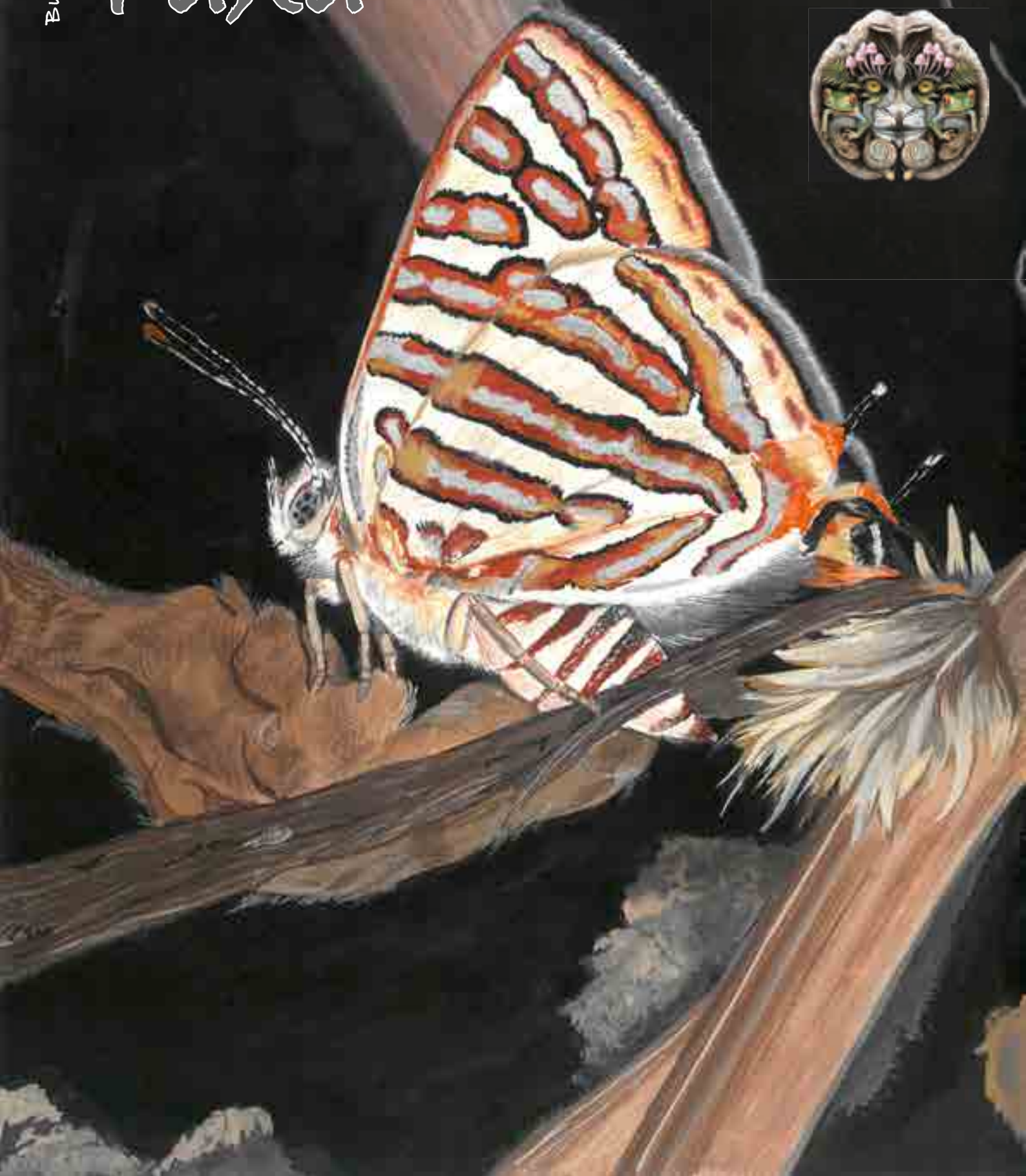






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43/2 Varadarajulu Nagar, 5th Street West, Ganapathy, Coimbatore, Tamil Nadu 641035, India
Ph: +91 9385339863 | www.threatenedtaxa.org
Email: sanjay@threatenedtaxa.org

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Cover: Common Silverline *Spindasis vulcanus vulcanus* in poster colours adapted from photograph by Kalpesh Tayade. © Pooja R. Patil.



A preliminary survey of moss flora of Chail Wildlife Sanctuary, Himachal Pradesh, India

Meenal Sharma¹ , Anju Rao² & S.S. Kumar³

¹⁻³ Department of Botany, Panjab University, Chandigarh 160014, India.

¹meenaljasra@gmail.com, ²anjuraoanju@rediffmail.com (corresponding author), ³sharmasudarshan455@gmail.com

Abstract: The present study aims to account for the moss flora of Chail Wildlife Sanctuary, district Solan, Himachal Pradesh (HP). Frequent field visits were made in different seasons to collect moss samples. Ecological data like temperature, humidity, and habitat preferences were also recorded at the time of collection. A total of 31 moss species belonging to 22 genera and 15 families were recorded so far. The family Pottiaceae (7 spp.) was the most dominant one, followed by Brachytheciaceae (4 spp.), Polytrichaceae, Fissidentaceae, and Entodontaceae with (3 spp.) each. In the acrocarpous mosses, family Pottiaceae was highly dominant, whereas, among the pleurocarpous mosses family, Brachytheciaceae was dominant. Among the genera, *Atrichum* P. Beauv. (Acrocarpous), *Fissidens* Hedw. (Acrocarpous), and *Entodon* C. Muell. (Pleurocarpous) were dominantly present, represented by three species each. This study provides baseline data of moss diversity and their ecological attributes in Chail Wildlife Sanctuary, which may prove beneficial in establishing policies for future exploration of bryodiversity with proper management and conservation in the sanctuary area.

Keywords: Acrocarpous, Bryodiversity, Bryophytes, Pleurocarpous, western Himalaya.

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Author details: MEENAL SHARMA is a research scholar in Department of Botany, Panjab University. ANJU RAO is an associate professor in Department of Botany, Panjab University. S.S. KUMAR is professor (Emeritus) in Department of Botany, Panjab University.

Author contributions: MS surveyed the study area, collected the plant material, prepared the slides for identification and written up the whole manuscript. SSK helped with the identification of the plant material. AR designed and supervised the whole experiment throughout. All authors read and approved the final manuscript.

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INTRODUCTION

Bryophytes constitute a vast group of land plants, only second after angiosperms. Approximately 20,000 species of bryophytes (Mosses, liverworts, and hornworts) are reported worldwide (Ismail et al. 2020). In India, the bryophytes are represented by 2,562 taxa (1,636 mosses, 887 liverworts and 39 hornworts (<http://www.bsienviis.nic.in/Database/Bryophytes>). Bryophytes can colonize a wide variety of habitats, including rocks, tree bark, wood, forest floors, and riverbank. Mosses constitute an important group of bryophytes with more species richness and wide geographic distribution than liverworts and hornworts. Moss flora of western Himalaya has been extensively studied by several authors such as Chopra & Kumar (1981), Vohra (1983); Tewari & Pant (1994), Joshi et al. (2001); Kumar & Singh (2002), Kapila & Kumar (2003), Saxena et al. (2006), Nath et al. (2008), Sahu & Asthana (2012), Alam (2013), Alam et al. (2013), Asthana & Sahu (2013), Riaz et al. (2015), and Sahu & Asthana (2015). In Himachal Pradesh (HP), several authors (Sharma & Choyal 2011) have documented the moss flora at the local scale. However, to the best of our knowledge, no study has explored the moss flora of a protected area in HP so far. Therefore, the present study aims to document the moss flora of Chail Wildlife Sanctuary supplemented with their habitat and ecological attributes.

MATERIAL AND METHODS

Study area

Chail wildlife sanctuary is situated in the Solan district of Himachal Pradesh (Figure 1), covering an area of ~110 km². The majority of the sanctuary area comes under the Kandaghat sub-division of Solan district, however, some part of it also falls under the jurisdiction of the Shimla wildlife division. It lies between 30.891 latitude & 77.138 longitude at an altitude range of 701–2,405 m. The study area experiences sub-tropical to temperate climatic conditions with temperatures ranging from 40°C in summer to -4°C in winters. The annual rainfall amounts to about 1,250 mm, most of which is procured during the monsoon season between July to September. The area receives occasional snowfall during winters.

Sampling and collection

The frequent field visits were made in different seasons to collect moss samples from the sanctuary. Samples were systematically collected from different

parts of the sanctuary to cover various aspects, topography, and forest types. The moss plants were collected from different habitats such as rocks, tree barks, trunks, and soil surfaces. The specimens were scraped out carefully with the edge of a knife and immediately placed in polybags. While collecting the samples, several field parameters such as habitat, host, geographic coordinates, surrounding vegetation, and substrate conditions were recorded. In addition, at each sampling site, soil, temperature and moisture data were also gathered. The collected moss samples were transferred to the laboratory and air-dried at room conditions. The dried material was then soaked in luke warm water for 5–10 minutes. The specimens are deposited in the herbarium of the Department of Botany, Punjab University, Chandigarh and a voucher number for each specimen was obtained. The geographical map of the study area was prepared using ArcGIS software version 10.8.

Identification and taxonomic treatment

For identification, anatomical studies were performed by soaking the plant material in luke warm water for 5–10 minutes to regain the turbidity and the permanent slides were prepared using 30% glycerine and DPX (Dibutyl phthalate Polystyrene Xylene) solvent. The selected mounts were observed and photographed under a microscope. Photographs of dry and wet plant material were also taken. The specimens were identified with the help of previously published floras such as Gangulee (1969–1980), Chopra (1975), Chopra & Kumar (1981), and Kumar (1980). The families are arranged according to Goffinet & Buck (2004) system of classification.

