the number of collected mosquitoes, *M. uniformis* has the highest frequency both indoor and outdoor, abundance in indoor, and dominance in both condition. It was followed by *M. annulifera* and *M. indiana* which become the second and third species

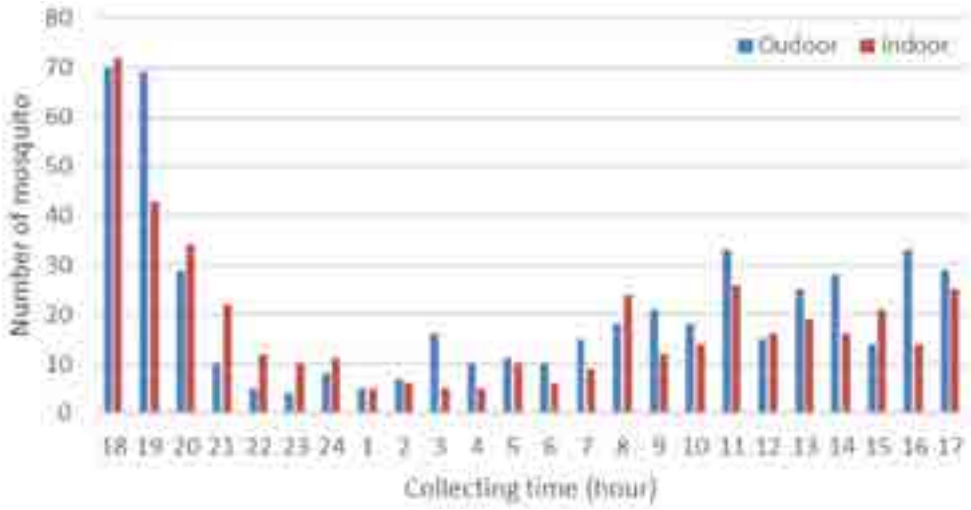

Figure 2. The biting activity of *Mansonia uniformis*.

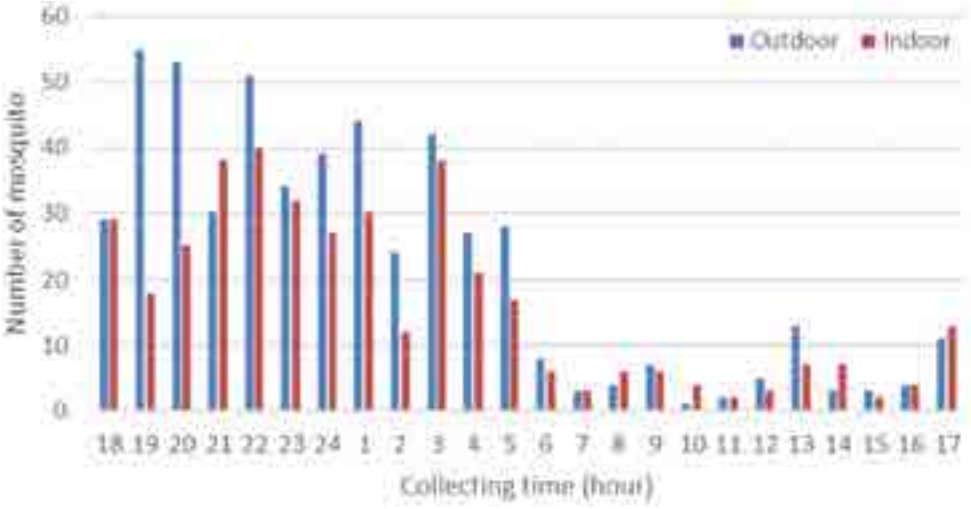

Figure 3. The biting activity of *Mansonia annulifera*.

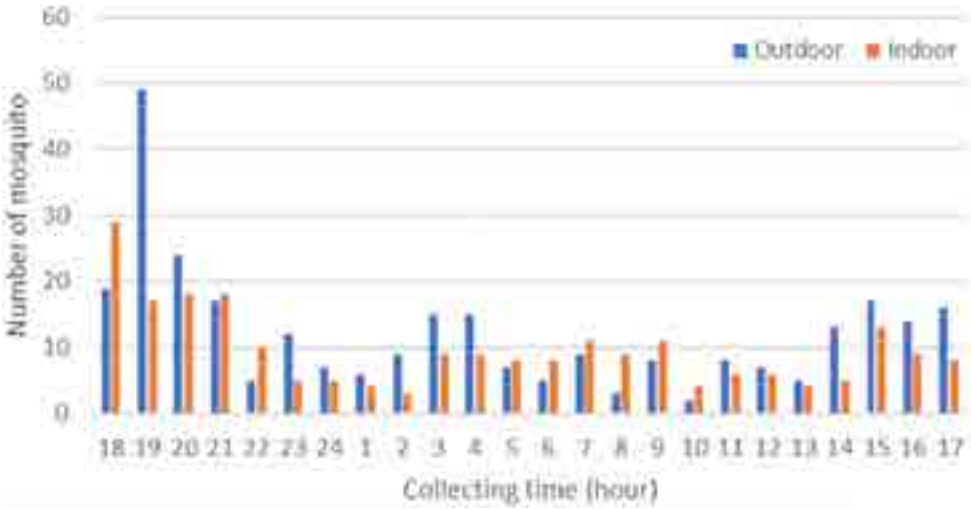

Figure 4. Biting activity of *Mansonia indiana*.

Table 3. Frequency, abundance, and dominance of Mansonia spp. biting activity in outdoor and indoor in Sedang Village.

Species	Outdoor Frequency	Indoor Frequency	Outdoor Abundance	Indoor abundance	Outdoor dominance	Indoor dominance
M. uniformis	0.50	0.42	37.90	41.15	19.06	17.51
M. annulifera	0.44	0.42	39.18	36.72	17.40	15.63
M. indiana	0.38	0.38	22.00	21.56	8.32	8.15
M. bonneae	0.03	0.009	0.68	0.38	0.02	0.003
M. annulata	0.003	0.003	0.15	0.09	0.0004	0.0003
M. dives	0.50	0.42	0.07	0.09	0.04	0.04

Table 4. Frequency, abundance, the dominance of Mansonia resting indoor and outdoor in Sedang village.

Species	Outdoor Frequency	Indoor Frequency	Outdoor abundance	Indoor abundance	Outdoor dominance	Indoor dominance
M. uniformis	0.41	0.46	39.96	46.88	16.41	21.49
M. annulifera	0.42	0.44	35.24	30.40	14.68	13.30
M. indiana	0.34	0.36	23.52	21.57	7.98	7.83
M. bonneae	0.02	0.03	0.79	0.86	0.02	0.03
M. annulata	0.009	0.009	0.003	0.3	0.027	0.002
M. dives	0.006	0.0	0.2	0.0	0.001	0.0

having the highest abundance and dominance under *M. uniformis*. But, *M. annulifera* was found as the highest outdoor abundance compared to all *Mansonia* spp. including *M. uniformis* as the highest collected mosquito.

In term of resting activities, there was a correlation between the biting activity and resting activity. Table 4 showed that *M. uniformis*, *M. annulifera* and *M. indiana* were the species that also had the highest outdoor and indoor resting frequency, abundance, and dominance compared with other species. In general, we can say that the high biting activity was positively followed by t high resting activity. However, *M. annulifera* showed a difference where it had high biting behavior and less resting activity.

The hourly biting behavior of most collected *Mansonia* spp.

The study was aimed to investigate the detailed biting time of *M. uniformis, M. annulifera,* and *M. indiana* as the highest species collected and active during the research period. Figure 2 showed that *M. uniformis* as the most collected species had the highest activity in the evening in both conditions (indoor and outdoor). It began at 1800 h and slightly decreased after 1900 h. However, the biting activity fluctuated and relatively increased in the early morning (after 0600 h) and continuously increased until the highest peak at 1800 h and 1900 h for indoor and outdoor activities, respectively. Moreover, the biting behavior patterns in outdoor and indoor was quite similar, instead the number of mosquitoes caught are different. The outdoor biting activity was higher than the indoor biting activity, indicating the *M. uniformis* was categorized as the exophage species.

Figure 3 showed the biting activity as a function of time of *M. annulifera* for 24 hours of collecting period. The outdoor biting activity began at 1800 h with the highest biting activity at 1900 h. The biting activity slowly decreased till midnight and then again slightly decreased after 0400 h. During noon, most of *M. annulifera* had low biting activity until 1600 h and started to increase after 1700 h. The biting activity was different with the indoor biting behavior of *M. annulifera* which began the biting activities at 0600 hoand had the highest peak of biting activity at 0300 h. The indoor biting activity started to drop at 0400 h to 1100 h and fluctuated between 1200 h and 1700 h. The biting behavior pattern of *M. annulifera* was different in the highest biting activity in outdoor and indoor condition, but had similar behavior in the low biting activity. Based on the number of collected species, *M. annulifera* identified as exophage species which had a higher number of collected mosquitoes in outdoor compared to the number of catch mosquito in indoor condition.

Figure 4 showed the biting activities of *M. indiana*

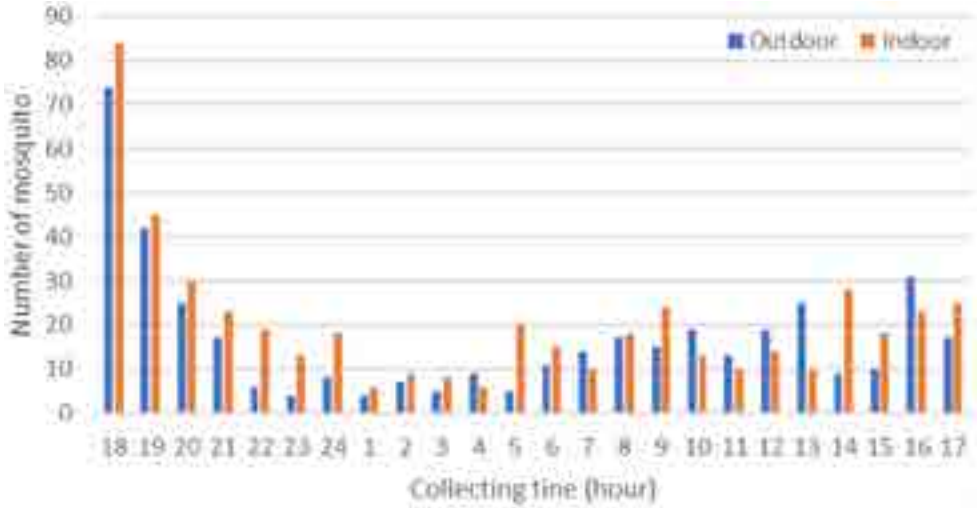

Figure 5. Resting activity of *Mansonia uniformis*.

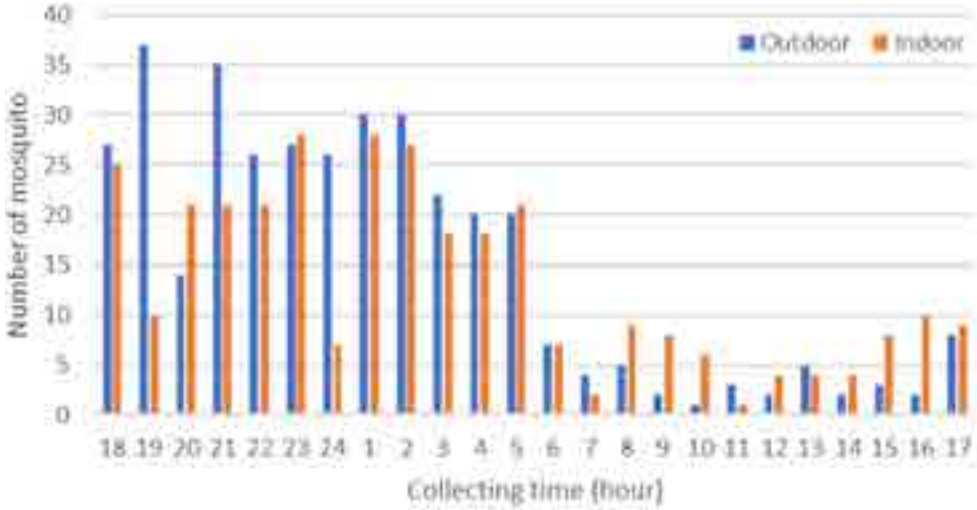

Figure 6. Resting rythme of *Mansonia annulifera*.

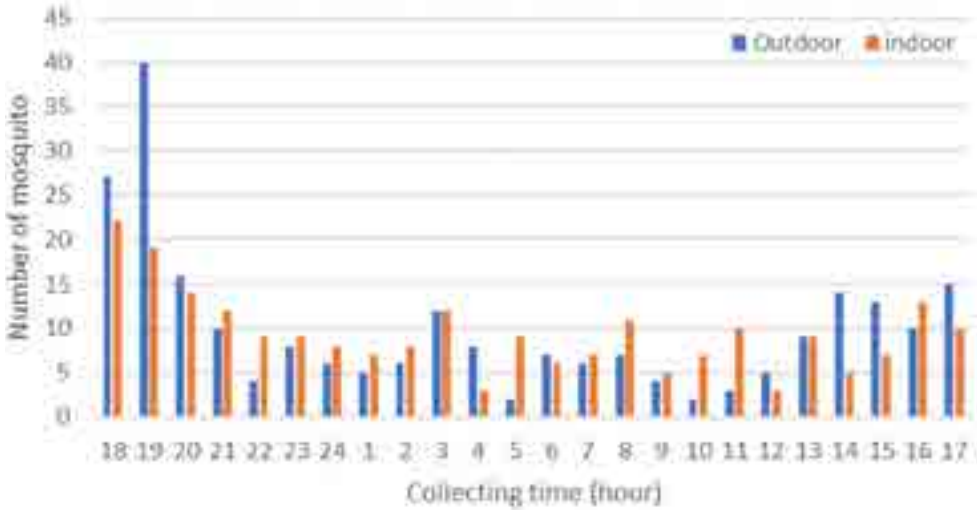

Figure 7. Resting rythme of *Mansonia indiana*.

as the third most collected mosquitoes in this present study. The highest peak of biting activity of *M. indiana* conducted at 1800 h and 1900 h for indoor condition and outdoor condition, respectively. However, there were an extremely decreased after the highest biting activity for outdoor condition, while the indoor condition showed a slightly decrease after the highest peak activity. The biting behavior of *M. indiana* was relatively different in outdoor and indoor condition at the highest biting activity was at 1800 h to 2300 h, but having the similar activity after the highest peak period. Based on the number of biting activity, *M. indiana* was the exophage species which had higher outdoor biting activity than indoor biting activity.

In this study, *Mansonia* spp. had a higher outdoor biting activity than indoor biting activity. It was in accordance with the study reported by Supranelfy et al. (2012) where *Mansonia* spp. bites more frequently outdoor than indoor condition. However, as the fraction of indoor biting behaviour remained high, we would still have to take them into account. The availability of the main indoor host (humans), or reservoir hosts (i.e., pets), attracted the adult mosquitoes to do more activities indoors. Besides, the environmental factors including climate, geography, and geology, the socio-economic-cultural environment (the environment produced by interpersonal interactions) potentially modify the outdoor/indoor ratio of biting activity of *Mansonia*.

Study the resting behavior of most collected *Mansonia* spp.

The bionomic study was continued by investigating the resting behavior of *Mansonia* species. In Figure 5, the resting activity of *M. uniformis* was quite similar with the biting activity (Figure 2). The highest peak of highest resting peak was conducted in the similar time of highest peak of biting activity. The result indicated that most of *M. uniformis* did resting activity when doing biting activity. The high number of resting activity also indicated that there was a high number of mosquito population in those range of time (1800–1900 h). Moreover, the resting mosquitoes was slightly down in number after the highest peak of resting activity until 2300 h and become fluctuating after 2300 h until 1700 h the next day. The resting paths for indoor and outdoor condition were relatively similar, indicating the condition (indoor or outdoor) did not affect resting behavior. In terms of number, the number of resting activities was found higher in indoor condition than outdoor condition. However, it could not be said that *M. uniformis* was categorized as endophage species since it was an exophage species based on biting behavior. The most possibility reason why there were high number of *M. uniformis* found in indoor condition was because of the presences of suitable place for resting.

Table 5. The density of collected *Mansonia* spp.

Species	Outdoor biting	Man hour density (MHD)	Indoor biting	Man hour density (MHD)
M. uniformis	503	10.43	437	9.06
M. annulifera	520	10.78	390	8.08
M. indiana	292	6.05	229	4.75
M. bonneae	9	0.19	4	0.08
M. annulata	2	0.04	1	0.04
M. dives	1	0.02	1	0.04

Table 6. Man biting rate data.

Species	Outdoor Biting	Indoor Biting	Total	MBR
M. uniformis	503	437	940	156.67
M. annulifera	520	390	910	151.67
M. indiana	292	229	521	86.83
M. bonneae	9	4	13	2.17
M. annulata	2	1	3	0.50
M. dives	1	1	2	0.33

The resting behavior of *M. annulifera* was found similar with the resting behavior of *M. uniformis* where the pathway was correlated with the biting activity. To be more specific, the outdoor resting activity began at 1800 h and had the highest peak at 1900 h. The outdoor resting behavior fluctuated between 2000 h and 0500 h the next day. However, the indoor resting behavior was completely different with the outdoor resting behavior where *M. annulifera* had the highest peak of resting activity at 2300 h. The resting behavior was similar with its biting activity, indicating *M. annulifera* relatively did the resting after biting activity in both condition (outdoor and indoor). In case of number, the resting activity in outdoor condition was higher than the number of indoor resting behavior, meaning *M. annulifera* was categorized as the exophage species. However, the result was different with the study reported by Kumar (1992) who reported *M. annulifera* as the endophage species.

Figure 7 shows the resting forms of *M. indiana* for 24 hours of collecting time. The result showed that the outdoor resting activity started at 1800 h with the highest peak at 1900 h. After the highest resting activity, the number of resting mosquitoes decreased

Table 7. Parity rate and longevity.

Species	Number of dissection	Parous	Nulliparous	Parity rate	Longevity (days)
M. uniformis	636	508	128	0.79	13.35
M. annulifera	680	491	189	0.72	9.21
M. indiana	544	443	101	0.81	14.61
M. bonneae	9	3	6	0.33	2.7
M. annulata	5	2	3	0.4	3.3
M. dives	4	4	0	1	-

and relatively fluctuated until the next day. In indoor condition, the resting time started 1800 h, which also become the highest peak of resting time. The resting activity dropped after the high peak and continuously fluctuated until the next day. The outdoor and indoor resting activity was quite similar but having the different in quantity. The number of resting *M. indiana* in outdoor condition was found higher than the number of resting *M. indiana* in indoor resting activity, indicating *M. indiana* as the exophage species.

From the study of resting behavior of most collected *Mansonia* spp., most of mosquitoes had the similar rhythm with the biting activity, indicating the biting activity was always followed by the resting activity before continuing doing their activity. However, it was only a hypothetic theory based on the rhythm of biting and resting activity.

Mosquitos density

Table 5 reveals that in Sedang village, the outdoor man hour density of *M. uniformis* was 10.43 mosquitos per person-hour in which *M. annulifera* and *M. indiana* were 10.78 and 6.05 mosquito per person-hour, respectively. On the other hand, the indoor man hour density of *M. uniformis*, *M. annulifera, M. indiana* were 9.06, 8.08, and 4.75 mosquitos per person-hours, respectively. It should be a concern because the potential for filariasis transmission is very high. The result was different with the one reported by Sabesan et al. (1991) where the average of man hour density for *M. annulifera, M. uniformis*, and *M. indiana* indoor were 3.29, 0.25, and 0.01, respectively.

Santoso et al. (2016) conducted a study of Mansonia species in Jambi Province, Indonesia and reported that the man hour density was below five (Santoso et al. 2016). In this study, we found that outdoor and indoor MHD of *Mansonia* spp. have more than five which meant that the potential for filariasis transmission was very high. It was supported by the regulation of the Minister of Health of the Republic of Indonesia No. 50 of 2017 where the value of man hour density should be under five. In addition, the high the man density was supported by the data of man biting rate (Table 6). The result showed that the highest man-biting rate was *M. uniformis* (156.67) followed by *M. annulifera* (151.67), and *M. indiana* (86.83), which correlated with the biting activities of *Mansonia* spp.

Dissection was performed on the ovaries of mosquitos to find out whether the mosquitos had laid their eggs or not. In Sedang village, the dissection was carried out using 1,878 *Mansonia* spp. Table 7 explains that 1,878 *Mansonia* spp. mosquitos had a dissection in which 1,451 and 427 were parous and nulliparous, respectively. The longevity was performed to see how long the mosquito life expectancy. To obtain the longevity, the parity rates was calculated. The result showed that the paruty rate of *M. uniformis, M. annulifera,* and *M. indiana* were 0,79, 0,72, and 0,81, respectively, indicating there are 79%, 72%, and 81% of these mosquitos have oviposited their eggs.

Based on the parity rate, the population longevity of *M. uniformis*, *M. annulifera,* and *M. indiana* was found to be 13.35 days, 9,21 days and 14,61 days, respectively. The result found that *Mansonia* was ideal as the host of filariasis transmission where the growth period of microfilariae in the body of mosquitoes that become hosts ranges from 10–14 days. To be more specific, *Brugia* species need 8–10 days, the *Wuchereria* species takes 10–14 days (Ministy Heath of Republic of Indonesia 2016). According to Gilles & Warrel (1993), the cycle of mosquitoes and the age are obtained to support the development of the parasite cycle in the body of mosquitoes. The number of longevity determined how long the host could transmit the disease, when associated with the parasite life cycle. Observation of the age of life was one of the most important factors in determining the discrimination of vectors so that transmission can be detected somewhere (Mardiana 2009).

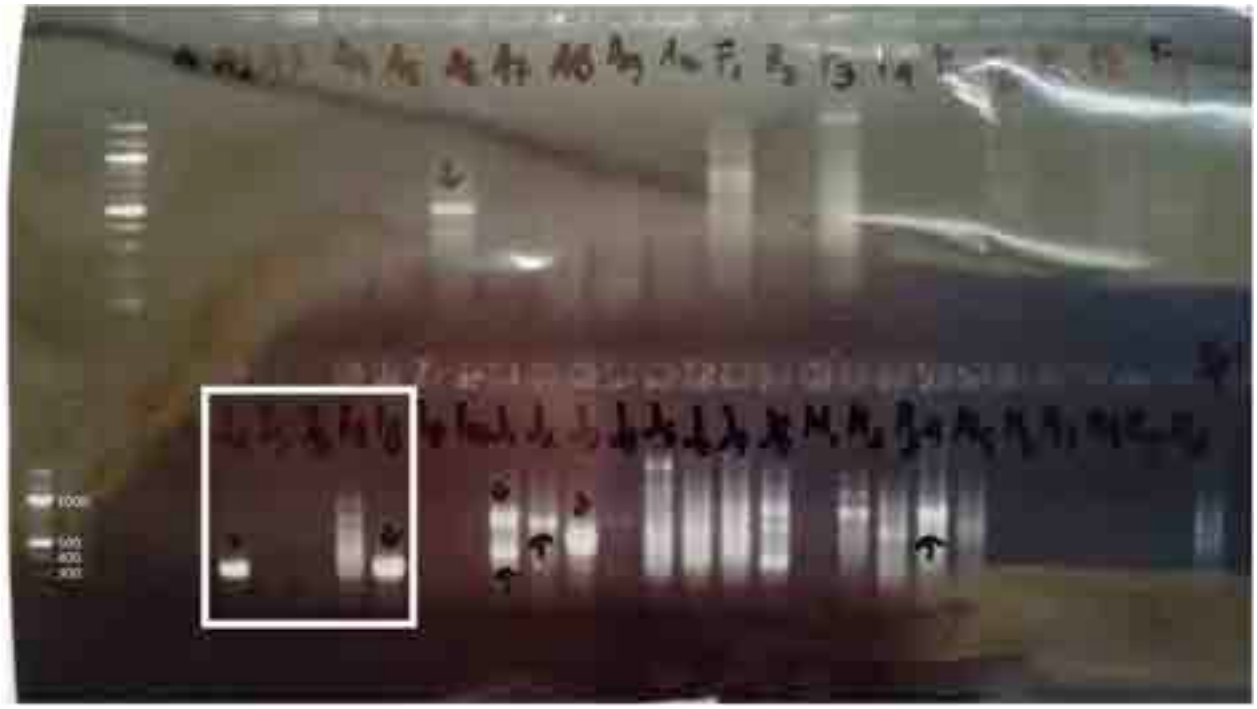

Image 3. Agarose gel with ethidium bromide electrophoresis UV visualization of sample's pools. Positive PCR amplicons wells of *Brugia malayi* were at 324 bp. Note: PCR process produces false-positive result, where the white band was in the wrong base pair size.

The PCR study was performed to support the longevity and the potential of filariasis transmission. In Figure 10, there was a correct band size of *Brugia malayi* which detected in I4 sample which come from *M. annulifera*. There was no positive band size of *Brugia malayi* detected in the other sample, indicating only *M. annulifera* potentially transmitted the filariasis. However, the result could not be a final conclusion since the other *Mansonia* species was potential as the host for filariasis transmission.

CONCLUSION

In conclusion, *M. uniformis, M. annulifera*, and *M. indiana* have the highest frequency, abundance, and dominance. The biting activity and resting rhythm are available in 24 hours and they also had a big parity rate and longevity. They eventually had the greatest number of MHD and MBR, which could be contributed to the high rate of filariasis transmission. *M. annulifera* was confirmed as the potential filariasis vector based on PCR examination.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21055–21067

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7549.14.5.21055-21067

#7549 | Received 30 June 2021 | Final received 18 April 2022 | Finally accepted 15 May 2022

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COMMUNICATION

Plant species diversity in a tropical semi-evergreen forest in Mizoram (northeastern India): assessing the effectiveness of community conservation

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Abstract: Community conservation of forest as a means of biodiversity conservation has gained broad acceptance in recent years. However, there are not many studies in India on how effective they really are for conservation of plants and how they compare to formal protected areas. This study was carried out in Reiek forest, a community conserved forest protected for more than a century, initially by the village Chiefs and after the abolishment of chieftainship, by the community of the nearby villages. An attempt was made to study the plant species diversity of this forest which falls under the Indo-Myanmar diversity hotspot and it was compared to two ecologically similar formal protected areas within Mizoram. A total of 265 species belonging to 213 genera and 89 families were recorded. Two vulnerable species *Eleocarpus rogusus* and *Saraca asocas* were identified. It was found that this community conserved forest contained more plant species than the two protected areas. But endemic and threatened species were found to decline in the community conserved forest.

Keywords: Biodiversity, community conservation, life forms, plant diversity, protected area.

Editor: Anonymity requested.

Date of publication: 26 May 2022 (online & print)

Citation: Lalzarzovi, S.T. & Lalnuntluanga (2022). Plant species diversity in a tropical semi-evergreen forest in Mizoram (northeastern India): assessing the effectiveness of community conservation. *Journal of Threatened Taxa* 14(5): 21055–21067. https://doi.org/10.11609/jott.7549.14.5.21055-21067

Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: STL and L developed the hypotheses and designed the methodology. STL collected the data in the field.. STL and L prepared the manuscript and gave approval for publication.

Acknowledgements: H. Lalhmachhuana, Scientist, Mizoram Remote Sensing Application Centre, Directorate of Science and Technology, Government of Mizoram for map preparation.

INTRODUCTION

Tropical forest contains the most diverse plant communities on earth and are disappearing at an alarming rate due to wide-spread land use changes with detrimental consequences for biodiversity, climate, and other ecosystem services (Givnish 1999; Lambin & Geist 2006). This ongoing loss of biodiversity has led to many studies which explores how effective the various approaches are for preventing ecosystem degradation and species extinction while providing sustainable use of resources (Shahabuddin & Rao 2010). The most important and commonly used measure for conserving biodiversity and reducing deforestation is the use of formal protected areas (Millennium Ecosystem Assessment 2005; Bajracharya et al. 2005) which has proven to be effective by studies such as Naughton-Treves et al. (2005) and Oliveira et al. (2007). However, while previous research has estimated the effectiveness of formal protected areas in reducing deforestation rates to be 65%, more recent studies in Costa Rica suggest only a 10% reduction within the protected areas (Andam et al. 2008).

In the last few decades, community conservation of biodiversity rich area, whether partial or complete as an effective method to prevent species extinction has gained broader acceptance (Kothari 2006). Various studies have shown that within the same region, forests which are conserved and managed by local or indigenous communities can be as effective in reducing deforestation as compared to officially designated protected areas committed to sole protection without community involvement (Porter-Bolland et al. 2012; Bray et al. 2008; Nepstad et al. 2006). Hayes (2006) found that the state of a forest in formally protected areas and community conserved forest were similar and suggested that the forest was in a better state when the rules of management were set and enforced by locals as compared to those without such rules. However, the use of community-based conservation for tropical forests is disputed with many prominent conservationists advocating for authoritarian enforcement of protected areas (Brockington 2007; Wilshusen et al. 2002).

Mizoram, situated in the north eastern part of India is composed of steep, rugged hill ranges and interspersed valleys. It has rich flora and fauna and the highest percentage of forest cover (84.53%) in the country (FSI 2021). The forests of the state are under a three tier management viz. those owned and controlled by the state, district councils, and village councils. The extent of forest under community control is 20.53% (FSI 2019). Traditionally, forest management in Mizoram was carried out by the 'Chieftain', helped by his advisors, who had the absolute decision making authority. Under the Mizo District (Land and Revenue) Act of 1956, the Chief was made the Chairman of the Village Authority without any discretionary authority. Another important traditional institution is 'Zawlbuk', a bachelor dormitory run by an important official of village government called 'Val Upa' (youth commander). Val upa through Zawlbuk imparted discipline and training in the art of tribal warfare and defence to male youth of the village. Zawlbuk no longer exists, and this traditional institution is now represented by Young Mizo Association (YMA) which may be considered the modern form of Val Upa. With people still depending on resources of forests and common land, village level YMA plays an important role in managing common property resources. YMA with the support of village council take the responsibility for management of community forest. (Tiwari et al. 2013)

Reiek forest in Mizoram is one such community conserved forest which is managed by the Young Mizo Association (YMA) and the village council of the two villages falling within the forest area. Shifting cultivation, being the main mode of agriculture in Mizoram, has destroyed much of the virgin forest and led to formation of secondary communities in the disturbed sites .However, this forest has been protected and conserved by the descendants of Sailo Chiefs since the 1890's. The Village Chief prohibited the killing of animals and plants in the forest and introduced a modern method of conservation with stringent protection. Shifting cultivation in this area was banned and as a result, while most of the area around this conserved area is degraded, this forest represents a forest ecosystem relatively less degraded by anthropogenic disturbances. There is an ongoing debate on what measures are the best for the forest and biodiversity conservation with some in favour of strict protection and others advocating for a more community driven form of conservation. The question remains on whether community conservation of forest is as effective as designating them as protected areas. With this in mind, the present study has been undertaken. The plant species diversity of a community conserved tropical semi-evergreen forest in Mizoram was determined and compared with the plant diversity of protected areas in the state and another community conserved forest outside the state.

MATERIALS AND METHODS

Study area

This research study was conducted in Reiek forest located between longitude 92.6039908 and latitude 23.6994866 in Mamit district of Mizoram, northeast India. This forest corresponds to Champion & Seth's (1968) Cachar Tropical Semi-evergreen Forest (2B/C2*)* and covers an area of 10 km^2. The highest point of Reiek Mountain is at 1485m asl. The annual temperature in Mamit district ranges between 8–22 ^{0}C in winter and 20–28 ^{0}C in summer. Average annual rainfall received during the study period from 2008–2012 was 2,585 mm which is mainly brought in by the southwest monsoon. Rainy season starts in early April, with interrupted showers, but incessant rain begins in June and continues until September, often stretching until October. The soil is composed of silt-loam in the upper portion and medium grain sandstone stone plates in the peak region and the rest of the area is mostly sandy-loam to black humus top-soils depending on thickness of the vegetation and nature of landscape.

Methods

Vegetation analysis was carried out using the methods outlined by Misra (1968) and Domboise & Ellenberg (1974) during the year 2008–2012. To study the woody species, 50 quadrats of 10 m^2 in area were laid randomly and diameter at breast height (dbh) of trees were measured and recorded. Within each quadrat, five smaller quadrats of 1m^2 were laid down for herbs and shrubs, one in each corner and one in the centre. All the understory plants viz. herbs (non-woody small plants *1–1.5 m tall), shrubs (*1.5–3 m tall with thick stem and branching at ground level without a distinct trunk) and herbaceous climbers were enumerated. Species diversity was determined by computing the Shannon diversity index (Shannon and Weaver 1949). Species identification was carried out using regional flora publications (Kanjilal et al., 1940; Singh et al.,2002; Lalramnghinglova, 2003; Sawmliana, 2003) and counterchecked with the herbarium of the Botanical Survey of India, Eastern Circle, Shillong. The conservation status of the identified species were assessed using Red Data book of India (Nayar & Sastry 1987–1990) and Red List of Threatened Vascular Plant Species in India (Rao et al. 2003). The results were compared with the plant diversity of two protected areas in Mizoram which are

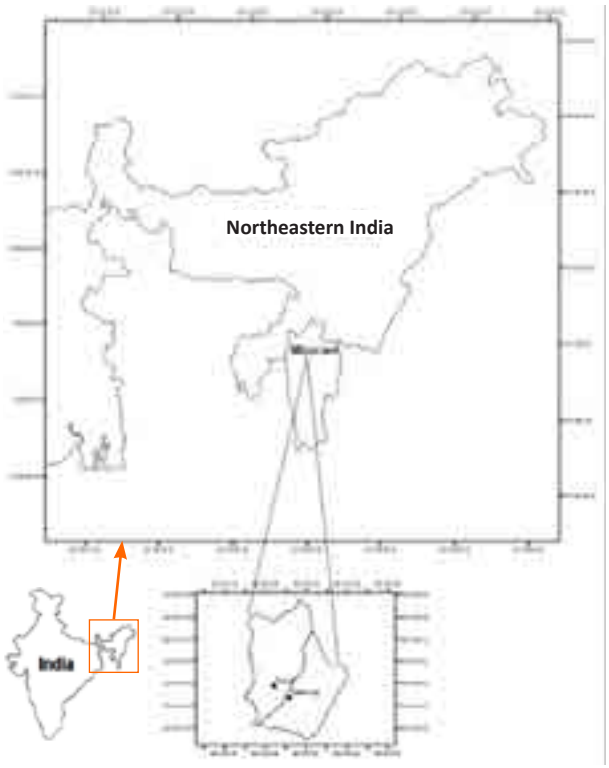

Figure 1. Location map of Reiek forest, a community conserved forest in Mizoram.

Figure 2. Map of the study area.

ecologically similar- Phawngpui which was declared a National park in 1997 with an area of 50 km^2 and Tawi Wildlife Sanctuary notified in 1999 with an area of 35.75 km^2.

RESULTS

Family

A total of 89 families were recorded out of which 84 were native while 5 were non-native. Out of the native families, 76 were angiosperms, 2 families were gymnosperms and 6 families were pteridophytes while the non-native families were all angiosperms. Dicotyledons comprised of 69 families (65 native and 4 non-native) and monocotyledons comprised of 12 families (11 native and 1 non-native). Five families with the highest species diversity (dominant families), accounting only 5.43% of total families represented 28.27% of the total species, and 26% of genera. Family with highest number of species was Orchidaceae (23 native species) followed by Poaceae (19 native and 2 non-native species), Arecaceae (11 native species and 3 non-native species) and Rubiaceae (11 native species and 1 non-native species) In contrast to the dominant families, 42 families (38 native and 4 non-native) were represented by only one species each.