RESULTS AND DISCUSSION

In the present study, 31 species of mosses belonging to 22 genera and 15 families were recorded so far. The family Pottiaceae accounts for the highest number of species (7), followed by Brachytheciaceae (4 spp.) and Polytrichaceae, Entodontaceae, & Fissidentaceae with (4 spp.) each. Rest of the recorded families were represented by one species each (Figure 2). All the recorded families along with their respective species and their habitat preferences are depicted in Table 1. Among the recorded families, nine are acrocarpous and six are pleurocarpous. The coloured pictures of all the collected taxa are provided in photo Images 1–31. A detailed description of recorded families with diagnostic

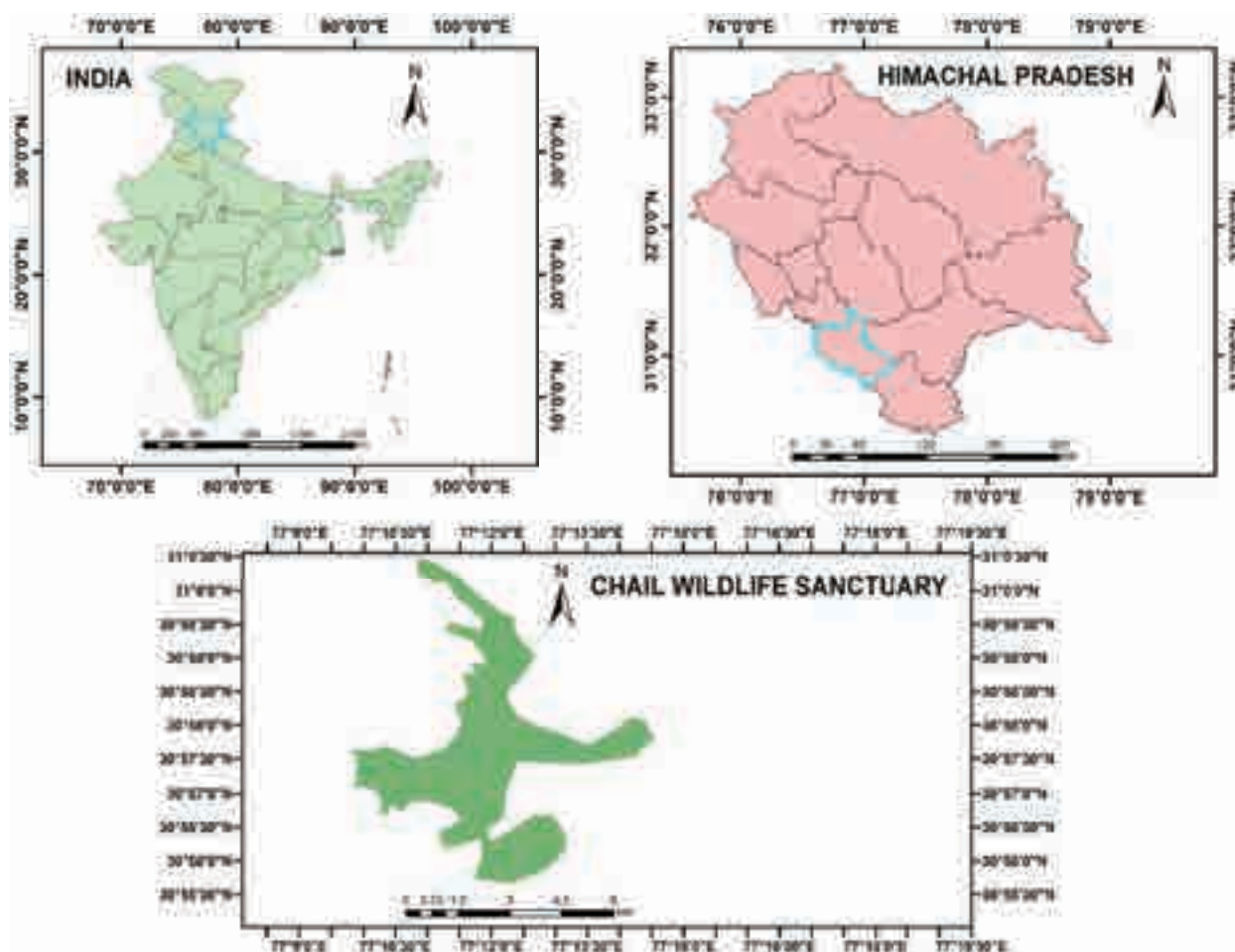


Figure 1. Location map of study area.

features is discussed below:

1. Polytrichaceae: Polytrichaceae includes 25 genera, of which four are commonly found in the Western Himalaya (Alam 2013). In the present study, only one genus, i.e., *Atrichum* (3 species, i.e., *Atrichum undulatum* (Hedw.) P. Beauv., *A. flavisetum* Mitt. and *A. obtusulum* (Müll. Hal.) A. Jaeger) was recorded. All three species are terricolous in habitat. Plants are usually small with an erect, unbranched stem. The characteristic feature of *Atrichum*, i.e., leaf lamina bisect with teeth and lamellae restricted to nerve portion only, helped in easy identification. The peristome teeth in the studied taxa are of nematodontous type.

2. Dicranaceae: Dicranaceae includes a total of 70 genera, of which 18 are present in the western Himalaya (Alam 2013). In our study area, this family is represented by only one taxon, i.e., *Dicranella divaricata* (Mitt.) A. Jaeger. This species is terricolous in its habitat. Plants are small, erect with the tomentose and usually branched stem. Leaves broader at the base and long distinctive

apex, leaf cells subquadrate or elongated in the apical region and alar cells are usually well differentiated. The sporophytic stage was not observed.

3. Fissidentaceae: This family comprises five genera, of which only one is reported from the western Himalaya (Alam 2013), i.e., *Fissidens*; this genus is also reported from the study area with three species viz. *Fissidens bryoides* Hedw., *F. crispulus* Brid. and *F. involutus* Wilson ex Mitt. *Fissidens bryoides* was recorded on the tree trunk and *F. crispulus* & *F. involutus* were found to be terrestrial. The plant body of *Fissidens* sp. is erect and ranges between 1–5 mm in height. This most distinctive taxon among mosses was easily recognizable by its distichous leaves and each leaf with lamina vaginata, lamina dorsalis and lamina apicalis. The sporophytic stage was not observed.

4. Ditrichaceae: Ditrichaceae includes 32 genera worldwide, of which only four are found in the western Himalaya (Alam 2013). In the present study, only one taxon, i.e., *Ditrichum tortipes* (Mitt.) Kuntze was

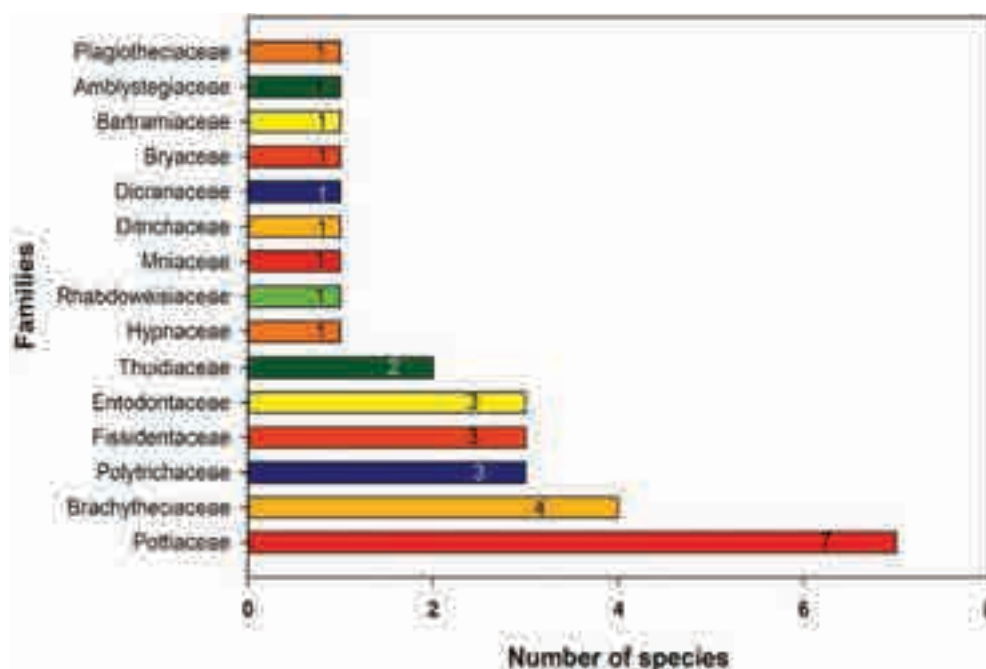


Figure 2. Family-wise number of species reported from the study area.

observed. The plants were found growing in loose tufts. Stem usually unbranched, with slightly dentate apex. The shape of the leaf, the deeper color of percurrent costa, leaves linear to almost square in shape with areolation helped distinction. The sporophytic stage was not observed.

5. Rhabdoweisiaceae: Rhabdoweisiaceae includes two genera, of which only one is found in the western Himalaya (Alam 2013). In the area under present study, only taxon, i.e., *Rhabdoweisia crenulata* (Mitt.) H. Jameson. was recorded. Like the other members of this acrocarpous family, plants are small and found growing in dense cushions. The long lingulate leaves with irregularly serrated margins, short, quadrate to elongated thin-walled areolation with undifferentiated alar cells, helped separation from other species. The sporophytic stage was not observed.

6. Pottiaceae: Pottiaceae includes 112 genera, of which 32 genera are found in the western Himalaya (Alam 2013). In present study, five genera i.e., *Anoetangium* (2 species, *A. stracheyanum* Mitt. and *A. bicolor* Renaud & Cardot), *Molendoa* [1 species, *M. roylei* (Mitt.) Broth.], *Hymenostylium* [1 species, *H. recurvirostrum* (Hedw.) Dixon], *Hyophila* [2 species, *H. involuta* (Hook.) A. Jaeger. and *H. rosea* R.S. Williams] and *Hyogonium* [1 species, *H. arcuatum* (Griff.) Wijk & Margad.] were recorded. *Anoetangium stracheyanum* and *Hymenostylium recurvirostre* were epiphytic and others were terrestrial. All members of the family

Pottiaceae are acrocarpic and grow in loose tufts. The plant body is erect, small and caespitose. The stem is well developed with a central hydroid strand. The most important identifying feature of this family is the multi papillose laminal cells. Leaves are of variable shape ovoid to lanceolate, broad at the apex and tapered at the base with entire or sometimes serrated margins. *Hyophila involuta* have serrated margins, while *Hyophila rosea* have smooth margins. *Anoetangium bicolor* can be easily identified by the presence of prominent costa, which extends beyond the tip. Peristome teeth are absent in all of the reported taxa.

7. Bartramiaceae: Bartramiaceae includes 11 genera, of which seven are found in the western Himalaya (Alam 2013). In the present study area, only one taxon, i.e., *Philonotis fontana* (Hedw.) Brid. is reported. Bartramiaceae is commonly called as a family of apple mosses because of the apple-like shape of the capsules. The plants grow in extensive tufts. Stems mostly tomentose, with whorls of subfloral innovations, leaves lanceolate and acuminate with single costa ending below the tip, laminal cells rectangular at the base, elongated in mid leaf with cell ends extended as mamillae which offer an additional feature of distinction. Capsules were found to be erect or slightly inclined with diplolepidous peristome teeth.

8. Mniaceae: Mniaceae includes 12 genera, of which seven are found in the western Himalaya (Alam 2013). In the present study area, only one taxon,

Table 1. A list of recorded moss species with habitat preferences and herbarium specimen numbers.

Genus	Species	Family	Habitat	Herbarium specimen number (PAN)
<i>Atrichum</i> P. Beauv.	<i>A. undulatum</i> (Hedw.) P. Beauv.	Polytrichaceae	RP, TR	6323
	<i>A. flavisetum</i> Mitt.		TR	6324
	<i>A. obtusulum</i> (Müll. Hal.) A. Jaeger		TR	6325
<i>Dicranella</i> Schimp.	<i>D. divaricata</i> (Mitt.) A. Jaeger	Dicranaceae	TR	6330
<i>Fissidens</i> Hedw.	<i>F. bryoides</i> Hedw.	Fissidentaceae	SX	6333
	<i>F. crispulus</i> Brid.		TR	6335
	<i>F. involutus</i> Wilson ex Mitt.		CT, RP, TR	6336
<i>Ditrichum</i> Hampe	<i>D. tortipes</i> (Mitt.) Kuntze	Ditrichaceae	TR	6341
<i>Rhabdoweisia</i> Bruch & Schimp.	<i>R. crenulata</i> (Mitt.) H. Jameson	Rhabdoweisiaceae	RP	6343
<i>Anoetangium</i> Schwägr.	<i>A. stracheyanum</i> Mitt.	Pottiaceae	CT, RP, TR, SX	6346
	<i>A. bicolor</i> Renauld & Cardot		TR	6348
<i>Molendoa</i> Lindb.	<i>M. roylei</i> (Mitt.) Broth.		TR	6349
<i>Hymenostylium</i> Brid.	<i>H. recurvirostrum</i> (Hedw.) Dixon		CT, RP, SX	6351
<i>Hyophila</i> Brid.	<i>H. involuta</i> (Hook.) A. Jaeger		RP, TR, SX	6358
	<i>H. rosea</i> R.S. Williams		RP, TR	6359
<i>Hydrogonium</i> (Müll. Hal.) A. Jaeger	<i>H. arcuatum</i> (Griff.) Wijk & Margad.		TR	6366
<i>Philonotis</i> Brid.	<i>P. fontana</i> (Hedw.) Brid.	Bartramiaceae	RP, TR	6370
<i>Plagiomnium</i> T. J. Kop.	<i>P. cuspidatum</i> (Hedw.) T.J. Kop	Mniaceae	TR	6375
<i>Ptychostomum</i> Hornsch.	<i>P. capillare</i> (Hedw.) D.T. Holyoak & N. Pedersen	Bryaceae	RP, TR	6384
<i>Cratoneuron</i> (Sull.) Spruce	<i>C. filicinum</i> (Hedw.) Spruce	Amblystegiaceae	TR	6386
<i>Thuidium</i> Schimp.	<i>T. glaucinum</i> (Mitt.) Bosch & Sande Lac.	Thuidiaceae	TR	6392
<i>Herpetineuron</i> (Müll. Hal.) Cardot	<i>H. toccoeae</i> (Sull. & Lesq.) Cardot		SX	6410
<i>Brachythecium</i> Schimp.	<i>B. buchananii</i> (Hook.) A. Jaeger	Brachytheciaceae	CT, RP, TR, SX	6394
	<i>B. populeum</i> (Hedw.) Schimp.		TR	6396
<i>Rhynchostegium</i> Schimp.	<i>R. planiusculum</i> (Mitt.) A. Jaeger		TR	6401
<i>Oxyrrhynchium</i> (Schimp.) Warnst.	<i>O. vagans</i> (A. Jaeger) Ignatov & Huttunen		RP, TR	6402
<i>Pseudotaxiphyllum</i>	<i>P. elegans</i> (Brid.) Z. Iwats.	Plagiotheciaceae	TR	6407
<i>Entodon</i> Müll. Hal.	<i>E. flavescens</i> (Hook.) A. Jaeger	Entodontaceae	RP	6403
	<i>E. myurus</i> (Hook.) Hampe		TR, SX	6404
	<i>E. rubicundus</i> (Mitt.) A. Jaeger		RP, TR, SX	6405
<i>Hypnum</i> Hedw.	<i>H. cupressiforme</i> Hedw.	Hypnaceae	RP	6409

RP—Rupicolous | TR—Terricolous | SX—Saxicolous | CT—Corticolous.

i.e., *Plagiomnium cuspidatum* (Hedw.) T.J. Kop. was observed. The species is characterized by large leaves, spatulate to oval in shape often present in rosette at stem apex, with sharp uniseriate teeth on the margins. The plants were found growing in loose tufts. The sporophytic stage was not observed.

9. Bryaceae: Bryaceae includes 33 genera, of which only six are reported from the western Himalaya (Alam 2013). In the present study, only one taxon, i.e.,

Ptychostomum capillare (Hedw.) D.T. Holyoak & N. Pedersen was recorded. The plants are terrestrial as well as epiphytic found growing in dense tufts under damp and shady conditions. Leaves ovate-lanceolate in shape with smooth margins, serrated at the tip and hexagonally elongated areolations helped recognition of this taxon. The sporophytic stage was not observed.

10. Amblystegiaceae: Amblystegiaceae includes 44 genera, of which only two are found in the western



Image 1–15. 1—*Atrichum undulatum* (Hedw.) P.Beauv. | 2—*A. flavisetum* Mitt. | 3—*A. obtusulum* (Mull.Hal.) A.Jaeger | 4—*Dicranella divaricata* (Mitt.) A.Jaeger | 5—*Fissidens bryoides* Hedw. | 6—*F. crispulus* Brid. | 7—*F. involutus* Wilson ex Mitt. | 8—*Ditrichum tortipes* (Mitt.) Kuntze | 9—*Rhabdoweisia crenulata* (Mitt.) H.Jameson | 10—*Anoetangium stracheyanum* Mitt. | 11—*A. bicolor* Renauld & Cardot | 12—*Molendoo roylei* (Mitt.) Broth. | 13—*Hymenostylium recurvirostrum* (Hedw.) Dixon | 14—*Hyophila involuta* (Hook.) A. Jaeger | 15—*H. rosea* R.S.Williams. © Meenal Sharma

Himalaya (Alam 2013). In the present study, only one taxon, i.e., *Cratoneuron filicinum* (Hedw.) Spurge was recorded. The stem leaves are broader than the branch leaves, lanceolate, acuminate, areolations elongated in the apical region and rhomboidal in the lower half. Peristome teeth are diplolepidous, hypnoid type.

11. Thuidiaceae: Thuidiaceae includes 25 genera, of which five are found in the western Himalaya (Alam 2013). In the present study, two taxa, viz., *Thuidium glaucinum* (Mitt.) Bosch. & Sande Lac. and *Herpetineuron toccoe* (Sull. & Lesq.) Cardot were recorded. In both these taxa, plants are yellowish-green growing in dense mats. Stem stoloniferous, branched pinnately with or without paraphyllia, dimorphic leaves viz. small leaves which are scale-like, large leaves ovate with broad apex, single costa usually ending below the leaf apex, laminal cells small, papillose helped distinction. The absence of paraphyllia and the tortuous costa of the leaves are the most characteristic features of the *Herpetineuron*. The sporophytic stage was not observed.

12. Brachytheciaceae: Brachytheciaceae includes 51 genera, of which 10 are reported from the western Himalaya (Alam 2013). In the present study, two taxa viz. *Brachythecium* [2 species, *B. buchananii* (Hook.) A. Jaeger & *B. populeum* (Hedw.) Schimp.] and *Rhynchostegium* [2 species, *R. planiusculum* (Mitt.) A.

Jaeger and *Oxyrrhynchium vagans* (A. Jaeger) Ignatov & Huttunen] were observed. These are the most common mosses among the plerocarpous, which are found on soil, tree trunks, and rocks. Plants are small, glossy, stem prostrate, irregularly branched. Stem leaves and branch leaves are well distinguished; branch leaves are relatively smaller, narrower, with more serrated margins and longer costa. Laminal cells linear, elongate, rhomboidal, costa reaching halfway to the apex of leaves of these taxa helped distinction. The capsules are slightly inclined in the case of *B. buchananii* and horizontal in *R. planiusculum*. Peristomes are hypnoid type.

13. Plagiotheciaceae: Plagiotheciaceae includes five genera worldwide, of which three are found in western Himalaya (Alam 2013). In the present study only one taxon, i.e., *Psuedotaxiphyllum elegans* (Brid.) Z. Iwats. was recorded. *P. elegans* is terricolous in habitat and is easily recognized by its glossy leaves with whitish tinges, apparently arranged in two rows. Irregularly and pinnately branched stems, ovate to ovate-lanceolate leaves with short and double costa. The sporophytic stage was not observed.

14. Entodontaceae: Entodontaceae includes 13 genera worldwide, of which four are found in the western Himalaya (Alam 2013). In the area under study, only one genus, i.e., *Entodon* [3 species, *E. flavescens*



Image 16–31. 16—*Hydrogonium arcuatum* (Griff.) Wijk & Margad. | 17—*Philonotis fontana* (Hedw.) Brid. | 18—*Plagiomnium cuspidatum* (Hedw.) T.J.Kop | 19—*Ptychostomum capillare* (Hedw.) D.T.Holyoak & N.Pedersen | 20—*Cratoneuron filicinum* (Hedw.) Spruce | 21—*Thuidium glaucinum* (Mitt.) Bosch & Sande Lac. | 22—*Herpatoneuron toccoeae* (Sull. & Lesq.) Cardot | 23—*Brachythecium buchananii* (Hook.) A.Jaeger | 24—*B. populeum* (Hedw.) Schimp. | 25—*Rhynchostegium populeum* (Hedw.) Schimp. | 26—*Oxyrrhynchium vagans* (A.Jaeger) Ignatov & Huttunen | 27—*Pseudotaxiphyllum elegans* (Brid.) Z.Iwats. | 28—*Entodon flavescens* (Hook.) A.Jaeger | 29—*E. myurus* (Hook.) Hampe | 30—*E. rubicundus* (Mitt.) A.Jaeger | 31—*Hypnum cupressiforme* Hedw. © Meenal Sharma

(Hook.) A. Jaeger, *E. myurus* (Hook.) Hampe, and *E. rubicundus* (Mitt.) A. Jaeger] were recorded. *Entodon flavescens* & *E. myurus* are terricolous and *E. rubicundus* is saxicolous in habitat. Plants thin, glossy, and found growing in mats. Branch leaves are smaller than stem leaves. Leaves lanceolate to ovate in shape. Laminal cells elongated rhomboidal, differentiated alar cells, double, short or absent costa is the most characteristic feature of the recorded taxa. Capsules are erect and cylindrical in shape. Peristome teeth are two-rowed.