Genera

A total of 213 genera were recorded (194 native and19 non-native) out of which 31 genera are multi-species while the rest were represented by only one species. Among the multi species genera, the largest genus was *Dendrobium* with seven species and among trees, *Ficus* and *Elaeocarpus* had five species each. The ratio of genera to species was 1:1.24 for native species which means that almost any one of the species of this site belongs to a different genus.

Species

A total of 265 species were recorded out of which 241 were native species and 24 were non-native species Habitat-wise analysis of flora showed 103 species of trees (97 native species and 6 non-native species), 32 species of shrubs (28 native species and 4 non-native species), 48 species of herbs (45 native species and 3 non-native species), 25 species of climbers/lianas (19 native species and 6 non-native species), 15 species of canes and palms(12 native species and 3 non-native species), 17 species of grasses(15 native species and 2 non-native species) and 25 species of epiphytes (native species) (Table 1)

Out of the total native plant species identified in the study site, 96% were found to be angiosperms, 3.5% gymnosperms and the rest were pteridophytes. All the non-native species identified were angiosperms. Among the native angiosperms, dicotyledons represented 74.2% while monocots represented 25.8% while for the non-native angiosperms, dicotyledons represented 80% while monocots represented 20%. The ratio of monocotyledons to dicotyledons was 1:2.89 for native species.

Diversity of life-form

Life forms of plants in Reiek forest were determined based on the classification of Raunkiaer (1934). All species were classified by life forms (Misra 1968; Domboise & Ellenberg 1974). The existence of a variety of life forms reflects the typically tropical characteristics of the flora of Reiek forest. Phanerophytes were the most dominant life form with about 50% of total plant species in the area. Out of the phanerophytes, Megaphanerophytes, i.e., trees exceeding 30 m were absent. Mesophenorophytes accounted for 32.45% of the total life form (78 native species and 8 non-native species), microphenarophytes accounted for 13.96% (36 native species and 1 non-native species), nanophanerophytes accounted for 6.04%, (15 native species and 1 non-native species), Chamaephytes accounted for 9.81% (24 native species and 2 non-native species), Hemicryptophytes accounted for 4.15% (9 native species and 2 non-native species) , Cryptophytes or geophytes accounted for 7.92 % (19 native species and 2 non-native species), Therophytes accounted for 5.66% (13 native species and 2 non-native species), Epiphytes accounted for 11.32 % (30 species) and lianas accounted for 8.68% (17 native species and 6 non-native species) of the total life form.

Species diversity index

The species diversity index (Shannon diversity H') for native species was highest among trees (3.9) followed by herb (3.45) and then shrubs (3.05)

Conservation status: Rare and threatened species

Out of the 265 species identified, only 15 have been assessed by the IUCN out of which two species have been identified as vulnerable which are *Elaeocarpus rogusus* and *Saraca asoca.* One species *Amomum dealbatum* is placed under Data Deficient.

Table 1. Comparison of plant diversity of Community conserved Reiek forest, Phawngpui National Park and Tawi Wildlife Sanctuary

Criteria	Reiek Forest (Native)	Reiek Forest (Non-native)	Phawngpui National Park	Tawi Wildlife Sanctuary
Number of families	84	5	71	83
Number of genera	194	19	150	167
Number of species	241	24	208	219
Trees	97	6	84	83
Shrubs	28	4	31	31
Herbs	45	3	45	41
Climbers and epiphytes	44	6	33	52
Grasses	15	2	10	17
Canes and palms	12	3	5	10
Species Diversity (Shannon diversity index)				
Trees	3.9		3.68	3.86
Shrubs	3.05		2.8	3.14
Herbs	3.45		2.96	3.26

DISCUSSION

Despite rampant deforestation for shifting cultivation in the state of Mizoram, the community conserved Reiek forest in Mamit district of Mizoram. It was found to have rich plant diversity comparable to protected areas under strict protection of the Forest Department, Government of Mizoram and to other community conserved sacred groves outside Mizoram. The climatic conditions of the area, its geographic proximity to the species-rich eastern Himalayas, Burma and the Malayan peninsula may be responsible for the formation of this rich biodiversity area but maintenance of this rich ecosystem may be attributed solely to its prolonged protection by the community.

Reiek forest containing 241 native species was found to support more plant species diversity than two formal protected area viz Phawngpui National Park and Tawi Wildlife Sanctuary. Phawngpui National Park was reported to have 208 species belonging to 150 genera and 71 families (Malsawmsanga 2011) while Tawi Wildlife Sanctuary was reported to have 219 species belonging to 167 genera and 73 families (Lallawmkimi 2011). Outside Mizoram, Namdapha National Park, a protected area with tropical wet evergreen vegetation was reported to have 200 species (Nath et al. 2005) and a community conserved sacred groves of Jaintia Hills was reported to have 395 species (Jamir and Pandey 2003). This is not an unusual finding. For example, Garcia and Pascal (2005) in their comparison of sacred groves to formal protected area in the Western Ghats of Karnataka, India found that the number of woody plant species were higher in the sacred groves than the adjacent Brahmagiri wildlife sanctuary. Similar results were also reported by Shackleton (2000) in their comparison of plant diversity in protected and communal lands in South Africa.

The percentage of angiosperms, gymnospersms and pteridophytes present in Reiek forest were almost similar to those reported in the sacred grove of Jaintia hill (Jamir & Pandey 2003) which have been under traditional community conservation for centuries. In Tawi Wildlife Sanctuary, 86.7% were angiosperms, 1.2% were gymnosperms, and 12.05% were pteridophytes (Lallawmkimi 2011).

The ratio of genera to species for native species was 1:1.24 while a ratio of 1:1.3 have been reported by Lallawmkimi (2011) for Tawi Wildlife Sanctuary.

The life form of Reiek forest closely resembles that of Tawi Wildlife Sanctuary where Megaphanerophyte were also absent and mesophanerophytes with 28.27% was the dominant life form followed by microphanerophyte 20.25%, nanophanerophyte 11.39%, chamaephyte 10.97 %, geophytes 3.38%, therophytes 3.80%, epiphytes 10.97% and climbers 10.97% (Lallawmkimi 2011). The dominance of Phanerophytes is a feature of tropical humid forest life form spectra (Richard, 1996). The life form spectrum of plant community of Reiek forest reveals that Hemicryptophytes and Therophytes were lower than the normal spectrum of Raunkiaers. Hemicryptophytes are characteristics of temperate region and therophytes are characteristics of desert climate (Cain & Castro 1959; Shimwell 1971).

Table 2. List a plant species recorded in community conserved Reiek forest of Mamit district in Mizoram, India.

	Name of species	Family	Native/ Non-native species
Tree species			
1	*Acer laevigatum* Wall.	Aceraceae	Native
2	*Acronychia pendunculata* (L.) Miq	Rutaceae	Native
3	*Aglaia spectabilis* (Miq.) S.S.Jain & S.Bennet.	Meliaceae	Native
4	*Alangium chinense* (Lour.) Harms	Alangiaceae	Native
5	*Alphonsea ventricosa* (Roxb.) Hook. f. & Thomson	Annonaceae	Native
6	*Alseodaphne petiolaris* (Meissn.) Hook. f.	Lauraceae	Native
7	*Amoora chittagonga* (Miq.) Hiern	Meliaceae	Native
8	*Anogeissus acuminata* (Roxb. ex DC.) Guillaumin et al.	Combretaceae	Native
9	*Betula cylindrostachys* Wall. ex Diels	Betulaceae	Native
10	*Bombax insigne* Wall	Bombacaceae	Native
11	*Bruinsmia polysperma* (C.B. Clarke) Steenis	Styracaceae	Native
12	*Calliandra umbrosa* (Wall.) Benth.	Mimosaceae	Native
13	*Calophyllum polyanthum* Wall. ex Choisy	Guttiferae	Native
14	*Camellia kissi* Wallich	Theaceae	Native
15	*Carallia brachiata* (Lour.) Merr.	Rhizophoraceae	Native
16	*Castanopsis echinocarpa* Miq.	Fagaceae	Native
17	*Castanopsis indica* (Roxb. ex Lindl.) A.DC.	Fagaceae	Native
18	*Castanopsis tribuloides* (Sm.) A.DC.	Fagaceae	Native
19	*Celtis timorensis* Span.	Ulmaceae	Native
20	*Cephalotaxus griffithii* Hook. f.	Cephalotaxaceae	Native
21	*Cinnamomum glanduliferum* (Wall.) Meisner	Lauraceae	Native
22	*Cinnamomum obtusifolium* (Roxb.) Nees.	Lauraceae	Native
23	*Cinnamomum verum* J.Presl	Lauraceae	Non-native
24	*Coffea khasiana* (Korth.) Hook.f.	Rubiaceae	Native
25	*Colona floribunda* (Wall. ex Kurz) Craib	Tiliaceae	Native
26	*Croton hookeri* Veitch	Euphorbiaceae	Native
27	*Cryptocarya amygdalina* Nees Bauch.Ham	Lauraceae	Native
28	*Cycas pectinata* Buch.-Ham	Cycadaceae	Native
29	*Debregeasia longifolia* (Burm. f.) Wedd.	Urticaceae	Native
30	*Diospyros lancifolia* Wallich ex Hiern	Ebenaceae	Native
31	*Drimycarpus racemosus* (Roxb.) Hook.f.	Anacardiaceae	Native
32	*Dysoxylum gobara* (Buch.-Ham.) Merr.	Meliaceae	Native
33	*Elaeocarpus floribundus* Blume	Tiliaceae	Native
34	*Elaeocarpus lanceaefolius* Roxb.	Tiliaceae	Native
35	*Elaeocarpus rugosus* Roxb.	Tiliaceae	Native
36	*Elaeocarpus tectorius* (Lour.) Poir.	Tiliaceae	Native
37	*Embelia tsjeriam-cottam* A.DC.	Myrsinaceae	Native
38	*Engelhardtia roxburghiana* Wall.	Juglandaceae	Native
39	*Engelhardtia spicata* Leschen, ex. Blume	Juglandaceae	Native
40	*Eriobotrya bengalensis* (Roxb.) Hook. f.	Rosaceae	Native
41	*Eurya cerasifolia* (D. Don) Kobuski	Pentaphylacaceae	Native
42	*Eurya loquaiana* Dunn	Pentaphylacaceae	Non-native
43	*Ficus benghalensis* L.	Moraceae	Native

	Name of species	Family	Native/ Non-native species
44	*Ficus benjamina* L.	Moraceae	Native
45	*Ficus prostrata* (Wall. ex Miq.) Miq.	Moraceae	Native
46	*Ficus religiosa* L.	Moraceae	Native
47	*Ficus semicordata* Buch.-Ham. ex Sm.	Moraceae	Non-native
48	*Garcinia xanthochymus* Hook. f. ex T. Anderson	Guttiferae	Native
49	*Glochidion khasicum* (Müll.Arg.) Hook. f.	Euphorbiaceae	Native
50	*Grevillea robusta* A. Cunn. ex R. Br.	Proteaceae	Non-native
51	*Gynocardia odorata* R. Br.	Flacourtiaceae	Native
52	*Helicia erratica* Roxb.	Proteaceae	Native
53	*Heteropanax fragrans* (Roxb.) Seem	Araliaceae	Native
54	*Holigarna longifolia* Buch.-Ham. ex Roxb	Anacardiaceae	Native
55	*Lithocarpus elegans* (Blume) Hatus. ex Soepadmo	Fagaceae	Native
56	*Lithocarpus pachyphyllus* (Kurz) Rehder	Fagaceae	Native
57	*Litsea lancifolia* Roxb. ex Nees	Lauraceae	Native
58	*Litsea monopetala* (Roxb.) Pers.	Lauraceae	Native
59	*Macaranga indica* Wight	Euphorbiaceae	Native
60	*Macropanax undulatus* (Wall. ex G.Don) Seem.	Araliaceae	Native
61	*Magnolia hodgsonii* (Hook.f. & Thomson) H.Keng	Magnoliaceae	Native
62	*Mallotus philippensis* (Lam.) Müll.Arg.	Euphorbiaceae	Native
63	*Mangifera sylvatica* Roxb.	Anacardiaceae	Native
64	*Memecylon celastrinum* Kurz	Melastomataceae	Native
65	*Mesua ferrea* Linn.	Guttiferae	Native
66	*Michelia champaca* Linn.	Magnoliaceae	Native
67	*Musa sylvestris* LA Colla	Musaceae	Non-native
68	*Neolamarckia cadamba* (Roxb.) Bosser	Rubiaceae	Native
69	*Olea dioica* Roxb.	Oleaceae	Native
70	*Olea salicifolia* Wall. ex G.Don	Oleaceae	Native
71	*Ostodes paniculata* Blume	Euphorbiaceae	Native
72	*Persea glaucescens* Nees.	Lauraceae	Native
73	*Persea villosa* (Roxb.) Kosterm.	Lauraceae	Native
74	*Phoebe lanceolata* (Nees) Nees	Lauraceae	Native
75	*Pithecellobium bigeminum* (L.) Mart.	Mimosaceae	Native
76	*Premna racemosa* Wall. ex Schauer	Lamiaceae	Native
77	*Prunus jenkinsii* Hook.f. & Thomson	Rosaceae	Native
78	*Pterospermum semisagittatum* Buch.-Ham. ex Roxb.	Sterculiaceae	Native
79	*Quercus glauca* Thunb.in A.Murray	Fagaceae	Native
80	*Quercus leucotrichophora* A.Camus	Fagaceae	Native
81	*Randia wallichii* Hook.f.	Rubiaceae	Native
82	*Rhus semialata* Murray.	Anacardiaceae	Native
83	*Rhus succedanea* (L.) Kuntze	Anacardiaceae	Native
84	*Sapium baccatum* Roxb.	Euphorbiaceae	Native
85	*Saraca asoca* (Roxb.) Willd.	Fabaceae	Native
86	*Schima wallichii* (DC.) Korthals	Theaceae	Native
87	*Securinega virosa* (Roxb. ex Willd.) Baill.	Euphorbiaceae	Native
88	*Stephegyne diversifolia* (Wall. ex G.Don) Brandis	Rubiaceae	Non-native

	Name of species	Family	Native/ Non-native species
89	*Sterculia hamiltonii* (Kuntze) Adelb.	Sterculiaceae	Native
90	*Sterculia villosa* Roxb.	Malvaceae	Native
91	*Stereospermum colais* Buch.-Ham. Ex Dillwyn	Bignoniaceae	Native
92	*Styrax serrulatum* (Roxb)	Styracaceae	Native
93	*Syzygium claviflorum* (Roxb.) Wall. ex A.M.Cowan & Cowan	Myrtaceae	Native
94	*Syzygium cumini* (L.) Skeels	Myrtaceae	Native
95	*Syzygium fruticosum* DC.	Myrtaceae	Native
96	*Trema orientalis* (L.) Blume	Ulmaceae	Native
97	*Ulmus lanceifolia* Roxb.	Ulmaceae	Native
98	*Vernonia arborea* Buch.-Ham	Asteraceae	Native
99	*Vernonia volkameriifolia* Bedd	Compositae	Native
100	*Vitex quinata* (Lour.) F. N. Williams	Verbenaceae	Native
101	*Wendlandia grandis* (Hook.f.) Cowan	Rubiaceae	Native
102	*Wightia speciosissima* (D. Don) Merr	Scrophulariaceae	Native
103	*Ziziphus incurva* Roxb.	Rhamnaceae	Native
Shrub species			
1	*Amomum dealbatum* Roxb.	Zingiberaceae	Native
2	*Antidesma diandrum* (Roxb.) B.Heyne ex Roth	Euphorbiaceae	Native
3	*Blumea lanceolaria* (Roxb.) Druce	Asteraceae	Native
4	*Callicarpa dichotoma* (Lour.) K. Koch	Lamiaceae	Non-native
5	*Chromolaena odorata* (L.) R.M.King & H.Rob.	Compositae	Non-native
6	*Clerodendrum viscosum* Vent.	Verbenaceae	Native
7	*Disporum cantoniense* (Lour.) Merr.	Liliaceae	Native
8	*Elaeagnus pyriformis* Hook.f	Elaeagnaceae	Native
9	*Ipomoea batatas* (L.) Lam.	Convolvulaceae	Non-native
10	*Lasianthus hookeri* C. B. Clarke ex J. D. Hooker	Rubiaceae	Native
11	*Leea indica* (Burm.f.) Merr	Vitaceae	Native
12	*Lepisanthes senegalensis* (Juss. ex Poir.) Leenh.	Sapindaceae	Native
13	*Maesa indica* (Roxb.) A. DC.	Primulaceae	Native
14	*Mallotus albus* (Roxb. ex Jack) Müll.Arg	Euphorbiaceae	Native
15	*Melastoma nepalensis* Lodd.	Melastomataceae	Native
16	*Murraya koenigii* (L.) Spreng.	Rutaceae	Native
17	*Mycetia longifolia* (Wall.) Kuntze	Rubiaceae	Native
18	*Osbeckia chinensis* L.	Melastomtaceae	Native
19	*Osbeckia crinita* Benth. ex Naudin	Melastomataceae	Native
20	*Polygonum chinense* L.	Polygonaceae	Native
21	*Randia fasciculata* (Roxb.) DC.	Rubiaceae	Native
22	*Rauvolfia densiflora* (Wall.) Benth. ex Hook. f.	Apocynaceae	Native
23	*Rhamnus nepalensis* M. Laws.	Rhamnaceae	Native
24	*Rubus buergeri* Miq	Rosaceae	Non-native
25	*Strobilanthes cusia* (Nees) Kuntze	Acanthaceae	Native
26	*Strobilanthes discolor* (Nees) T. Anderson	Acanthaceae	Native
27	*Strobilanthes parryorum* T. Anders.	Acanthaceae	Native
28	*Symplocos lancifolia* Siebold et Zucc.	Symplocaceae	Native
29	*Tabernaemontana divaricata* (L.) R. Br. ex Roem. & Schult.	Apocynaceae	Native

	Name of species	Family	Native/ Non-native species
30	*Toddalia asiatica* L.	Rutaceae	Native
31	*Viburnum foetidum* Wall	*Caprifoliaceae*	Native
32	*Woodfordia fruticosa* (L.) Kurz	*Lythraceae*	Native
Herb species			
1	*Adiantum caudatum* Linn	Adiantaceae	Native
2	*Arisaema album* N.E.Br.	Araceae	Native
3	*Arisaema speciosum* (Wall.) Mart.	Araceae	Native
4	*Asparagus racemosus* Willd.	Asparagaceae	Native
5	*Begonia dioica* Buch.-Ham. ex D.Don	Begoniaceae	Native
6	*Blumea alata* (D.Don) DC	Asteraceae	Native
7	*Boenninghausenia albiflora* Reichb.	Rutaceae	Native
8	*Centella asiatica* L.	Umbelliferae	Native
9	*Cheilocostus lacerus* (Gagnep.) C.D. Specht	Zingiberaceae	Native
10	*Chlorophytum khasianum* Hook.f	Liliaceae	Native
11	*Commelina benghalensis* Linn.	Commelinaceae	Native
12	*Conyza stricta* Willd.	Asteraceae	Native
13	*Costus speciosus* (J.König) Sm.	Zingiberaceae	Native
14	*Curculigo crassifolia* (Baker*)* Hook. f.	Hypoxidaceae	Native
15	*Curcuma caesia* Roxb. *'Ailaidum'*	Zingiberaceae	Native
16	*Dichrocephala integrifolia* (L.f.) Kuntze	Asteraceae	Native
17	*Diplazium dilatatum* Blume	Polypodiaceae	Native
18	*Diplazium maximum* (D.Don) Chatt 'Cha-kawk'	Polypodiaceae	Native
19	*Elatostema dissectum* Wedd.	Urticaceae	Native
20	*Elatostema sesquifolium* (Reinw. ex Blume) Hassk.	Urticaceae	Native
21	*Gleichenia linearis* (Burm.f.) C.B.Clarke 'Arthladawn'	Gleicheniaceae	Native
22	*Gnaphalium luteoalbum* Linn	Asteraceae	Native
23	*Hedychium coccineum* Buch.-Ham. ex Sm.	Zingiberaceae	Native
24	*Hedychium villosum* Wall.	Zingiberaceae	Native
25	*Houttuynia cordata* Thunb.	Saururaceae	Native
26	*Impatiens laevigata* Wall. ex Hook. f. & Thomson	Balsaminaceae	Native
27	*Kalanchoe integra* (Medik.) Kuntze. 'Kangdamdawi'	Crassulaceae	Native
28	*Leucas mollissima* Wall	Lamiaceae	Native
29	*Lindernia ruellioides* (Colsm.) Pennell 'Thasuih'	Linderniaceae	Native
30	*Lycopodium cernuum* Linn	Lycopodiaceae	Native
31	*Lygodium flexuosum* (Linn.) Swartz	Lycopodiaceae	Native
32	*Microlepia rhomboidea* (Wall.*ex* Kunze) Prantl, Arb.	Dennstaedtiaceae	Native
33	*Mimosa pudica* L. 'Hlonuar'	Mimosaceae	Non-native
34	*Ophiorrhiza mungos* L.	Rubiaceae	Native
35	*Ophiorrhiza oppositiflora* Hook.f.	Rubiaceae	Native
36	*Persicaria hydropiper* (L.) Opiz	Polygonaceae	Native
37	*Phaius mishmensis* (Lindl. & Paxton) Rchb.f.	Orchidaceae	Native
38	*Plantago major* Linn	Plantaginaceae	Non-native
39	*Plectranthus coetsa* Buch.-Ham. Ex D. Don	Lamiaceae	Native
40	*Polygonatum oppositifolium* (Wall.) Royle	Liliaceae	Native
41	*Polygonum barbatum* L. 'Dawngria'	Polygonaceae	Native
42	*Pouzolzia bennettiana* Wight	Urticaceae	Native

	Name of species	Family	Native/ Non-native species
43	*Pronephrium lakhimpurense* (Rosenst.) Holtt.	Thelypteridaceae	Native
44	*Pteridium aquilinum* (Linn.) Kuhn.	Polypodiaceae	Non-native
45	*Rhaphidophora decursiva* (Roxb.) Schott	Araceae	Native
46	*Scleria terrestris* (L.) Fass	Cyperaceae	Native
47	*Torenia violacea* (Azaola ex Blanco) Pennell	Linderniaceae	Native
48	*Urena lobata* Linn	Malvaceae	Native
Climbers and lianas			
1	*Acacia oxyphylla* Benth	Fabaceae	Native
2	*Aganope thyrsiflora* (Benth.) Polhill	Fabaceae	Native
3	*Bauhinia scandens* L.	Fabaceae	Native
4	*Caesalpinia cucullata* Roxb.	Fabaceae	Native
5	*Cissampelos pareira* L.	Menispermaceae	Native
6	*Cissus javana* DC	Vitaceae	Native
7	*Clematis siamensis* Drumm. et Craib	Ranunculaceae	Native
8	*Dioscorea glabra* Roxb.	Dioscoreaceae	Native
9	*Entada rheedei* Spreng. Subsp. Rheedei	Mimosaceae	Native
10	*Ipomoea hederifolia* L.	Convolvulaceae	Non-native
11	*Marsdenia formosana* Masam.	Apocynaceae	Non-native
12	*Mikania micrantha* Kunth	Asteraceae	Non-native
13	*Millettia pachycarpa* Benth.	Papilionaceae	Native
14	*Mucuna gigantea* (Willd.) DC.	Fabaceae	Native
15	*Passiflora edulis* Sims	Passifloraceae	Non-native
16	*Passiflora nepalensis* Wallich	Passifloraceae	Native
17	*Paederia foetida* L.	Rubiaceae	Native
18	*Piper betle* L	Piperaceae	Non-native
19	*Shuteria vestita* var. *glabrata* (Wight & Arn.) Baker	Fabaceae	Native
20	*Smilax glabra* Roxb.	Liliaceae	Native
21	*Smilax lanceifolia* Roxb.	Liliaceae	Native
22	*Tetrastigma dubium* (M. A. Lawson) Planch	Vitaceae	Native
23	*Tetrastigma leucostaphylum* (Dennst.) N.P. Balakr.	Vitaceae	Native
24	*Trichosanthes quinquangulata* A. Gray	Cucurbitaceae	Non-native
25	*Uncaria sessilifructus* Roxb.	Rubiaceae	Native
Grasses			
1	*Bambusa khasiana* Munro	Poaceae	Native
2	*Bambusa tulda* Roxb	Poaceae	Native
3	*Cephalostachyum latifolium* Munro	Poaceae	Native
4	*Dendrocalamus hamiltonii* Nees & Arn. ex Munro	Poaceae	Native
5	*Dendrocalamus longispathus* (Kurz) Kurz	Poaceae	Native
6	*Dendrocalamus sikkimensis* Gamble ex Oliv.	Poaceae	Native
7	*Dinochloa compactiflora* Kurz. Mc Clure	Poaceae	Native
8	*Drepanostachyum intermedium* (Munro) Keng f.	Poaceae	Native
9	*Erianthus longisetosus* Anderss. ex Benth	Poaceae	Native
10	*Eulalia trispicata* (Schult.) Henrard	Poaceae	Native
11	*Imperata cylindrica* (L.) Raeusch	Poaceae	Non-native
12	*Melocanna baccifera* (Roxb.) Kurz	Poaceae	Native
13	*Pseudostachyum polymorphum* Munro	Poaceae	Native

	Name of species	Family	Native/ Non-native species
14	*Schizostachyum dulloa* (Gamble) Majumdar 'Rawthla'	Poaceae	Native
15	*Setaria glauca* (L.) P. Beauv	Poaceae	Non-native
16	*Themeda villosa (Poir.)* A. Camus	Poaceae	Native
17	*Thysanolaena maxima* (Roxb.) Kuntze	Poaceae	Native
Epiphytes			
1	*Aerides odorata* Lour.	Orchidaceae	Native
2	*Aeschynanthus maculatus* Lindl.	Gesneriaceae	Native
3	*Bulbophyllum elatum* (Hook.f.) Sm	Orchidaceae	Native
4	*Bulbophyllum khasianum* Griff	Orchidaceae	Native
5	*Bulbophyllum umbellatum* Lindl	Orchidaceae	Native
6	*Cleisostoma filiforme* (Lindl.) Garay	Orchidaceae	Native
7	*Cleisostoma racemiferum* (Lindl.) Garay	Orchidaceae	Native
8	*Coelogyne prolifera* Lindl.	Orchidaceae	Native
9	*Dendrobium chrysanthum* Lindl.	Orchidaceae	Native
10	*Dendrobium chrysotoxum* Lindl.	Orchidaceae	Native
11	*Dendrobium densiflorum* Lindl.	Orchidaceae	Native
12	*Dendrobium formosum* Lindl	Orchidaceae	Native
13	*Dendrobium ochreatum* Lindl.	Orchidaceae	Native
14	*Dendrobium parishii* Reichb.f.	Orchidaceae	Native
15	*Dendrobium transparens* Wall. ex Lindl	Orchidaceae	Native
16	*Drynaria coronans* (Wall. ex Mett.) J. Sm. ex T	Polypodiaceae	Native
17	*Eria paniculata* Lindl	Orchidaceae	Native
18	*Eria pannea* Lindl.	Orchidaceae	Native
19	*Mycaranthes stricta* Lindk.	Orchidaceae	Native
20	*Oberonia iridifolia* (Roxb.) Lindl	Orchidaceae	Native
21	*Papilionanthe vandarum* (Rchb.f.)Garay	Orchidaceae	Native
22	*Pholidota imbricata* Hook	Orchidaceae	Native
23	*Premna coriacea* C.B.Clarke	Verbenaceae	Native
24	*Rhynchostylis retusa* (Lindl.) Bl.	Orchidaceae	Native
25	*Vanda coerulea* Griff. ex Lindl.	Orchidaceae	Native
Canes and Palms			
1	*Arenga pinnata* (Wurmb) Merr.	Arecaceae	Native
2	*Borassus madagascariensis* Bojer ex Jum. & H.Perrier	Arecaceae	Non-native
3	*Calamus inermis* Griff.	Arecaceae	Native
4	*Calamus khasianus* Kurz	Arecaceae	Native
5	*Calamus erectus* Roxb.	Arecaceae	Native
6	*Calamus flagellum* Griff. ex Mart	Arecaceae	Native
7	*Calamus guruba* Buch.-Ham. ex Mart.	Arecaceae	Native
8	*Calamus acanthospathus* Roxb.	Arecaceae	Native
9	*Caryota mitis* Lour. 'Mei-hle'	Arecaceae	Native
10	*Caryota urens* L.	Arecaceae	Non-native
11	*Livistona chinensis* (Jacq.) R.Br. ex Mart	Arecaceae	Non-native
12	*Pandanus odorifer* (Forssk.) Kuntze	Pandanaceae	Native
13	*Pinanga gracilis* Blume	Arecaceae	Native
14	*Wallichia nana* Griff.	Arecaceae	Native
15	*Zalacca secunda* Griff	Arecaceae	Native

Phanerophytes, Cryptophytes, and Epiphytes were higher than normal spectrum while Chamaephytes came the closest to normal spectrum. The abundance of epiphytes is indicative of tropical humid forest as epiphytes are so tightly associated with wet tropics, as definitions of tropical rain forests frequently include the presence of this growth form (Richards 1952, 1996; Webb 1959). Lianas are most abundant in tropical forests where wide array of dimensions, shapes and morphological characters of the trees provides support for them (Clark & Clark 1990). They form an important structural and functional component of tropical rain forests (Hegarty & Caballe 1991). The percentage of lianas was quite high which according to Whitmore (1990) it is another characteristic feature of tropical moist and humid forest.

The species diversity index (Shannon diversity H') in the study site were comparable to that of Tawi Wildlife Sanctuary and Phawngpui National Park. In Tawi wildlife sanctuary, Lallawmkimi (2011) reported species diversity index of 3.86 for trees, 3.26 for herbs and 3.14 for shrubs and in Phawngpui National Park, Malsawmsanga (2011) reported species diversity index to 3.68 for trees, 2.96 for herbs and 2.8 for reported for lower elevations (1500–1700 m) (Table 1) There may be several reasons for species richness in community conserved forests. Bajracharya et al (2005) studied the effectiveness of community based approached for conservation of biodiversity in Annapurna Conservation Area (ACA), Nepal which is an experimental model considered to be a pioneer in promoting the concepts of protected area using an integrated, community based conservation and development approach. They found that the forest basal area and tree species diversity were significantly higher inside ACA than in neighbouring areas outside which they have attributed to increased conservation awareness among the local people leading to a change in their behaviour and use of resource. Comparison of deforestation rates by various research have also shown no significance difference in community conserved areas and strictly protected areas (Nepstad et al. 2006; Bray et al. 2008) which suggests that community conservation is just as effective as state-controlled protected areas in reducing deforestation rates.

However, comparison of community conserved forest and formal protected areas reveal a change in species composition in areas that are ecologically comparable and endemic and threatened species tend to decline in community conserved forest (Shahabuddin & Rao 2010). This trend has been observed in this study which reveals only two vulnerable species in the community conserved Reiek forest while Lallawmkimi (2011) reported 3 endemic species which are critically endangered from Tawi wildlife sanctuary and Malsawmsanga (2011) reported 7 rare, endemic and endangered species and 3 critically endangered species from Phawngpui National Park.

The whole study area although protected jointly by the village councils of Reiek and Ailawng village and a non-governmental organisation viz Young Mizo Association of the two villages, is still not free from encroachment which is the main threat to the rich biodiversity of the area. Although a formal conservation action is desired from the Government, this study has shown that the community has carried out conservation that is locally effective in terms of species diversity.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21068–21075

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.6551.14.5.21068-21075

#6551 | Received 09 August 2020 | Final received 10 April 2022 | Finally accepted 01 May 2022

OPEN ACCESS

COMMUNICATION

Floristic studies on mangrove vegetation of Kanika Island, Bhadrak District, Odisha, India

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Abstract: This study pertains to the floristic composition of unexplored mangrove habitats of Kanika Island, Bhadrak District, Odisha, India. Six quadrats each measuring 31.62 x 31.62 m (0.1 ha) were laid at randomly selected sites in the Island between October 2019 and February 2020. Quantitative inventory yielded a total of 12 species across the sampled quadrats. Qualitative floristic inventory of the Island revealed a total of 20 species belonging to 17 genera and 13 families, including four true mangrove species, viz., *Avicennia alba*, *A. marina*, *A. officinalis*, and *Lumnitzera littorea* were evaluated as 'Least Concern' by the IUCN Red List. Out of 20 species, eight species were trees, followed by herbs (8 species), shrubs (3 species), and a climber. The study revealed that four species were true mangroves and 16 species were mangrove associates. *Avicennia alba* and *A. marina* were found dominant and have potential for regeneration in the island.

Keywords: Basal area, diversity index, importance-value, mangrove-associates, true-mangroves.

Editor: A.J. Solomon Raju, Andhra University, Visakhapatnam, India.

Date of publication: 26 May 2022 (online & print)

Citation: Poornima, P. (2022). Floristic studies on mangrove vegetation of Kanika Island, Bhadrak District, Odisha, India. *Journal of Threatened Taxa* 14(5): 21068–21075. https://doi.org/10.11609/jott.6551.14.5.21068-21075

Funding: None.

Competing interests: The author declares no competing interests.

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Acknowledgements: I thank Shri. Subash Chandra Nayak, Range officer, Chandbali, Shri. Sushanta Mohanty, Dy Ranger, Shri Gadadhar Biswal, Forester, Dhamra and members of Protection Squad, Bhadrak WL Division for helping in data collection.

INTRODUCTION

Mangroves are ecologically important coastal vegetation that is globally represented by 70 species (Polidaro et al. 2010), of which, 46 are present in India (Ragavan et al. 2016). The number of reported mangrove species in India varies from 34 to 69 (Untawale 1987; Banerjee 1984; Banerjee et al. 1989; Naskar 1993; Mandal et al. 1995). Such a variation in the number is mainly due to the inclusion of many mangrove associates in the list by various researchers. The mangrove vegetation of the east coast of India differs from that of the west coast. Mangroves of the east coast are larger with high diversity due to the presence of tidal creeks, canals, and brackish water bodies (Ahmed 1972). Mangroves are dynamic and its floristic composition reflects the health of the vegetation. Any changes in the floristic structure alter its functions and services (Alongi 2014; Krauss et al. 2014). Recent studies highlighted the importance of extensive floristic studies in understanding the diversity of true mangroves in different mangrove regions in India (Ragavan 2015; Saenger et al. 2019). Though conservation and management of mangroves have become a global priority, yet many of the mangrove patches within India are lacking baseline information (e.g., species diversity and threats) that are vital for their long-term management. Addressing such knowledge gaps will be imperative to improve our understanding of phytogeography, which can eventually contribute to the better management and conservation of this ecologically and economically important coastal ecosystem. I studied one such unexplored mangrove forests in Kanika Island, Odisha situated on the east coast of India. The objectives of the study were: (i) to quantitatively evaluate and document the species diversity, density, basal area, and IVI in the study area, (ii) to check whether there is any invasion by alien species into the island, (iii) to determine the dominant tree species, (iv) to enumerate the true mangroves and mangrove associates, and (v) to study the natural regeneration potential of dominant mangrove species in the Island.

MATERIALS AND METHODS

Study area

Kanika Island (20.830°N–86.985°E) is situated at the mouth of Dhamra River, which is the confluence of Baitarini and Dhamra rivers, in Bhadrak district of Odisha (Image 1). It has drying mud and sand flats covering an extent of 485 ha. The island has no human habitation. The district covers an area of 2,505 km^2 with the human population of c. 15,00,000 (2011 census) and agriculture and fishing are their major occupation. The average annual rainfall of the district is 1,530 mm and the average annual temperature is 26.8 °C

METHODS

The quantitative data on mangrove vegetation was collected by laying six quadrats (each 31.62 x 31.62 m) (0.6 ha) at sites (I to VI) on the Island by ensuring 300–500 m distance between each quadrat between October 2019 and February 2020. All the plant species were identified using Mandal & Bar (2018), www.plantlist.org, www.indiabiodiversity.org, & www.ipni.org and enumerated according to Manon (2006), such as trees (>1 m height) and juveniles (<1 m height). For trees and shrubs, the girth was measured at breast height using measurement tape and for herbs and climber, the girth was measured close to the ground. Vegetation data was quantitatively analyzed for species richness, density, basal area, and above-ground biomass based on the individual species enumerated in the quadrats. The excel data was used to compute community indices like Fischer's alpha diversity index, Simpson's diversity index (Simpson 1949) and importance value index (IVI). Data analysis was carried out using Biodiversity Pro Ver. 2.0. The dominance of species was calculated based on species IVI (Curtis & McIntosh 1950; Cintron & Novelli 1984). Above ground biomass (AGB) calculation was restricted only for trees and shrubs using diameter (D), height (H), and wood specific density (ρ) (species-specific) and using allometric equation of Chave et al. (2005). Study sites were determined using a Garmin Etrex 20x GPS device. Photography and videography was done using a Nikon P1000 digital camera. The collected data was tabulated, analyzed, and represented graphically.

RESULTS AND DISCUSSION

Qualitative diversity inventory yielded a total of 20 species belonging to 17 genera and 13 families in Kanika Island. Among 20 species, eight were trees, three were shrubs, eight were herbs and one was a climber. According to the classification of Barik and Choudhury (2014), four species were true mangroves and the remaining 16 species were mangrove associates. Fabaceae, with four species was the most species-rich family. Avicenniaceae was represented by three species, whereas families such as Malvaceae and Convolvulaceae had two species each.

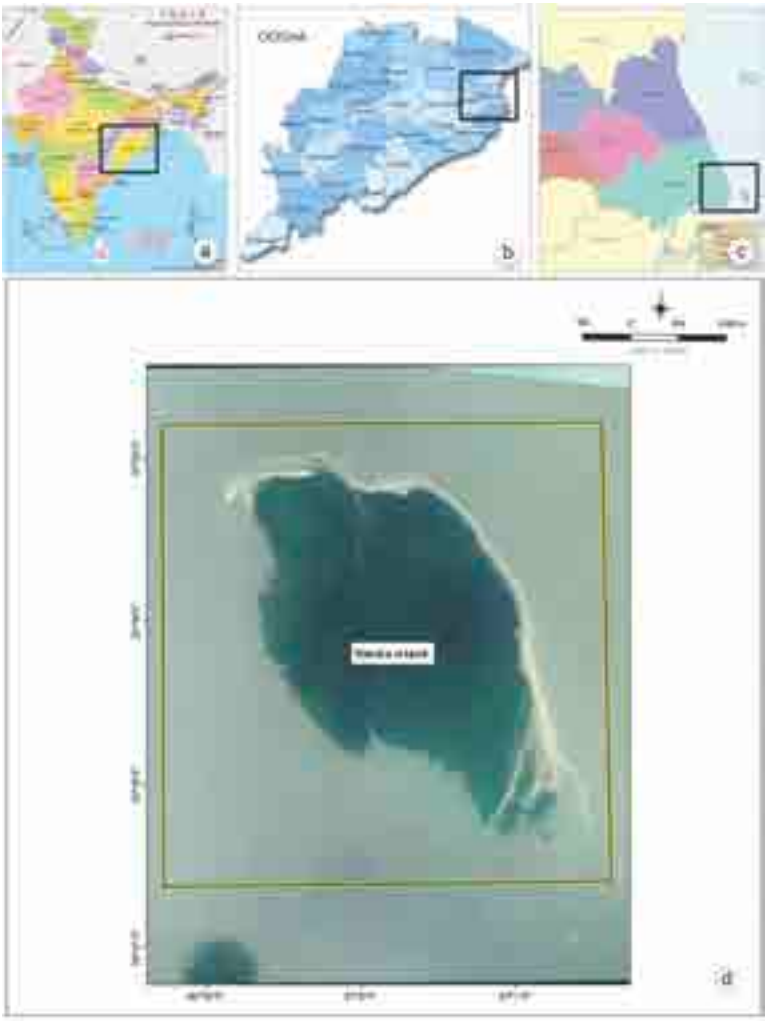

Image 1. Study area map: a—India map showing Odisha state | b—Odisha state map showing Bhadrak District | c—Bhadrak District map | d—Kanika Island map.

The remaining nine families were represented by only one species each. The genus *Avicennia* had contributed three species, *Ipomoea* represented two species and other 15 genera had contributed one species each. The study revealed that Kanika Island harbors only five mangrove species probably due to the small land cover (Table 1).

Species diversity

In the study area, sites V and VI with seven species each were the species-rich plots. The dry sandy elevated area of these plots supported more species of herbs and shrubs than the other plots that are primarily established on marshy areas. The probable reason is that true mangrove species grow in saline marshy areas, whereas sandy elevated areas support mangrove as well as associated species. *Acanthus ilicifolius* was found along the borders of creeks, canals, and marshes whereas the creeper *Ipomoea pes-caprae* and the herb *Sesuvium portulacastrum* were observed on the exposed elevated sand bars. Individuals of *Pongamia pinnata*, *Thespesia populnea*, and *Hibiscus tiliaceus* have grown on dry sandy areas in sites V–VI. *Derris scandens* was observed under the vegetations of *E. agallocha*. The remaining quadrats (I–IV) were laid in the marshy regions having creeks and canals, which naturally had homogeneous vegetation containing dominant species such as *A. alba* and *A. marina*. Blasco (1975) considered *A. marina* as the most salt tolerant mangrove species. The occurrence of *A. marina* as a dominant species on the island has corroborated the views of Blasco (1975).

Density

Maximum density of 4,750 individuals/ha was found in site-IV, followed by site-III with 1,670 /ha due to the predominant representation of juveniles of *A. marina* and *A. alba*. *Avicennia marina* had contributed to density of 246 individuals/ha in Bhitarkanika Wildlife Sanctuary (Upadhyay & Mishra 2014), 884 individuals/ha in Godavari delta mangroves (Venkatana & Rao 1993), 1,107

Table 1. List of plant species enumerated by qualitative survey in Kanika Island and their IUCN Red List Status

	Binomial	Family	Life forms	True mangrove (TM) or mangrove associate (MA)	IUCN Red List status	Native / Non-native
01	*Excoecaria agallocha* L.	Euphorbiaceae	Tree	MA	Least Concern	Native
02	*Avicennia alba* Blume.	Avicenniaceae	Tree	TM	Least Concern	Native
03	*Avicennia marina* (Forsk.) Vierh.	Avicenniaceae	Tree	TM	Least Concern	Native
04	*Avicennia officinalis* L.	Avicenniaceae	Tree	TM	Least Concern	Native
05	*Lumnitzera littorea* (Jack) Voigt.	Combretaceae	Tree	TM	Least Concern	Native
06	*Acanthus ilicifolius* L.	Acanthaceae	Shrub	MA	Least Concern	Native
07	*Thespesia populnea* L. Solander ex Correa	Malvaceae	Tree	MA	Least Concern	Native
08	*Hibiscus tiliaceus* L.	Malvaceae	Shrub	MA	Least Concern	Native
09	*Suaeda maritima* (L.) Dumort.	Chenopodiaceae	Herb	MA	Not assessed	Native
10	*Clerodendrum inerme* Gaertn	Verbenaceae	Shrub	MA	Not assessed	Native
11	*Ipomea pes-caprae* (L.) Sweet	Convolvulaceae	Herb	MA	Not Evaluated	Native
12	*Ipomea tuba* (Schl.) G. Don	Convolvulaceae	Herb	MA	Not assessed	Native
13	*Sesuvium portulacastrum* L.	Aizoaceae	Herb	MA	Least Concern	Native
14	*Pongamia pinnata* (L.) Pierre	Fabaceae	Tree	MA	Least Concern	Native
15	*Sauropus bacciformis* (L.) Airy Shaw	Phyllanthaceae	Herb	MA	Not assessed	Native
16	*Prosopis juliflora* (SW.) DC.	Fabaceae	Tree	MA	Invasive alien species	Non-native
17	*Derris scandens* Benth	Fabaceae	Climber	MA	Not assessed	Native
18	*Launea sarmentosa* (Willd.) Sch. Bip.	Asteraceae	Herb	MA	Not assessed	Native
19	*Canavalia rosea* (Sw.) DC.	Fabaceae	Herb	MA	Least Concern	Native
20	*Aeluropus lagopoides* (L.) Thwaites	Poaceae	Herb	MA	Invasive alien species	Non-native

individuals /ha in south-west coast of India (Sreelakshmi et al. 2018), and density of 1,731 individuals/ha in Coringa Wildlife Sanctuary (Satyanarayan et al. 2002). It indicates that the density of *A. marina* (662 individuals/ha) is lesser in Kanika Island than the other mangroves studied by Venkatana & Rao (1993), Sreelakshmi et al. (2018), and Satyanarayan et al. (2002).

Basal area

Basal area is an indicator for measuring forest-stand development, biomass, and productivity (Twilley 1998). The mean basal area of all the six plots was 3.137 (0.52±0.41). The maximum basal area (11.63 m^2/ha) was observed in site-I and the minimum basal area (0.14m^2/ha) was recorded in site-V. Site-I had more basal area than the other sites due to the occurrence of *A. marina* and *A. alba*. Out of 12 species enumerated, *A. marina* (0.993 m^2/0.6 ha), *A. alba* (0.889 m^2/0.6 ha), and *A. ilicifolius* (0.403 m^2/0.6 ha) had contributed to maximum basal area. Upadhyay & Mishra (2014) had recorded the basal area of *A. marina* (0.89 m^2/ha), *A. alba* (1.23 m^2/ha) in Bhitarkanika, whereas Venkatana & Rao (1993) had observed 3 m^2/ha for the same two species in

Table 2. Basal area, importance value index, and above ground biomass of mangroves on Kanika Island.

	Name of the species	Total BA (m^2)	IVI	Total AGB (Kg)
1	*Acanthus ilicifolius*	0.403	26.69	0
2	*Avicennia alba*	0.889	45.59	3757.53
3	*Avicennia marina*	0.993	126.91	3331.78
4	*Avicennia officinalis*	0.027	05.63	32.81
5	*Derris scandens*	0.011	11.53	0
6	*Excoecaria agallocha*	0.101	12.78	89.66
7	*Hibiscus tiliaceus*	0.080	12.78	59.23
8	*Ipomoea pes-caprae*	0.001	12.21	0
9	*Pongamia pinnata*	0.254	17.89	302.23
10	*Prosopis juliflora*	0.196	11.03	446.46
11	*Sesuvium portulacastrum*	0.002	07.16	0
12	*Thespesia populnea*	0.182	15.58	241.36
Total		**3.139**	-	**8261.06**

Note: Woody climber: Allometric equation by Schnitzer et al. 2006 (AGB= exp [-1.484+2.657 (ln (D)].
Trees: Allometric equation by Chave et al. 2005 for moist mangrove stands [GB= exp (2.977+ln ($_p$d2H)].
(AGB—above ground biomass | D—diameter | $_p$—wood specific density | H—height).

Godavari delta. George et al. (2017) had observed that the total stand basal area of mangroves of Kerala state was 20.33 m^2/ha. In the present study, the average stand basal area measured was 31.37 m^2/ha. It indicates that the mangrove stand density, biomass, regeneration capacity, and productivity of mangrove forest in Kanika Island is higher than the mangrove ecosystems studied by Upadhyay & Mishra (2014), Venkatana & Rao (1993), and George et al. (2017) and hence this basal area work is important for the present study.

Above-ground biomass

The present study on the mean above ground biomass (AGB) of true mangrove in Kanika Island reveals that the highest AGB of 3.757 tons/0.6 ha was recorded in *A. alba*, while the lowest AGB value of 0.032 tons/0.6 ha was found in *A. officinalis* (Table 2). But in Sunderbans, the AGB of *Avicennia* spp. was 101.9–118.7 tons/ha in 1991 (Choudhry 1991), whereas in 2014 it was 8.9 to 50.9 tons/ha (Joshi & Ghose 2014) and 104.1 tons/ha in Australia (Woodroffe 1985). The AGB of Thane creek, Maharashtra was estimated as 54.9 tons/ha (Pachpande & Pejaver 2015). Authors such as Chmura et al. (2003), Bouillon et al. (2008), Duarte et al. (2009), Murdiyarso et al. (2009), Kennesy et al. (2010), Chen et al. (2012), and Kauffman & Donato (2012) have highlighted that though mangroves represent small geographical areas, they are capable of high potential carbon sequestration. The average AGB of Kanika Island was 8.261 tons/ha which is less than the above said mangroves.

Diversity indices

Alpha diversity index was highest for site-VI (2.113) due to the occurrence of seven different species and site-II had the lowest value of 0.198 due to the homogeneous vegetation consisting of *A. marina*. Simpson's Index shows the highest value of 1.000 in site-II due to occurrence of large woody trees consisting of *A. marina*. Site-V had the lowest index value 0.297 due to presence of large number of herbs with less girth. Diversity indices revealed that the vegetation of all the study sites was varied and hence the vegetation is heterogeneous in nature (Table 2).

Importance Value Index (IVI)

Out of 12 species enumerated, *A. marina* had the highest IVI of 126.91, followed by *A. alba* (45.59), *A. ilicifolius* (26.69), and *P. pinnata* (17.89) while the remaining eight species had IVI of less than 15.58. *Avicennia officinalis* had the lowest IVI of 5.63. The IVI depicts the ecological importance of a species in a given ecosystem and also helps give conservation priority to the species (Malimbwi et al. 2005; Kacholi 2013). In the present study, *A. marina* and *A. alba* showed high IVI due to their higher relative frequency, relative density, and relative basal area. The IVI of *A. marina* and *A. alba* in Thakurdia mangroves in Odisha were 8.2 and 19.73, respectively (Mishra et al. 2005), whereas the IVI of *A. marina* and *A. alba* were found to be 126.91 and 45.59 on Kanika Island, respectively. It indicates that *A. marina* and *A. alba* enjoy pre-dominance in Kanika island and the reasons for their dominance needs further study.

The analysis of community structure of mangroves at six study sites (0.6 ha) revealed that the relative density of *A. marina* and *A. alba* were higher than the remaining 10 species. It clearly indicates that the mangrove vegetation in Kanika island is characterized by the dominance of *A. marina* and *A. alba.* Eight mangrove associate species were recorded in addition to the species recorded in all six quadrats. *Derris scandens* was observed in close association with mangroves. *Ipoemea pes-caprae*, *H. tiliaceus*, *C. inerme*, *P. pinnata*, *T. populnea*, *S. bacciformis*, *S. maritima*, *S. portulacastrum*, *C. rosea*, and *L. sarmentosa* occurred on the sand dunes and sand bars in the elevated region of the island. Individuals with stunted growth of *P. pinnata* and *T. populnea* were found in the elevated areas. *Sauropus bacciformis* was found in the dried muddy regions of the island. Only one species of grass *A. lagopoides* (Poaceae) was found in the dried muddy areas (Table 2).

True Mangrove vs. Mangrove Associates

Ragavan et al. (2016) had stated that there are 35 true mangrove species in Sunderbans, 35 species in Odisha, 16 species in Karnataka, 19 species in Kerala, 22 species in Andhra Pradesh (Swain et al. 2008), 17 species in Pichavaram, Tamil Nadu (Salachandran et al. 2009), and 16 species in Goa (Sanjappa et al. 2011). But in the present study, out of 20 species counted, only four species—*A. alba*, *A. marina*, *A. officinalis*, and *L. littorea*—were found to be true mangroves and the remaining 16 species were only mangrove associates (Table 1). The occurrence of less number (4) of true mangrove species may be due to the small extent of Kanika Island. In the present study, *P. juliflora* trees also occur in the elevated sandy beach (site-VI). Since there is no human habitation in Kanika Island, the possibility of introduction of these species by humans is very less. Hence, there might be possibility of dispersal of seeds from inland to this island by river water. The impact of *P. juliflora* on the mangrove ecosystem in the study area requires sustained monitoring and further studies (Image 2).

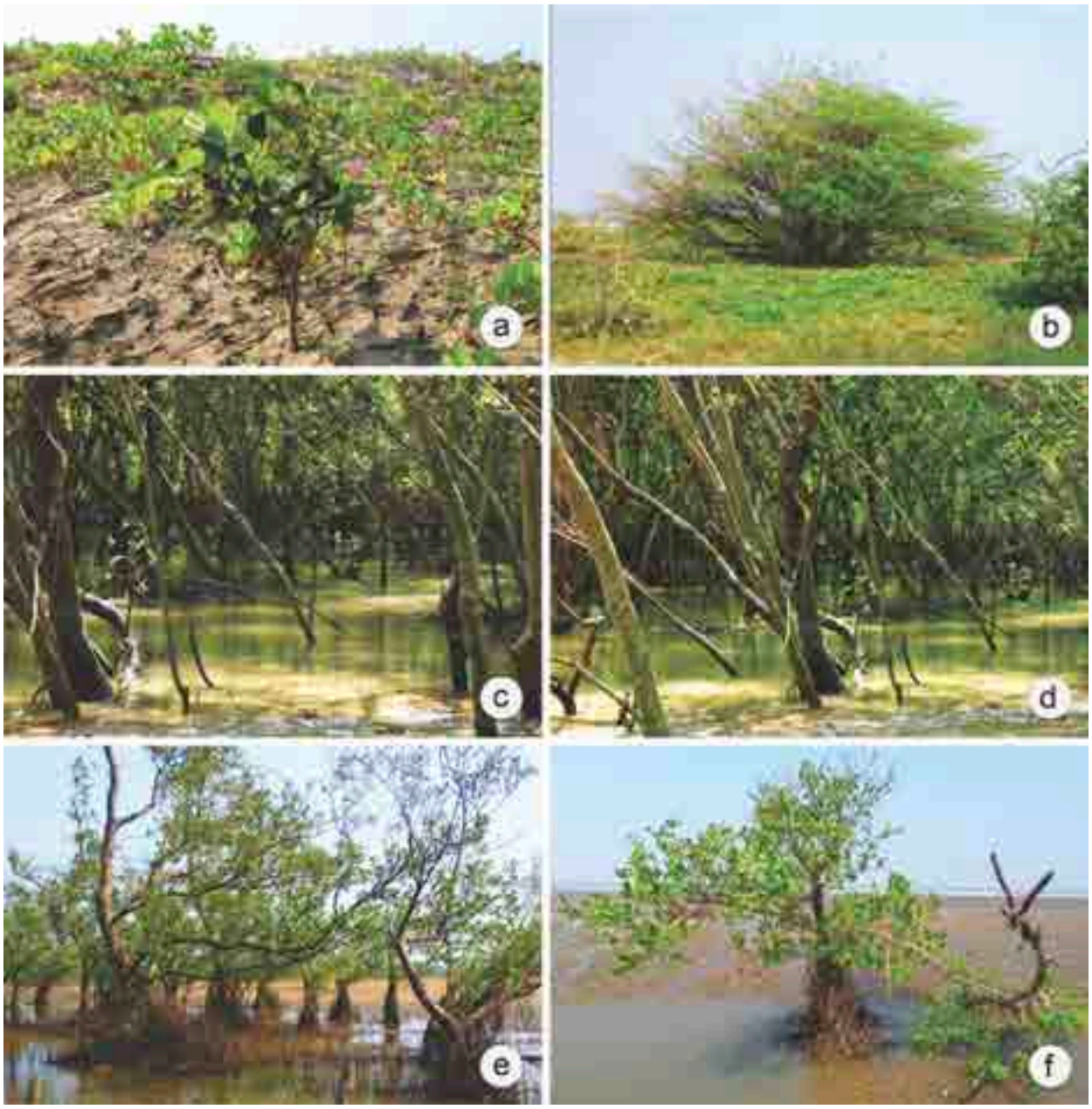

Image 2. Mangrove and mangrove associates in Kanika Island: a—Spread of *Ipomoea pes-caprae* and *Pongamia pinnata* individuals on sand bar | b—Growth of *Prosopis juliflora* tree | c & d—*Avicennia alba* and *Avicennia marina* trees | e & f—Damaged and dead trees of *Avicennia marina* due to erosion. © P. Poornima.

Regeneration of mangroves

The study on the mature (≥1 m height) and juvenile (≤1 m height) individuals of *A. marina* and *A. alba* revealed that *A. marina* had the highest percentage of juveniles than *A. alba*. No juveniles of *A. officinalis* were found in the study sites. Most of the juveniles were found in the exposed areas adjacent to dense mangroves. This may be due to the fact that the seedlings found in open areas grow faster than those under canopy (Ellison & Farnsworth 1993, 1996). If the number of seedlings/saplings of a particular mangrove species is greater than 50% of their mature trees/ha, it is considered to possess good regeneration potential (Gan 1995). In the present study, 92% (n= 609) individuals of *A. marina* and 58.1% (n= 18) individuals of *A. alba* were found to be juveniles. It indicates that these two species were found dominant and have potential for regeneration in the Island as stated by Gan (1995). Apart from natural regeneration some seedlings also found washed into the shore by waves. Seedlings found in open areas grow faster than those

Image 3. Regeneration of mangrove on Kanika Island: a—Deposition of seeds of *Avicennia marina* on sandy beach | b—Germination of a seed | c & d—Natural growth of juveniles in the peripheral muds. © P. Poornima.

under canopy (Ellison & Farnsworth 1993, 1996). In the present observations of seedlings found in the exposed areas corroborate the findings of Ellison & Farnsworth (1993, 1996).

The regeneration potentials of such seeds in inter tidal zones require further studies. It was observed that 13 matured *A. marina* trees were found damaged due to erosion of waves and of them, four were found dead. Since there is no anthropogenic pressure in the Island, the sea erosion related threats to the mangrove may pose a major management challenge in the near future (Image 3).

IUCN status

The IUCN Red List of Threatened Species has classified the status of *A. alba, A. officinalis, A. marina*, and *L. littorea* as 'Least Concern'. These four mangroves are a common occurrence in all the mangrove vegetations of eastern and western coasts and Andaman & Nicobar Islands. Apart from India, *A. marina* is distributed in Australia, Indonesia, Thailand, Malaysia, Singapore, Philippines, and China. *Avicennia alba* is found in all the above said countries except China (Ragavan et al. 2016). Seven species were not evaluated and *P. juliflora* is an invasive alien species. Kanika Island has been considered ecologically sensitive as it provides habitat for several mangrove species (Table 1).

CONCLUSION

My study provides an overview of mangrove species diversity in Kanika Island, Odisha. This Island harbors four true mangroves and 16 mangrove associates. The dominance of *A. marina* and *A. alba* is indicative of species robustness where other species have failed to colonize. The status and species composition of mangrove forest is a basic requirement and a pre-requisite for effective long-term monitoring, management and conservation of mangrove resources. It is suggested that immediate attention should be given for strengthening research activities to build database on true mangrove and mangrove associates. Since the island is uninhabited and free from cattle, there is no anthropogenic pressure on this mangrove vegetation. However, the fishing activities around the island should be regulated without causing

any harm to the fragile mangrove ecosystem.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21076–21083

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.6721.14.5.21076-21083

#6721 | Received 17 September 2020 | Final received 09 March 2022 | Finally accepted 01 April 2022

OPEN ACCESS

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Two new varieties of *Russula* Pers. (Basidiomycota: Russulaceae) from Sal forests of Shiwaliks, India

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Abstract: This paper deals with two new varieties of *Russula* species, *R. camarophylla* var. *reticulospora* var. nov. and *R. aurea* var. *minuta* var. nov. These were collected from the Shiwalik range of northwestern India, in association with *Shorea robusta*. *Russula aurea* var. *minuta* differs from *R. aurea* in having small sized sporophores, dentate to wavy gill edges with golden or yellow deposition instead of smooth and much smaller spores. Whereas, mushroom *R. camarophylla* var. *reticulospora* is close to *Russula camarophylla* except for the larger carpophores that have white cream pileus surface and larger spores. In basidiospores warts are connected to form mostly complete reticulum instead of mostly isolated warts reported in *Russula camarophylla*. In view of the presence of some unique varied features in the presently examined collections two new varieties of *Russula* has been proposed.

Keywords: Diversity, Ectomycorrhiza, *R. camarophylla* var. *reticulospora* var. nov., *R. aurea* var. *minuta* var. nov., scanning electron microscopy, taxonomy.

Editor: Richard Mibey, Nairobi, Kenya.

Date of publication: 26 May 2022 (online & print)

Citation: Kumar, J. & N.S. Atri (2022). Two new varieties of *Russula* Pers. (Basidiomycota: Russulaceae) from Sal forests of Shiwaliks, India. *Journal of Threatened Taxa* 14(5): 21076–21083. https://doi.org/10.11609/jott.6721.14.5.21076-21083

Funding: This study was supported by Council of Scientific and Industrial Research, New Delhi, India (Grant number 09/140 (0159) 2014-EMR-1).

Competing interests: The authors declare no competing interests.

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Author contributions: Both the authors carried out the research work. The first draft of the manuscript was written by Jitender Kumar and Narender Singh Atri commented on previous versions of the manuscript. Both the authors read and approved the final manuscript.

Acknowledgements: We would like to express our sincere gratitude to the Head, Department of Botany, Punjabi University, Patiala for providing laboratory facilities and to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for financial assistance under CSIR-JRF fellowship scheme. To UGC, DST and DBT we are indebted for financial assistance under different schemes to the Department during the course of the present work.

INTRODUCTION

Genus *Russula* is one of the dominant basidiomycetous fungi genus which grow in a wide variety of habitats. These are mostly found in mycorrhizal association with variety of plants including trees (Corrales et al. 2016). Studies on taxonomy and diversity of genus *Russula* are inviting more attention now a days primarily because of their importance in human welfare, ecosystem functioning and stability. These macro-fungi are important source of food, medicine, nutraceuticals and also play a pivotal role in ecosystem strengthening and maintenance as mycorrhizal associates (Manoharachary et al. 2005). It is reported that *Russula* spp. can form EcM with many temperate and tropical plant families, including *Leguminosae*, *Fagaceae*, *Cistaceae*, *Dipterocarpaceae*, *Salicaceae*, *Betulaceae*, *Nothofagaceae*, *Myrtaceae*, and *Pinaceae* (Tedersoo et al. 2010; Wang et al. 2017). The compounds derived from these mushrooms are reported to boost up immune system and avert diseases thereby improving human health (Wasser 2002). Different species of *Russula* are known to possess anti-inflammatory, antiviral, antibacterial, antiparasitic, antioxidant, hepatoprotective, anticancer, and antidiabetic properties (Wasser 2011).

To date, approximately 1,100 *Russula* species have been reported worldwide (Kirk 2014) and distributed across a wide range of habitats from the tropics to arctic zones (Riviére et al. 2007; Ba et al. 2012). *Russula* is one of the dominant ectomycorrhizal genera in Indian Himalaya (Saini & Atri 1984, 1989; Atri & Saini 1986; Atri et al. 1994; Kumar & Atri 2016, 2019; Sharma et al. 2016) and is represented by ca. 158 taxa from India (Sharma et al. 2017). While investigating the EcM diversity of Sal forest, two varieties of *R. camarophylla* Romagn. and *R. aurea* Pers. were documented, which upon investigation were found to be new to science based on detailed macro- and micro-morphological examination. In the present study sporocarps and their EcM colonised roots were collected by tracing the hyphal or rhizomorphs connections in association with *Shorea robusta* from pure Sal forests. These species are fully illustrated and described in this paper.

MATERIALS AND METHODS

Study area

Area selected for the present investigation is Sal forests of Shiwalik mountain range of northwestern India (Figure 1), which represent the geologically lowest and youngest mountain range of Himalaya. The study area

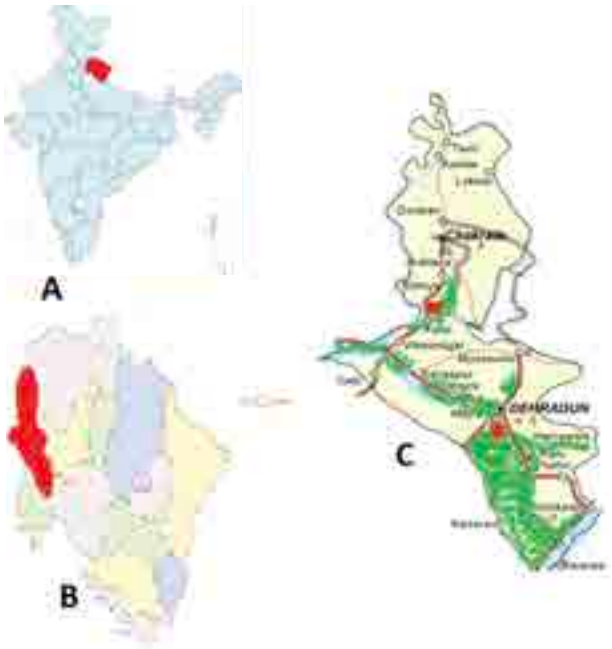

Figure 1. Location map of Study area (Red).

is located between 30.316N, 78.032E. Elevation range of the area is 400–1500 m and vegetation of the area is typical of tropical moist deciduous type (Champion & Seth 1968).

Sampling, identification and characterization

Sporocarps were collected from different localities of pure Sal forests, during the rainy season of 2013–2015. Macromorphological features were recorded from fresh collections in the field and colour codes used are that of Kornerup & Wanscher (1978). After noting down morphological characters on the field key (Atri et al. 2005) some pieces of sporocarps from cap and stipe were preserved in liquid preservative (25 ml rectified alcohol (95%) + 5 ml formalin (37%) + 70 ml distilled water) for studying the microscopic characters. By adopting the standard procedures spore deposit was taken after bringing the specimens to the temporary laboratory setup. Sporocarps were air dried at 40–45 ^{0}C in a drier specially designed for drying mushroom specimens (Atri et al. 2005) which were finally packed in a cellophane paper packet for permanent preservation in Punjabi University Herbarium under PUN. The cross section of pileus and longitudinal section of stipe were stained in congo red for examination, drawn under a compound microscope and photographed under digital microscope (Leica DM4000 B LED). Observation of basidia, cystidia, and elements of pileipellis and stipitipellis were recorded for further use in taxonomic categorization. Melzer's reagent was used

to observe the amyloidy in basidiospore ornamentation. The microscopic details were worked out as per standard methodology (Singer 1986; Atri et al. 2000, 2017).

Scanning electron microscopy

Scanning electron microscopic (SEM) studies of basidiospores were carried out with JSM6610LV GEOL scanning electron microscope. For SEM examination basidiospores from spore print and lamellae tissue were mounted on a double-sided adhesive tape pasted on a metallic specimen holder or stub. The material was scanned at different magnification ranging 3,000–15,000 X in high vacuum mode to observe pattern of spore ornamentation.

TAXONOMY

***Russula aurea* Pers. var. *minuta* var. nov.**
(Image 1a–h, Figure 2A–G)
MycoBank number: MB834095

Diagnosis: *Russula aurea* Pers. var. *minuta* var. nov. is characterised by small golden to brightly yellow pileus with more darker brownish-yellow centre; dentate to wavy gill edges with golden deposition instead of smooth; sour taste , much smaller spores size and presence of pilocystidia.

Etymology: The variety name is based on the smaller size of sporophore and basidiospore as compared to *Russula aurea.*

Holoype: PUN 9112, Male, 27 July 2013, Rajban, Dehradun, Uttrakhand, India, 30.316N, 78.032E, 800 m, coll. J. Kumar.

Paratype: PUN 9113, 1 ex., Male, 21 August 2015, Kalsi, Dehradun, Uttrakhand, India, 30.316N, 78.032E, 1,190 m, coll. J. Kumar.

Taxonomic description

Sporophores 2.0–2.5 cm in height. Pileus 1.3-2.0 cm broad, convex to hemispherical when young, flattened depressed at maturity; centre umbonate when young, golden (6C7) to brightly yellow with more darker brownish-yellow centre; margin regular to slightly irregular, nonsplitting at maturity, moist, unchanging, apex depressed at maturity with slight umbo; cuticle half peeling; flesh 0.1 cm thick in the centre, almost absent along the margin, white (1A1), changes to light brown on bruising and cutting, brittle; taste sour, odour mild. Lamellae adnexed to slightly adnate, equal, moderately broad (2–3 mm), crowded (12–16 gills/cm), white with golden edges; gill edges not smooth, eroded or wavy. Stipe central, 1.5–2.0 cm in length, 0.3–0.5 cm broad, cylindrical to slightly tapering downward, white (1A1) in the upper half, yellowish to pale white in the lower half, unchanging, first solid, than hollow, smooth. Spore deposit deep ochre.

Basidiopores 5.0–6.5 (7.5) × 4.0–5.0 (6.0) µm (excluding ornamentation), broadly ellipsoidal to ellipsoid (Q =1.2–1.3), warty; warts up to 0.8 µm high, mostly connected by thick and thin lines to form partial to complete reticulum, ornamentation type IIIa, IIIb, IV, amyloid; plage hyaline, indistinct; apiculate, apiculus up to 1.6 µm long. Basidia 19.5–32.6 × 6.5–9.0 µm, clavate, bisporic to tetrasporic, hyaline, abundant; sterigmata up to 3.5 µm long; pleurocystidia 26.0–40.9 × 6.5–9.8 µm, clavate to ventricose granulated; cheilocystidia 22.5–37.4 × 4.1–13.1 µm, similar to pleurocystidia. Pileus cuticle clearly differentiated, epicutis gelatinised, heteromerous, palisade having interwoven projecting septate 3–5 µm broad hyphae mixed with 5–10 µm broad sphaerocyst and dermatocystidia, cuticle hyphae and cellular mass having dark yellow content throughout; pilocystidia

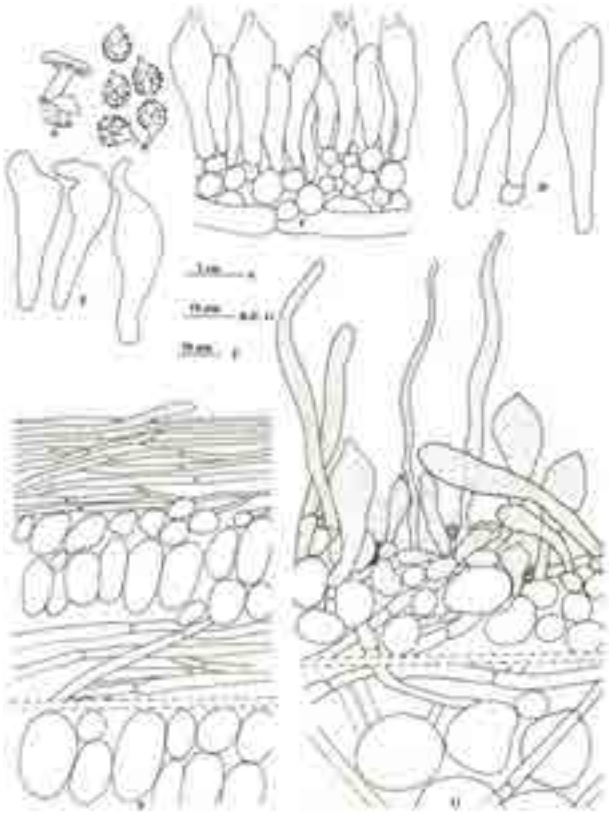

Figure 2. *Russula aurea* var. *minuta* var. nov: A—Sporophores | B—Basidiospores | C—Hymenophore showing basidia | D—Pleurocystidia | E—Cheilocystidia | F—Cross section through stipe showing cuticular details and context | G—Cross section through pileus showing cuticular details and context.