15. Hypnaceae: Hypnaceae includes 60 genera, of which 15 are found in the western Himalaya (Alam 2013). In the area under study, only one taxon, i.e., *Hypnum cupressiforme* Hedw. was recorded. These green-glossy plants were found to be saxicolous in habitat. Leaves are ovate to lanceolate with smooth margins. The costa is indistinct. Areolations linear, differentiated at angles helped distinction. Capsule mostly erect. Peristome teeth are observed to be hypnoid type.

The number of reported species (31 spp.) in our study is comparatively less than other studies in the Himalayan region. In a similar study conducted at

Kedarnath Wildlife Sanctuary, Bahuguna et al. (2016) reported 113 species of mosses belonging to 65 genera. The lesser number of moss species in our study could be attributed to the small geographical area of the sanctuary. Alam (2013) provided an updated list of moss flora of western Himalaya, comprising three states, namely, Jammu & Kashmir, Uttarakhand, and Himachal Pradesh. He reported a total of 745 species of mosses across the three Himalayan states.

In the present study, acrocarpous mosses were found in greater numbers. The plerocarpic mosses were mostly observed as epiphytes or saxicolous. The relatively lower occurrence of plerocarpic mosses than the acrocarpous mosses appears to be due to the lack of shade and moisture availability in the epiphytic and saxicolous conditions.

Among the acrocarpous mosses, Pottiaceae (7 species) is most commonly found, followed by Polytrichaceae (3 spp.) and Fissidentaceae (3 spp.), suggesting that it can exploit more diverse habitats and can also withstand relatively more bryologically xeric conditions. Among the plerocarpous mosses,

Brachytheciaceae (4 spp.) and Entodontaceae (3 spp.) are more dominant. Among genera, the most dominating are *Atrichum*, *Fissidens* and *Entodon*, each represented with three species. As evident from the table 1, the substrate preference of most of the taxa is terricolous, a few are found to be epiphytic and some of them were present in both types of habitats. It is further observed that the acrocarpous mosses are better adapted to the arid and exposed habitats, whereas the plerocarpous mosses are scarce in such environmental conditions. In short, 19 acrocarpous (63.3%) and 11 plerocarpous (36.6%) mosses were collected from the site, which indicates the dominance of the acrocarpous mosses.

CONCLUSION

Although bryophytes are the second largest group of plants after angiosperms, detailed information about their number and distribution is still scarce. The present study provides a preliminary assessment of the moss flora of the Chail Wildlife Sanctuary with a total of 31 moss species. The most dominating family was found to be Pottiaceae (7 species). Acrocarpous mosses dominate the study area compared to pleurocarpous mosses, suggesting that the former possesses varied ecological adaptability than the latter. The habitat preferences data provided can be used in niche modelling and conservation programs. Regional and local plant inventories of mosses, especially in protected areas, can be an important tool for national database preparation and keeping a record of species for future management and conservation practices. Therefore, this study will undoubtedly act as baseline information for futuristic researchers. Further studies are recommended to understand the relationships between moss flora, associated tree species, and substrate conditions.

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New distribution record and DNA barcoding of *Sapria himalayana* Griff. (Rafflesiaceae), a rare and endangered holoparasitic plant from Mizoram, India

Laldinfeli Ralte¹ , Hmingremhlua Sailo² , Sagolshem Priyokumar Singh³ , Laldinliana Khiangte⁴
& Y. Tunginba Singh⁵

^{1–5} Laboratory of Molecular Ecology and Genetics, Department of Botany, Mizoram University, Tanhril, Aizawl, Mizoram 796004, India.

¹ lucylaldinfeli39@gmail.com, ² hmingremhlua94@gmail.com, ³ priyosagolshem@gmail.com, ⁴ eleda.kh@gmail.com,

⁵ tunginba@mzu.edu.in (corresponding author)

Abstract: *Sapria himalayana* Griff. is a rare and endangered holoparasitic plant that prefers a specific host (*Tetrastigma* sp.). It is one of the lesser-known and poorly understood plants facing threats of extinction owing to human interference in the evergreen forests of Mizoram. The flower is the only visible part of this endophyte and blooms from November to December. The plant was encountered for the first time in the evergreen forest near Rullam village in the Serchhip District of Mizoram, India. In the present study, DNA barcoding was used to identify the plants, and the internal transcribed spacer 2 (*ITS2*) region of *S. himalayana* was amplified and sequenced. The *ITS2* sequence could accurately identify up to the species level for this endangered species. The absence of the ribulose-biphosphate carboxylase gene (*rbcl*) region in the genome supports its holoparasitic nature. Hence, DNA barcoding can help in taxonomic and biodiversity research and aid in selecting taxa for various molecular ecology and population genetics studies. The phylogenetic tree was analyzed using the maximum-likelihood method, and our findings showed that species from different families were clearly discriminated in a phylogenetic tree. To the best of our knowledge, this is the first report of DNA barcoding using *ITS2* region of *S. himalayana* from Mizoram, India.

Keywords: DNA barcoding, endangered species, endophyte, holoparasitic, *ITS2*, Mizoram, *Sapria himalayana*.

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Author details: LALDINFELI RALTE is a CCRAS post-doctoral fellow at the Department of Botany, Mizoram University, Aizawl, Mizoram. She is interested in the bioprospecting of natural products from underutilized plants. HMINGREMHLUA SAILO is a PhD student at the Department of Botany, Mizoram University. SAGOLSHEM PRIYOKUMAR SINGH is a PhD student at the Department of Botany, Mizoram University. LALDINLIANA KHIANGTE is a PhD student at the Department of Botany, Mizoram University. Y. TUNGINBA SINGH is a professor at the Department of Botany, Mizoram University. He is interested in the molecular genetics studies of rare and endangered species.

Author contributions: LR—data collection, data handling, data curation, writing, review and editing of manuscript; HS—writing, review, and editing of manuscript; SPS—review, and editing of manuscript; LK—statistical analysis; YTS—supervision, review, and editing of the manuscript.

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INTRODUCTION

Angiosperms, commonly known as flowering plants, are the most diverse group of land plants, and this group also includes parasitic plants. Parasitic plants lack chlorophyll and depend on the host plants for water and nutrition (Osathanunkul 2019). Rafflesiaceae comprises holoparasitic plants (Rubiales et al. 2011) and includes three genera, namely *Rafflesia* (28 species), *Rhizanthus* (four species) and *Sapria* (three species) (Trần et al. 2018). *Sapria himalayana* Griff. (Rafflesiaceae) is also a holoparasite with a preference for specific hosts-*Tetrastigma* species (Elliott 1990).

Sapria himalayana consists of endophytic vegetative tissues with microscopic strands called haustoria, ramifying through the root cambium of the host plant. The flowers (Image 1A) are about 20 cm across, bright red and mottled with yellow spots. They appear above the ground, emitting a putrid odour. The flowers usually remain in bloom for two-three days and eventually decompose. The flowering stalks are short, erect and unbranched. The flower buds (Image 1B) are globose and covered basally by light pink bracts. The fruit is swollen, blackish-brown, and crowned with perianth remnants. The flowering and fruiting of *S. himalayana* occur during winter, usually during December-February. The seeds have been reported to be the size of grapes and blackish-brown in colour (Borah & Ghosh 2018).

Sapria himalayana has been reported to have a preference for specific hosts, so the removal of the host plants might eventually result in the death of this parasitic plant (Osathanunkul 2019). In addition, fragmentation and loss of habitat, intensive agriculture to meet human needs and other anthropogenic activities threaten the existence of this holoparasitic plant (Osathanunkul 2019). Apart from biodiversity conservation, accurate taxonomic assignment is important for this rare species as it may be accidentally collected, adding to the threat of its existence.

Traditionally, the taxonomic assignment has mainly been the responsibility of taxonomic experts (Yang et al. 2018). Above that, population genetic studies are also restricted because of their limited distribution (Elliott 1990). DNA barcoding using nucleotide comparisons of approved gene regions allows simple, rapid and reliable identification of species (Cosaic et al. 2016; Saddhe & Kumar 2018). The internal transcribed spacer Two (ITS2) region of nuclear ribosomal DNA is considered one of the candidate DNA barcodes since it has several desirable characteristics, including conserved regions for designing universal primers, ease of amplification, and

adequate variability, to distinguish even closely related species (Kang et al. 2017).

Global distributions of *S. himalayana* have been reported from Myanmar, northeastern India, southeastern Tibet, Thailand and Vietnam (Elliott 1990; Hajra 1996). In India, William Griffith first reported *S. himalayana* in 1847 from the tropical wet evergreen forests of Mishmi Hills of Lohit District in Arunachal Pradesh. Since then, *S. himalayana* has also been reported from Assam, Manipur, and Meghalaya (Borah & Ghosh 2018; Ahmad et al. 2020). In Mizoram, *S. himalayana* was first reported by Lakshminarasimhan et al. (2013) from Tawi Wildlife Sanctuary in Aizawl, Mizoram. However, no molecular analysis has been undertaken thus far on *S. himalayana* plants found in Mizoram.

Recently, *S. himalayana* was spotted in an evergreen forest near Rullam village in Serchhip District of Mizoram, India. The plant is locally called 'lei pangpar,' meaning flower without a stalk. This study aimed, for the first time, to identify *S. himalayana* using DNA barcoding combined with morphological characterization. The genome of *S. himalayana* collected from Thailand has recently been published by Cai et al. (2021). However, to our knowledge, DNA barcoding of Indian materials of this rare species has not been conducted so far.

MATERIALS AND METHODS

Study area

Flowering buds of *Sapria himalayana* were collected from an evergreen forest near Rullam village in Serchhip District, Mizoram, India (Figure 1). The locality has an average elevation of 888 m and is situated at 23.44°N & 92.99 °E. The annual daily average temperature ranges 15–27 °C with moderate rainfall.