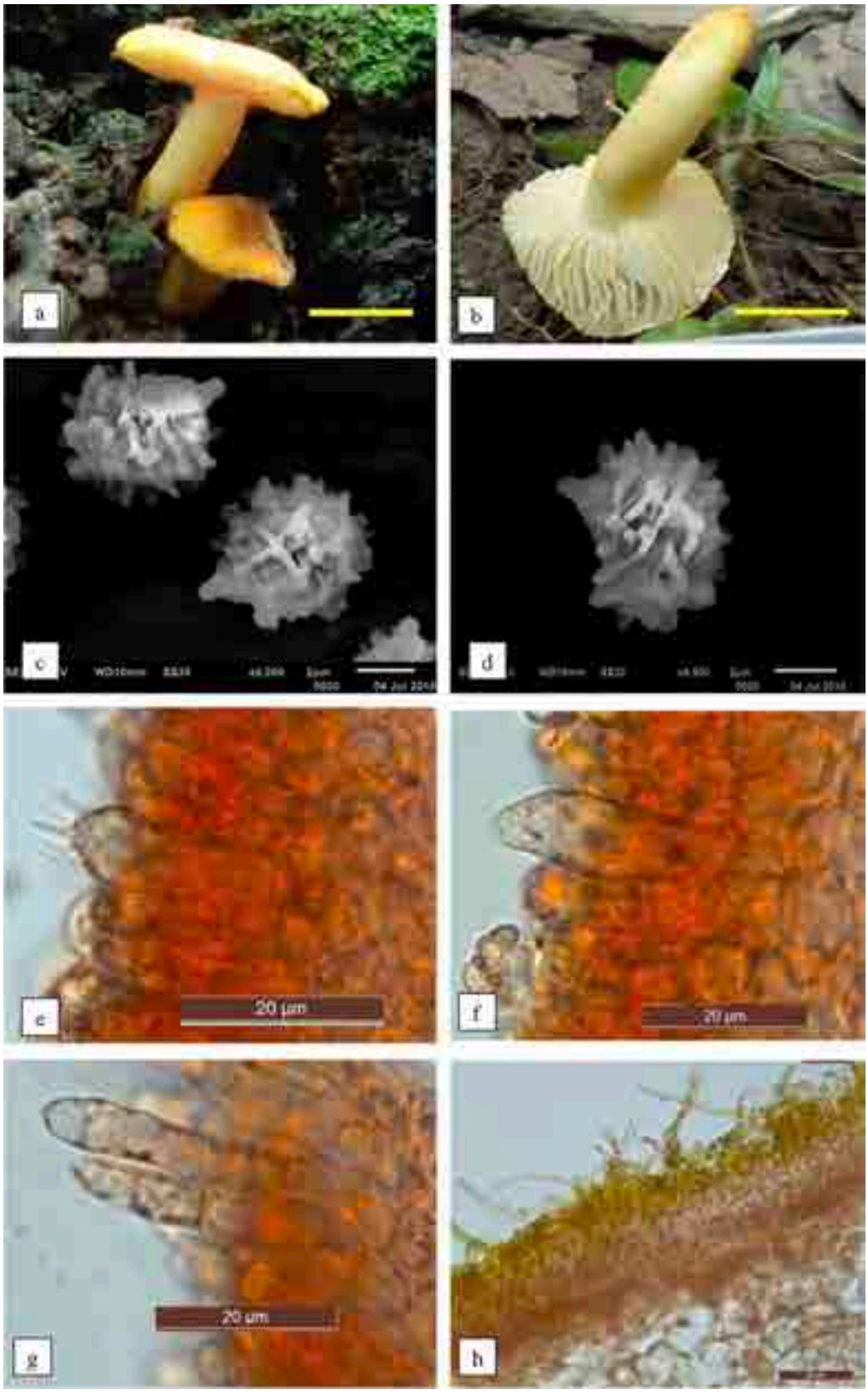

Image 1. *Russula aurea* Pers. var. *minuta* var. nov. a–b—Sporophores | c–d—Scanning electron photographs of basidiospores | e—Hymenophore showing basidia and cystidia | f—Pleurocystidia | g—Cheilocystidia | h—Cross section through pileus showing cuticular details and context. Scale bar a–b= 1 cm.

24.0–40.8 × 4.9–8.2 µm, thin walled, clavate, fusiform to fusoid ventricose with acute to blunt end; context heteromerous having multiseptate 3.0–6.5 µm broad hyphae intermingled with 8.0–36.0 × 8.0–32.6 µm rosettes of sphaerocysts. Hymenophoral trama 16–100 µm, heteromerous with up to 3.3–5.0 µm broad hyphae intermingled with 8.0–36.0 × 8.0–32.6 µm sphaerocysts. Stipe cuticle gelatinised with more or less parallel arranged 1.6–6.5 µm broad septate hyphae having yellowish content; context made up of 12–28 × 12–24 µm sized rosettes of sphaerocysts and 4–6 µm broad septate hyphae in alternate manner. Clamp connections absent.

Chemical colour reaction: Stipe surface pinkish with $FeSO_4$, Gills turns carmine red in Sulphovanillin.

Habitat: Sporophores directly attached to the roots at the base of *Shorea robusta* tree.

Collections examined: Uttrakhand: Dehradun, Rajban (800 m), in groups in Sal forest in association with *Shorea robusta*. Jitender Kumar, PUN 9112, 27 July 2013. Dehradun, Kalsi (1,190 m), solitary in Sal forest in association with *S. robusta* tree. Jitender Kumar PUN 9113, 21 August 2015.

Remarks: The overall diagnostic characters of the presently examined collection are in agreement with *Russula aurea* (Rayner 1970; Romagnesi 1967; Das & Marstad 2014) except that the carpophores are much smaller in size (2.0–2.5 cm instead of 4–9 cm), gill edges not smooth (dentate to wavy with golden or yellow deposition instead of smooth), much smaller spores size (5–7.5 × 4–6 µm instead of 7.5–10 × 6–8 µm) and presence of pilocystidia which are absent in case of *Russula aurea*. In view of the presence of some unique varied features in the presently examined collections in comparison to *R. aurea*, a new variety *minuta* has been named.

Russula camarophylla Romagn. var. *reticulospora* var. nov.

(Image 2a–h, Figure 3A–G)

MycoBank number: MB834095

Diagnosis: *Russula camarophylla* Romagn. var. *reticulospora* var. nov. is characterised by larger sporophore with creamish-white pileus surface, distantly spaced lamellae, very hard and compact flesh and larger spore size. Also in basidiospores warts are connected to form mostly dense complete reticulum.

Etymology: The variety name is based on the densely reticulated basidiospores.

Holoype: PUN 9124, Male, 30 August 2013, Kalsi, Dehradun, Uttarakhand, India, 1,190 m, 30.316N, 78.032E, coll. J. Kumar.

Taxonomic description

Sporophore 7.5 cm in height. Pileus 10 cm broad, umblicate with a depressed disc and irregular margin; pileus surface moist, glabrous, cream white to white (1A1), not peeling; flesh 5 mm thick in the centre, off white to slightly creamish, unchanging. Lamellae unequal broadly adnate to decurrent, distant (3–4 gills/cm), broad (11 mm at the centre), creamish-white to orange white (5A2), forked near the base, lamellulae present, gill edges smooth, normal. Stipe 2 cm long and up to 2 cm broad, central, solid, white, fleshy, concolorous with the pileus, unchanging on cutting and bruising; flesh taste spicy; odour fruity, spore deposit yellowish-white.

Basidiospores 6.5–8.0 (9.0) × 5.0–7.0 (7.5) µm, subglobose to broadly ellipsoid (Q= 1.12–1.33), densely ornamented, warty, warts up to 0.5 µm, connected to form mostly complete reticulum, superahilar area usually with low ornamentation, ornamentation type IIIa, IIIb; apiculate, apiculus up to 1.6 µm in size. Basidia 35–57 × 5.0–8.5 µm, clavate to subcylindric, 2–4 spored, sterigmata 6.5–9.8 µm long. Pleurocystidia 39.0–86.5

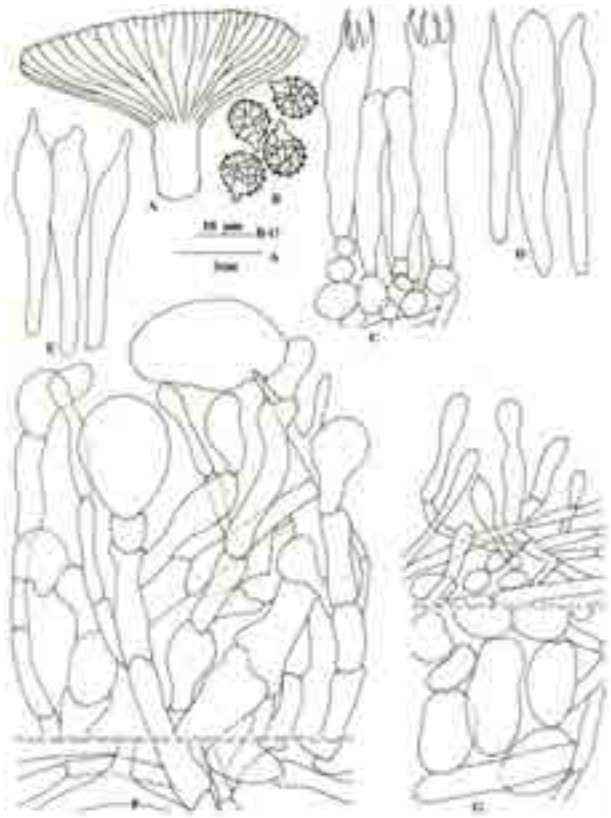

Figure 3. *Russula camerophylla* Romagn. var. *reticulospora* var. nov: A—Sporophore | B—Basidiospores | C—Hymenophore showing basidia | D—Pleurocystidia | E—Cheilocystidia | F—Cross section through pileus showing cuticular details and context | G—Cross section through stipe showing cuticular details and context.

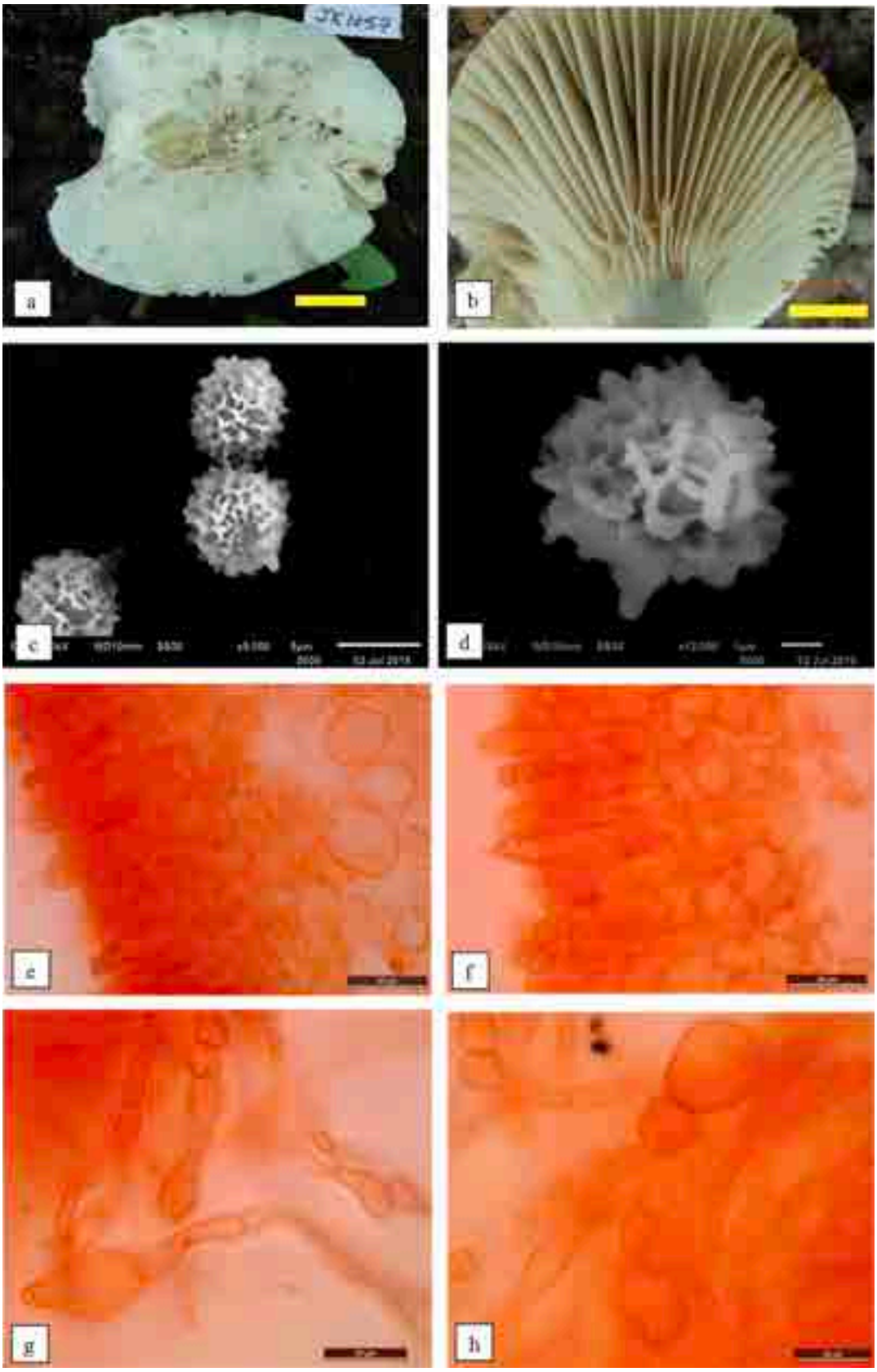

Image 2. *Russula camarophylla* Romagn. var. *reticulospora* var. nov.: a–b—Sporophores | c–d—Scanning electron microphotographs of basidiospores | e—Hymenophore showing basidia and cystidia | f—Pleurocystidia | g–h—Cross section through pileus showing cuticular details and context. Scalebar a–b= 2 cm.

× 4.8–9.8 µm, clavate, fusiform, fusoid clavate to fusoid ventricose, often acute to blunt ended, granulated to hyaline; arising usually from the subhymenium, nonprojecting. Cheilocystidia 35.8–48.9 × 4.8–9.0 µm, clavate to fusiform, apically acute to blunt, mostly hyaline, gill edges heteromorphic; subhymenium 31.4–47.2 µm, heteromerous with 3–13 µm broad hyphae interspersed with 3.0–11.5 × 3.0–10.0 µm sphaerocyst; hymenophoral trama 471–942 µm, mostly cellular with 6.5–11.5 µm hyphae and 6.5–42.5 × 4.9–42.5 µm sphaerocyst. Pileus cuticle up to 240 µm broad, trichoderm of nongelatinous, 6.5–18.2 µm interwoven septate projecting hyphae, hyphae ending attenuate or rounded and some ending with inflated or rounded cells, hyphal cells cylindric to ampullate at both sides of the septum, septa mostly constricted, some hyphae with large inflated cells in the intercalary position. Subcutis not clearly differentiated, made up of interwoven, nongelatinous, 6.5–18.5 µm broad hyphae with a few inflated scattered cells. Stipe composed of parallel arranged interwoven septate up to 4.9 µm broad hyphae; caulocystidia 21–41 × 3–5 µm, cylindric to clavate, rounded apically.

Chemical colour reactions: Stipe surface pinkish with $FeSO_4$, gills turns carmine red in Sulphovanillin.

Collection examined: Uttarakhand: Kalsi (1,190 m), in association with *Shorea robusta*. Jitender Kumar, PUN 9124, 30 August 2013.

Habitat: Solitary on the ground in monsoon under *Shorea robusta* tree.

Remarks: The external and internal characters of the presently examined collection are in agreement with *Russula camarophylla* (Romagnesi 1968) except that the carpophores are larger in size with cream white pileus surface and larger spore size (6.5–9 × 5–7.5 µm instead of 5–6.2 (7) × 3.6–4.8 µm). In basidiospores warts are connected to form mostly complete reticulum instead of mostly isolated warts in case of *Russula camarophylla* as documented in literature.

DISCUSSION

During the present study, *R. aurea* var. *minuta* and *R. camarophylla* var. *reticulospora* were found forming direct organic connection with *Shorea robusta*. The overall diagnostic characters of the presently examined collections of *R. aurea* var. *minuta* are in agreement with *Russula aurea* Pers. which is commonly known as the gilded brittle gill or golden *Russula* and is an uncommon species of mushroom found in deciduous woodland forests. Its specific epithet aurea has been derived from the Latin word *aurum*, which means golden. Unlike many red-capped members of the genus, *Russula aurea* is edible and mild-tasting and is easily characterised in the field by its golden pileus, free to adnexed broad fairly distant golden gills, cylindrical smooth light yellow stipe and brittle yellow flesh. Mostly it is reported to grow solitary or scattered forming mycorrhizal association with pine trees (Romagnesi 1967; Rayner 1970; Das & Marstad 2014). *Russula aurea* var. *minuta* differs from *R. aurea* except in having small sized sporophores, dentate to wavy gill edges with golden or yellow deposition instead of smooth, much smaller spores and presence of pilocystidia which are absent in case of *Russula aurea*. In view of this a new variety *Russula aurea* var. *minuta* has been proposed. *Russula aurora* probably appears to be morphologically closest species to this undescribed taxon from which it differs in having fairly crowded pale cream lamellae with abundant forkation near the stipe, mild taste, and absence of dermatocystidia in pileipellis and low warted spores (0.25–0.5 µm) with few connections (Romagnesi 1967). Another close taxon is *Russula aurantiaca* which differs from *R. aurea* var. *minuta* in having usually brick-orange, copper to carmine coloured cap, widely spaced rather thick bright yellow ochre strongly interveined lamellae and presence of mild to slightly acrid taste (Romagnesi 1967). Earlier *Russula aurea* was known as *R. aurata* and under this name it was documented from different localities of northwestern Himalaya from coniferous and angiospermic forest (Saini & Atri 1984, 1989; Atri & Saini 1986; Atri et al. 1994). The present collection is found in pure Sal forest in close vicinity to *Shorea robusta* tree from Uttarakhand.

Russula camarophylla, a rare western Mediterranean European representative of section *Archaeinae* is characterized by its camarophylloid habit, pale ochre or creamish sporophores with distant lamellae, very hard and compact flesh, hygrophoroid basidia and tiny spores with barely visible ornamentation (Romagnesi 1968). The present collection of *R. camarophylla* var. *reticulospora* is close to *Russula camarophylla* (Romagnesi 1968) except that the carpophores are larger in size with white cream pileus surface and larger spore size. In basidiospores warts are connected to form mostly complete reticulum instead of mostly isolated warts reported in case of *Russula camarophylla* (Romagnesi 1968). In view of this a new variety *Russula camarophylla* var. *reticulospora* has been proposed. The apical swelling of hyphal terminations in the pileipellis is an important feature that is very common within *Russula camarophylla* (Buyck et al. 2003) and presently examined collection. *R. camarophylla* var. *reticulospora* also resembles the recently described

Russula capillaris, by Buyck (in Wang et al. 2019) from Madagascar. The latter species is not only very similar in the field, but it also possesses similar apical swellings in the hyphal terminations of pileipellis. Spores, however, are much smaller with isolated and very low warts (0.1–0.2 µm) in *R. capillaris* and, again, the pileocystidia are not septate. *Russula camarophylla* is a very rare species and has been found only a few times in France (Buyck et al. 2003), northern Italy (Setti & Bigoni 1998; Boffelli 2012) and Austria (Pidlich-Aigner & Klofac 2018).

CONCLUSION

Two new varieties of *Russula* species, viz. *R. camerophylla* var. *reticulospora* var. nov. and *R. aurea* var. *minuta* var. nov. have been described based upon detailed macro- and micromorphological comparison with already existing *Russula* species. The newly proposed varieties are putative mycorrhizal associates of Sal and were found in direct organic connection with *Shorea robusta* roots.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21084–21090

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7606.14.5.21084-21090

#7606 | Received 04 August 2021 | Final received 15 May 2022 | Finally accepted 18 May 2022

OPEN ACCESS

COMMUNICATION

New additions to the lichen biota of Assam from Dhubri district, northeastern India

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Abstract: The present study deals with the exploration of lichen diversity in Dhubri district of Assam state. A total of 42 lichen species belonging to 10 families and 16 genera were recorded, the majority of which were crustose (93%) with Graphidaceae as the dominant family. Eleven of the lichen species under eight genera are new additions to the lichen biota of Assam.

Keywords: Biodiversity, Brahmaputra River, Corticolous, crustose, Graphidaceae, Indo-Bangladesh border.

সাংক্ষিপ্তসাৰ: এই গৱেষণা পত্রখনত ভাৰতবর্ষৰ অসম ৰাজ্যৰ ধুবুৰী জিলাৰ পৰা ৪২ টা লাইকেন প্রজাতিৰ উল্লেখ কৰা হৈছে। ইয়াৰে ১১ টা লাইকেন প্রজাতি ৮ টা গণ আৰু ৭ টা গোত্রৰ অন্তর্গত, অসমত প্রথমবাৰৰ বাবে পোৱা গৈছে।

Editor: Anonymity requested.

Date of publication: 26 May 2022 (online & print)

Citation: Biswas, S., R. Daimari, P. Islary, S. Nayaka, S. Joseph, D.K. Upreti & P.K. Sarma (2022). New additions to the lichen biota of Assam from Dhubri district, northeastern India. *Journal of Threatened Taxa* 14(5): 21084–21090. https://doi.org/10.11609/jott.7606.14.5.21084-21090

Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: Biswas, S., R. Daimari & P. Islary—concept and documentation of manuscript. S. Nayaka, S. Joseph & D.K. Upreti—identification of specimens. P.K. Sarma—mapping.

Acknowledgements: We are thankful to the Department of Botany, Bodoland University, Kokrajhar, Assam for providing the facility to carry out the research works and director of CSIR-NBRI, Lucknow for permitting access to LWG herbarium and library. Rebecca Damari is thankful to DST-SERB, New Delhi for financial assistance under EMEQ scheme (EEQ/2019/000547).One of the author Siljo Joseph would like to thank financial assistance under DST-INSPIRE Faculty scheme (IFA 18- LSPA 124).

INTRODUCTION

Lichens are highly cosmopolitan in nature. Lichenogeographically, India is divided into eight regions (Nayaka & Asthana 2014). Among these, the Western Ghats, the eastern Himalaya and northeastern India are regarded as biodiversity hotspots both for higher plants and lower cryptogams. The physical structures as well as the climatic conditions of the region support the luxuriant growth of lichens. From the state of Assam, there is a report on lichen which covers 20 out of 34 districts (Behera et al. 2021; Gupta & Sinha 2018). However, extensive exploration of most of the districts for lichen diversity study is indispensable. Literature on lichenology from Dhubri district is very limited. Recently Gupta & Sinha (2018) reported six lichen species—*Graphis subasahinae* Nagarkar & Patw., *Lecanora alba* Lumbsch, *Lecanora helva* Stizenb., *Parmotrema saccatilobum* (Taylor) Hale, *Protoparmelia hesperia* (Kantvilas & Elix) Kantvilas, Papong & Lumbsch, and *Letrouitia flavocrocea* (Nyl.) Hafellner & Bellem—from various parts of the district. Therefore, the present study was undertaken to explore and enumerate the lichen diversity of Dhubri district. The district is situated in the extreme western part of Assam in the Indo-Bangladesh border and on the northern bank of the river Brahmaputra.

MATERIALS & METHODS

For the present study, about 700 lichen specimens were collected from January to December 2020 from 13 different localities of Dhubri district of Assam (Figure 1). All the specimens were collected from the bark of trees, air-dried and stored in paper packets. The lichen specimens were identified morphologically, anatomically and chemically. The morphological characters were studied under stereozoom microscope Leica EZ4W. For anatomical details, thin sections of the apothecia or perithecia were mounted in water and observed under the compound microscope Leica DM 750. The presence of chemical substances was analysed by performing colour tests using K, P, and C solutions and thin layer chromatography (Orange et al. 2001). The lichen thallus was also observed under the UV cabinet. The specimens were identified following relevant literature (Nayaka 2004; Awasthi 2007; Lücking et al. 2009; Ram et al. 2009; Aptroot 2012; Sharma et al. 2012). The families of the identified species were assigned as per the literature of Lücking et al. (2016). Specimens were identified up to the species following relevant literature and updated as per the databases available for lichen taxonomy.

The identified specimens are housed in the Bodoland University Botanical Herbarium (BUBH), Department of Botany, Bodoland University. A set of voucher specimens is deposited in the herbarium of CSIR-National Botanical Research Institute, Lucknow (LWG), Uttar Pradesh, India.

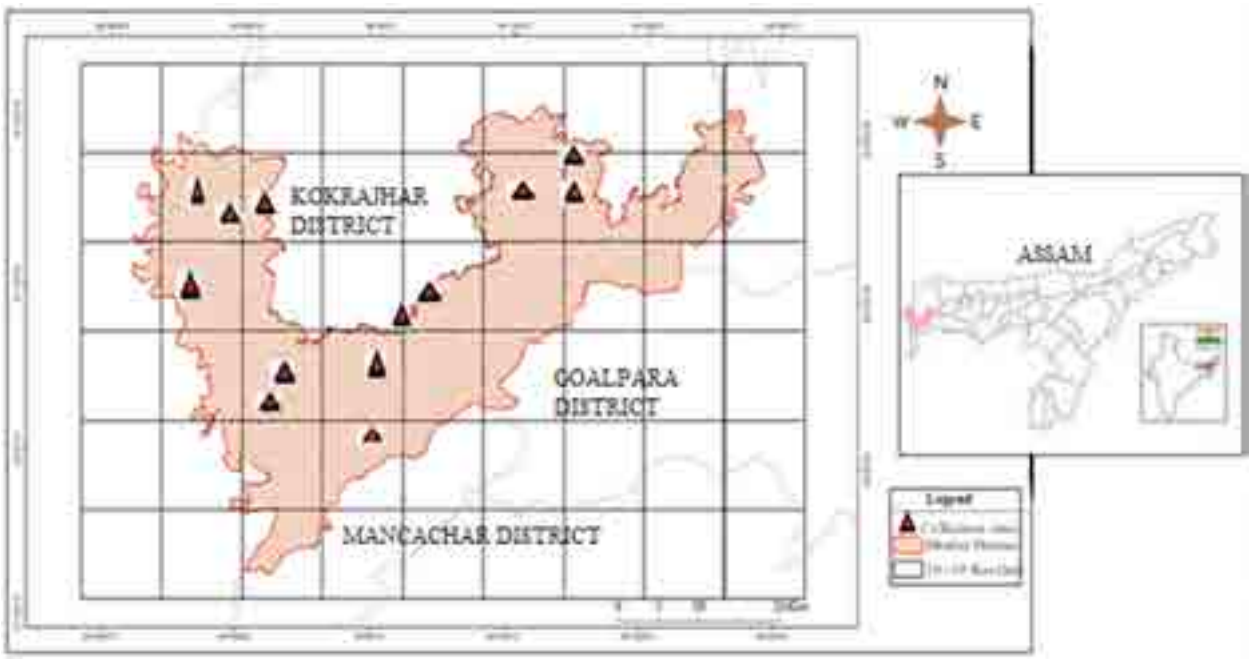

Figure 1. Map of Dhubri district, Assam showing the collection sites.

RESULTS

The present study identified 42 lichen species under 10 families and 16 genera (Table 1). The majority of the lichen species are crustose (93%) followed by 7% foliose. Among the lichen families Graphidaceae emerged as the dominant family with 15 species, followed by Caliciaceae with nine species.

DISCUSSION

Based on Joseph et al. (2020), the annotated checklist by Singh & Sinha (2010) and literature available on lichens for Assam state (Awasthi 1961; Rout et al. 2005, 2010, 2012; Das et al. 2013; Gupta et al. 2013; Daimari et al. 2014; Gogoi et al. 2019; Gupta & Sinha 2018; Behera et al. 2021), 11 species under eight genera and seven families are listed as new records to Assam and brief descriptions of these species are provided.

A comparative study of the six lichen species reported by Gupta & Sinha (2018) from Dhubri district with the present study reveals that only two of the species are found to be common and therefore, till date the district records a total of 46 species. However, the list may further go up with the exploration of more locations for the lichen study.

Enumeration of the newly recorded lichen species

Family: Arthoniaceae

Herpothallon himalayanum Jagadeesh Ram & G.P. Sinha (Image 1D)

Distribution: India (West Bengal, Darjeeling district), Endemic.

Specimen examined: 2020-0169 (BUBH), India, Assam, Dhubri district, Khajurbari part 1, on the bark of *Lannea coromandelica*, 24.xii.2020, 39 m, 26.262 N, 90.179 E, coll. S. Biswas & P. Biswas.

Family: Caliceaceae

Pyxine isidiophora (Müll. Arg.) Imshaug (Image 2H)

Distribution: India (West Bengal), Sri Lanka, Columbia.

Specimen examined: 2020-0170 (BUBH), India, Assam, Dhubri district, Debotar hasdaha part 4, on the bark of *Lannea coromandelica*, 22.xi.2020, 27.73 m, 26.050 N, 89.893 E, coll. S. Biswas & P. Biswas.

Family: Graphidaceae

Allographa stictilabiata (Patw. & C.R. Kulk.) J. Kalb & Kalb (Image 1C)

Distribution: India (Karnataka and Maharashtra), Endemic

Specimen examined: 2020-0171 (BUBH), India, Assam, Dhubri district, Alokjhari, on the bark of *Shorea robusta*, 12.i.2020, 52.82 m, 26.253 N, 89.860 E, coll. S. Biswas & P. Biswas.

Graphis asahinae Patw. & C.R. Kulk. (Image 1A)

Distribution: India (Kerala and Tamil Nadu), Brazil.

Specimen examined: 2020-0172 (BUBH), India, Assam, Dhubri district, Gopigoan part 3, on the bark of *Lannea coromandelica*, 26.Xii.2020, 44.14 m, 26.257 N, 90.232 E, coll. S. Biswas & P. Biswas.

Graphis modesta Zahlbr. (Image 1B)

Distribution: India (Maharashtra), Brazil, Mexico, Papua New Guinea, Seychelles.

Specimen examined: 2020-0173 (BUBH), India, Assam, Dhubri district, Rangamati part 3, on the bark of *Artocarpus heterophyllus*, 27.Xi.2020, 28.84 m, 26.161 N, 90.059 E, coll. S. Biswas & P. Biswas.

Family: Lecanoraceae

Lecanora leproplaca Zahlbr. (Image 1E)

Distribution: India (Madhya Pradesh), Australia, Brazil, Central and South America, Dominica, El Salvador, Fiji, Hawaiian Islands, Jamaica, Seychelles, South Africa, Thailand.

Specimen examined: 2020-0174 (BUBH), India, Assam, Dhubri district, Gauripur Matiabag Hawakhana, on the bark of *Michelia champaca*, 8.ii.2020, 44.82 m, 26.097 N, 89.975 E, coll. S. Biswas & P. Biswas.

Family: Parmeliaceae

Parmotrema mesotropum (Müll. Arg.) Hale. (Image 1F)

Distribution: India (Arunachal Pradesh, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Manipur, Uttarakhand), Argentina, Brazil, Bolivia, Central & South America, China, Colombia, Costa Rica, Guyana, Mexico, Paraguay, Venezuela.

Specimen examined: 2020-0175 (BUBH), India, Assam, Dhubri district, Alomganj part 9, on the bark of *Lannea* sp., 27.xii.2020, 43.48 m, 26.135 N, 90.036 E, coll. S. Biswas & P. Biswas.

Family: Physciaceae

Physcia abuensis D.D. Awasthi & S.R. Singh (Image 2G)

Distribution: India (Rajasthan), Endemic

Specimen examined: 2020-0176 (BUBH), India, Assam, Dhubri district, Dhubri town, on the bark of *Litchi chinensis*, 10.i.2020, 41.43 m, 26.022 N, 89.959 E, coll. S.

Table 1. Distribution of lichen species in the study site along with their growth form.

	Species	GF	Locations												
			1	2	3	4	5	6	7	8	9	10	11	12	13
Arthoniaceae															
1	*Coniocarpon cinnabarinum* DC.	C	-	-	-	+	-	-	+	-	-	-	-	-	-
2	*Cryptothecia lunulata* (Zahlbr.) Makhija & Patw.	C	-	-	-	-	-	-	-	-	-	+	-	-	+
3	* *Herpothallon himalayanum* Jagad. Ram & G.P. Sinha	C	-	-	-	-	-	-	-	-	-	-	+	-	-
Caliciaceae															
4	*Cratiria lauri-cassiae* (Fée) Marbach	C	-	-	-	-	-	-	-	-	-	-	-	+	-
5	*Dirinaria applanata* (Fée) D.D. Awasthi	F	-	+	+	+	-	-	-	-	-	-	+	-	-
6	*D. consimilis* (Stirt.) D.D. Awasthi	F	-	-	-	-	-	-	-	-	-	-	-	-	+
7	*D. papillulifera* (Nyl.) D.D. Awasthi	F	-	-	-	-	-	+	+	-	-	+	+	-	-
8	*D. picta* (Sw.) Clem. & Shear.	F	-	+	-	-	-	-	-	-	-	-	+	-	-
9	*Pyxine cocoes* (Sw.) Nyl.	F	-	+	+	+	-	+	+	-	-	-	+	+	-
10	*P. coralligera* Malme.	F	-	-	-	-	-	+	-	-	-	-	-	-	-
11	* *P. isidiophora* (Müll. Arg.) Imshaug	F	-	-	-	-	-	+	-	-	-	-	-	-	-
12	*P. reticulata* (Vain.) Vain.	F	-	-	+	-	-	-	-	-	-	-	-	-	-
Graphidaceae															
13	**Allographa stictilabiata* (Patw. & C.R. Kulk.) J. Kalb & Kalb.	C	+	-	-	-	-	-	-	-	-	-	-	-	-
14	*Diorygma junghuhnii* (Mont. & Bosch) Kalb, Staiger & Elix	C	+	-	-	-	-	-	-	-	-	-	-	-	-
15	*D. soozanum* (Zahlbr.) M. Nakan. & Kashiw.	C	+	-	-	-	-	-	-	-	-	-	-	-	-
16	*Graphis analoga* Nyl.	C	-	-	-	-	-	-	+	-	-	-	-	-	-
17	*G. arecae* Vain.	C	-	-	-	-	-	-	-	+	-	-	-	-	-
18	* *G. asahinae* Patw. & C.R. Kulk.	C	-	-	-	-	-	-	-	-	+	-	-	-	-
19	*G. furcata* Fée	C	+	-	-	-	-	-	-	-	-	-	+	-	-
20	*G. glaucescens* Fée	C	+	-	-	-	-	-	-	-	-	-	-	-	-
21	* *G. modesta* Zahlbr.	C	-	-	-	-	-	-	-	-	-	-	-	+	-
22	*G. pyrrhocheiloides* Zahlbr.	C	+	-	-	-	-	-	+	-	-	-	-	-	-
23	*G. sayeri* Müll. Arg.	C	-	-	-	-	-	-	-	-	-	+	-	-	-
24	*G. scripta* (L.) Ach.	C	-	-	-	-	-	-	-	-	-	-	-	+	-
25	*G. sulphurella* (Zahlbr.) Lücking	C	-	-	-	-	+	-	-	-	-	-	-	-	-
26	*G. sundarbanensis* Jagad. Ram & G.P. Sinha	C	-	-	-	-	-	-	+	-	+	-	-	-	-
27	*G. xanthospora* Müll. Arg.	C	+	-	-	-	+	-	-	-	-	-	-	-	-
Lecanoraceae															
28	*Lecanora helva* Stizenb.	C	-	+	+	-	-	+	-	-	-	+	-	+	-
29	* *L. leproplaca* Zahlbr.	C	-	-	-	-	-	-	-	+	-	-	-	-	-
Parmeliaceae															
30	* *Parmotrema mesotropum* (Müll. Arg.) Hale	F	-	+	-	-	-	-	-	-	-	-	-	-	-
31	*P. saccatilobum* (Taylor) Hale	F	-	-	-	-	-	-	-	-	-	+	-	-	-
Physciaceae															
32	* *Physcia abuensis* D.D. Awasthi & S.R. Singh	F	-	-	-	-	-	-	+	-	-	-	-	-	-
Porinaceae															
33	*Porina suhibernica* Upreti	C	+	-	-	-	-	-	-	-	-	-	-	-	-
Pyrenulaceae															
34	*Pyrenula aggregata* (Fée) Fée	C	-	-	+	-	-	-	-	-	-	-	-	-	-
35	*P. aspistea* (Afzel. Ex Ach.) Ach.	C	-	-	-	-	-	-	-	+	-	-	-	-	-
36	* *P. mastophora* (Nyl.) Müll. Arg.	C	-	-	-	+	-	-	-	-	-	-	-	-	-
37	* *P. minor* Fée	C	-	-	-	-	-	-	-	-	-	-	-	-	+
38	*P. thelomorpha* Tuck.	C	-	-	-	-	-	-	-	-	-	+	-	-	-
39	* *P. welwitschii* (Upreti & Ajay Singh) Aptroot	C	-	-	-	-	-	-	-	-	-	+	-	-	-
Ramalinaceae															
40	*Bacidia medialis* (Tuck.) Zahlbr.	C	-	-	+	-	-	-	-	-	-	-	-	-	-
41	*B. rubella* (Hoffm.) A. Massal.	C	-	-	-	-	-	-	-	-	-	-	+	-	-
Trypetheliaceae															
42	*Trypethelium eluteriae* Spreng.	C	-	-	-	-	-	-	-	-	-	+	-	-	-

GF—Growth form | C—Crustose | F—Foliose | 1—Alokjhari | 2—Alomganj part 9 | 3—Barobalurchar | 4—Bhasani goan | 5—Bidyadabri part 5 | 6—Debotar hasdaha part 4 | 7—Dhubri town | 8—Gauripur Matiabag Hawakhana | 9—Gopigoan part 3 | 10—Kismat hasdaha part 2 | 11—Khajurbari part 1 | 12—Rangamati part 3 | 13—Satrasal. (*) denotes new records to Assam, (+) present and (–) absent.

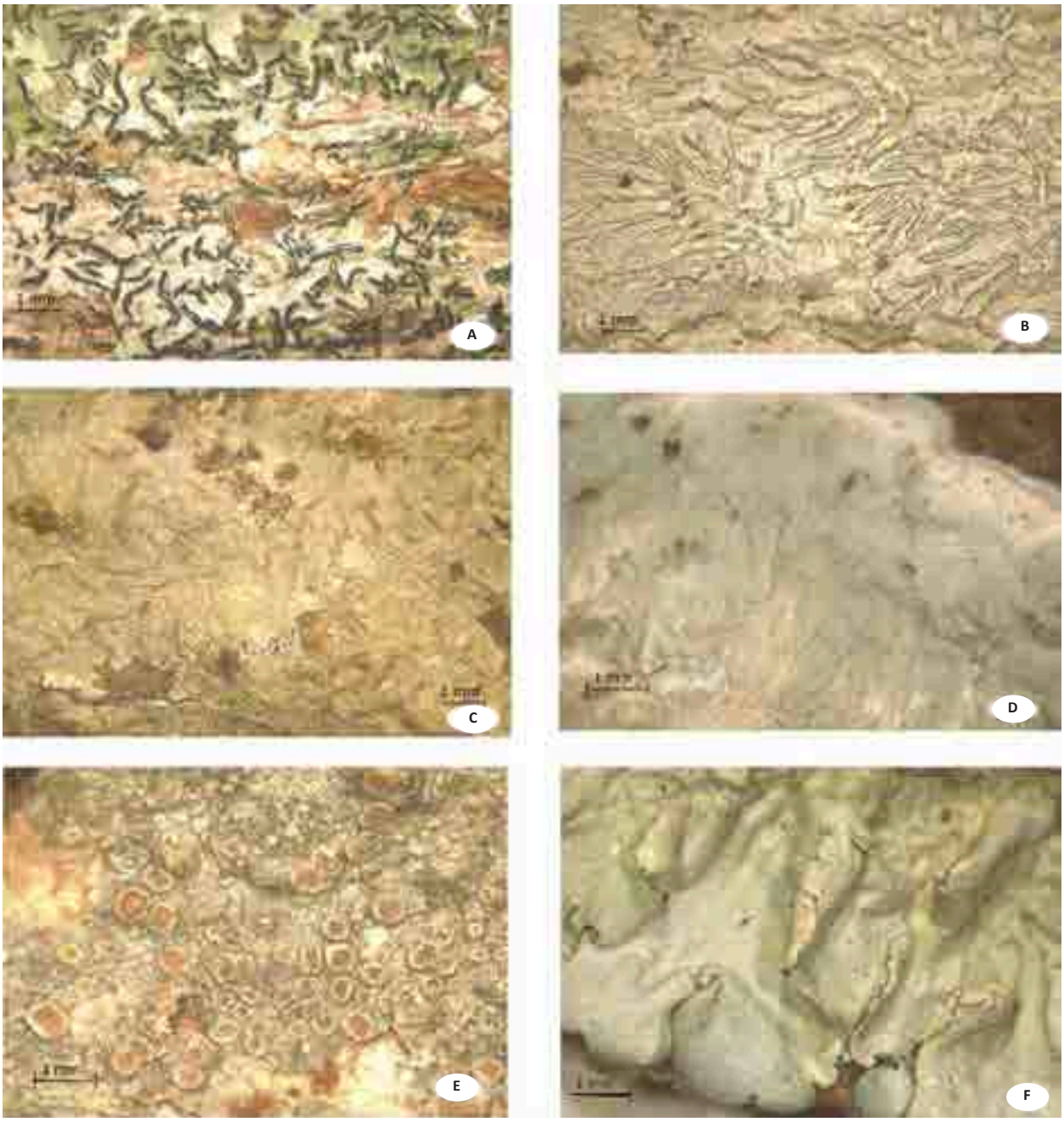

Image 1. Habits of lichen new records: A—*Graphis asahinae* Patw. & C.R. Kulk. | B—*Graphis modesta* Zahlbr. | C—*Allographa stictilabiata* (Patw. & C.R. Kulk.) J. Kalb & Kalb | D—*Herpothallon himalayanum* Jagad. Ram & G.P. Sinha | E—*Lecanora leproplaca* Zahlbr. | F—*Parmotrema mesotropum* (Müll. Arg.) Hale | (Scale bar = 1mm).

Biswas & P. Biswas.

Family: Pyrenulaceae

Pyrenula mastophora (Nyl.) Müll. Arg. (Image 2K)

Distribution: India (Tamil Nadu), Philippines

Specimen examined: 2020-0177 (BUBH), India, Assam, Dhubri district, Bhasani goan, on the bark of *Lannea coromandelica*, 26.xii.2020, 36.22 m, 26.301 N, 90.224 E, coll. S. Biswas & P. Biswas.

Pyrenula minor Fée (Image 2I)

Distribution: India (Andaman and Nicobar Islands), Brazil, El Salvador, French Guiana, USA

Specimen examined: 2020-0178 (BUBH), India, Assam, Dhubri district, Satrasal, on the bark of *Lannea coromandelica*, 4.i.2020, 36.89 m, 26.131 N, 89.734 E,

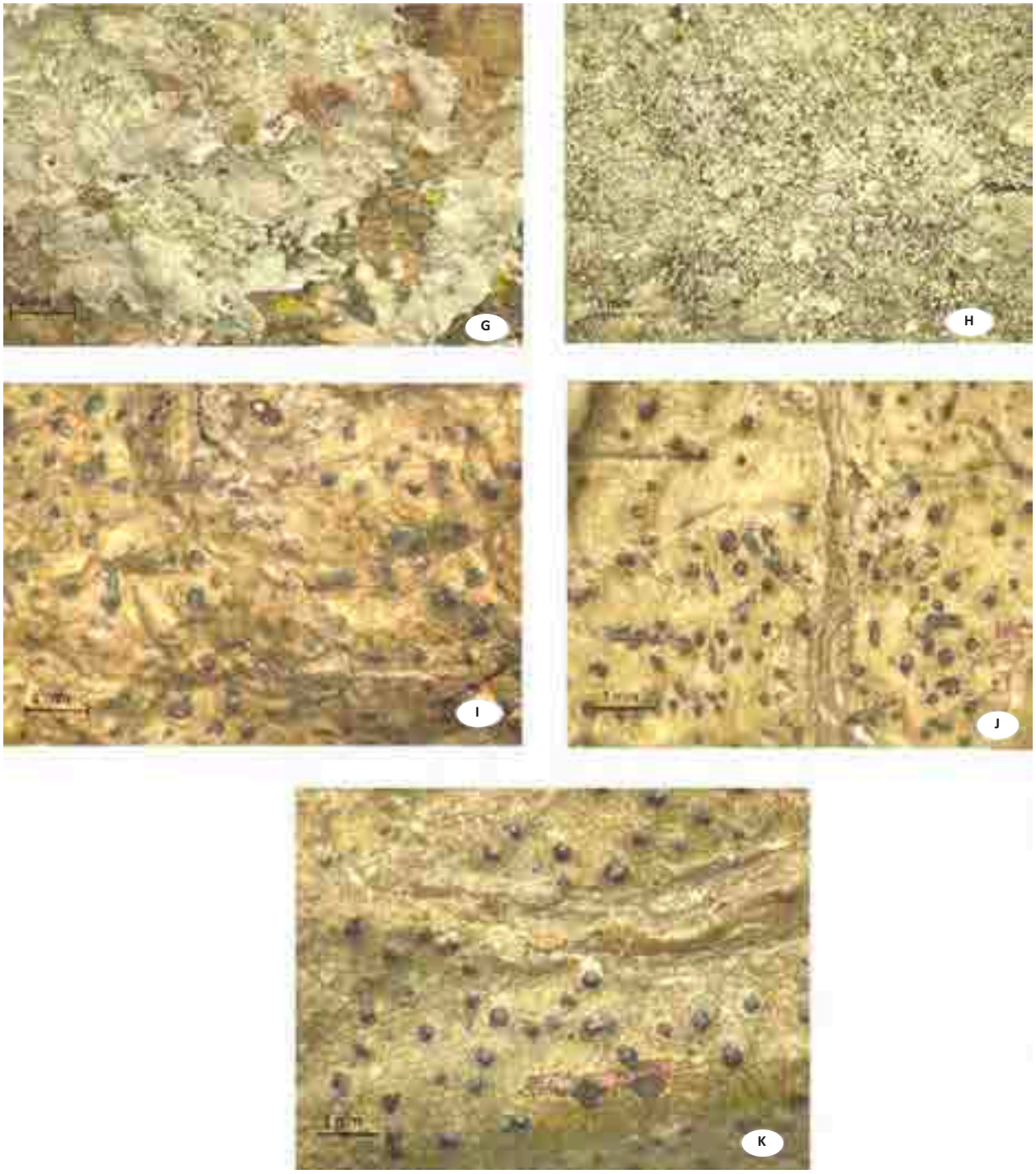

Image 2. Habits of lichen new records: G—*Physcia abuensis* D.D. Awasthi & S.R. Singh | H—*Pyxine isidiophora* (Müll. Arg.) Imshaug | I—*Pyrenula minor* Fée | J—*Pyrenula welwitschii* (Upreti & Ajay Singh) Aptroot | K—*Pyrenula mastophora* (Nyl.) Müll. Arg. | (Scale bar = 1mm).

coll. S. Biswas & P. Biswas.

Pyrenula welwitschii (Upreti & Ajay Singh) Aptroot (Image 2J)

Distribution: India (Uttarakhand), Angola

Specimen examined: 2020-0179 (BUBH), India, Assam, Dhubri district, Kismat hasdaha part 2, on the bark of *Lannea coromandelica*, 22.xi.2020, 37.07 m, 26.050 N, 89.893 E, coll. S. Biswas & P. Biswas.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21091–21101

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.6928.14.5.21091-21101

#6928 | Received 25 November 2021 | Final received 27 April 2022 | Finally accepted 01 May 2022

OPEN ACCESS

COMMUNICATION

Genus *Gymnopilus* (Agaricales: Strophariaceae): addditions to the agarics of India

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Abstract: The present study deals with the diversity of the genus *Gymnopilus* collected from Kashmir Himalaya. Frequent fungal forage were undertaken during spring, summer, and autumn seasons as a result of which a systematic account of various taxa of the genus *Gymnopilus* was compiled. In the present paper six species of the genus are taxonomically described and identified as *G. decipiens*, *G. aeruginosus, G. fuscosquamulosus, G. crocias, G. junonius*, and *G. liquiritiae*. Out of all described species *G. decipiens* is reported for the first time from India while the other four are reported for the first time from northern India. In addition, only *G. aeruginosus* is reported for the first time from Bangiward, southern Kashmir. Detailed morpho-anatomical characters of these species with habitat photographs, line drawings of macro and microscopic features are given. An identification key to the described species are also given.

Keywords: Clamp connections, Cystidia, Dextrinoid basidiospores, habitat, Kashmir Himalaya, line drawings, macrofungi.

Editor: Anonymity requested.

Date of publication: 26 May 2022 (online & print)

Citation: Wani, N.A., M. Kaur & N.A. Malik (2022). Genus *Gymnopilus* (Agaricales: Strophariaceae): addditions to the agarics of India. *Journal of Threatened Taxa* 14(5): 21091–21101. https://doi.org/10.11609/jott.6928.14.5.21091-21101

Funding: None.

Competing interests: The authors declare no competing interests.

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Author contributions: MK—developed the research idea and also helped in identification of the agaric species. NAW—led the manuscript writing with inputs from MK and NAM. All the authors approved final draft of the manuscript for submission.

Acknowledgements: Thanks are due to the head, Department of Botany, Punjabi University, Patiala for providing laboratory facilities during the course of this work.

INTRODUCTION

Jammu & Kashmir has different climate varying from tropical deciduous forests to temperate and coniferous forests which provide compatible habitat for the growth of macrofungal species. The macrofungal richness of the union territory is directly related to its diverse weather patterns and expansive forest communities. The genus *Gymnopilus* P. Karst under the order Agaricales includes interesting and important saprotrophic, usually lignicolous inhabiting fungi occurring all over the world (Holec 2005). The important characters of the genus are the bright coloured yellow, ferruginous, or purple fruiting bodies, adnexed to decurrent lamellae, along with cortinoid to membranaceous veil and a rusty-brown spore print. Microscopically, the genus is identified by the rough basidiospores having a verrucose to rugulose ornamentation lacking a germ pore or plage and mostly dextrinoid wall, gill edges are sterile with cheilocystidia, which are more or less ventricose below and possess subcapitate to capitate apex and clamp connections present on almost all kinds of hyphae (Kühner 1980; Singer 1986). The size and shape of the basidiospores and cystidia are considered important characters for differentiation among the species (Rees et al. 2004).

Gymnopilus was considered a member of Cortinariaceae by Hesler (1969) & Singer (1986) and under Strophariaceae by Kühner (1984) & Guzmán-Dávalos et al. (2003). Presently, this genus is placed under the family Strophariaceae purely on the basis of non-ectomycorrhizal associations. According to (Høiland 1990) this genus may be linked to a hypothetical primitive, saprophytic ancestor of both Cortinariaceace and Strophariaceae. In this line the first family developed the ectomycorrhizal mode of life while the second maintained the saprophytic mode. According to the review, globally *Gymnopilus* is represented by 200 species (Kirk et al. 2008) while MycoBank (https://www.mycobank.org/) documents 289 legitimate species. In India, 30 species of the genus were recorded (Berkeley 1851; Sathe & Rahalkar 1975; Manjula 1983; Natrajan & Raman 1983; Dhancholia et al. 1991; Chadha & Sharma 1995; Natarajan et al. 2005; Farook et al. 2013; Kaur et al. 2015; Upadhyay et al. 2017).

MATERIALS & METHODS

Study Area

Jammu & Kashmir is the second largest union territory of India, located in the extreme north of the country. The area is geographically divided into two regions namely Kashmir valley and Jammu region. The Kashmir valley lies between the coordinates 34.166N & 74.500E, is situated between Pir Panjal range & Zanskar range; and has a total area of 15,948 km^2 (Qazi 2005). Northern and southern Kashmir, presently selected as the areas of investigation harbour a rich floristic diversity. Due to the varied climatic and topographic conditions, the area is considered a hot spot of fungal diversity.

Morpho-anatomical observations

Collections of agarics were made on routine mycological field visits to the forests of northern and southern Kashmir. Basidiomes were collected with care using a sharp knife, waste newspapers, hand lens, camera, paper & pen, field notes regarding locality, GPS position, altitude, date of collection, collection number, habit, habitat, substrate, and their association with the surrounding forest vegetation. The basidiomes collected for the purpose of taxonomic studies were fresh and healthy and wherever possible in the field, the whole range of developmental stages were collected. The collected species were taken to the laboratory for further analysis such as microscopic observations, drying, and packing. The study also examines the data with respect to the seasonal availability, habit, habitat, edibility status and the range of distribution of studied taxa as described in Table 1.

The morphological characters and chemical tests were carried out in the field as well as in the laboratory as per the standard protocol given by Atri et al. (2005, 2017), further the colour names and codes were followed as given by Kornerup & Wanscher (1978).

Macro-morphological characters were observed from fresh specimens considering all the available basidiomes. A small portion of the cap, stipe and volva were preserved in liquid preservative (25% rectified alcohol + 5 % formalin + 70 % distilled H_2O (Hawksworth et al. 1983). The microscopic details were studied by cutting free hand sections of the revived parts (revived with KOH) of the dried specimen and staining them either in cotton blue or Congo red and the internal details of the pileus cuticle, stipe cuticle, hymenophore trama and various cystidial elements were observed. The basidiospores were studied from the spore print as well as from the crush mounts of the lamellae and their reaction with Melzer's reagent were checked. The basidiospore quotient (Q) was calculated by ratio of mean length divided by mean breadth of 30 as per Singer (1986). Properly dried and preserved specimens of the described species were deposited in the

Table 1. The data of described species regarding localities, seasonal availability, habitat, edibility status, and the allotted herbarium numbers.

Name of the species	Locality of the species along with altitude	Date, Month & Year of collection	Growing habit	Habitat	Edibility	Herbarium numbers
G. decipiens	PanzullaTakya (1,807 m)	17 May 2013	Groups	Growing on soil around burnt stalk of *Pinus*	Poisonous	PUN 9290
G. aeruginosus	Bangiward (2,700m)	19 August 2015	Solitary	Growing on wood of *Cedrus deodara*	Hallucinogenic	PUN 9068
G. liquiritiae	Pahalgam (2,650m)	5 August 2014	Caespitose	Growing on burnt and rotten wood of *Cedrus deodara*	Unknown	PUN 9070
G. junonius	Naugam (2,100 m)	22 June 2015	Groups	Growing on burnt wood of *Pinus*	Inedible	PUN 9292
G. fuscosquamulosus	Naugam (2,125 m) Bangiward (2,700m)	06 August 2014 20 August 2015	Caespitose	Growing on dead wood stump of *Cedrus deodara* and on dry peat moss of *Pinus wallichiana*	Poisonous	PUN 9291 9069
G. crocias	Dazna Rafiabad (2,215 m)	08 August 2014	Caespitose	Growing on humicolous soil around the scattered needles of *Pinus*	Unknown	PUN 9289

Key to the investigated species of the genus *Gymnopilus*

1 Cuticle half peeling; Stipe annulate with rhizomorphs usually present at the base of the stipe ..... *G. decipiens*
- Cuticle fully peeling; Stipe exannulate without any rhizomorphs present at the base of the stipe 2

2 Cap with a bluish tinge; Pileal veil appendiculate ..... *G. aeruginosus*
- Cap without any bluish tinge; Pileal veil absent 3

3 Gill edges sterile; Caulocystidia present ..... *G.liquiritiae*
- Gill edges heteromorphous; Caulocystidia absent..... 4

4 Gill edges serrate; Cap with areolate cracking exposing the flesh below ..... *G. junonius*
- Gill edges smooth; Cap without areolate cracking 5

5 Basidiome growing on dead wood stump of Cedrus deodara; Flesh changing; Taste acrid ..... *G. fuscosquamulosus*
- Basidiome growing on humicolous soil; Flesh unchanging; Taste mild ..... *G. crocias*

Herbarium, Department of Botany, Punjabi University, Patiala (Punjab) India, under the Accession No. PUN as given in Table 1.

RESULTS

The taxonomic descriptions of six species of genus *Gymnopilus—decipiens, aeruginosus, liquiritiae, junonius, fuscosquamulosus*, and *crocias*—are provided as per the sequence of segregation in the identification key given below.

Taxonomic study

Gymnopilus decipiens (Sacc.) P.D. Orton, *Transactions of the British Mycological Society* 43(2): 176 (1960). **(Image 1– 4)**

[Mycobank No. 331590; Legitimate]

Basidiome up to 4.0 cm in height, pileus up to 3.6 cm broad, convex to applanate with uplifted margin; umbo absent; margin irregular, splitting at maturity, non-striate; surface pale orange (5A3); moist; scaly, scales squamulose, light orange (5A4) to brownish-orange (5C6), cover the entire pileus, more concentrated towards the centre; cuticle half peeling; context up to 0.4 cm thick, creamy white, unchanging; odour mild. Pileal veil absent. Lamellae free to adnexed, distant, unequal, not in series; moderately broad (up to 0.3 cm); pale orange (5A3) to greyish orange (5B5), unchanging; gill edges serrate, white; lamellulae present. Stipe central, up to 4.0 cm long, up to 0.3 cm broad above, 0.2 cm broad at middle and up to 0.4 cm broad at the base, unequal in diameter with a slightly bulbous base; surface creamy white, light yellow (4A4) towards apex, light orange (5A4) in the middle, orange (5B8) to brownish-yellow (5C8) towards base, unchanging; scaly, scales appressed fibrillose, brownish-yellow (5C7); hollow; rhizomorphs present at the base of the stipe; annulate, annulus scaly, attached, evanescent. Spore print brownish-orange (7C4).

Basidiospores 7.47–9.13 x 4.15–5.81µm, Q = 1.8, ellipsoidal, dextrinoid, ornamented, outer wall thick,

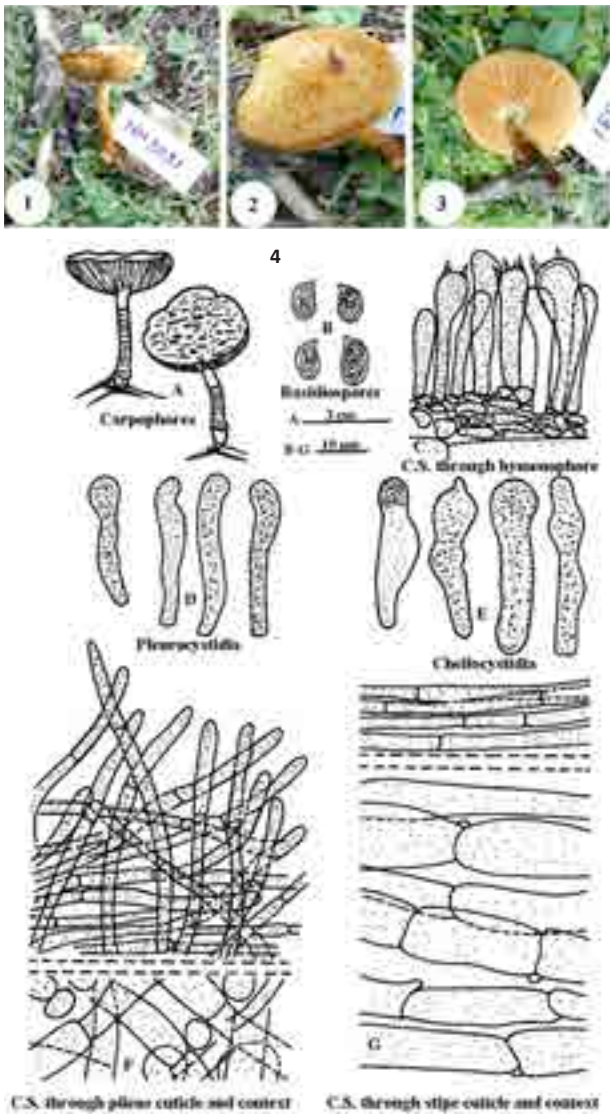

Image 1–4. *G. decipiens* (Sacc.) P.D. Orton: 1—Basidiome growing solitary in their natural habiat and the rhizomorphs are clearly visible at the base of the stipe | 2—Cap surface covered with light orange to brownish-orange squamulose scales | 3—Under view of cap showing distinct, free to adnexedlamellae | 4—Camera lucida drawings (A–G).

rough, thickly granular; apiculate, apiculus 0.83–1.66 µm long, excentric. Basidia 25.0–34.86 x 5.0–6.64 µm, claviform, granular; bisterigmate to tetrasterigmate, sterigmata 2.5–4.15 µm long, granular. Pleurocystidia 20.0–36.52 x 4.15–6.64 µm, cylindric to capitate, densely granular. Cheilocystidia 21.58–39.84 x 6.64–10.0 µm, claviform, capitate, lageniform to lecythiform, densely granular; gill edges heteromorphous. Hymenophoral trama regular. Pileipellis hyphal, ixocutis, made up of 1.66–5.0 µm broad, horizontally tangled septate, hyphae giving rise to scattered turf of 2.5–4.15 µm broad, septate, granular, projecting hyphae; pilocystidia absent; context made up of 6.64–9.13 µm broad, granular, septate, hyphae intermingled with 5.81–13.28 µm broad, granular, cellular elements. Stipe cuticle hyphal, made up of longitudinally arranged, 4.15–5.0 µm broad, septate, hyphae; context hyphal, made up of, 8.3–12.45 µm broad, septate, hyphae. Clamp connections present throughout the context.

Collection examined: Jammu & Kashmir, Baramulla, Panzulla Takya (1,807 m) 34.486N & 74.350E, growing in groups on soil around burnt stalk of *Pinus*, in mixed coniferous forest, Naseema Aqbar Wani, PUN 9290, 17 May 2013.

Edibility: O'Reilly (2016) listed it as a poisonous mushroom.

Distribution and Ecology: *Gymnopilus decipiens* was found growing solitary or in small groups on burnt soil and on burnt pine stumps from England by Orton (1960). Høiland (1990) reported this species growing on burnt dry sandy soil in open pine forest in the month of July from Norway. Holec (2005) reported this species from European countries and Czech Republic. This species was also found growing on hardwood stumps, burnt wood, and forest fire sites in the months of June to November from Britain, England, Scandinavia, France, and Italy by O'Reilly (2016).The present collection has been found growing in groups on soil around burnt stalk of *Pinus*, in mixed coniferous forests in the month of May from Jammu & Kashmir.

Remarks: The morphology and microscopic details of the above examined collection are in full conformity with the details given for *Gymnopilus decipiens* (Sacc.) P.D. Orton, by Orton (1960) and Høiland (1990). But in the recent work collection the encrustations and pigmentation pattern are lacking in the projecting hyphae of the turf of pileus cuticle which should be present as per Orton (1960). The species is recorded for the first time from India.

Gymnopilus aeruginosus (Peck) Singer, *Lilloa* 22: 560, 1951. **(Images 5–7)**

[Mycobank No. 298026; Legitimate]

Basidiome up to 7.0 cm in height. Pileus up to 8.0 cm broad, convex; umbonate, umbo acute with a bluish tinge; surface light yellow (4A4), orange or deep orange (6B6) near margin with reddish tinge; scaly, scales, appressed fibrillose, deep orange (6A6); margin, involute, not splitting at maturity; dry; cuticle fully peeling; contextup to 0.2 cm thick, brown, unchanging; taste bitter observed from dry specimen, odor mild. Pileal veil appendiculate, dry. Lamellae up to 0.5 cm broad, adnate to adnexed, close, unequal, non furcate,

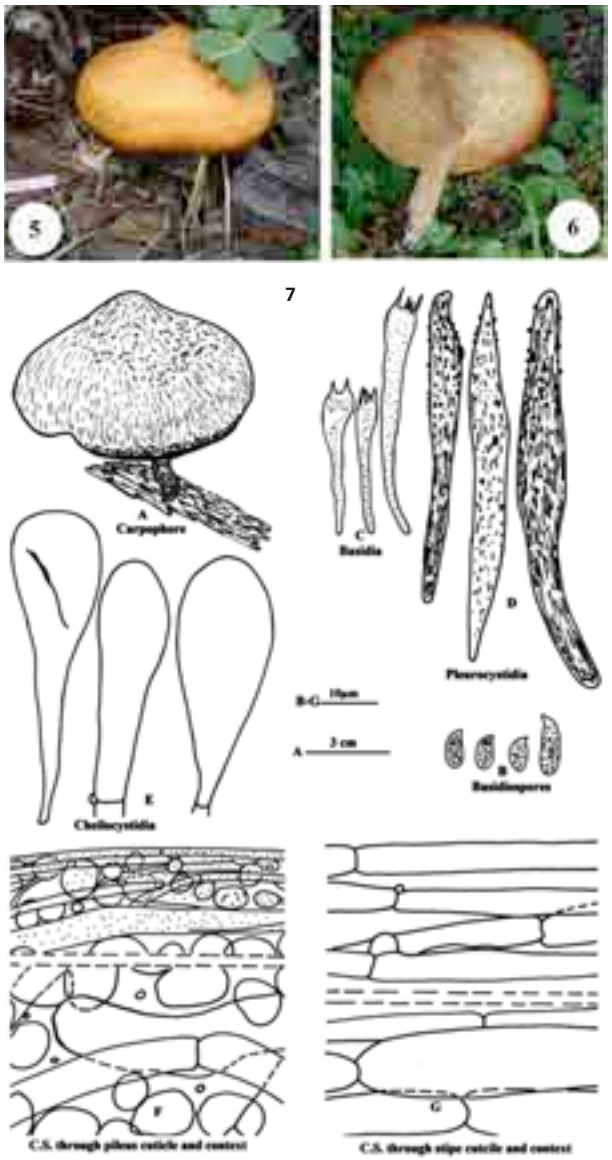

Image 5–7.*G. aeruginosus* (Peck) Singer: 5—Basidiome in solitary habit with acute umbo with light yellow, orange to deep orange surface and reddish tinge near margin | 6—Under view of Basidiome with dentate lamellae edges and excentric stipe | 7—Camera Lucida drawings (A–G).

creamy white, changing to deep orange or dark brown (7F8); lamellulae present. Gill edges dentate. Stipe excentric, up to 6.5 cm long, up to 0.6 cm broad, equal in diameter; surface off white with orange (5A6) shade; scaly, scales fibrillose, floccose near apex; changing to yellow on handling; white mycelium present at base; first solid then hollow; exannulate. Spore print greyish orange (6B3).

Basidiospores 6.4–9.6 × 3.2–4.0 µm; Q = 1.7, ellipsoidal to oblong, single thick walled, ornamented, ornamentation very fine, punctuate, granular; amyloid; apiculate, apiculus up to 0.8 µm long. Basidia 24.0–40.0 × 2.4–4.8 µm, clavate, narrow, granular, tetrasterigmate, sometimes bi-sterigmate; sterigmata 1.6–3.3 µm long, granular, apices pointed. Pleurocystidia 80.0–118.4 × 5.6–6.4 µm, clavate, ventricose with beaked, pointed to rounded tips, thickly granular, encrustated, filled with yellow shiny content, protruding beyond the basidia, deeply seated. Cheilocystidia 48.0–72.0 × 14.4–24.0 µm, broadly clavate, hyaline, rarely clamped at the base, abundant. Hymenophoral trama regular. Gill edge sterile. Pileus cuticle hyphal, ixocutis, made up of 2.0–3.32 µm broad, narrow, septate, granular hyphae; context hyphal, made up of 2.0–10.52 µm broad, septate, irregularly placed, hyaline, hyphae, intermixed with clavate to globose sphaerocysts. Stipe cuticle hyphal, made up of 3.2–9.6 µm broad, longitudinally placed, hyaline, septate, clamped hyphae; caulocystidia absent; context hyphal, made up of 8.0–13.6 µm broad, longitudinally placed, hyaline, inflated hyphae.

Collection examined: Jammu and Kashmir, Bangiward (2,700m), 33.670N & 75.074E, growing solitary on wood of *Cedrus deodara* in coniferous forest, Nazir Ahmad Malik, PUN 9068, 19 August 2015.

Edibility: *Gymnopilus aeruginosus* is a hallucinogenic species (Arora 1986).

Distribution and Ecology: Arora (1986) found *Gymnopilus aeruginosus* growing gregarious in caespitose habit on logs, stumps, woodchip or sawdust on hardwood and conifers during spring and winter in the Pacific Northwest. Sharma et al. (2019) has reported this species from the plains of Jammu. In the present study *G. aeruginosus* has been found growing solitary on stumps of *Cedrus deodara* in coniferous forest.

Remarks: The macroscopic and microscopic observation of present collection matches and fits well the description provided for *Gymnopilus aeruginosus* (Peck) Singer by Arora (1986) and Barnhart (1994). The present PUN 9068 falls under the section *Gymnopilus* of subgenus *Gymnopilus* (Hesler 1969) and is characterized by acute umbo with bluish tinge, broadly convex cap with orange or deep orange (6B6), involute margin, deep orange (6A6) appressed fibrillose scales, bitter taste observed from dry specimen, pleurocystidia are shiny encrustated, cheilocystidia are rarely clamped at the base and pileus context is intermixed with clavate to globose sphaerocysts. However, the microscopic observations both from dry as well as wet specimen does not revealthe presence of caulocystidia on the stipe as described by Barnhart (1994) for this species. *Gymnopilus aeruginosus* has been recorded from India (Gogoi & Parkash 2015) while Sharma et al. (2019)

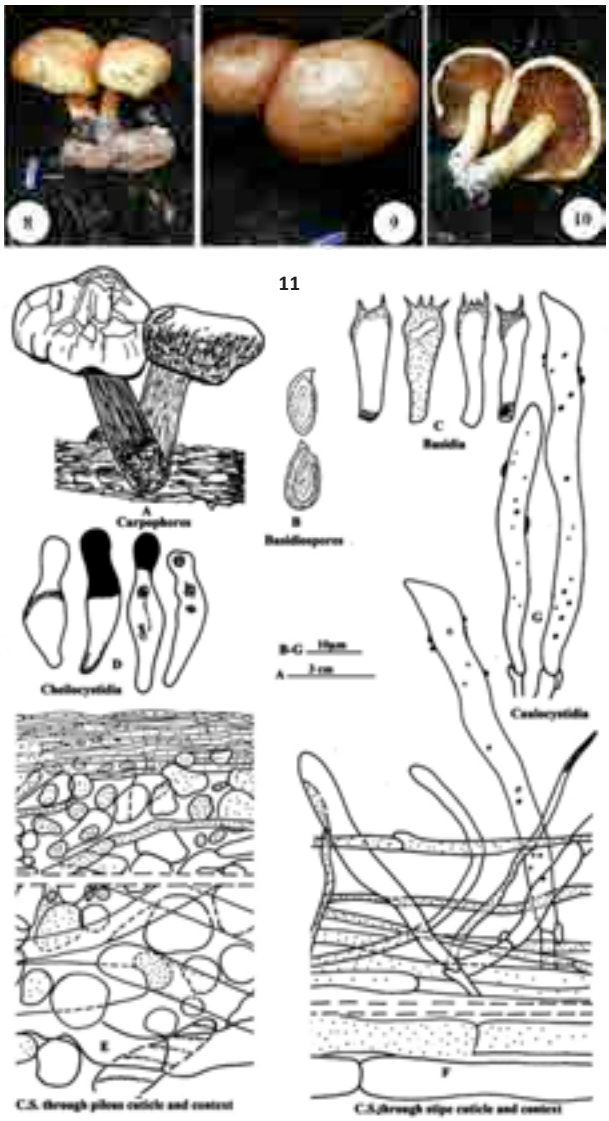

Image 8–11. *G. liquiritiae* (Pers.) P. Karst.: 8—Basidiomata caespitose on burnt and rotten wood of *Cedrus deodara* with rusty brown powdery mass both on cap and stipe surface | 9—Cap surface bearing squamose scales | 10—Underview of cap showing involute margin and dentate, wavy lamellae edges | 11—Camera lucida drawings (A–G).

reported this species from the Jammu plains. Presently this species is reported for the first time from the Kashmir Himalaya.