Collection of samples and Isolation of DNA

The samples were found attached to the roots of *Tetrastigma* species (*T. obovatum* Gagnep, *T. pachyphyllum* (Hemsl.) Chun, *T. cruciatum* Craib & Gagnep). The collected samples were brought to the Department of Botany, Mizoram University, for further investigation. Isolation of genomic DNA was done using the standard CTAB method (Doyle & Doyle 1990) with some modifications. Briefly, 200 mg of two flower buds was ground and analysed separately using a sterile mortar and pestle with 500 µl of extraction buffer (100 mM TrisHCl, 1.4M NaCl, 2 mM EDTA, 2% CTAB, 1% PVP at pH 8), and incubated at 60°C for 30 mins followed by



Figure 1. Political map of Mizoram, India, showing the collection site, Rullam. Source: www.mapsofindia.com.

centrifugation at 11,000 rpm for 15 mins. After RNase A treatment, the sample was incubated at 30°C for 30 mins. Then, 500 µL Chloroform Isoamyl was added to the sample and centrifuged at 11,000 rpm for 1 min. A 0.7 volume of ice-cold isopropanol was added to precipitate the genomic DNA at -20°C. The DNA was washed with 70% ethanol and dissolved in 30 µL TE buffer (10 mM TrisHCl, 1 mM EDTA).

Amplification of DNA, sequencing and analysis

The isolated DNA was amplified using *ITS2* primers: F – GAAGGAGAAGTCGTAACAAGG, R – TCCTCCGCTTATTGATATGC and *rbcL* primers: F- CTGTATGGACCGATGGACTTAC, R-CGGTGGATGTGAAGAAGTAGAC (Zahra et al. 2016) in a Veriti 96-Well Thermal Cycler (ABI, Thermo Fisher Scientific).

The amplified DNA products were cleaned and sent for commercial sequencing to AgriGenome (Cochin, India). The resultant sequence was analyzed using NCBI BLAST (ncbi.nlm.gov), and the similarity indices with the reference sequences from GenBank database were used

for the species identification of the samples.

Phylogenetic analysis

A phylogenetic tree was constructed in MEGA X (Kumar et al. 2018) using the maximum likelihood (ML) method. The model suggested by Bayesian information Criterion (BIC) was T92 + G, with the lowest BIC score. The models with the lowest BIC scores were considered to describe the best substitution pattern (Posada & Crandall 2001). The phylogenetic tree was constructed using similar sequences identified from BLASTn analysis from Genbank. Species of closely related families from the same order (Malpighiales)- *Euphorbia canariensis* (Euphorbiaceae), *Chaetocarpus echinocarpus* (Peraceae) were also used, and a non-photosynthetic plant *Conopholis americana* (Orobanchaceae) was taken as an outgroup. Only when conspecific and congeneric species in the study formed a single clade with bootstrap P >50, the ML tree was considered successful.

RESULT AND DISCUSSION

Morphological characters of *S. himalayana* and the host plant

Sapria himalayana flowers and flower-buds were found growing on the roots of *T. cruciatum*. (Vitaceae). The host plant had leaves with tendrils arising from the bases of the petioles.

The collected flowers of *S. himalayana* (Image 1) were dark-red, mottled with yellowish-white dots, and had a bowl-shaped disk. Leaves were absent. The flowering occurs during winter, from November to February.

DNA - Isolation, Amplification, Sequencing and Analysis

The genomic DNA from *S. himalayana* was successfully isolated and amplified using *ITS2* primer (Image 2). However, the *rbcl* primer failed to amplify the DNA.

The amplified DNA of *S. himalayana* was subjected to sequencing and the sequence was submitted to the GenBank database (MW788913). The amplicon (731 bp) also showed a high percentage similarity (97.44%) with the reference sequence (EU882286) from GenBank database.

Phylogenetic analysis

The ML-based phylogenetic tree of *ITS2* showed high bootstrap values, and species of each genus were clustered on different branches and nodes as monophyletic taxon and clustered with the genus of other clades. The taxonomic units were statistically branched from their nodes with bootstrap $P > 70$ for most of the sub-trees. Thus, the present study revealed that

Table 1. Flower description of *Sapria himalayana*.

Floral parts	Size
Flower	8 cm high; 14.5–15.5 cm in diameter
Outer Perigone Lobes	3.5–4.5 cm long; 3–4 cm wide
Inner Perigone Lobes	2.5–3 cm long; 2–2.5 cm wide
Disk	3.5–4 cm in diameter
Host Plant's Root	1–2 cm in diameter

ITS2 had a high-resolution potential for the molecular taxonomy of *S. himalayana*. The collected sample was clustered together with other *Sapria* species. Here, *S. himalayana* formed a monophyletic group (bootstrap value = 100), and *S. himalayana* individuals showed coalescent stochasticity with high branch support value (bootstrap value = 100) (Figure 2) while other species were grouped into a different clade. Our study showed that the species from different families were discriminated clearly in a phylogenetic tree. Therefore, *ITS2* locus-based ML phylogenetic tree can be used to identify unknown samples for molecular taxonomy and identification of rare and endangered species.

The identification and classification of plants based on their morphological characteristics are an integral part of taxonomy; however, identifying plants based on their morphology alone may sometimes be inaccurate (Feng et al. 2017). DNA barcoding is a valuable taxonomic tool for the identification of species. A study was conducted to identify *S. himalayana* from Thailand using *ITS2* employing environmental DNA (eDNA) (Osathanunkul 2019). In our study, DNA barcoding of *S. himalayana* gDNA was successfully done using *ITS2* primers,

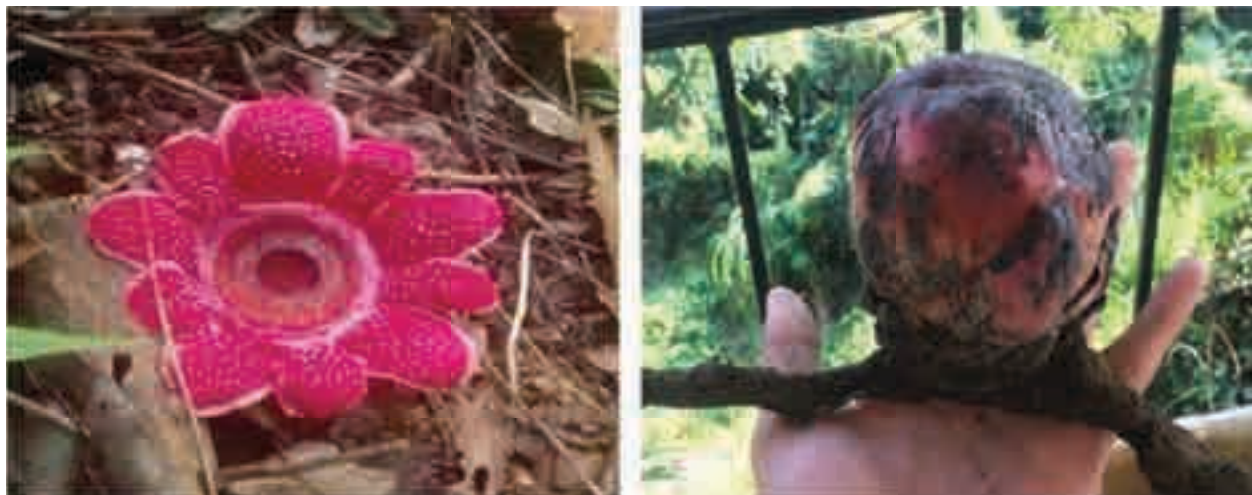


Image 1. *Sapria himalayana*: A—Flower | B—Flower bud with the root of its host (*Tetrastigma* sp.) collected from Rullam forest, Serchhip District. © Hmingremhlua Sailo.

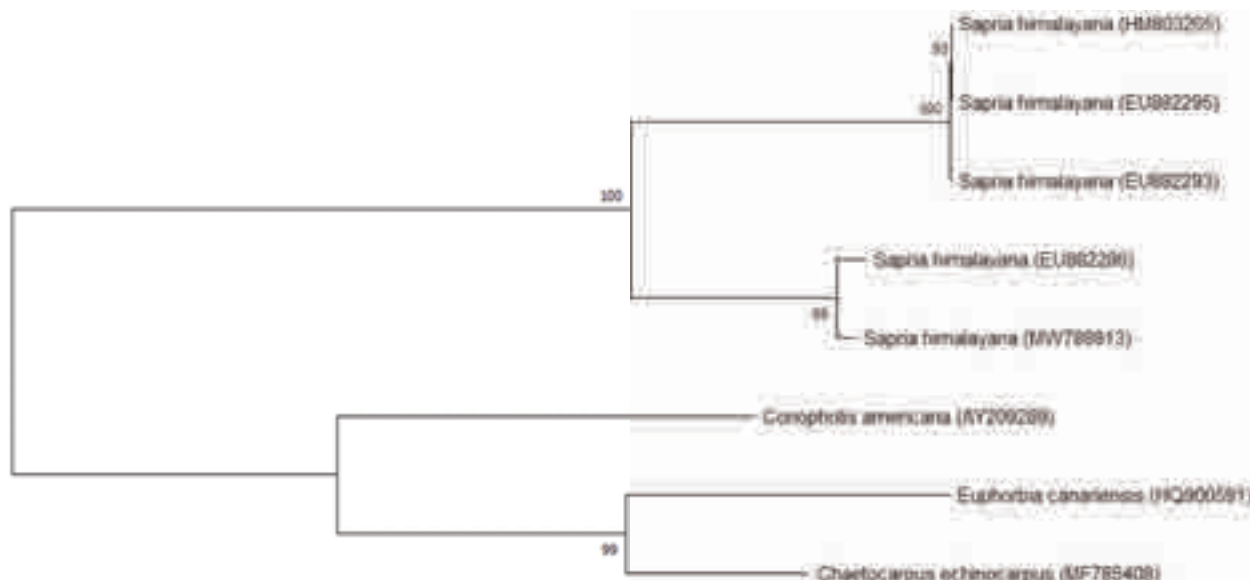


Figure 2. A maximum likelihood phylogenetic tree of *Sapria himalayana* using ITS2 region.

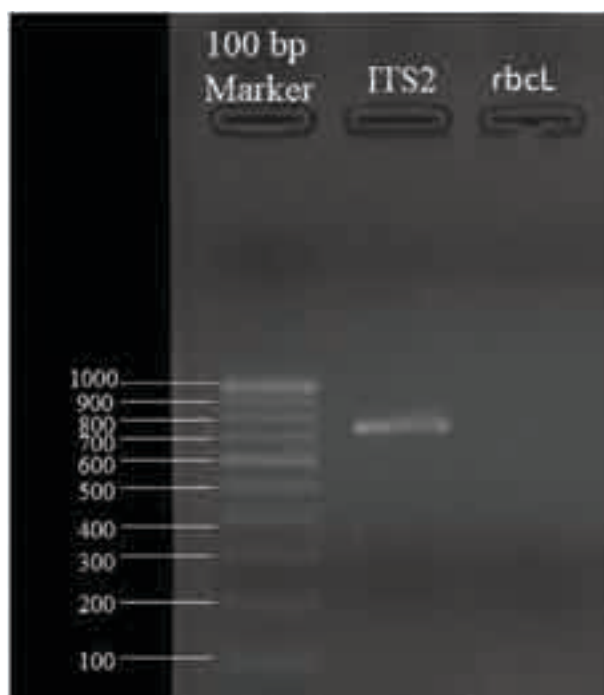


Image 2. A 1.5% agarose gel electrophoresis of amplified products of *S. himalayana* using ITS2 and *rbcL*.

resulting in a 97.44% similarity with the reference sequences from the GenBank database; this confirms the identification of the studied plant sample. Another interesting observation was the failure to amplify the *rbcL* region of the species; this could be primarily due to heavy gene loss, including the plastid genome, as already reported for the genus *Sapria* (Cai et al. 2021) and hence

loss of its photosynthetic activity. Thus, DNA barcoding can help derive an accurate phylogenetic classification. Therefore, the identification and classification of plants based on their morphology and DNA complement each other to attain accurate species identification.