Gymnopilus liquiritiae (Pers.) P. Karst., *Bidr. Finl. Nat. Folk* 32: 400, 1879.[MycoBank No. 415197; Legitimate] **(Images 8–11)**

Basidiomata 6.5–8.0 cm in height. Pileus 4.0–6.5 cm broad, hemispherical when young, obtuse convex at maturity; surface orange (5A6), light yellow (4A9) near margin with light rusty tinge; scaly, scales appressed fibrillose, squamose, reddish-brown (8D8), rusty brown powdered depositions present; cracked; margin regular, involute at maturity; dry; glabrous; cuticle fully peeling; context up to 0.8 cm thick, brown, unchanging; odor mild, taste bitter. Pileal veil absent. Lamellae up to 0.8 cm broad, adnexed, crowded, unequal, brownish orange (7C6), yellowish shade near stipe, lamellulae present. Gill edges dentate, wavy. Stipe central, 5.0–7.0 cm long, up to 1.5 cm broad, equal in diameter; surface orange (5A6) with light orange ($5A_4$) tinge, stains after handling; rusty brown powdery mass covering the entire stipe; white mycelium with yellow tinge present at base; solid; exannulate.

Basidiospores 8.8–11.2 × 4.8–6.4 µm; Q = 1.7, ellipsoidal to amygdaliform, thick double walled, rough, ornamented, verrucose; amyloid; apiculate, apiculus 0.8 µm long. Basidia 18.26–23.24 × 5.0–8.3 µm, clavate to subcylindrical, granular, bi to tetrasterigmate; sterigmata 1.66–3.32 µm long, granular, apices pointed. Pleurocystidia absent. Cheilocystidia 16.6–25.0 × 5.0–8.3 µm, lecythiform, capitate, rarely granular, abundant. Hymenophoral trama regular. Gill edge sterile. Pileus cuticle hyphal, ixocutis made up of 1.66–2.5 µm broad, narrow, compact hyphae, consisting yellowish content; context hyphal, made up of 5.0–11.62 µm broad, septate, irregularly placed, hyaline, gelatinized, hyphae, intermixed with clavate, inflated cells. Stipe cuticle hyphal, ixocutis, made up of 1.66 - 2.49 µm broad, longitudinally placed hyphae, giving rise to irregular turf of hyaline to granular filled with yellowish black content, clamped hyphae caulocystidia; caulocystidia 72.0–96.0 × 6.4–8.0 µm broad, elongated, granular, encrustated, clamped at the base, rare; context hyphal made up of 5.0–11.62 µm broad, longitudinally placed hyphae with inflated to beaked hyphal ends, hyaline to granular hyphae.

Collection examined: Jammu & Kashmir, Pahalgam (2,650 m), 34.076N & 75.425E, growing in caespitose habit on burnt and rotten wood of *Cedrus deodara* in pure *Cedrus* forest, Nazir Ahmad Malik, PUN 9070, 5 August 2014.

Edibility: Unknown.

Distribution and Ecology: *Gymnopilus liquiritiae* is a widely distributed species growing in caespitose habit on wood of conifer. Guzman-Davalos & Guzman (1991) have found this species growing in cespitose habit on dead wood of *Pinus* and *Quercus* in the forests of Mexico. Natarajan & Raman (1983) found this species on living or dead wood in South India. The present Indian collection has been found growing in caespitose habit on burnt and rotten wood of *Cedrus deodara* in pure *Cedrus*

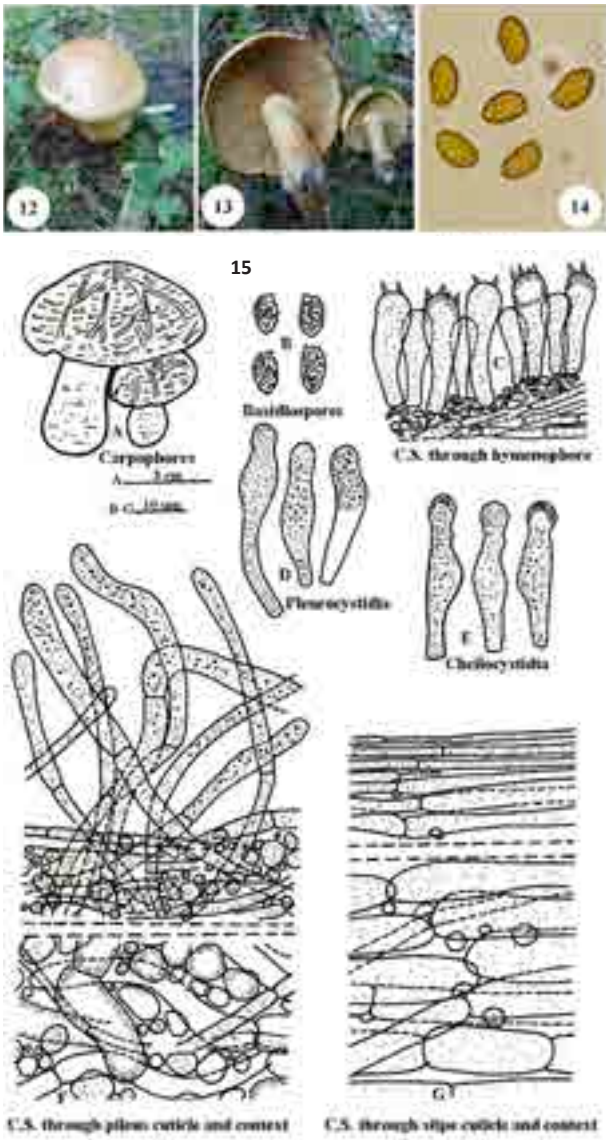

Image 12–15. *G. junonius*(Fr.) P.D. Orton: 12—Basidiomata growing in groups on burnt wood of *Pinus* and the cap surface with prominent areolate cracking | 13—Underview of cap showing pale orange to brownish orange lamellae with serrate edges | 14—Microphotograph of verrucose, dextrinoid basidiospores | 15—Camera lucida drawings (A–G).

forests during August at an altitude of 2,650 m.

Remarks: The present collection due to the absence of annular ring on the stipe falls under the section *Gymnopilus,* further on the basis of morphological and internal details it matches well with *G. liquiritiae* (Pers.) P. Karst. as described by Arora (1986), Natarajan & Raman (1983) and Barnhart (1994). This PUN 9070 is characterized by reddish-brown (8D8) scales on hemispherical to convex cap, bitter taste, pileus surface consists clavate, inflated cells and presence of elongated caulocystidia with clamps at base on stipe. This collection was compared with an allied species viz. *G. penetrans* (Fr.) Murrill and *G. flavidellus* Murrill which has a whitish veil that makes it differ from the present collection. Present PUN 9070 grows on burnt and rotten wood but *G. sapineus* (Fr.: Fr.) Maire does not grow on burnt wood or debris, hence makes it differ from the present collection. Holec (2005) reported similar species from the Czech Republic and described as a *G. picreus* (Pers.: Fr.) P. Karst but Bon & Roux (2002) described similar species as *G. liquiritiae. G. liquiritiae* is first time reported from northern India.

Gymnopilus junonius (Fr.) P.D. Orton,*Transactions of the British Mycological Society* 43(2): 176 (1960)**. (Images 12–15**) [Mycobank No. 331593; Legitimate]

Basidiomata 3.2–7.0 cm in height. Pileus 3.0–6.2 cm broad, convex, with inrolled margin; umbonate, umbo broad; margin irregular, splitting at maturity, non striate; surface greyish-orange (5B5) at centre, light orange (5A4) towards margin; moist; areolate cracking, flesh exposed beneath the cracks; glabrous; scaly, scales appressed fibrillose especially when young, cuticle half peeling; context up to 0.4 cm thick, creamy white to pale yellow (3A3), unchanging;odor mild. Pileal veil absent. Lamellae adnate to decurrent, subdistant, unequal, not in series; moderately broad (up to 0.6 cm); pale orange (5A3) to brownish-orange (6C4), unchanging; gill edges serrate, floccose white, gills forming striations on the stipe apex; lamellulae present. Stipe central to eccentric, 2.4–5.5 cm long, up to 1.6 cm broad above, up to 2 cm broad at the base, short stout, equal in diameter throughout with a bulbous base; surface pale orange (5A3) towards apex, brownish orange (5C6) towards base; solid; scaly, scales fibrillose; white mycelial mat present at the base of the stipe; annulate, annulus patchy, evanescent in mature basidiomata.

Basidiospores 7.47–9.13 x 4.98–5.81µm, Q = 1.5, ellipsoid, dextrinoid, ornamented, warts low, rough, thick; apiculate, apiculus up to 0.83 µm long. Basidia 20.0–28.22 x 5.81–7.5 µm, clavate, granular; bisterigmate to tetrasterigmate; sterigmata 2.5–4.2 µm long, granular. Pleurocystidia 25.0–40.0 x 5.8–7.5 µm, clavate to lecythiform with rounded capitate apex, densely granular, non encrustrated. Cheilocystidia 30.0–36.5 x 6.64–7.5 µm, lecythiform with rounded capitate apex, densely granular, non encrustrated; gill edges heteromorphous. Hymenophoral trama regular. Pileus cuticle hyphal, ixocutis, made up of 1.66–4.15 µm broad, horizontally tangled hyphae giving rise to a scattered turf of 3.32–6.64 µm broad, septate, thickly granular, projecting hyphae; pilocystidia absent; context

made up of 2.5–10.0 µm broad, granular, septate, hyphae intermingled with 3.32–11.62 µm broad, granular, cellular elements. Stipe cuticle hyphal, made up of longitudinally arranged, 2.5–4.15 µm broad, septate hyphae; context hyphal, made up of, 5.0–13.3 µm broad, septate, hyphae. Clamp connections present throughout.

Collection examined: Jammu & Kashmir, Kupwara, Naugam (2,100 m), 34.424N & 74.450E, growing in groups on burnt wood of *Pinus*, in mixed coniferous forest, Naseema Aqbar Wani, PUN 9292, 22 June 2015.

Edibility: Due to its very bitter taste it is recommended as inedible by Orton (1960).

Distribution and Ecology: Orton (1960) reported *Gymnopilus junonius* growing solitary to caespitose on deciduous trees, coniferous stumps or on ground mostly attached to buried wood from Great Britain and Ireland. Orton (1960) reported it growing in clusters on logs and stumps of hardwoods and conifers during early to midwinter from Great Britain. Arora (1986) found this growing usually in clusters but occasionally solitary on old pine stumps and trees on *Eucalyptus* during early spring and fall, winter, and favors conifers from North America. Phillips (2001–2016) found this species growing on stumps or logs of deciduous trees during late summer to early winter from America and Europe. This species has also been reported by Kuo (2018) growing in caespitose clusters on decomposed hardwoods and conifers from the western coast in North America during summer and spring. Natarajan & Raman (1983) found this species growing in groups on decaying wood of *Eucalyptus* trees from September to November from Tamil Nadu. The presently examined collection has been collected from coniferous forests of Jammu and Kashmir growing in groups on burnt wood of *Pinus* in the month of June.

Remarks: The morphology and microscopic details of the above examined collection are in full conformity with the details given for *Gymnopilus junonius* (Fr.) P.D. Orton, by Orton (1960), Arora (1986), Phillips (2001–2016) and Kuo (2018). This species is characterized in possessing large sized convex cap, evanescent annulus, gills forming striations on the stipe apex, gill edges floccose white, cheilocystidia and pleurocystidia present, spore size similar and in their habitat the present species too was found growing on burnt wood as reported by Orton (1960). The present collection was also compared with an allied taxa *G. odini* (Fr.) Bon & P. Roux, but due to the smaller size of basidiospores and the shape of cheilocystidia given by Orton (1960) it was ruled out. *G. junonius* was earlier reported from southern India by Natarajan & Raman (1983) and Mohanan (2011). Presently, it has been recorded for the first time from north ernIndia.

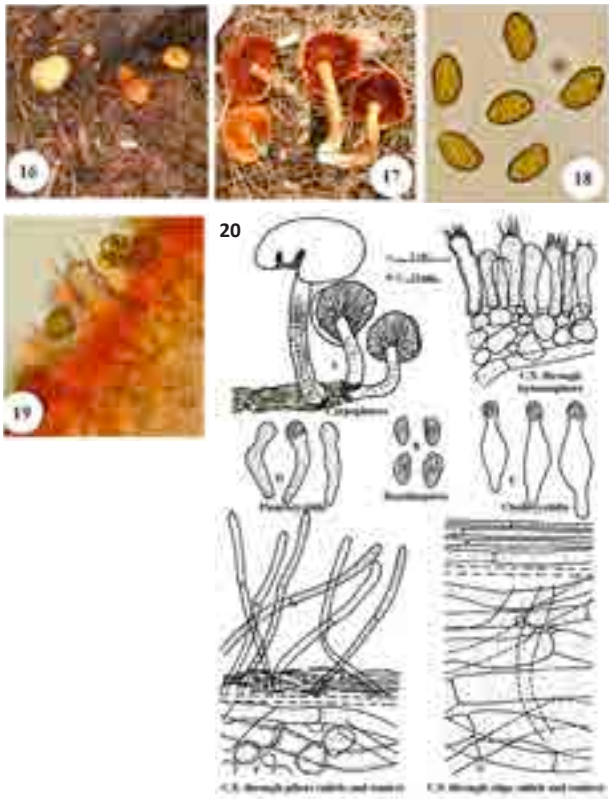

Image 16–20. *G. Fuscosquamulosus* Hesler: 16—Basidiomata growing on dead wood stump of *Cedrus deodara* | 17—Underview of cap showing adnexed to adnate, orange to brownish-orange lamellae | 18—Microphotograph of verrucose basidiospores | 19—C.S. through lamellae showing tetrasterigmate basidia | 20—Camera Lucida drawings (A–G).

Gymnopilus fuscosquamulosus Hesler, *Mycologia Memoirs* 3: 78, 1969. [Mycobank No. 314786; Legitimate] **(Images 16–20)**

Basidiomata 9.5–10.5 cm in height. Pileus up to 5.5–6.5 cm broad, convex to plano-convex; umbonate, umbo broad; margin irregular, splitting at maturity, non striate; surface pale yellow (4A4) to reddish orange (7B8) to light brown (7D6); dry; cuticle fully peeling; context up to 0.2 cm thick, creamy white, changing;odor mild; taste acrid. Pileal veil absent. Lamellae adnexed to adnate, distant, unequal, not in series; moderately broad (up to 0.7 cm); orange (6A6) (6B7) to brownish-orange (7C6), unchanging; gill edges smooth; lamellulae present. Stipe central to eccentric, 9.5 cm long, up to 0.8 cm broad above, up to 1.0 cm broad at the base, equal in diameter throughout with a slightly bulbous base; surface light orange (5A5) to brownish-orange (7C5), unchanging;

scaly, scales appressed fibrillose, white mycelial mat present at the base of the stipe; solid; exannulate.

Basidiospores 7.47–9.13 (9.96) x 4.15–4.98 µm, Q = 1.8, ellipsoidal, dextrinoid, ornamented, verrucose, beaded, thick-walled, rough,; apiculate, apiculus up to 0.83 µm long, excentric. Basidia 18.26–34.86 x 5.0–6.64 µm, clavate, granular, without clamp connections at the base; tetrasterigmate, rarely bisterigmate; sterigmata 4.15–6.64 µm long, granular. Pleurocystidia 20.0–33.2 x 5.0–6.64 µm, clavate to lecythiform with capitate apex, granular, not much protruding out of the basidial layer, densely granular towards apices. Cheilocystidia 25.0–34.86 x 7.5–9.13 µm, lageniform to lecythiform with rounded capitate apices, thickly granular, filled with yellowish content towards the apex; gill edges heteromorphous. Hymenophoral trama regular. Pileus cuticle hyphal, ixocutis, made up of 1.66–2.5 µm broad, horizontally tangled septate hyphae giving rise to sparcely populated regular turf of 1.66–3.32 µm broad, septate, clamped, projecting hyphae; pilocystidia absent; context made up of 4.15–13.3 µm broad, densely granular, septate, clamped, hyphae intermingled with 5.0–11.62 µm broad, granular, cellular elements. Stipe cuticle hyphal, made up of longitudinally arranged, 2.5–3.32 µm broad, septate hyphae; caulocystidia absent; context hyphal, made up of 6.64–11.62 µm broad, septate, hyphae. Clamp connections present throughout.

Collection examined: Jammu & Kashmir, Kupwara, Naugam (2,125 m) 34.424N & 74.450E, growing in caespitose clusters on dead wood stump of *Cedrus deodara*, in coniferous forest, Naseema Aqbar Wani, PUN 9291, 06 August 2014; Jammu & Kashmir, Bangiward (2,700 m), 33.670N & 74.450N, growing in caespitose on dry peat moss on *Pinus wallichiana* tree in coniferous forest, Nazir Ahmad Malik, PUN 9069, 20 August 2015.

Edibility: It is poisonous, hence inedible as reported by Pushpa & Purushothama (2012).

Distribution and Ecology: *Gymnopilus fuscosquamulosus* was found growing on the roots of Buckeye and Rhododendron in the month of June from North America and North Carolina by Hesler (1969). Natarajan & Raman (1983) found this species growing in groups on wood in the month of October from southern India. Pushpa & Purushothama (2012) collected this species from Karnataka. The presently examined collection was made from Jammu & Kashmir growing in caespitose clusters on dead wood stump of *Cedrus deodara* and on dry peat moss of *Pinus wallichiana* tree in the month of August.

Remarks: The present collection falls under section *Gymnopilus*, as the annular ring on the stipe is lacking.

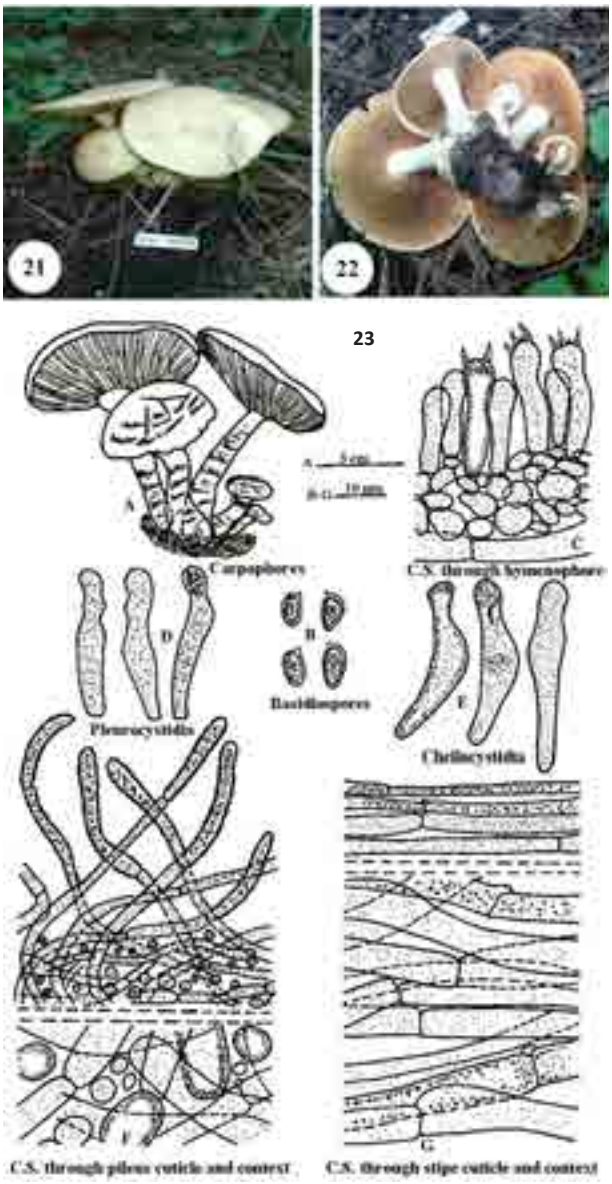

Image 21–23. *G. crocias* (Berk. & Broome) Singer: 21—Basidiomata growing in caespitose clusters on humicolous soil around the scattered needles of *Pinus* in their natural habitat | 22—Under view of cap adnexed, furcate lamellae | 23—Camera lucida drawings (A–G).

Further this matches well with the description provided for *Gymnopilus fuscosquamulosus* Hesler by Hesler (1969), Natarajan & Raman (1983) and Pushpa & Purushothama (2012). *G. fuscosquamulosus* has been earlier reported from India by Natarajan & Raman (1983) from Tamil Nadu and by Pushpa & Purushothama (2012) from Karnataka. Presently, it has been recorded for the first time from northern India.

Gymnopilus crocias (Berk. & Broome) Singer, *Sydowia* 9(1–6): 412 (1955).[Mycobank No. 298031; Legitimate]

(Images 21–23)

Basidiomata 5.7–7.4 cm in height. Pileus 3.7–6.5 cm broad, convex to applanate with inrolled margin; umbo absent; margin irregular, splitting at maturity, non striate; surface orange white (5A2) to pale orange (5A3); dry; scaly, scales appressed fibrillose, cuticle half peeling; flesh up to 0.5 cm thick, creamy white, exposed below the cracking, unchanging; taste mild. Pilealveil reduced to cortinoid zone in young basidiomata. Lamellae adnexed, close to subdistant, unequal, not in series; moderately broad (up to 0.6 cm); greyish-orange (6B3) to brownish-orange (6C3), unchanging, furcate; gill edges smooth; lamellulae present. Stipe central to eccentric, 4.5–6.2 cm long, up to 0.6 cm broad above, up to 1.0 cm broad at the base, equal in diameter throughout with a bulbous base; surface creamy white, pale orange (6A3) towards apex and base, unchanging; scaly, scales appressed fibrillose, white; white mycelial mat present at the base of the stipe; solid; annulate, annulus patchy, evanescent in mature basidiomata.

Basidiospores 6.64–8.3 x 4.15–4.98 µm, Q = 1.6, elliptical, dextrinoid, ornamented, verrucose, wall rough, thick; apiculate, apiculus up to 0.83 µm long, eccentric. Basidia 21.58–36.25 x 5.0–6.64 µm, clavate, granular; bisterigmate to tetrasterigmate, rarely bisterigmate; sterigmata 3.32–5.0 µm long, thickly granular. Pleurocystidia 25.0–34.86 x 5.0–6.64 µm, clavate to lecythiform with rounded capitate apex, thickly granular. Cheilocystidia 26.56–34.86 x 5.0–7.5 µm, subcapitate to lecythiform, densely granular towards the apex, abundant; gill edges heteromorphous. Hymenophoral trama regular. Pileus cuticle hyphal, ixocutis, made up of 1.66–2.5 µm broad, horizontally tangled septate, hyphae giving rise to a regular turf of 1.66–4.15 µm broad, septate, granular, heavily encrustrated projecting hyphae, few hyphae with transverse thick encrustations; pilocystidia absent; context made up of 4.5–10.8 µm broad, clamped, granular, septate, hyphae intermingled with 6.64–11.62 µm broad, granular, cellular elements. Stipe cuticle hyphal, made up of longitudinally arranged 2.5–4.15 µm broad, septate, densely granular, hyphae; context hyphal, made up of loosely arranged, 5.81–11.62 µm broad, septate, hyphae. Clamp connections present throughout.

Collection examined: Jammu & Kashmir, Baramulla, Dazna Rafiabad (2,215 m) 34.366N & 74.466E, growing in caespitose clusters on humicolous soil around the scattered needles of *Pinus*, in coniferous forest, Naseema Aqbar Wani, PUN 9289, 08 August 2014.

Edibility: Unknown.

Distribution and Ecology: *Gymnopilus crocias* was found growing on dead wood in the month of February from Thwaites by Pegler (1986). The presently examined collection was collected from Jammu & Kashmir, growing in caespitose clusters on humicolous soil around the scattered needles of *Pinus* in the month of August at an altitude of 2,215 m.

Remarks: The details of the presently examined collections agree well with the description of *Gymnopilus crocias* (Berk. and Broome) Singer given by Pegler (1986). *Gymnopiluscrocias* is easily recognized by convex to applanate cap with in-rolled margin, veil reduced to a cortinoid zone in young basidiomata, spore size similar, shape of cheilocystidia and pleurocystidia similar and the gill edges are hetromorphous with crowded cheilocystidia. Further, the clamp connections are present on both pileus and stipe cuticle. From India, this species has been found reported from Kerala by Mohanan (2011). Presently, it has been recorded for the first time from northern India.

CONCLUSION

Amongst the six keyed out species of the genus *Gymnopilus* documented in this manuscript *G. decipiens*and *G. aeruginosus* are the first time reports from India while as *G. fuscosquamulosus, G. crocias, G. junonius*, and *G. liquiritiae* are reported for the first time from northern India. Based on the results obtained from this study, it is clear that there are still a lot of macrofungal species that have not been explored yet. So it is advisable to do more investigations on the other locations of the Kashmir valley in order to complete the list of the macrofungi from the area.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21102–21116

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7837.14.5.21102-21116

#7837 | Received 19 January 2022 | Final received 28 April 2022 | Finally accepted 05 May 2022

OPEN ACCESS

REVIEW

Environmental DNA as a tool for biodiversity monitoring in aquatic ecosystems – a review

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Abstract: The monitoring of changes in aquatic ecosystems due to anthropogenic activities is of utmost importance to ensure the health of aquatic biodiversity. Eutrophication in water bodies due to anthropogenic disturbances serves as one of the major sources of nutrient efflux and consequently changes the biological productivity and community structure of these ecosystems. Habitat destruction and overexploitation of natural resources are other sources that impact the equilibrium of aquatic systems. Environmental DNA (eDNA) is a tool that can help to assess and monitor aquatic biodiversity. There has been a considerable outpour of research in this area in the recent past, particularly concerning conservation and biodiversity management. This review focuses on the application of eDNA for the detection and relative quantification of threatened, endangered, invasive and elusive species. We give a special emphasis on how this technique developed in the past few years to become a tool for understanding the impact of spatial-temporal changes on ecosystems. Incorporating eDNA based biomonitoring with advances in sequencing technologies and computational abilities had an immense role in the development of different avenues of application of this tool.

Keywords: eDNA, non-invasive, biomonitoring, endangered, eutrophication, anthropogenic

Tamil: நீர்சூழியலமைப்புகளில் மானுட செயற்பாடுகளால் ஏற்படும் மாற்றங்களைக் கண்காணித்தல், நீர்வாழ் பல்லுயிர் இனங்களின் நல்வாழ்வை உறுதி செய்தல் மிகமுக்கியம். மானுட இடையூறுகளால் நீர்நிலைகள் தூர்ந்துபோதல், நீர்சூழல்களில் ஊட்டச்சத்து வெளியேற்றத்திற்கு முக்கிய காரணியாக செயல் படுவதுடன், அதன் விளைவாக உயிரியல் உற்பத்தித்திறன் மற்றும் பல்லுயிர் சமூகக்கட்டமைப்பை மாற்றுகிறது. வாழ்விட அழிப்பு மற்றும் இயற்கை வளங்களின் அளவு கடந்த சுரண்டல் ஆகியன நீர்மண்டலங்களின் சமநிலையை எதிர்மறையாக தாக்கும் மூலங்கள் ஆகும். சூழல் மரபணு என்பது, நீர்வாழ் பல்லுயிர்களை மதிப்பிடவும்,கண்காணிக்கவும் உதவும் ஒரு முறை. இந்த ஆய்வு களத்தில், குறிப்பாக பல்லுயிர் பாதுகாப்பு மற்றும் மேலாண்மை குறித்த பகுதிகளில், அண்மை காலங்களில் கணிசமான அளவில் ஆய்வுகள் நடந்து வருகின்றன. இந்த மதிப்பாய்வுரை, அழிவின் விளிம்பில் இருக்கும் அச்சுறுத்தப்பட்ட மற்றும் அருகிவரும் உயிரினங்கள், கண்டறிய கடினமான மற்றும் ஆக்கிரமிப்பு உயிரினங்கள் ஆகியவற்றை, கண்டறிதல் மற்றும் ஒப்பிட்டு அளவிடுதலில் சூழல் மரபணுவின் பயன்பாடுகள் குறித்து கவனம் செலுத்துகிறது. சுற்றுச்சூழல் மண்டலங்களில் பரப்பு-காலம் சார்ந்த மாற்றங்களின் தாக்கங்களைப் புரிந்து கொள்ள ஒரு வழிமுறையாக மாற, கடந்த சில ஆண்டுகளில் இந்த நுட்பம் எவ்வாறு மேம்பட்டுள்ளது என்பதற்கு நாங்கள் ஒரு சிறப்பு முக்கியத்துவம் கொடுத்திருக்கிறோம். மரபணு வரன்முறையிடல் நுட்பங்கள் மற்றும் கணக்கீட்டுத்திறன்களில் ஏற்பட்டுள்ள முன்னேற்றங்களை சூழல் மரபணு சார்ந்த பல்லுயிர் கண்காணித்தலுடன் ஒருங்கிணைத்தது, இவ்வழிமுறையின் பன்முகப் பயன்பாடுகளை மேம்படுத்துவதில் மாபெரும் பங்காற்றியுள்ளது.

Editor: Anonymity requested.

Date of publication: 26 May 2022 (online & print)

Citation: Ray, M. & G. Umapathy (2022). Environmental DNA as a tool for biodiversity monitoring in aquatic ecosystems – a review. *Journal of Threatened Taxa* 14(5): 21102–21116. https://doi.org/10.11609/jott.7837.14.5.21102-21116

Funding: Department of Biotechnology (DBT) grant no. BT/PR29032/FCB/125/4/2018 and Council of Scientific & Industrial Research (CSIR), Govt. of India.

Competing interests: The authors declare no competing interests.

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Acknowledgements: We are thankful to S. Manu and Gopi Krishnan for their constant suggestions to improve the manuscript.

INTRODUCTION

Earth is an abode of numerous living organisms which exist in varying environmental conditions and all are ultimately interconnected. Major unknowns in estimating global biodiversity are: how many species inhabit Earth, and what is their rate of extinction. Only a fraction of total biodiversity is known, and a substantial number of species that have not yet been accounted for and are vanishing without our knowledge. Since all species are dependent on each other in some way or another, the removal of one drastically affects other species. Unravelling each point in this network of life is important to study how an ecosystem at large functions and also to understand the life history of a species and how new communities get established.

Aquatic ecosystems comprising freshwater, brackish, and marine water in nature are the sources of a lot of species diversity ranging from microbes to mammals. The impact of human activities on these life forms is multifactorial. An increase in the emission of carbon from anthropogenic actions is leading to an increase in water temperature, acidification and oxygen deprivation of aquatic systems (Jiao et al. 2015). The changes in the abiotic parameters of the ecosystem is accompanied with impacting the cycling and efflux of nutrients. These changes in turn regulates the geographic distribution of the life forms in that habitat (Nazari-Sharabian et al. 2018). According to the special report of IPCC (The Intergovernmental Panel on Climate Change) on changing ocean and cryosphere 2019, by the year 2100, the ocean will witness an increase in temperature by 2 to 4 times and oxygen levels will decline further resulting in increase in the volume of oxygen-deficient zones (OMZ). These changes will impact ecosystem services with a projected decrease in fish catch potential and global marine biomass, which will further impact revenue generation, food security and threaten livelihood. Analysing the world's biodiversity becomes a critical aspect of learning about the distribution of these "biodiversity hotspots" and applying conservation practices to protect these areas.

The traditional practices of estimating biodiversity are biased towards the sampling of particular species (Gunzburger 2007) or can also pose a risk to sensitive organisms. In recent times, molecular techniques are gaining importance in the estimation of biodiversity and its conservation in the world. One such molecular tool is the study of environmental DNA (eDNA), which has tremendous potential to develop our understanding of biodiversity science and provide implications for conservation practices with census data of species present at a comprehensive scale in real-time.

What is environmental DNA?

The term 'environmental DNA' (eDNA) was introduced in the field of microbiology for the detection of microbial communities in sediments by Ogram et al. (1987). eDNA has been classified based on particulate size: aggregates of eDNA greater than 0.2 µm were termed as particulate DNA (P-DNA) while eDNA less than 0.2 µm is termed as dissolved DNA (D-DNA) by (Paul et al. 1987). DNA extracted non-invasively from environmental sources like soil, air, or water is termed environmental DNA (eDNA). It has a polydisperse nature, i.e., the origin of eDNA can have several sources like sloughed cells, faecal matter, spores, slimy coating (in amphibians), or dead carcasses. Based on the source of origin of eDNA, it undergoes selective decay and thus complicates the evaluation of decay rates (Wilcox et al. 2015). eDNA has been used in the aquatic system to either detect the presence or absence of a species or for quantitative estimation of a particular species. Its application varies between lotic and lentic ecosystems as their nature varies. The lotic ecosystem is flowing and can transport eDNA directionally downstream from the correct location of the target organism, whereas the lentic ecosystem is stagnant. eDNA is released into the environment and subsequently undergoes progressive decay due to many biotic and abiotic factors.

Factors governing the concentration of eDNA in the aquatic environment:

Based on the literature review, it has been perceived that there can be numerous factors that can govern the concentration of eDNA at a particular time and space, but can be primarily divided into three categories:

1) eDNA released by the organism
2) Persistence of eDNA in different environmental conditions
3) Capture protocols for eDNA and sensitivity of detection assay

eDNA release by the organism

The concentration of eDNA released by an organism and the degradation rate of DNA in a particular environment are the two attributes on which the concentration of eDNA varies on a given spatial-temporal scale. The release of eDNA is a complex interaction between environmental conditions, the natural history of an organism, its metabolic rate, and the developmental stage. With an increase in the temperature of the water,

Table 1. Few key studies on the applications of eDNA as a tool.

	Study details	References
A)	**Detection of species:**	
1)	Detection of alien invasive species *Procambarus clarkii* (crayfish) in water from the natural pond and artificial aquarium	(Geerts et al. 2018)
2)	Detection of a threatened species *Glyptemys insculpta* (wood turtle) using qPCR by designing species-specific primers and Taq man probe	(Lacoursière-Roussel et al. 2016c)
3)	Detection of endangered Shasta crayfish (*Pacifastacus fortis*) and invasive crayfish (*Pacifastacus leniusculus*) in river water	(Cowart et al. 2018)
4)	Comparing the sensitivity of detection of alien invasive species- American bullfrog (*Lithobates catesbeianus*)	(Dejean et al. 2012)
5)	Detection of invasive species, African jewelfish (*Hemichromis letourneuxi*) and determine the lower limit of detection and effect of fish density and time on detection in an artificial aquarium	(Díaz-Ferguson et al. 2014)
6)	Detection of invasive species, New Zealand mud snails (*Potamopyrgus antipodarum)* and to find the time till which eDNA remains detectable in the aquatic system	(Pilliod et al. 2013a)
7)	Detection of invasive submerged aquatic plant, *Egeria densa* in pond water	(Fujiwara et al. 2016)
8)	Differentiating between endemic species, Japanese giant salamander (*Andrias japonicum*) and exotic species, Chinese giant salamander (*Andrias davidianus*) using eDNA	(Fukumoto et al. 2015)
9)	eDNA detection rate has a positive relationship with flow volume in waterways and has a more pronounced effect on eDNA detection probability than other co-variates like temperature, dissolved oxygen concentration, pH	(Song et al. 2017)
10)	Detection of transient pelagic marine fish, Chilean devil ray (*Mobula tarapacana*)	(Gargan et al. 2017)
B)	**Estimation of biomass/abundance:**	
1)	Effect of water temperature and eDNA capture method on altering the relationship between eDNA concentration and fish biomass of economically important salmonid, Brook Charr (*Salvelinus fontinalis*)	(Lacoursière-Roussel et al. 2016b)
2)	Killer whale (*Orcinus orca*) eDNA quantification using ddPCR from seawater	(Baker et al. 2018)
3)	Estimation of transport distance of eDNA of brown trout (*Salmo trutta, L.*) using a dual-labelled probe for relative quantification	(Deutschmann et al. 2019)
4)	Comparison of detection probability, density, biomass and occupancy with traditional methods of sampling of Rocky Mountain tailed frog (*Ascaphus montanus*) and Idaho giant salamander (*Dicamptodon aterrimus*)	(Pilliod et al. 2013b)
5)	Salmon DNA was measured from water samples during the spawning season using species-specific quantitative PCR probes and factors affecting the correlation between eDNA concentration and biomass of these fishes were also studied.	(Tillotson et al. 2018)
C)	**Studying the communities in the ecosystem**	
1)	The direct impact of an anthropogenic activity like an oil spill on the coastal marine ecosystem was observed. The succession of communities after the event was monitored which included bacteria, metazoans and protists. Certain communities were found to be resistant to the effect of this incidence whereas few others were conferred with the sensitivity to this.	(Xie et al. 2018)
2)	The community-level response in cyanobacteria, diatoms and microbial eukaryotes were correlated to physicochemical parameters of Lake Constance like rising phosphorus and air temperature. Major environmental perturbations like eutrophication during the 20th century were found to align with the reversion of resilience demonstrated by the communities.	(Elberri et al. 2020)
3)	The change in community structure of bacterial, protistan, and metazoan communities in response to pollution status of the river using eDNA metabarcoding. The varying level of nutrients in the ecosystem was shown to be the main driving factor in the relative abundance of OTUs and community structure.	(Li et al. 2018)
4)	The spatial distribution of bacterial communities was studied using metabarcoding. The change in the richness of these communities and the abundance was shown to be a measure of the degree of anthropogenic contamination and can be an area to focus on for biomonitoring of coastal ecosystems.	(Garlapati et al. 2021)
5)	The study focuses on identifying the association between the fish assemblages in the ecosystem and invasive species and how these get affected by environmental co-variates and human-induced disturbance.	(Pukk et al. 2021)

the mobility of fish has been reported to increase (Petty et al. 2012) hence the metabolic rate also increases (Xu et al. 2010) until a physiological limit of tolerance is attained. The timing of sample collection plays a vital role because it can help in capturing the presence of the migratory species based on its natural history or seasonal variability in levels of resident species (Lesley et al. 2016). It has been found that with different developmental stages, eDNA released also varied. eDNA release rate per fish body weight is slightly more in the juvenile group when compared to that of an adult group due to factors related to ontogeny. But, the rate of eDNA release per individual is more from adult fish than juveniles because of the larger body size of adult fish (Maruyama et al. 2014). Hence, it is difficult to infer if the source of eDNA is from a higher number of juveniles or a lesser number of adults.

Persistence of eDNA in different environmental conditions

DNA has limited chemical stability (Lindahl 1993) and once it is shed into the environment, it can either persist in free form or get adsorbed to organic or inorganic matter or else get sedimented or degraded (Dejean et al. 2011). The persistence of eDNA depends on factors which are divided into three categories - abiotic (temperature, salinity, pH, oxygen, & light), biotic (extracellular enzyme & microbial community), and DNA characteristics (length, conformation, & membrane-bound) reviewed by Barnes et al. (2014).

Capture protocols for eDNA and sensitivity of the assay

Most efficient capture protocols are a combination of a selection of the most appropriate filter materials which allows filtering the maximum amount of water using powerful automatic motors along with optimized isolation protocols and preservation techniques to maximize the yield of eDNA. The pore size of the filter is also an important feature that decides which source of DNA shall be enriched- gametes, sloughed cells free DNA, etc, and also the target group of organisms. If microorganisms are the target, then very low pore size filters will capture most of them. Renshaw et al. 2015 found that there was no significant difference in copy number in the case of 0.8 µm cellulose nitrate (CN) filter or 0.8 µm polyether sulphone (PES) filters. In contrast to this, (Hinlo et al. 2017) and (Liang & Keeley 2013) found a CN filter to have a significant difference in DNA yield. This difference could be due to a different combination of isolation and preservation protocol.

Precipitation and filtration are the two methods that have been used to extract eDNA from water samples. Precipitation is generally used for smaller volumes by using salt and ethanol to precipitate extracellular DNA by using centrifugal forces (Maniatis et al. 1982). Filtration is more size-dependent and is based on the property of filter material to keep eDNA. Filtration had shown more yield of eDNA in combination with isolation protocols for DNA (Deiner et al. 2015). DNA isolation: three protocols generally have been used to extract DNA from filters, namely the phenol chloroform Isoamyl alcohol method (PCI), Qiagen's DNeasy® blood and tissue kit, and MoBio'sPowerwater® DNA isolation kit. PCI method has been shown to yield more targeted DNA compared to Qiagen's DNeasy® blood and tissue kit using a 0.45 µm CN filter. While MoBio'sPowerWater® DNA isolation kit has shown more yield than the PCI method using a 1.5 µm glass membrane filter (GMF) (Renshaw et al. 2015). However, filtration along with Qiagen's DNeasy® blood and tissue kit has shown a higher diversity of eukaryotes being detected compared to that of limited species being detected in the case of the PCI method with filtration (Deiner et al. 2015). We believe that skipping the use of lysis buffer during isolation of eDNA from filter membranes will help in reduction of the microbial eDNA part as it will limit the lysis of microbial cell. This method will help in studying the non-microbial or eukaryotic taxa. The flow rate through filters had also been seen as a crucial step, as eDNA might start the process of degradation if the filtration time is too much. Hence, filters with higher flow rates have been preferred (Hinlo et al. 2017).

Preservation of DNA and storage is also a very crucial step in the case of detection of very low abundant species or quantification of the abundance of any species, as even a slight degradation in copy numbers might give faulty results. Freezing of filters at a very low temperature cannot always be workable in field conditions hence 95% ethanol (Minamoto et al. 2015), Longmire buffer (Renshaw et al. 2015; Williams et al. 2016), and CTAB (Renshaw et al. 2015) has been shown as alternatives. It was found that both the Longmire buffer and CTAB preserved filtered eDNA for over two weeks at 20˚C but at 45˚C Longmire, buffer outperformed CTAB buffer (Renshaw et al. 2015). Enhanced CTAB buffer has shown to have better inhibitor removal activity while Longmire buffer has the property to preserve eDNA for a longer time (Hunter et al. 2019). It is recommended to choose the best preservation buffer according to one's requirement by conducting a pilot experiment.

PCR inhibitors can be responsible for incorrect estimation of abundance or failure in the detection of very low copy number species. These inhibitors can either be co-extracted along with the extraction of eDNA or during isolation protocols. These inhibitors, like phenol and proteinase K, are removed by adding BSA to the PCR master mix (Deiner et al. 2015). These inhibitors might also be removed using inhibitor removal columns available in some commercial kits (McKee et al. 2015).

The specificity of primer and sensitivity of PCR is crucial. Nested PCR has been shown to improve detection compared with conventional PCR (Jackson et al. 2017). Detection rates of eDNA are greater with digital droplet PCR (ddPCR) than real-time PCR (qPCR) at lower concentrations (Doi et al. 2015). Quantitative estimation of biomass was shown to be more accurate by using ddPCR than qPCR. ddPCR was suitable for measurement of the natural sample as inhibitory substances have little effect on DNA quantification, as endpoint PCR amplification in each droplet can be

detected independent of amplification efficiency in ddPCR (Doi et al. 2015). There have been reports that base pair mismatches in the primer have more impact than that of the probe and the location of the mismatch also plays an important role. Base pair mismatch near the 3' end has shown a larger impact on specificity than in the 5' end or any other region (Wilcox et al. 2013).

Applications of eDNA as a tool in conservation and biodiversity monitoring

From deciphering single species to documenting entire communities, our understanding of eDNA study has progressed over the years. There is a multitude of applications of eDNA ranging from detection of invasive species, elusive species or any other ecologically important or threatened species to unravelling community dynamics and their response to changing spatial-temporal changes. This has paved new avenues in ecosystem management. In the case of microbes, less than two per cent of the total are culturable (Wade 2002). This necessitates the implementation of culture-independent methods for understanding their genomic and functional aspects . The eDNA technique has found a host of new applications over several years in the field of ecosystem monitoring and management.

1) Detection of species

Its advent revolved around the uncovering of single species like the detection of invasive species, Crayfish *Procambarus clarkia* (Geerts et al. 2018), endangered or vulnerable species, Wood Turtle *Glyptemys insculpta* (Lacoursière-Roussel et al. 2016c), or some elusive species, Oriental Weather Loach *Misgurnus anguillicaudatus* (Hinlo et al. 2017). A brief methodology for the detection of species from environmental aquatic samples using the eDNA method has been depicted in Image 1. eDNA technology along with occupancy modelling has been utilised for monitoring the presence of endangered species of Northern Tidewater Goby species *Eucyclogobius newberryi* and Southern Tidewater Goby species *Eucyclogobius kristinae* across the entire coast of 1,350 km (Sutter & Kinziger 2019). They found that eDNA technology showed double the rate of detection compared to the seining method, which resulted in improved site occupancy estimates as Northern Tidewater Goby was detected at two sites where their presence was never known before. A positive correlation was observed between eDNA concentration and catch per unit effort (CPUE). The implication of such objectives paves the path towards improved conservation goals. A list of key studies, along with the primers used in the detection and monitoring of different species, is summarised in Table 2.

2) Population genetics studies

Population genetics has been a significant aspect in the study of ecology as it gives information about evolutionary history. But, research in this sector with the use of eDNA has just begun and is in its initial stage. Sampling in the case of population genetics has been a major challenge, especially in threatened organisms. eDNA approach helps to mitigate such challenges and helps in the study of organisms that are difficult to sample. Researchers have used eDNA that was extracted from sea water to examine the haplotype frequencies and genetic diversity at population level in Whale Shark *Rhincodon typus* (Sigsgaard et al. 2017). They used high throughput sequencing of two mitochondrial control region sequences and compared it with tissue samples from 61 individuals at the same locality from when samples for eDNA were collected. It was found that relative frequencies in both were similar. The more current study of elusive Harbour Porpoise *Phocoena phocoena* used high throughput sequencing for studying haplotype diversity and found eight unique mitochondrial DNA sequences from seawater sampling (Parsons et al. 2018). In another study, species and ecotypes of Killer Whales (*Orcinus orca* were identified following encounters using digital droplet PCR and subsequently were sequenced. It was identified that the killer whale encounter was from a southern resident community (Baker et al. 2018). In a more recent study by Stepien et al. (2019), Silver Carp *Hypophthalmichthys molitrix* which is an invasive species in the U.S was studied for its introduction and spread using eDNA and mitochondrial markers targeting cytochrome b and c oxidase and nuclear DNA microsatellite markers.

3) Estimation of relative abundance

The scope of eDNA is more than just detecting the presence/absence of an organism. Estimation of copy number or biomass has been the major focus and extrapolation of avenues in which an eDNA study can be helpful. The information about an organism's relative abundance in the spatial-temporal scale helps to document the seasonal variations due to its response to the environment or due to other external forces like inter or intra-species competition. Estimation of abundance can have economic value in aquaculture if yield in a particular season can be known beforehand by studying the history of a few years about its seasonal variations. Even though numerous factors play a role in the persistence of eDNA in the environment along with its polydisperse nature, as discussed in the earlier section, if all protocols related to filtrations, isolation,

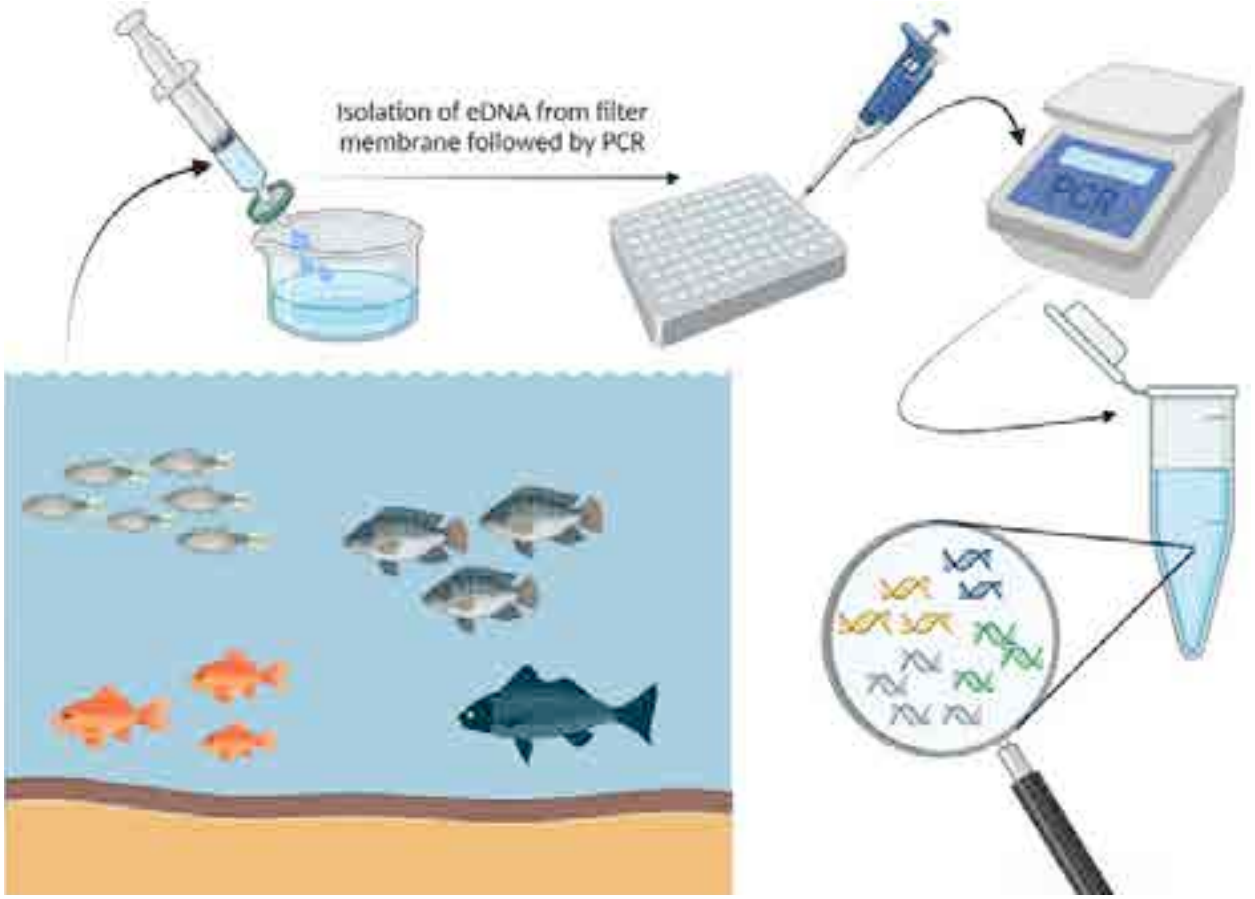

Image 1. Illustrative representation of methodology of the detection of the different species residing in an aquatic ecosystem. The collected environmental sample (water) can be syringe-filtered using membrane filters of desired pore size and material type, followed by amplification using PCR to detect the target species. In the case of metabarcoding based approaches, the entire biodiversity of the ecosystem can be deciphered. A repetitive sampling at the same site can give an idea about the quantitative change in biodiversity due to seasonal variations, anthropogenic disturbances, or some other abiotic attribute. As the abundance of a particular species varies, the same will be reflected in the copy number of eDNA which can give an understanding of how population dynamics are controlled by multiple factors. (Illustration created with BioRender.com)

and preservation are followed the same way for all samples across all seasons, then it can give an insight of its relative abundance. eDNA concentration of Lake Trout *Salvelinus namaycush* was estimated in 12 natural lakes and its abundance was compared to that of standardized gill net catches (catch per unit effort -CPUE and Biomass per unit effort- BPUE (Lacoursière-Roussel et al. 2016a) . Another study showed that the eDNA released from the target organism is a measure of its biomass for which laboratory and field-based experiments were conducted on Common Carp. This highlighted that the concentration of eDNA positively correlated to its biomass and can serve to understand its distribution in natural systems (Takahara et al. 2013).

An endangered amphidromous fish, Ryukyu ayu *Plecoglossus altivelis ryukyuensis*, was monitored to estimate abundance using qPCR with specific primers to amplify the mtDNA ND4 region. The visual snorkelling surveys by individually fish counting positively correlated to eDNA copies/ml (Akamatsu et al. 2020). In another recent study by (Capo et al. 2019), digital droplet PCR was used to detect as well as quantify Brown Trout *Salmo trutta* and Arctic Char *Salvelinus alpinus* populations. While they compared between fish population estimated by conventional Catch per unit effort (CPUE) from gill netting method and eDNA concentration from digital droplet PCR, no significant correlation could be deduced, yet this paves a promising path for future research in this aspect by focussing on challenges and limitations which need to be overcome. This study also focuses on probable problems of stand-alone methods and how a congregation of various approaches, together with optimised protocols, can yield the desired result. In another method of individual

Table 2. Key studies for detection of important species in aquatic ecosystem.

	Aim of Study	Primer sequence used in the study					Reference
1.	Detection of invasive rusty crayfish *(Orconectes rusticus)* in inland lakes using specific qPCR primers targeting the cytochrome c oxidase subunit 1 (COI) sequences	Forward primer	Reverse primer			Amplicon length (in bp)	(Dougherty et al. 2016)
		5′-CAGGGGCGTCAGTAGATTTAGGTAT-3′	5′-CATTCGATCTATAGTCATTCCCGTAG-3′			128	
2.	Detection of invasive common Atlantic slipper limpet *(Crepidula fornicate)* from environmental seawater sample using species-specific primers targeting COI gene	Forward primers	Reverse primers			Amplicon length (in bp)	(Miralles et al. 2019)
		5′-GATGATCAACTATACAATGTA-3′	5′- TAAACCGTTCAACCGG-3′			239	
3.	Detection of invasive signal crayfish (*Pacifastacus Leniusculus)* in river and lake water samples using Taqman probe and species-specific primers targeting the COI gene	Forward primer	Reverse primer	Probe		Amplicon length (in bp)	(Harper et al. 2018)
		5′-ATAGTTGAA AGAGGAGTGGGTACT-3′	(5′-TAA ATCAACAGAAGCCCCTGCA-3′)	FAM-5′-CCTC CTCTAGCAGCGGCTATTGCTCATGC-3′-BHQ1		87	
4.	Studying the distribution of silver carp *(Hypophthalmichthys molitrix)* and developing of novel methodology for on-site detection of the species	Forward primer	Reverse primer	Probe			(Doi et al. 2021)
		5′-GCAATTAACTTCATCACCACAACTATTA-3′	5′-TCCAGCAGCTAAAACTGGTAAGG-3′	5′-[FAM]-AAACACCTCTCTTTGTTTGAGCTGTGC-[TAMRA]-3′			
5.	Detection and quantification of European weather loach *(Misgurnus fossilis)* using digital droplet PCR targeting the COI gene. This species is cryptic and is facing population decline in recent times.	Forward primer	Reverse primer	Probe		Amplicon length (in bp)	(Brys et al. 2021)
		5′-CCCCCGACATAGCATTTCCG-3′	5′-AACTGTTCAGCCTGTCCCAG-3′	5′- (6-FAM) CTCGTTCCTCCTTCTGCTGG(ZEN/IBFQ)-3′		119	
6.	Detection of endangered freshwater or Spectacle case Mussel *(Margaritifera monodonta)* using species-specific qPCR primers.	Forward primer	Reverse primer	Probe			(Lor et al. 2020)
		5′-AGTGGGTGATACCWGTATCT-3′	5′-TACCCCTAGCACCATTTGAT-3′	5′-5HEX/TCTAGCCCT/ZEN/AAGACTATGACAACTTTTCC/3IABkFQ-3′			
7.	Monitoring of river systems for detection of invasive Eastern mosquitofish *(Gambusia holbrooki)* and the consequent decline of two endemic species of killifish (*Valencia letourneuxi* and *Valencia robertae)* using species-specific qPCR targeting the COI region.	Species	Forward primer (5′–3′)	Reverse primer (5′–3′)	Probe (5′–3′)	Amplicon length (in bp)	(Mauvisseau et al. 2020)
		Valencia letourneuxi	TGGGGGTTTTGGCAACTGAC	GGAGGAGAAGAAACGAGGGGGG	CATAGCCTTCCCTCGGATAAAC	113	
		Valencia robertae	ATGGCCTTCCCCCGAATGAA	GCTAAGTTTCCGGCCAGAGG	CTTCCTCTGGCGTCGAGGC	137	
		Gambusia holbrooki	GTGCCCCAGACATAGCCTTT	TACAGAAGGTCCGGCATGTG	AAGATGCGAGGAGGAGGAGA	167	
8.	Detection of endangered Hay's Spring Amphipod *(Stygobromus hayi)* and a co-occurring species of *S. tenuis potomacus* in groundwater using species-specific qPCR targeting the COI region.	Species	Forward primer (5′-3′)	Reverse primer (5′-3′)	Probe (5′-3′)		(Niemiller et al. 2018)
		Stygobromus hayi	GCATCTGTCGACTTAGCTATT	CGGCACTTGGTCTATAGTTATT	6-FAM-TCACTTCATTTAGCAGGAGCCTCCTC-TAMRA		
		S. tenuis potomacus	CTGAACAGTATATCCACCACT	CATTCCAGGTCTCCGTATATT A	-6-FAM-TGCAGTAGCCCATAGTGGAGCATCT-TAMRA		

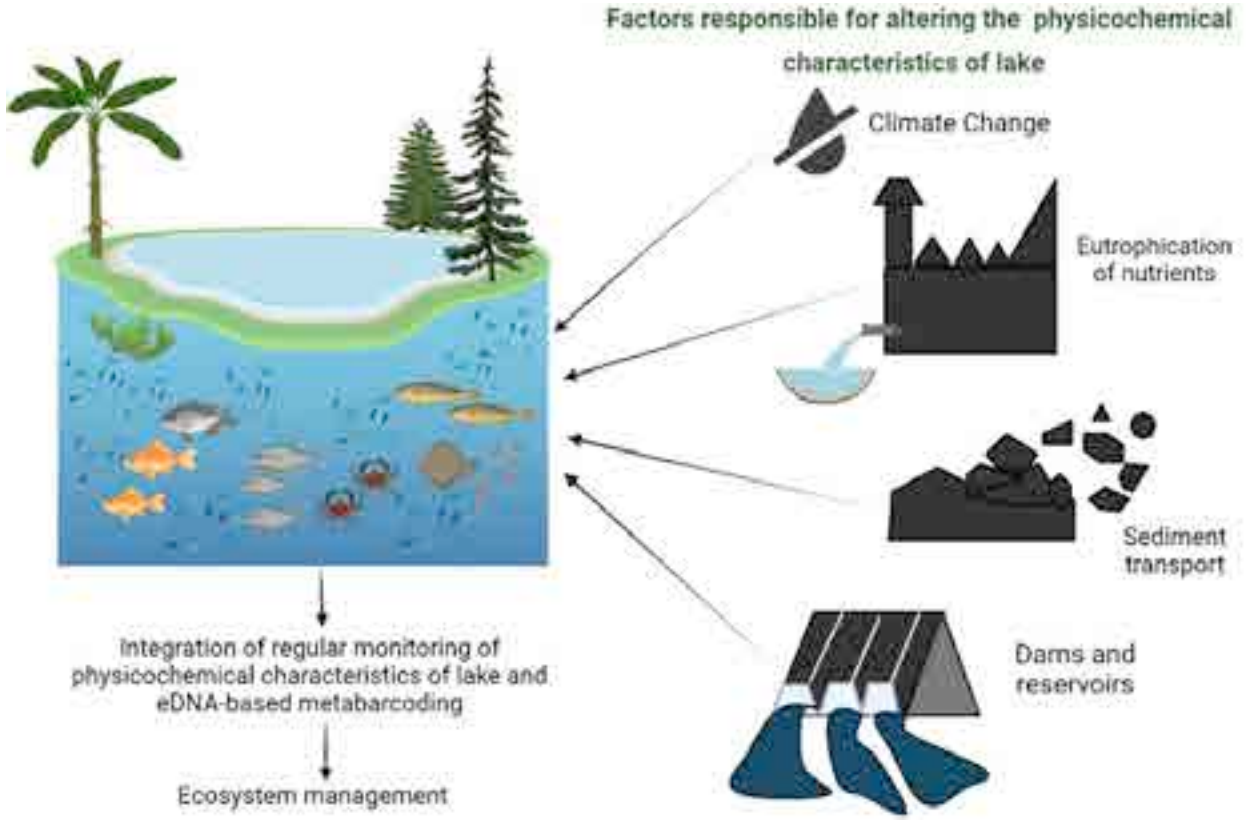

Image 2. The change in physicochemical parameters of the waterbody can be due to either seasonal variations or due to anthropogenic disturbances like the building of dams and reservoirs, sediment transport due to erosion of topsoil or eutrophication of nutrients from industrial and agricultural outflow which lead to subsequent climate change. These changes in physicochemical factors can be monitored along with the changes in species found in the ecosystem using eDNA metabarcoding. Continuous monitoring over all the seasons at a spatial scale might offer a link among these changes in biotic and abiotic components of an ecosystem and hence help in future restoration processes. (Illustration created with BioRender.com)

estimation in a population, a novel NGS based strategy was used which counted haplotypes in the mitochondrial D-loop sequence of eel. This method was named HaCeD-Seq and it was claimed to be better and more accurate in quantification than conventional qPCR. However, its accuracy decreased when the number of individuals increased because of lesser unique haplotypes and more overlap of sequence among individuals (Yoshitake et al. 2019). A much deeper understanding of factors affecting the abundance of eDNA copies in natural environments can help to boost this technology and can be of extreme importance, especially in fisheries management and has direct implications on increasing its economic value.

Though there are substantial volumes of research in this field of eDNA our understanding is still limited. There have been enormous volumes of reports concerning the release and persistence of eDNA in various environments, but there has been no noticeable research on the effect of stressed environments like human activities or predation pressure on the release rate of eDNA and how it brings changes in our overall understanding of species abundance.

4) Studying the communities in the ecosystem:

Holistic study of ecosystem and metabarcoding gives more inferential insights and hence an upheaval in the use of eDNA has led to transitioning from DNA barcoding to metabarcoding, hence from studying single species to communities and their interactions. This in turn has enabled extracting more information and data using less time and manpower under field conditions.

Understanding ecosystem health in aquatic bodies

In the last few years, a new paradigm has got an increasing focus that aids in the understanding of the health of ecosystems using metabarcoding. This can be accomplished by establishing the link between changing abiotic factors and the ecology of the ecosystem to that of changing biotic interactions among communities inferred from metabarcoding data. Eutrophication is a process of enrichment of nutrients like nitrogen and

phosphorus (Conley et al. 2009) in water bodies. Although natural eutrophication occurs at a very slow pace due to the ageing of water bodies (Carpenter 1981), in the past century cultural eutrophication due to anthropogenic actions has led to rapid nutrient efflux into the water bodies (Smith 1998). Eutrophication is one of the major indicators of anthropogenic means of changing the physicochemical parameters of aquatic bodies along with the construction of dams, channelisation and sediment transport as depicted in Image. 2 (Bianchi & Morrison 2018). This can change the biological productivity and community structure composition of the water bodies (Sawyer 1966). There are manifold effects of eutrophication, algal blooms being the most noticeable of them. The change in Nitrogen (N): Phosphorus (P) ratio or dissolved organic carbon (DOC): dissolved organic nitrogen (DON) has been found as a variable in case of such blooms (Anderson et al. 2002). The major component of these blooms is Cyanobacteria and they produce cyanotoxins which act as neurotoxins and hepatotoxins for fishes, mammals and also humans (Oberemm et al. 1999; Carmichael 2001). Literature search shows that though there are some studies in this area using metabarcoding to find the community structure of bacteria and planktons (Wan et al. 2017; Banerji et al. 2018) in aquatic systems, we find very few studies relating how anthropogenic disturbances might affect the ecosystem services.

One such study by (Craine et al. 2017), showed the relation among the changing environmental variables like dissolved nutrient concentration with four taxonomic groups namely bacteria, phytoplankton, invertebrates, and vertebrates. Further, they found that increasing eutrophication of nutrients and river size were the crucial variables that changed the abundances of these broad taxa. Clark et al. (2020) demonstrated the impact of enrichment with fertilizers on the benthic communities in two estuaries that differed in its environmental attributes. The effect was studied using eDNA metabarcoding on bacterial (16sRNA), eukaryotic (18sRNA) and diatom only (rbcL) communities after seven months of nutrient enrichment. They found that there were clear changes in the case of bacterial and eukaryotic taxa but more obscure in the case of diatoms. Also, they found that these changes could be observed within 150 g N m-2 of fertiliser treatment, suggesting that early signs of ecosystem degradation could be studied and the restoration process could be initiated using such shifts in the structure of communities as cues. Such methods were used initially for species detection and quantification, now it has been used for ecosystem assessment and monitoring for its health. The focus on studying community structure as a measure of predicting ecosystem health has advantages as it brings about a holistic view of the same and helps acknowledge the fact how species interdependency is linked to abiotic factors as well. One such study in this regard was by Yang & Zhang (2020), where they used zooplankton community to assess the quality of the ecosystem. They showed across three seasons, i.e., dry, normal and wet, the species detected remained the same but their relative abundances changed at the temporal scale. The study also emphasised that though eDNA based abundance studies are the semi-quantitative presence of species along with changing relative abundances of indicator zooplankton species at spatial-temporal scale. The water quality index correlated with 60 different zooplankton indices which were both qualitative and quantitative. But such correlations need not always be direct/correct due to other confounding factors like interaction with other species communities, which in turn influence the zooplankton community. Such studies aren't limited to only aquatic systems but also have seen recent applications in analysing sediment pollution from coastal regions at a spatiotemporal scale. In a study by Lee et al. (2020), the changes in microbial diversity at phylum level showed variation concerning 13 environmental variables of sediment pollution and toxicity. Although certain phyla remained dominant others showed shifts in community structure.

Whole-genome or metagenome-assembled-genomes (MAGs) based studies

Most of the above-mentioned studies are based on the amplification of the universal marker regions of DNA or amplicon-based 16sRNA sequencing and can have bias during PCR (Jovel et al. 2016). They are based on the single-gene approach of identification of its taxa. One way to solve this is to bring in a multi-gene or whole genome-based taxonomic approach. This also helps in functional prediction of genes like those involved in the biogeochemical cycling by microorganisms and can be of great significance in studying ecosystem services. Another method used in recent times to study taxonomy based on phylogenetic reconstruction is by assembling the metagenomes also called metagenome-assembled genomes (MAGs) and is of immense importance in culture-independent microbial molecular studies. In a study by Tran et al. (2021), the role of specific taxa of microbes in biogeochemical processes in the lake, they had assembled 24 samples individually by de novo method and generated 24 MAGs which were then binned, then

finally used for the construction of concatenated gene phylogeny using single-copy ribosomal proteins. They found that MAGs showed an abundant genomic capacity for nitrogen and sulphur cycling. In another similar work by Reji & Francis (2020), MAGs were constructed for a lineage of Thaumarchaeota, a phylum of Archaea from the marine ecosystem. This lineage seemed to be devoid of genomic repertoire only for chemoautotrophy as it did not have ammonia-oxidising machinery and other pathways related to the same as in other archaeal lineages. This highlights the metabolic diversity among the microbial communities for nutrient acquisition and processing, which is generally not possible in the case of culture-dependent molecular studies as most of the bacteria are non-culturable.

We find studies in eDNA are becoming broader in perspective rather than only species detection, but this holds much more potential in coming years in terms of answering some basic ecological questions about the effect of anthropogenic disturbances that lead to changes in abiotic factors of an ecosystem which changes community structure composition at spatial and temporal scales and threatens ecosystem services and ecosystem health.

Also, there has been very little emphasis on understanding the functional role of eDNA studies i.e., how it can be used to compare eDNA and eRNA and decipher the active constituent of the genome which might have an important role in ecological functioning like genes responsible for biogeochemical cycling of various nutrients in nature. Since RNA has lesser stability than DNA, it is a better and more reliable measure for studying the presence of an organism or its abundance and hence has been used in forensic science to estimate the time since deposition of biological material (Bremmer et al. 2012).

Technical Challenges of eDNA-based methods

Although eDNA technology has provided a plethora of its applications and helped to understand nature in a holistic view, it still suffers from a few challenges which require more refinement and troubleshooting.

PCR Bias

The foremost problem arises in the estimation of relative abundance using a metabarcoding approach where PCR bias serves as a major issue. Those taxa having organisms that are not affected by seasonal variations and are more abundant in number having high dispersal ability tend to be over-represented during sampling than sedentary and seasonal ones. Even the copy number of target loci may vary among taxa, individuals, or tissue types. There can be several possibilities that can cause bias in PCR amplification during metabarcoding. PCR is a stochastic process hence can become a source of bias like the number of PCR cycles, mismatch in primer binding site, annealing temperature, secondary structures in template DNA, multiple templates in the sample, more selectivity of primers for some specific taxa and copy number of target loci (Pinto & Raskin 2012; Elbrecht & Leese 2015; Fonseca 2018). Nichols et al. (2018), showed that polymerase can show bias toward GC sequence and can alter the relative abundance of molecules dramatically during metabarcoding and that this bias can be removed experimentally using a molecular identifier (MID) where starting material is disambiguated bioinformatically following PCR.

Unknown source of eDNA

There have been reports of transport of undigested material of higher organisms or their dead carcasses, which gives a false implication of their presence at that particular site (Song et al. 2017).

Problems with single-species detection and bias in eDNA extraction protocols

Single species detection in the marine environment is challenging due to increased dilution, higher salinity, and more intermixing of constituents (Cristescu & Hebert 2018). Higher salt concentration can also inhibit PCR and give false implications about the absence of the target organism. Continuous sample collection either monthly or seasonal depending on the research question might serve as a way to overcome false detections. Enrichment of extracellular DNA can help in reducing the signal from non-target microbial cells as they are more abundant in natural ecosystems.