CONCLUSION

Sapria himalayana, a rare and endangered holoparasitic plant, was collected from Mizoram. The results of DNA barcoding confirms the identification of this species. However, the distribution of this little known taxon is highly restricted in the region. This study suggests that focused explorations must be conducted in similar habitats to assess the population size. Suitable conservation measures are needed to protect this rare and interesting species from threat of extinction from the region.

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Species distribution modeling of a cucurbit *Herpetospermum darjeelingense* in Darjeeling Himalaya, India

Debasruti Boral¹ & Saurav Muktan²

^{1,2} Department of Botany, University of Calcutta, 35, B.C. Road, Kolkata, West Bengal 700019, India.

¹debasruti.boral@gmail.com, ²smbot@caluniv.ac.in (corresponding author)

Abstract: *Herpetospermum darjeelingense* (C.B. Clarke) H. Schaef. & S.S. Renner is a rare cucurbit found in Darjeeling, Himalaya. It is known for its use as food and medicine with possible pharmaceutical applications. Here we assess the current and future habitat suitability of *H. darjeelingense* in the study area using MaxEnt modeling. In order to obtain accurate results for future models, the ensemble method was used. The current suitable habitat covers only 13% of the study area, while the future models for 2050 and 2070 show zero habitat suitability for the species. This strongly indicates a possible local extinction of the species indicating a need for rapid and decisive conservation efforts.

Keywords: BioClim, climate change, ecology, elevation, ensemble, habitat suitability, MaxEnt, population, taxonomy, vulnerable.

Abbreviations: AUC—Area Under the Curve | CCSM4—Community Climate System Model 4 | CMIP5—Coupled Model Intercomparison Project 5 | GCM—General Circulation Model | GFDL-CM3—Geophysical Fluid Dynamics Laboratory- Climate Model 3 | IPCC—International Panel on Climate Change | LPT—Least Presence Threshold | MIROC5—Model for Interdisciplinary Research on Climate 5 | RCP—Representative Concentration Pathways | ROC—Receiver Operating Curve | SDM—Species Distribution Modeling | SEM—Scanning Electron Microscopy | TSS—True Skill Statistic.

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Author contributions: DB—field data collection, data analysis, writing original draft; SM—conceptualization, reviewing, editing and supervision.

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INTRODUCTION

The Himalaya biodiversity hotspot is one of the 36 currently recognised by CEPF (2021). The eastern region of the hotspot stands out in its global significance as it contains several centres of plant diversity (CEPF 2005). The complex landscape of the region has contributed to its floristic diversity, which includes several threatened plants (Kandel et al. 2019). In particular, the political boundary of India harbours an estimated 5,800 species of plants from the eastern Himalaya (Pande & Arora 2014).

The Darjeeling Himalaya is a part of the extension with its characteristic vegetation & landscape (CEPF 2005). Some of the major threats to this region include rapid urbanisation and climate change (Pandit et al. 2014). The impact of climate change on plants results in changes in phenology (Hart et al. 2014) and geographic ranges (Gómez-Ruiz & Lacher Jr. 2019). A distinctive pattern of upward altitudinal shift is also observed in mountainous regions (Dullinger et al. 2012). Another impact of climate change includes invasion by alien species which are hardier and more competitive (Pandit et al. 2014).

As the effects of climate change become more drastic, there is an urgent need to study consequences for significant species such as *H. darjeelingense*, which have vulnerable status. SDM functions on the principle of comparing the environmental conditions of the known location of the species to novel climatic conditions (Pearson 2007). Several different algorithms have been developed to model species distribution, such as MaxEnt (Elith et al. 2011), BIOCLIM (Beaumont et al. 2005), and GARP (Peterson et al. 2007). The accuracy of each modeling system is dependent on the sampling size and ecology of the species. Ultimately, species distribution models are an effective tool that can provide focus to possible practical applications (Hernandez et al. 2006). Among these tools, MaxEnt has been used widely for many different species such as *Picrorhiza kurroa* Royle ex Benth. (Chandra et al. 2021), *Podophyllum hexandrum* (Royle) T.S. Ying (Banerjee et al. 2017), *Rhododendron niveum* Hook.f. (Chhetri & Badola 2017) and including vulnerable species such as *Ornduffia calthifolia* (F.Muell.) Tippet & Les, *O. marchantii* (Ornduff) Tippet & Les (Ball et al. 2020), and *Lavatera acerifolia* Cav. (Villa-Machío et al. 2020). MaxEnt uses presence-only data to create a probability map predicting the distribution of a species across a spatial dimension (Elith et al. 2011). Thus, the objectives of the present study were to: i. characterize the taxonomy and habitat ecology of the

taxa in Darjeeling Himalaya and, ii. identify current and future potential habitat and environmental variables determining distribution.

MATERIALS AND METHODS

Study Area

The study encompassed the Darjeeling Himalayan region that extends between 27°13'10"–26°27'05" N & 88°53'–87° 30" E covering an altitudinal range between 130–3,636 m in the lap of the eastern Himalaya hotspot. The region is bordered by Bangladesh to the south-east, Nepal to the west, and Bhutan to the east. The region is also flanked by the state of Sikkim (Figure 1).

As an extended part of the Himalayan hotspot, the region boasts several types of vegetation ranging from tropical to sub-alpine (Das 1995). A combination of topography & climate along with its location makes the region floristically diverse. The region harbours vegetation of Indo-Chinese, Indo-Malaysian, and western Himalayan origin including rare species such as *Gastrochilus corymbosus* A.P. Das & Chanda, *Liparis tigerhillensis* A.P. Das & Chanda, *Globba teesta* S. Nirola & A.P. Das to mention a few (Nirola & Das 2017).

The Species

The present study uses MaxEnt to explore the distribution of *Herpetospermum darjeelingense* (C.B. Clarke) H. Schaef. & S.S. Renner, a member of the family Cucurbitaceae in Darjeeling Himalaya (Image 1). The genus *Herpetospermum* comprises of four known species found restricted in the Himalaya and southeastern Asia (POWO 2021), of which three are found in the Darjeeling Himalayan region (Renner & Pandey 2013). *H. darjeelingense* (syn. *Edgaria darjeelingensis* C.B. Clarke) is one of the species found in the eastern Himalaya (Renner & Pandey 2013). The presence of this species has been recorded in Bhutan (Grierson & Long 1991), southern China, and Nepal (Renner & Pandey 2013). In India, the species is distributed sparsely in the states of Sikkim and Arunachal Pradesh. Threat search classified the species as Vulnerable in 2017 (BGCI 2021).

Species Occurrence Data

The occurrence points were gathered through a field study conducted during 2019–2020 within the Darjeeling Himalaya. The coordinate points in the locations were recorded using Garmin eTrex H. The collected coordinates were first converted to decimal degrees and then thinned using spThin package in R in order to

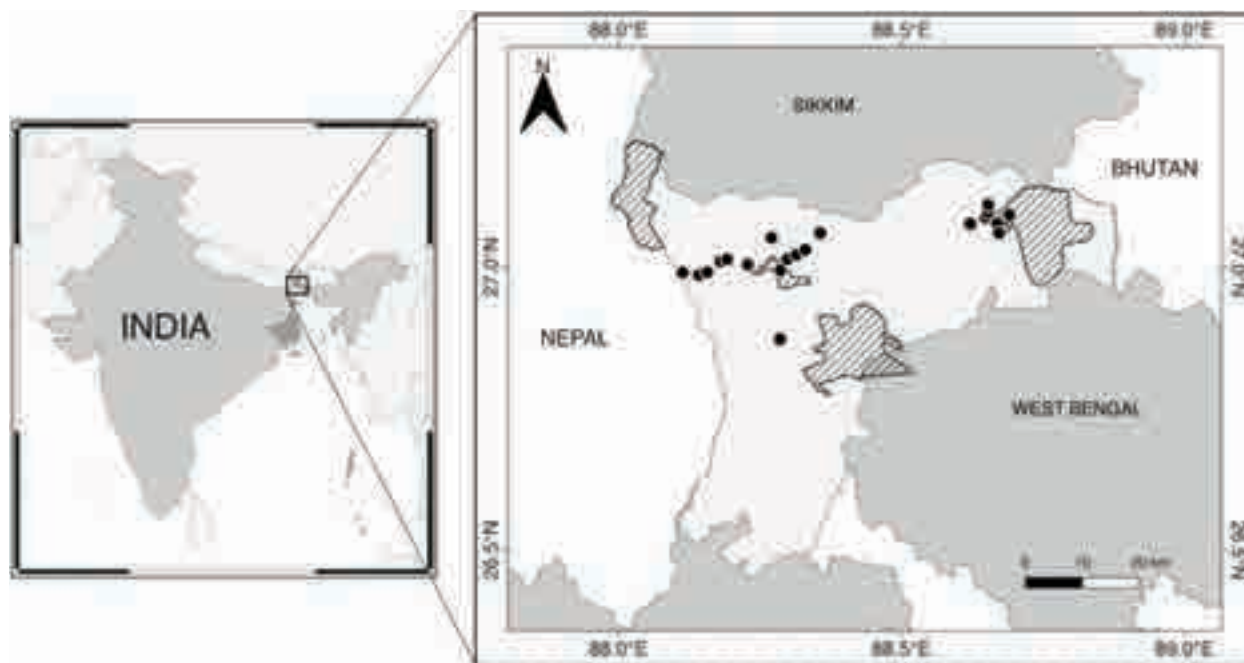


Figure 1. Study area. Shaded area indicates protected areas; black dots indicate occurrence points.

remove duplicates and to remove any coordinates with a distance of less than 1 km between them. The resulting 21 coordinates were used for modeling suitable habitat. The taxonomy of the species was studied through the collection of voucher specimens. Pollen grains were collected from the partially opened bud, and the process of acetolysis was followed (Erdtman 1960) and thereby, SEM observations were made. The population of the species was assessed along with its habitat ecology and the associated species.

Environmental variables

Elevation data were sourced at 30-arc second (~ 1 km²) resolution from WorldClim 2.1 (Fick & Hijmans 2017). From this, slope and aspect data were generated using QGIS 3.4 Madeira software in ASCII format. The elevation, slope, and aspect constituted the three topographic predictors used in this paper. The current bioclimatic variables were obtained from WorldClim 2.1 at 30-arc second (~ 1 km²) resolution (Fick & Hijmans 2017). The future bioclimatic variables were based on CMIP5, obtained from WorldClim 1.4 (Hijmans et al. 2005). The selected dataset were the GCMs (General Circulation Models) GFDL-CM3 (Griffies et al. 2011; Chaturvedi et al. 2012), CCSM4 (Meehl et al. 2012; Purohit & Rawat 2021) and MIROC5 (Watanabe et al. 2010) for years 2050 & 2070 for three different Representative Concentration Pathways (RCPs), RCP 2.6, RCP 4.5, and RCP 8.5. The RCP 2.6, RCP 4.5, and RCP 8.5

represent three different carbon emission levels (IPCC, 2014). All data were trimmed to the appropriate size and converted to ASCII format using QGIS 3.4 Madeira.

Modeling Procedure

First, highly correlated variables (variables with Pearson's coefficient r value > 0.9) were identified and removed using ENM Tools 1.3 (Warren et al. 2010) (Figure 2). The remaining list of environmental variables is given in Table 1. Overall, seven bioclimatic variables and three topographic variables, i.e., elevation, slope, and aspect, were used for modeling. Models were run on MaxEnt ver.3.4.1 (Phillips et al. 2006). As there were merely 21 occurrence points, only linear and quadratic features were applied. Five replicated models were run using the random test percentage of 25% (Srivastava et al. 2018; Qin et al. 2020). For predictions based on future climate, current occurrence data was projected onto future climatic variables. These were from the datasets GFDL-CM3, CCSM4, and MIROC5 for years 2050 & 2070; for RCP 8.5, 4.5 & 2.6. This resulted in 18 different future models to consider. An ensemble approach was applied wherein; the three different models from each GCM for each RCP of a particular year were combined (Araújo & New 2007; Khanum et al. 2013).

Model Validation

The area under the curve (AUC) values were used to assess individual models. Along with AUC, models were

Table 1. Site characteristics of *Herpetospermum darjeelingense* in different habitats.

Latitude (N)	Longitude (E)	Altitude (m)	Aspect	Slope (°)	Habitat	Population
26.99395	88.28557	2449	SW	0–15	Hilly slope	5
27.00408	88.22867	2176	SE	15–30	Roadside	1
27.05187	88.27033	1830	NE	15–30	Roadside	5
26.98553	88.1428	2246	SE	15–30	Roadside	1
26.99013	88.1141	2170	SW	15–30	Hilly slope	3
26.9908	88.15693	2197	SE	0–15	Hilly slope	3
27.00958	88.17978	2151	SE	15–30	Hilly slope	8
27.01318	88.19185	2188	SE	15–30	Hilly slope	23
26.87283	88.28515	1457	SW	15–30	Jhora	1
27.01334	88.29806	2134	SE	15–30	Roadside	2
27.02173	88.31473	1951	SE	15–30	Roadside	4
27.03048	88.3302	1799	SE	30–45	Stream bank, Hilly slope	8
27.0599	88.3569	1628	NE	15–30	Hilly slope	7
27.0764	88.62195	1847	NE	30–45	Hilly slope	2
27.08777	88.64798	2079	SE	15–30	Hilly slope	3
27.08153	88.67965	2219	SE	0–15	Hilly slope	5
27.09168	88.69067	1910	SW	15–30	Hilly slope	1
27.09618	88.6527	2150	NE	15–30	Hilly slope	4
27.10963	88.65315	1938	NE	15–30	Scrub	1
27.07688	88.66888	2090	SW	15–30	Scrub	1
27.06005	88.67223	1772	SW	15–30	Hilly slope	4

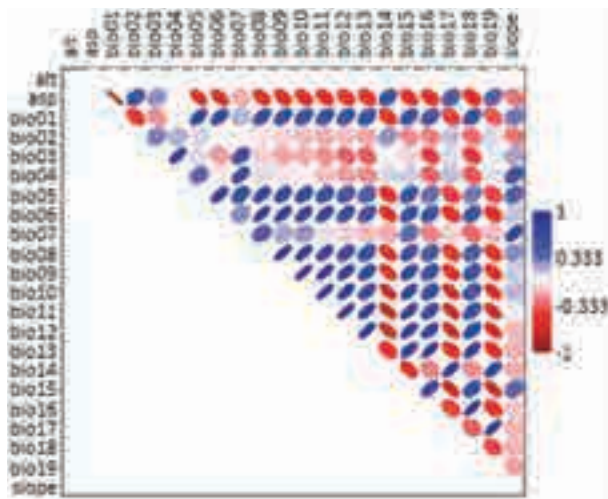


Figure 2. Pearson correlation of independent variables. Dark blue represents correlation > 0.9).

also appraised by true skill statistic (TSS) values (Allouche et al. 2006). TSS values were calculated for each model iteration with the lowest presence threshold (LPT). The value of LPT is equal to the lowest probability at a species occurrence point. LPT thus excludes all areas

that are at least not as suitable as locations where the species occurred (Pearson et al. 2007).

RESULTS

Taxonomy and Ecology of *H. darjeelingense*

H. darjeelingense is described as being an annual with a climbing habit, bifid tendrils, deeply cordate-ovate, and unlobed leaves. The leaves were pubescent with undulate and denticulate margin. The plant is dioecious with male flowers being paired. Bracts are absent or inconspicuous. Both male and female flowers have elongated calyx tube, teeth subulate; corolla is rotate, bright yellow, with deep lobes. Male flowers carry three stamens, anthers connate, single-celled. Female flowers are solitary, with ellipsoid ovary, three stigmas. Fruits are broadly fusiform, carrying about three-six seeds. SEM analysis of the pollen grains revealed that they are spherical, triplicate, with distinctly spinous exine (Image 1).

Ecologically, the species is found to grow on roadsides, hilly slopes, stream banks, jhoras, and scrubs

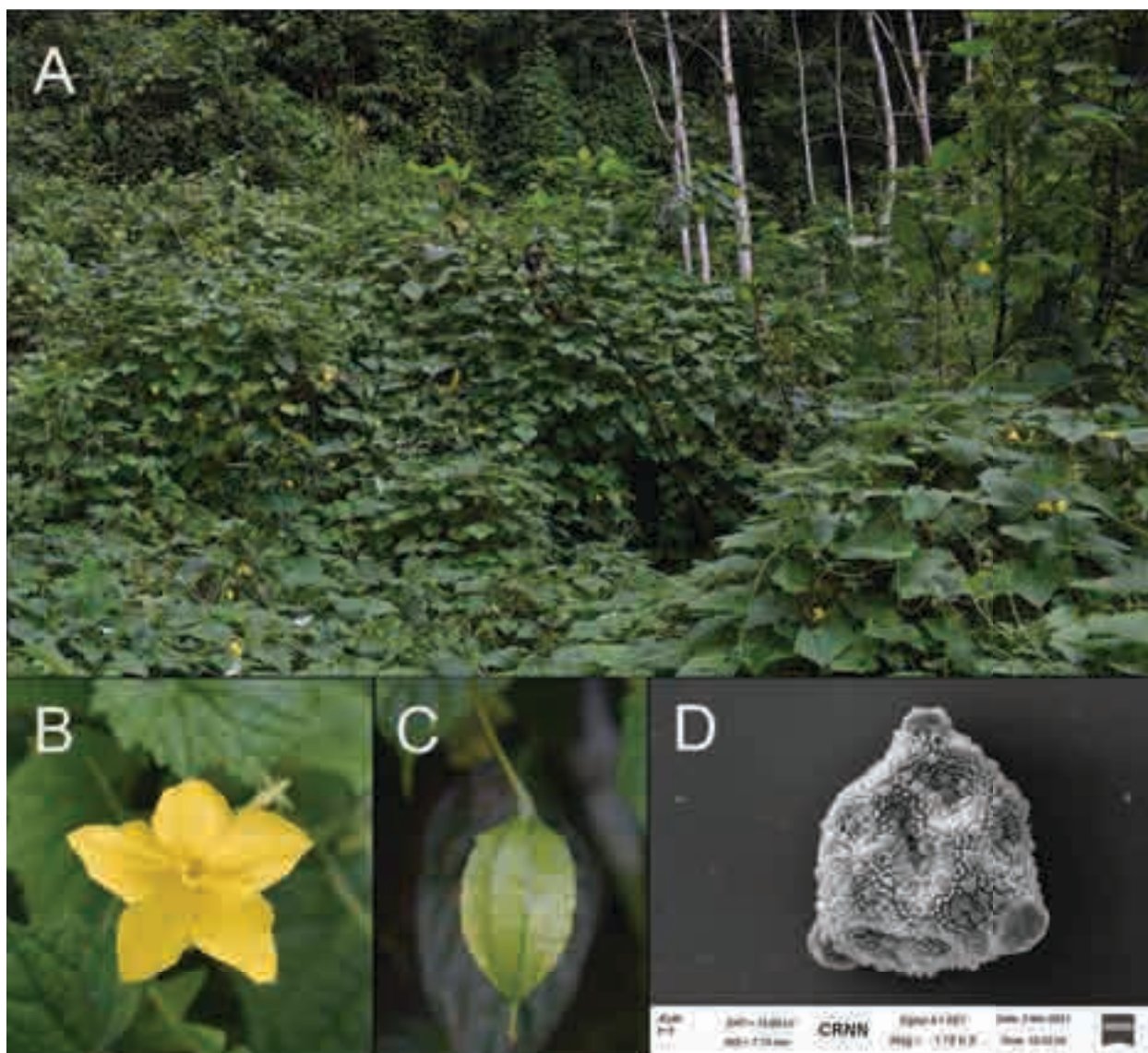


Image 1. *Herpetospermum darjeelingense*: A—Habit and habitat | B—Flower | C—Fruit | D—Pollen grain under SEM. © D. Boral.

Table 2. Variables used for species distribution modeling in MaxEnt.