Chances of false positives and false negatives

False positives errors (Type-I) arise when there is no actual presence of the target organism, but still, it is detected at that site which can be due to contamination issues or problems in PCR optimization or sequencing (Schmidt et al. 2013). The specificity of primers also plays a vital role in minimizing picking up related species having very little sequence variance than the target species. False-negative errors (Type-II) arise when a target organism fails to get detected even though it is present there. This can be attributed to reasons like inefficient sample preservation, faulty sampling practices, or less sensitivity of detection assay in the

case of low-abundant organisms.

Measuring the absolute abundance of the species is practically not possible

Factors governing the quantification of eDNA are dependent on countless factors. Many juvenile organisms or a lesser number of adult organisms, might release an equal amount of eDNA. Hence, biomass estimation can be made but estimating abundance can be difficult with PCR-based methods (Elbrecht & Leese 2015). Change in eDNA concentration due to seasonal variation has been reported by many, which can lead to difficulty in estimation of true abundance (Barnes et al. 2014). Maintaining many replicates for PCR and DNA isolation can increase the probability of capturing many taxa by the metabarcoding approach (Leray & Knowlton 2017).

eDNA shedding and decay rates in a particular environment govern the quantification of particular species. In a study by Sassoubre et al. (2016), eDNA decay and shedding rates in seawater mesocosm were assessed for three economically and ecologically important marine fishes- *Engraulis mordax* (Northern Anchovy), *Sardinops sagax* (Pacific Sardine), and *Scomber japonicas* (Pacific Chub Mackerel) by Taqman® qPCR assay. In another similar study, Round Goby *Neogobiusme lanostomus*, an elusive species, was assessed for the shedding and decay rate of eDNA. eDNA shedding was measured after fixed time intervals, and the effect of temperature on shedding rate was also studied. First order decay constants were calculated and the decay rate was found to be slightly lower in cold water than in warm water. A most significant part of the study was that a positive correlation between eDNA concentration and the number of round gobies collected using two capture methods could be established (Nevers et al. 2018). Knowledge about these factors together with factors affecting abundance can act as a lead in abundance estimation studies. The effect of various environmental factors affecting the persistence of eDNA and indirectly the abundance has been shown by (Barnes et al.2014).

Potential solutions to the challenges:

We have developed a few reflections that might be helpful for future eDNA research:

PCR- free methods

As mentioned in the previous section, PCR introduces several kinds of biases. Hence developing a new methodology to overcome this step during the metabarcoding approaches can be of immense value in future. Following the same optimized capture and isolation protocols for all collected samples along with maintaining appropriate controls, increasing the number of replicates at each site of sample collection, seasonal collection of samples at the same points throughout the year and developing of PCR-free approach can help to give a picture of near-absolute abundance of organisms. Manu & Umapathy (2021), designed a novel metagenomic workflow which used PCR-free library preparation during Next-generation sequencing (NGS) and performed an ultra-deep sequencing and pseudo taxonomic assignment to get the biodiversity of an ecosystem across the entire tree of life.

Source of eDNA can be both from live and dead organisms: In aquatic systems, transport of eDNA has been observed for tens of kilometres (Andruszkiewicz et al. 2019), hence mere detection of eDNA at a particular time neither confirms the exact location nor the source since eDNA can persist in systems for approximately 48 hours (Collins et al. 2018). A probable way of accounting for this issue is by an increase in both the number of biological and technical replicates as well as sampling continuously for a minimum of three days at the same locations which might add more confidence to the data acquired.

Sampling criteria, filtration of samples and isolation of eDNA protocols

It should be based on the research question. The standardisations of all the protocols should consider the main hypothesis of the research. For example, if the purpose of the research question is only addressed towards deciphering prokaryotic diversity, then all the protocols should be tweaked to get enriched eDNA from that community and also to get maximum diversity of that taxa. This might help to get a better and more focused results. The enrichment of extracellular DNA should be targeted if the question needs studying the entire biodiversity of the system.

Reducing false positives and false negatives

It has been reported that increasing the number of replicates during PCR can minimize the chances of false negatives. The inclusion of positive control during PCR can help check the optimization of PCR conditions. To limit the detection of false absence, the number of replicates should be a minimum of six for a detection probability of 0.5, and for even lower detection probability, a minimum of eight replicates are needed (Ficetola et al. 2015). When both detection probability

and the number of replicates has been too low, it was found that this underestimated occupancy and overestimated the detection rate (Ficetola et al. 2015).

Only relative abundance can be quantified

Since eDNA yield depends on the developmental stage and size of an individual (Petty et al. 2012), mesocosm or aquarium-based studies can be standardised for a particular developmental stage or size of an individual of a species to get an estimate of the actual number of individuals, but mimicking natural environmental conditions of an ecosystem is very difficult and prone to errors. Also, since every ecosystem has its own abiotic and biotic features, the results might not be reproducible.

CONCLUSION

The use of eDNA and its multitude of applications has become a fast-developing area. This outpour comes in the light of the increasing need to monitor changes in our environment and how living organisms are affected by them. This helps to have better conservation focus on regions or species of special importance. In this era of unprecedented climate change and the concerns possessed by it, eDNA can help assist in the monitoring of biodiversity alongside other conventional methods to yield better results. Any new technology calls for new challenges and room for improvement, so is with eDNA where chances of contamination and bias for the detection of abundant species are higher. But with more stringent methodology and computational advancements, the risks are getting minimised. It has the potential to answer many deeper questions of research in this area.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21117–21121

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.6708.14.5.21117-21121

#6708 | Received 13 September 2020 | Final received 02 November 2021 | Finally accepted 28 April 2022

OPEN ACCESS

SHORT COMMUNICATION

New record and update on the geographic distribution of the Egyptian Tomb Bat *Taphozous perforatus* (E. Geoffroy, 1818) in Cameroon

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Abstract: The Egyptian Tomb Bat *Taphozous perforatus* is an emballonurid whose presence in Cameroon is least known. While there are previous records in the country from Waza and Yabassi, they are contested. Available publications fail to mention the presence of this species in the country, thus more information is needed to evaluate its presence. Recently, five specimens were captured near a cave entrance in Bocklé, Garoua in the Sahelian zone of northern Cameroon, adding a new locality to the distribution of the species. The discovery of this roost unequivocally confirms the presence of this species in Cameroon and indicates that the species has a wide distribution in the country as a resident species. Detailed geographical distribution of this species in Africa, along with detailed descriptions and photographs of available specimens are provided.

Keywords: Chiroptera, Emballonuridae, Garoua, Sahelian zone, Waza, Yabassi.

The chiropteran or bat fauna of Cameroon has received much attention in recent years (Bakwo Fils 2009, 2010, 2014; Bakwo Fils et al. 2012, 2014, 2018; Hassanin 2014; Lebreton et al. 2014; Atagana et al. 2018; Mongombe et al. 2019; Waghiiwimbom et al. 2019; Manfothang et al. 2020). Additionally, many voucher specimens from the country are deposited in museums around the world. Despite the increase in knowledge of bats, most publications do not provide detailed distribution of species. As a result, information on most species remains fragmentary and this hinders the development of sustainable conservation plans (Bakwo Fils 2010; Bakwo Fils et al. 2018). Most of the recent studies have yielded new records and even new species (Sedlacek 2006; Bakwo Fils 2009; Bakwo Fils et al. 2012, 2014; Hassanin 2014; Lebreton et al. 2014). Yet, information on the ecology of many species in Cameroon remains scarce or unavailable (Bakwo Fils et al. 2018).

Taphozous perforatus is an insectivorous bat of the family Emballonuridae, suborder Yangochiroptera (Teeling et al. 2005). According to African Chiroptera Report (2018), the genus *Taphozous* comprises five species, of which three occur in Cameroon; these include *Taphozous mauritianus*, *T. nudiventris,* and *T. perforatus*. The Egyptian Tomb Bat *Taphozous perforatus* is a medium-sized bat with a grey-brown dorsal and ventral pelage and a gular pouch in males (Happold 1987). It is distributed in western and eastern Africa where it has

Editor: Paul Racey, University of Exeter, UK.

Date of publication: 26 May 2022 (online & print)

Citation: Bakwo Fils, E.M., K.Z.J. Flora, M.D. Ervis, M.M. Aaron & J. Decher (2022). New record and update on the geographic distribution of the Egyptian Tomb Bat *Taphozous perforatus* (E. Geoffroy, 1818) in Cameroon. *Journal of Threatened Taxa* 14(5): 21117–21121. https://doi.org/10.11609/jott.6708.14.5.21117-21121

Funding: None.

Competing interests: The authors declare no competing interests.

Acknowledgements: We would like to thank the staff at Zoological Research Museum Alexander Koenig (ZFMK) and the Royal Museum of Central Africa (RMCA) for providing information on specimens of *T. perforatus* previously recorded from Cameroon. We are also grateful to the local guides Mr. Ngoukap Abdelaziz and Mr. Hamadou who helped us locate the cave. The research permit was granted by the Cameroon Ministry of Scientific Research and Innovation (Ref: 0000011 / MINRESI /B00 /C00/C10/C14).

been recorded from Egypt, and through Sudan to South Africa (Taylor 2013; African Chiroptera Report 2018).

The geographic distribution of this species in Cameroon is subject to contradiction according to literature. According to Taylor (2013) and the IUCN Red List (Monadjem et al. 2020), this species has not been recorded in Cameroon. However, the African Chiroptera Report (2018) mentions one specimen of *T. perforatus* captured in Yabassi, Littoral Region of Cameroon, collected by Thys Van den Audenaerde & Opdenbosch on 15 April 1970**.** The specimen was deposited at the Royal Museum for Central Africa (RMCA 1973 029-M-0094). Moreover, two of the three specimens collected by W. Böhme & W. Hartwig in Waza, northern Cameroon (Eisentraut 1975) were examined at the Museum Alexander Koenig of which, one female was captured on 10 February 1974, and one male was captured on 12 February 1974 (ZFMK MAM 1974-0331, ZFMK MAM 1974-0330).

Although the species is widely distributed throughout sub-Saharan Africa, the Arabian Peninsula, and the Indian subcontinent, its presence in Cameroon was uncertain. Here, we report evidence of a new locality to the distribution of the species in the country and provide the first verifiable record of this species in Cameroon. We also provide a morphometric comparison between the recent specimens and museum vouchers. Details on its geographical distribution in Africa, with descriptions and photographs of our specimens are provided.

Material and Methods

The specimens were captured at Bocklé, a locality near Garoua, north region of Cameroon (09.303°N & 013.575°E) during surveys to determine the species of bats occurring in the area. The climate is described as Sudano-Sahelian, with low savanna, characterized by a long dry season and short rainy season. A main rainfall peak generally occurs in October (Suchel 1988).

Mist nets (four 12 × 2.5 m - Ecotone Poland), were deployed and left open from 18.00–24.00 h at the entrance of the cave. The nets were checked every 15 minutes to reduce severe entanglement of any captured bats. Captured bats were carefully removed and placed individually in airy cloth bags and weighed using an electronic balance (500 x 0.1 g, Ohaus). Morphometric measurements (mm) were taken in the field using a dial calliper (Ecotone-Poland 150/0.1 mm) and were used for identification: head body length (HBL), tail length (TL), forearm length (FA), ear length (EL), tragus length (TrL), and tibia length (TIB). The presence or absence of a gular pouch was used to determine the species' identity (Rosevear 1965; Hayman & Hill 1971) (Image 1). The coordinates of the capture site were recorded using a handheld GPS (Garmin eTrex 10). Additionally, the presence of a hairy chin, wings attached to the tibia, hairs on the lower belly, and posterior back were also used for identification (Bates & Harrison 1997; Taylor 2013).

After identification in the field, three individuals

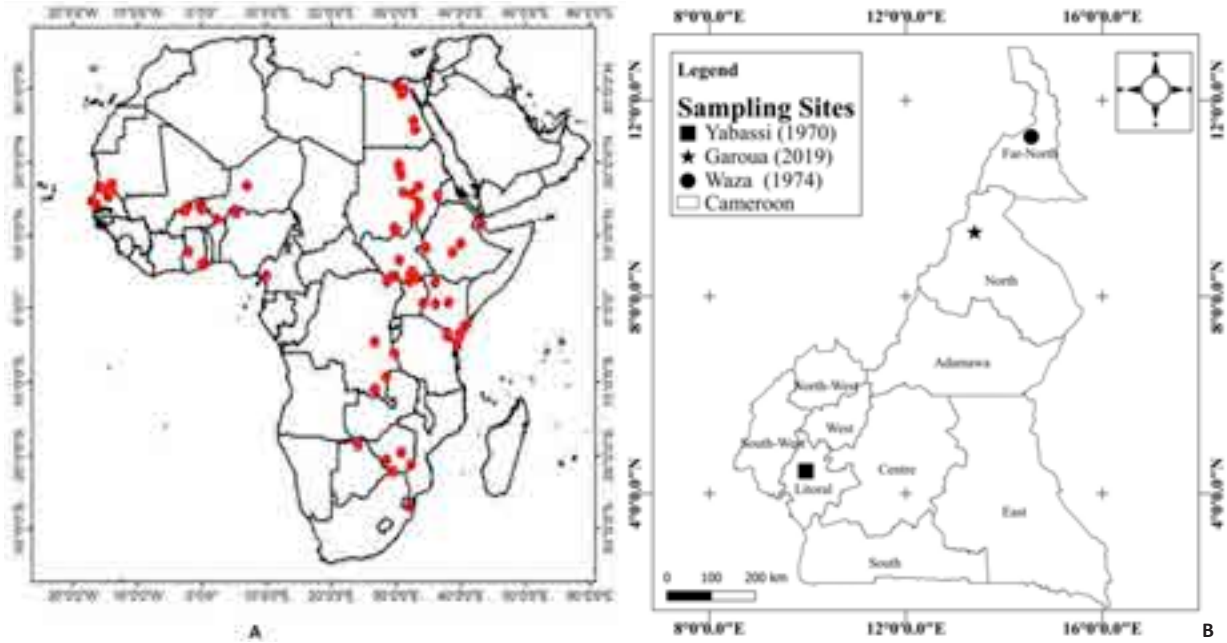

Figure 1. A—Distribution of *Taphozous perforatus* in Africa according to ACR (2018), including the records of Waza (indicated by a triangle) and the recent record in Garoua (indicated by a star) | B—Current known distribution of *Taphozous perforatus* in Cameroon.

were released and two specimens (one female and one male) were euthanized and kept in 70% alcohol for further examination of craniodental characteristics. The greatest length of the skull (GLS), condylo-canine length (CCL), condylo-basal length (CBL), mastoid breadth (MB), maxillary tooth row (C–M^3), mandibular tooth row (C–M3), mandible length (ML), interorbital constriction (IC), mandible length (ML), width across canines (C-C), and the cranio-canine length (CrnC) were measured. These specimens were deposited in the collection of the Laboratory of Zoology of the University of Maroua, Maroua, Cameroon under the voucher number GAR568 and GAR570. The measurements of our specimens were compared with those in the Chiroptera collection of the Zoological Research Museum Alexander Koenig (ZFMK), Bonn, Germany (Table 1).

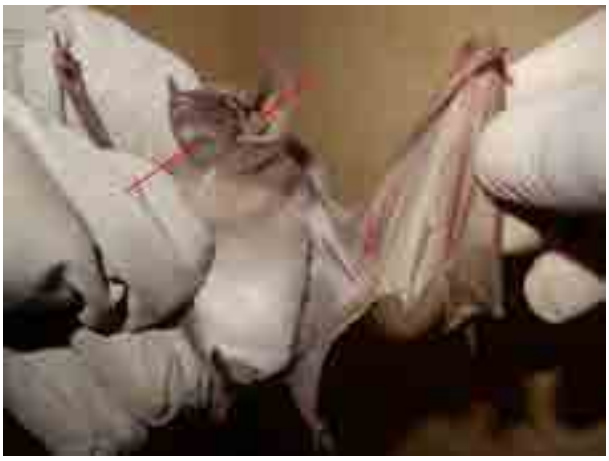

Image 1. Ventral view of the *Taphozous perforatus* collected from "Grotte de Bocklé" Sahelian zone of northern Cameroon. The upper arrow indicates the hatchet-shaped tragus; the lower arrow indicates the absence of gular pouch. © Kingha 2019.

Image 2. The side view of *Taphozous perforatus* showing the hatchet-shaped tragus and the poorly developed lobule at base of posterior margin of the ear. © Kingha 2019.

Results and Discussion

Five individuals of *Taphozous perforatus* were captured using a mist-net on 13 October 2019 at about 08.23 h at the entrance of a cave in Bocklé, near Garoua, northern Cameroon. Our specimens had a greyish-brown dorsal pelage (Image 2) and greyish-white ventral pelage (Image 1), with forearm length between (FA: 60.92–65.16 mm). The head was pointed, large pointed ears with a hatchet-shaped tragus, and a poorly developed lobule at the base of the posterior margin (Image 2).

The Tomb Bat (*Taphozous* E. Geoffroy, 1818) is different from all other emballonurids by the presence of a radio-metacarpal pouch and tragus not parallel-sided (Monadjem et al. 2010). *Taphozous* bats can also be distinguished from other emballonurids by the profile of the forehead being strongly concave; FA: 56–79 mm, and the upper incisors being minute and often absent (Rosevear 1965; Taylor 2013). Within Africa, the genus *Taphozous* comprises five species, of which three species occur in Cameroon (African Chiroptera Report 2018) namely the Mauritian Tomb Bat *Taphozous mauritianus* E. Geoffroy, 1818; the Naked-rumped Tomb Bat *Taphozous nudiventris* Cretzschmar, 1830, and the Egyptian Tomb Bat *Taphozous perforatus* E. Geoffroy, 1818. The Egyptian Tomb Bat *Taphozous perforatus* occurs throughout western and eastern Africa. It is recorded continuously from Egypt, Sudan to South Africa (Taylor 2013; African Chiroptera Report 2018). The geographic distribution of this species in Cameroon is subject to confusion. Taylor (2013) and Monadjem et al. (2020) did not mention the species in previous records in Cameroon. However, the African Chiroptera Report (2018) mentioned one specimen of *T. perforatus* collected in Yabassi, by Thys Van den Audenaerde & Opdenbosch in 1970 (Figure 1A), which is housed in the Royal Museum of Central Africa (RMCA 1973 029-M-0094).

Simmons (2005) showed that there are four subspecies of *T. perforatus* recognized in Africa. *T. perforatus sudani* is the subspecies found in Cameroon (ZFMK MAM 1974-0331 & MAM 1974-0330). This species is listed as Least Concern (LC) on the IUCN Red List of Threatened Species. We identified the specimens collected in the Bocklé cave as *T. perforatus* based on morphometric and cranio-dental measurements. The Egyptian Tomb Bat is a medium-sized insect-eating bat in Africa and is distinguished from others *Taphozous* by the absence of a pronounced gular pouch; radio-metacarpal

Table 1. External and craniodental measurements (mm) and mass (g) for *Taphozous perforatus* including specimens from our recent surveys and ZFMK specimens. Males and females presented separately. (n—number of specimens)

	Males		Females	
Measurements (mm)	**Present study (GAR570)**	**ZFMK MAM 1974-0330**	**Present study (GAR568)**	**ZFMK MAM 1974-0331**
Head Body (HBL)	60.07	80	56.87–69.7 (n = 5)	[82]
Forearm (FA)	63.29	[64.2]	60.92–65.16 (n = 5)	[59.15]
Tail length (TL)	24.11	29	17.67–26.78 (n = 5)	[24.26]
Ear length (EL)	17.02	18	16.5–18.9 (n = 5)	[14.4]
Tragus length (Trl)	4.03	[3.91]	4.78–5.07 (n = 5)	[4.71]
Tibia length (TIB)	24.26	-	23.8–26 (n = 5)	-
Weight (W) in grams (g)	32	-	27–35 (n = 5)	-
Greatest length of skull (GLS)	21.04	19.66	20.12	20.61
Condylo-canine length (CCL)	18.26	19.64	19.32	19.52
Condylo-basal length (CBL)	20.21	-	18.39	-
Mastoid breadth (MB)	9.33	11.08	8.81	10.99
Mandibular tooth row (C–M_3)	8.96	10.3	9.24	9.23
Maxillary tooth row (C–M^3)	8.31	8.73	8.28	8.34
Interorbital constriction (IC)	5.82	5.17	7.38	5.13
Mandible length (ML)	15.37	15.73	15.19	15.55
Width across canines (C-C)	3.53	-	3.84	-
Cranio-canine length (CrnC)	19.68	-	19.17	-

[] skin measurements on dry specimen

pouch present in both sexes; body weight of 20-39g; sexes similar; and wings attached to the tibia (Rosevear 1965; Roberts 1997; Mahmood-ul-Hassan et al. 2009, 2012; Taylor 2013). The braincase is rounded and elevated above the level of the rostrum, with distinct frontal depression, flanked by anterior (lachrymal), and posterior (postorbital bar) inflations of bones on each side of the rostrum (Monadjem et al. 2010). The body and cranial measurements of our specimens do not exceed the published range as reported by Rosevear (1965), Hayman & Hill (1971), Patterson & Webala (2012), and Taylor (2013).

The cave from where the present specimens were captured contained two species of bat, the upper entrance is occupied by *Nycteris arge*, and the deep and lower entrance is occupied by *Taphozous perforatus*. The same roost was visited twice, on 25 October 2019 and 25 November 2019, when these species were observed. The area is covered by moist savanna with ambient temperature varied between 28–45 °C. This is in line with studies conducted by Bohra (2011) who reported that *T. perforatus* avoids areas with low temperatures. Indeed, Monadjem et al. (2010) and Taylor (2013) mentioned this species in open woodland savannas and flooded savannas (e.g., Sahel Savanna, Sudan Savanna, Guinea Savanna) where suitable day-roosts are present. It roosts by crawling into dark crevices in rocky outcrops and caves (Smithers 1971; Smithers & Wilson 1979), where it forms small groups (Monadjem et al. 2010). The discovery of the roost of *Taphozous perforatus* in Garoua is therefore not surprising and re-affirms the species presence in Cameroon (Figure 1B). According to Taylor (2013), records of this species' distribution in Africa are extremely scarce.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21122–21124

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

https://doi.org/10.11609/jott.7864.14.5.21122-21124

#7864 | Received 03 February 2022 | Final received 07 May 2022 | Finally accepted 14 May 2022

OPEN ACCESS

SHORT COMMUNICATION

First definite record of Collared Pratincole *Glareola pratincola* Linnaeus, 1766 (Aves: Charadriiformes: Glareolidae) from Goa, India

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Abstract: Collared Pratincole *Glareola pratincola* has been recorded from various regions in southern India and is known to be a two way passage migrant to Western Ghats. However, this species had not been included in Goa's avifaunal checklist since the old records for the bird could not be confirmed. Hence, the present sighting of this bird at Maina-Curtorim fields, Goa qualifies as the first definite record for Goa. During the period 2013–2021, three birds namely the Spot-billed Pelican *Pelecanus philippensis*, Lesser Flamingo *Phoeniconaias minor* and Eurasian Bittern *Botaurus stellaris* were spotted within one-kilometer radius of the Maina-Curtorim wetlands and added to the avifaunal checklist of Goa. This offers an opportunity for additional surveys to establish the migratory route and also highlights the conservational value of the region.

Keywords: Avifaunal diversity, Collared Pratincole, conservation, Goa, Maina-Curtorim, new record, wetland.

This note describes the first definite sighting of Collared Pratincole *Glareola pratincola* at Maina-Curtorim fields situated in Curtorim, Salcete taluka of South Goa district, Goa (Dharwadkar 2021).

The Maina-Curtorim fields admeasuring approximately 40 ha are located opposite the Sonbem Lake in Curtorim (15.300°N 74.007°E) (Image 1). These fields locally known as 'Aadis', are cultivated twice a year where high yielding varieties of paddy crops are grown using stored water from the adjoining Sonbem lake. The water in the lake gets collected during the monsoons showcasing age old water management system. The said area serves as a habitat for more than 250 species of birds, both residents and migratory such as Bar-headed Goose *Anser indicus*, Jack Snipe *Lymnocryptes minimus*, Spotted Redshank *Tringa erythropus*, and White Stork *Ciconia ciconia*.

On 13 December 2021 at approximately 0830 h, a single bird was spotted resting motionless on a mud bund. The overall pale brown upper parts of the bird makes it all the more difficult to spot when the bird is resting as the bird gets completely camouflaged with the color of the mud (Image 2). The bird was not too shy and was later seen preening. It was also seen feeding on insects by taking short flights. A few good photographs and a video was taken immediately. Initially, it was presumed to be an Oriental Pratincole *Glareola maldivarum*. However, with the help of good photographs and identification guides (Ali & Ripley 1981; Driessens & Svensson 2005; Grimmett et al. 2011), several ID features (as listed below) matched with the Collared Pratincole *Glareola pratincola* and not with Oriental Pratincole *Glareola maldivarum*.

1. Tern-shaped, short-legged plover like bird (Image 3).
2. Olive-brown head and back (Image 3).
3. Slit-shaped nostril, as compared to the egg-shaped nostril in Oriental (Image 3).
4. Black lores continued under eye forming a narrow

Editor: H. Byju, Coimbatore, Tamil Nadu, India.

Date of publication: 26 May 2022 (online & print)

Citation: Pandit, R., M. Dharwadkar & J. Rebello (2022). First definite record of Collared Pratincole *Glareola pratincola* Linnaeus, 1766 (Aves: Charadriiformes: Glareolidae) from Goa, India. *Journal of Threatened Taxa* 14(5): 21122–21124. https://doi.org/10.11609/jott.7864.14.5.21122-21124

Funding: Self-funded.

Competing interests: The authors declare no competing interests.

Acknowledgements: We wish to express our deepest gratitude to Tim Inskipp and Gerald Driessens for confirming the ID of the bird.

Image 1A–B. A—Location of Maina-Curtorim fields | B—Satellite view of Maina-Curtorim fields (marked in green) and Sonbem Lake (marked in blue) with observation point of Collared Pratincole *Glareola pratincola* (marked in red).

black band/gorget around the pale rufous-buff chin and throat (Image 3).

5. Brown upper-breast, passing downwards into rufous (Image 3).

6. White abdomen and under-tail coverts (Image 3).

7. The tail-to-wing length ratio is considered as one of the best features to identify the species on field (Rajeevan & Thomas 2013). When the bird is in resting position, the tip of the outermost tail feathers (tail-tip) reaches the tip of closed wings unlike in the case of Oriental wherein the tail-tip falls well short of wing-tip at rest (Image 3).

8. Broad white trailing-edge to the secondaries which the Oriental lacks (Image 4).

9. White upper tail-coverts; black tail (Image 4).

10. Deep and pronounced fork tail in flight unlike the Oriental which has a shallow fork-tail (Image 5).

Apparently, since there is a significant overlap of features between Oriental and Collared pratincoles and to avoid further confusions, Dr. Tim Inskipp, author of 'The Birds of the Indian Subcontinent" (Dr. Tim Inskipp in litt. 13.xii.2021) and Gerald Driessens, the Belgian authority on pratincoles (Driessens & Svensson 2005) (Gerald Driessens in litt. 21.xii.2021), were contacted wherein both confirmed the ID of the bird as Collared Pratincole.

Although known to breed in the Mediterranean region, Middle East, southwestern Asia, Afghanistan, southern Europe, Pakistan, and northwestern India (Peters & Vinay 2020) various records from southern India also exist. Besides being a migratory bird (Peters & Vinay 2020), this species is a winter visitor not only in Africa and India but also to Sri Lanka in southern Asia (Rajeevan & Thomas 2013) and is considered to be a two-way passage migrant to Western Ghats (Rasmussen & Anderton 2012). As per e-bird, one individual photographed from Kelgeri Lake, Dharwad, Karnataka on 06 January 2021, happens to be the closest record to Goa (Sahana 2021).

Although four records exist in Goa between 1998 and 2009 (Lainer & Alvares 2013), this species has not been included in Goa's avifaunal checklist since the old records could be confused with the Oriental Pratincole which is a commoner (Baidya & Bhagat 2018, 2021). Hence, the present sighting qualifies as the first definite record for Goa. The place was revisited on 21 December 2021 at 1020 h where the bird was spotted and photographed once again at the same location.

Although this species has an extremely large range

Image 2–5. 2—Collared Pratincole *Glareola pratincola* camouflaged in its habitat | 3—ID features mentioned at pt.1–pt.7 | 4—ID features mentioned at pt.8 & pt. 9 | 5—ID feature mentioned at pt. 10. © 2&3—Mangirish Dharwadkar | © 4&5—Justino Rebello.

and has been listed in the Least Concern category of the IUCN Red List, the population trend is decreasing as the species is threatened not only by the use of herbicides and insecticides but also due to changes to its preferred habitats (Birdlife International 2022). Surveys and monitoring projects, therefore, need to be conducted to ascertain the status of this rare bird in Goa and to further establish its migratory route.

Additionally, stringent steps are required to be taken to protect these wetlands considering the fact that during the period 2013–2021, three new records for the state of Goa, namely, Lesser Flamingo *Phoeniconaias minor* (Dumadag 2020) was spotted from Maina-Curtorim wetlands; whereas, the Spot-billed Pelican *Pelecanus philippensis* (Dharwadkar 2013) and Eurasian Bittern *Botaurus stellaris* (Rebello & Dharwadkar 2021) were added from within one-kilometer radius of the Maina-Curtorim wetlands, highlighting the conservational aspect of the region.

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Journal of Threatened Taxa | www.threatenedtaxa.org | 26 May 2022 | 14(5): 21125–21126
ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)
https://doi.org/10.11609/jott.7915.14.5.21125-21126
#7915 | Received 09 March 2022 | Final received 16 April 2022 | Finally accepted 20 May 2022
OPEN ACCESS
NOTE

Nectar robbing by sunbirds on the flowers of *Morinda pubescens* J.E. Smith (Rubiaceae)

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The genus *Morinda* is represented by only eight species, namely, *M. angustifolia* Roxb., *M. persicaefolia* Ham., *M. villosa* Hook.f., *M. umbellata* L., *M. trimera* Hillebr., *M. elliptica* (Hook.f.) Ridl., *M. citrifolia* L., and *M. pubescens* J.E. Smith, in India (Arya et al. 2014). Recently, *M. tinctoria* Roxb. and *M. tomentosa* Heyene ex Roth. are treated as synonyms to *M. pubescens* and accordingly this is followed in this work and the published work reported on these species is cited as related to *M. pubescens*. *M. tomentosa* which is now known as *M. pubescens* is widely distributed in India, Sri Lanka, China, central Myanmar, southern Thailand, Vietnam, and Indonesia (Kesonbuaa & Chantaranothai 2013). It is a small evergreen tree adapted to grow successfully in arid/semi-arid to mesophytic conditions (Bermer & Manen 2000).

Morinda pubescens distributed naturally with a few individuals in Kadiri Reserve Forest with arid ecosystem (14.0667°N, 78.1967°E and altitude 761 m) in Anantapur District, Andhra Pradesh, India, was used for the study during February–June 2019. This tree grows here in cracks and crevices of stony rocks and in partially weathered rocks accumulated with soil. Leaves are petiolate, simple, opposite, decussate and broadly elliptic with acute apex. Flowers are borne in leaf-opposed solitary, terminal, globose heads. They are sessile, greenish-white, fragrant and hermaphroditic with distyly. Calyx tube is connate at base with denticulate lobes. Corolla is greenish-white and salver-shaped with slender tube extended into five abruptly spreading flat lobes exposing the sex organs. The stamens here are five attached to the corolla throat and linearly extended beyond the rim of the corolla tube. The ovary is bi-locular, 4-ovuled with linear style which is extended into a bi-lobed stigma. The fruit is a green globose syncarp with four oblong pyrenes.

In Kadiri Reserve Forest, *M. pubescens* blooms in foliate state during dry season from March to May (Image 1a). Mature buds open during early morning and expose the sex organs out of the corolla tube (Image 1b–e). The flowers are nectariferous. However, the nectar is concealed at the base of the corolla tube which is accessible only to legitimate foragers probing the flower-opening side. Thrips use the floral buds for breeding and open flowers for pollen and nectar. They move out during flower-opening. Sunbirds, *Nectarinia asiatica* and *N. zeylonica* were seen

Editor: Analinda C. Manila-Fajardo, University of the Philippines Los Banos, Laguna, Philippines. **Date of publication:** 26 May 2022 (online & print)

Citation: Raju, A.J.S., S.S. Kumar, G. Nagaraju, C.V. Reddy, T.P. Raliengoane, L.K. Grace, K. Punny, K. Prathyusha & P. Srikanth (2022). Nectar robbing by sunbirds on the flowers of *Morinda pubescens* J.E. Smith (Rubiaceae). *Journal of Threatened Taxa* 14(5): 21125–21126. https://doi.org/10.11609/jott.7915.14.5.21125-21126

Funding: Self-funded.

Competing interests: The authors declare no competing interests.

Acknowledgements: We thank the Andhra University, Visakhapatnam, for providing physical facilities to carry out this work.

Image 1. *Morinda pubescens*: a—Flowering tree | b–e—Different stages of bud and flower | f—*Nectarinia asiatica* feeding on thrips | g—*Nectarinia zeylonica* feeding on thrips | h & i—*Nectarinia zeylonica* puncturing corolla base to rob nectar. © A.J. Solomon Raju.

at the flowers of *M. pubescens* foraging on thrips and nectar. These birds pick up thrips (Image 1f,g) from the flowers by probing the flowers legitimately during which they occasionally effect pollination and collect nectar illegitimately by making a puncture/slit at the base of corolla tube from outside (Image 1h,i).

The flowers with tubular corolla are vulnerable to nectar robbing. Maloof & Inouye (2000) and Irwin et al. (2010) reported that nectar robbing is very frequent in plant species producing flowers with long corollas and abundant nectar production. Irwin & Maloof (2002) reported that nectar robbing involves primary and secondary robbing with the first one as the most common. In primary robbing, the flower forager makes a slit or hole or tear in petal tissue to rob nectar bypassing the floral opening used by legitimate pollinators. In secondary robbing, the flower forager acquires nectar via slit/hole/tear made by primary robbers again bypassing the floral opening used by legitimate pollinators. Specialization in floral architecture is vulnerable to exploitation by flower visitors which remove or steal nectar without effecting pollination (Navarro 2001). Nectar robbing by sunbirds on the flowers of *M. pubescens* is an indication of primary robbing which does not effect pollination but this robbing phenomenon reduces nectar reward and increases variability in nectar standing crop. Such a situation is expected to promote pollination rate in general and cross-pollination in particular when legitimate pollinators visit *M. pubescens* flowers for nectar. Therefore, *M. pubescens* is an important source of thrips as insect food and nectar as instant drink for sunbirds during dry season in the arid ecosystem.

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Journal of Threatened Taxa is indexed/abstracted in Bibliography of Systematic Mycology, Biological Abstracts, BIOSIS Previews, CAB Abstracts, EBSCO, Google Scholar, Index Copernicus, Index Fungorum, JournalSeek, National Academy of Agricultural Sciences, NewJour, OCLC WorldCat, SCOPUS, Stanford University Libraries, Virtual Library of Biology, Zoological Records.

NAAS rating (India) 5.64

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Saravanampatti, Coimbatore, Tamil Nadu 641035, India
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Journal of Threatened Taxa

www.threatenedtaxa.org

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

May 2022 | Vol. 14 | No. 5 | Pages: 20951–21126
Date of Publication: 26 May 2022 (Online & Print)
DOI: 10.11609/jott.2022.14.5.20951-21126

Publisher & Host

WILD ZOOREACH
Threatened Taxa