Variable abbreviation	Variable Name	Units
BIO02	Mean diurnal range	°C
BIO03	Isothermality	%
BIO07	Temperature annual range	°C
BIO11	Mean temperature of coldest quarter	°C
BIO15	Precipitation seasonality	mm
BIO18	Precipitation of warmest quarter	mm
BIO19	Precipitation of coldest quarter	mm
ALT	Altitude	m
ASPECT	Aspect	NA
SLOPE	Slope	(°)

within an elevation range of around 1,400–2,600 m. The associated species in the niche includes major trees like *Magnolia cathcartii* (Hook.f. & Thom.) Noot., *Symplocos glomerata* King ex Clarke, *Alnus nepalensis* D. Don, and *Cryptomeria japonica* (Thunb. ex L.f.) D. Don. The associated undershrubs are *Tetrastigma serrulatum* (Roxb.) Planch., *Aconogonon molle* (D. Don) Hara, *Boehmeria macrophylla* Hornem., *Yushania maling* (Gamble) Majumdar & Karth., *Ageratina adenophora* (Spreng.) King & Rob, *Girardinia diversifolia* (Link) Friis, while the ground covers include *Galium elegans* Wall. ex Roxb., *Strobilanthes divaricata* (Nees) T. Anders., *Persicaria chinensis* (L.) H. Gross, *Drymaria cordata* (L.) Willd. ex Schult., *Pouzolzia hirta* Blume ex Hassk., *Lecanthus peduncularis* (Wall. ex Royle) Wedd., and

species of *Pilea*. It is difficult to tally number of individuals of *H. darjeelingense* as it has climbing/creeping habit and thus in some cases forms dense sprawling clumps. The site characteristics revealed 48% of the population was distributed towards south-east, followed by south-west with 28% and north-east with 24% aspect location. Majority of the populations was distributed on the hilly slope with around 15°–30° inclination followed by roadside while only few populations were distributed at steep habitat.

Reportedly, *H. darjeelingense* is used both as food (Mueller-Boeker 1993) and as medicine to treat cattle (Shrestha & Khadgi 2019), traditionally among different communities from the Himalayan belt. A recent study also reports the presence of 13 antioxidants from leaf material, indicating the pharmaceutical potential of the species (Chakraborty et al. 2021). The species is classified as Vulnerable (BGCI 2021) regionally in China. However, information regarding its current status in the study area is scant.

Habitat Suitability for Present Day

The different variables used for predicting suitable habitat for *H. darjeelingense* included temperature, precipitation data, altitude, slope, and aspect. The present-day model with the predicted suitable habitat is shown in Figure 3 along with the ROC curve and the jackknife in Figure 4. The current model performed very robustly with the AUC value at 0.986 and the TSS value 0.948. The potential distribution of *H. darjeelingense* was stretched over an area of 416.25 km² (13.21%) after application of LPT. The percentage of contribution is highest for the bioclimatic variable mean temperature in the coldest quarter (BIO11) at 61.2 %, followed by precipitation of seasonality (BIO15) at 24.5%, mean diurnal range (BIO02) at 4.4% and precipitation of warmest quarter (BIO18) at 4.4%. The jackknife also reveals that BIO11 is the most important environmental variable while the other influential variable according to the jackknife is precipitation of seasonality (BIO15) (Figure 4b).

Response to Variables

The species response curve of *H. darjeelingense* to each variable is depicted in Figure 5. The probability of the presence of the species increases with ALT sharply peaking at 2,000 m (Figure 5a) with the range 1,500–3,000 m. The altitude of almost all sample points fell within this range. For aspect, the response increases with an increase in degree (Figure 5b). For BIO02, BIO03, BIO18, response decreases with increase in variable

while, the response increases as BIO15 increases. For BIO11, suitable habitat requires a mean temperature ranging from 5°C–12°C in the coldest quarter. For BIO19, suitable habitat required mean precipitation between 40–90 mm for the coldest quarter.

Habitat Suitability for Future Models

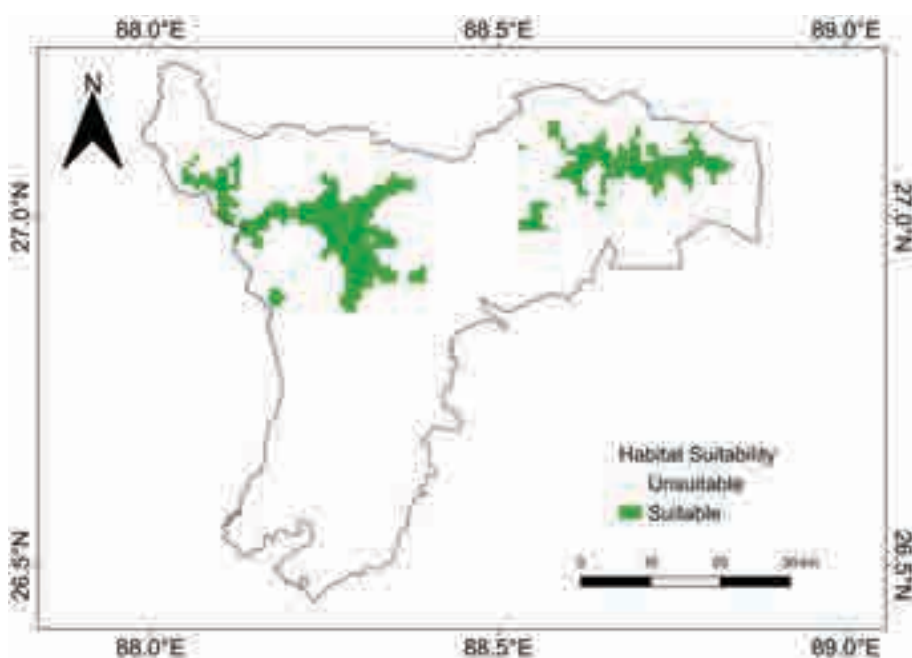
The six future ensemble models have an AUC value ranging from 0.99–0.985. The TSS value ranges from 0.903–0.944. The highest percentage of contribution is mean temperature in the coldest quarter (BIO11) for all six ensemble models. Similarly the altitude (ALT) has the highest permutation of importance for both the current and future models. The jackknife shows some difference in the results for the future models where ALT has the highest training gain when used in isolation in some models while mean temperature in the coldest quarter (BIO11) has the highest training gain when used in isolation in other models. The prediction accuracy details of the individual models, along with the ensemble models, are given in Table 3. After the LPT value (0.49) was applied for all future models, probable spatial distribution was 0 km² for all.

DISCUSSION

The present study explores the ecological status and assesses the habitat distribution of *H. darjeelingense* in current and future climate scenarios. Previous studies on other species have been conducted using MaxEnt, such as *Angelica glauca* Kitam. (Singh et al. 2020), *Rosa arabica* (Crép. ex Boiss.) Déségl. (Abdelaal et al. 2019), *Ixora* sp. (Banag et al. 2015), *Berkheya cuneata* (Thunb.) Willd. (Potts et al. 2013), *Acer cappadocicum* subsp. *lobelia* (Ten.) A.E. Murray (Sumarga 2011), *Pterocarpus santalinus* L.f. (Babar et al. 2012), *Aglaia bourdillonii* Gamble (Irfan-Ullah et al. 2006). MaxEnt has also been used to explore the distribution of endangered species such as *Dioscorea* sp. (Hills et al. 2019). MaxEnt is one of several modeling algorithms available for species distribution modeling. MaxEnt predicts probable distribution using presence-only data and a set of climatic grids generating output where each grid cell has a value ranging from 0 (least suitable) to 1 (most suitable) (Phillips et al. 2017). MaxEnt is also effective even with small sample sizes making it suitable for studying endangered species (Pearson et al. 2007). Concerning the performance of MaxEnt models, both AUC and TSS values were used. Swets (1988) classified model performance into failing (0.5–0.6), bad (0.6–0.7),

Table 3. Prediction accuracy with important variables of *Herpetospermum darjeelingense* models.

			AUC	TSS	Percentage contribution		Permutation importance		Jackknife training gain	
					Variable	Value	Variable	Value	In isolation	In absence
Current			0.986	0.949	BIO11	61.2	ALT	59.5	BIO11	BIO15
2050	RCP 2.6	CCSM4	0.988	0.946	BIO11	59.9	ALT	66.2	ALT	BIO15
		GFDL-CM3	0.987	0.942	BIO11	63	ALT	46.2	BIO11	BIO15
		MIROC5	0.987	0.933	BIO11	60.3	ALT	61.2	BIO11	BIO15
		Ensemble	0.987	0.94	BIO11	61.1	ALT	57.9	-	-
	RCP 4.5	CCSM4	0.985	0.957	BIO11	63.6	ALT	46.7	BIO11	BIO15
		GFDL-CM3	0.986	0.93	BIO11	63.6	ALT	56.8	BIO11	BIO15
		MIROC5	0.99	0.93	BIO11	57.9	ALT	43.7	BIO11	BIO15
		Ensemble	0.987	0.939	BIO11	61.7	ALT	49.1	-	-
	RCP 8.5	CCSM4	0.989	0.966	BIO11	60.3	ALT	57.8	BIO11	BIO15
		GFDL-CM3	0.989	0.946	BIO11	58.7	ALT	63.1	BIO11	BIO15
		MIROC5	0.985	0.921	BIO11	60.5	ALT	45.5	BIO11	BIO15
		Ensemble	0.987	0.944	BIO11	59.8	ALT	55.5	-	-
2070	RCP 2.6	CCSM4	0.988	0.954	BIO11	61.7	ALT	48.4	BIO11	BIO15
		GFDL-CM3	0.989	0.94	BIO11	56.3	ALT	60.7	BIO11	BIO15
		MIROC5	0.988	0.906	BIO11	61.5	ALT	52.7	ALT	BIO15
		Ensemble	0.988	0.933	BIO11	59.8	ALT	53.9	-	-
	RCP 4.5	CCSM4	0.988	0.926	BIO11	60.9	ALT	32	BIO11	BIO15
		GFDL-CM3	0.989	0.891	BIO11	62.5	ALT	48.9	BIO11	BIO15
		MIROC5	0.986	0.892	BIO11	61	ALT	54.8	BIO11	BIO15
		Ensemble	0.987	0.903	BIO11	61.4	ALT	45.2	-	-
	RCP 8.5	CCSM4	0.985	0.949	BIO11	59.4	ALT	61.6	BIO11	BIO15
		GFDL-CM3	0.988	0.955	BIO11	60.9	ALT	47.4	BIO11	BIO15
		MIROC5	0.988	0.918	BIO11	60.4	ALT	58.6	BIO11	BIO15
		Ensemble	0.987	0.941	BIO11	60.2	ALT	55.9	-	-

Figure 3. Current climate model of *Herpetospermum darjeelingense* showing potential distribution.

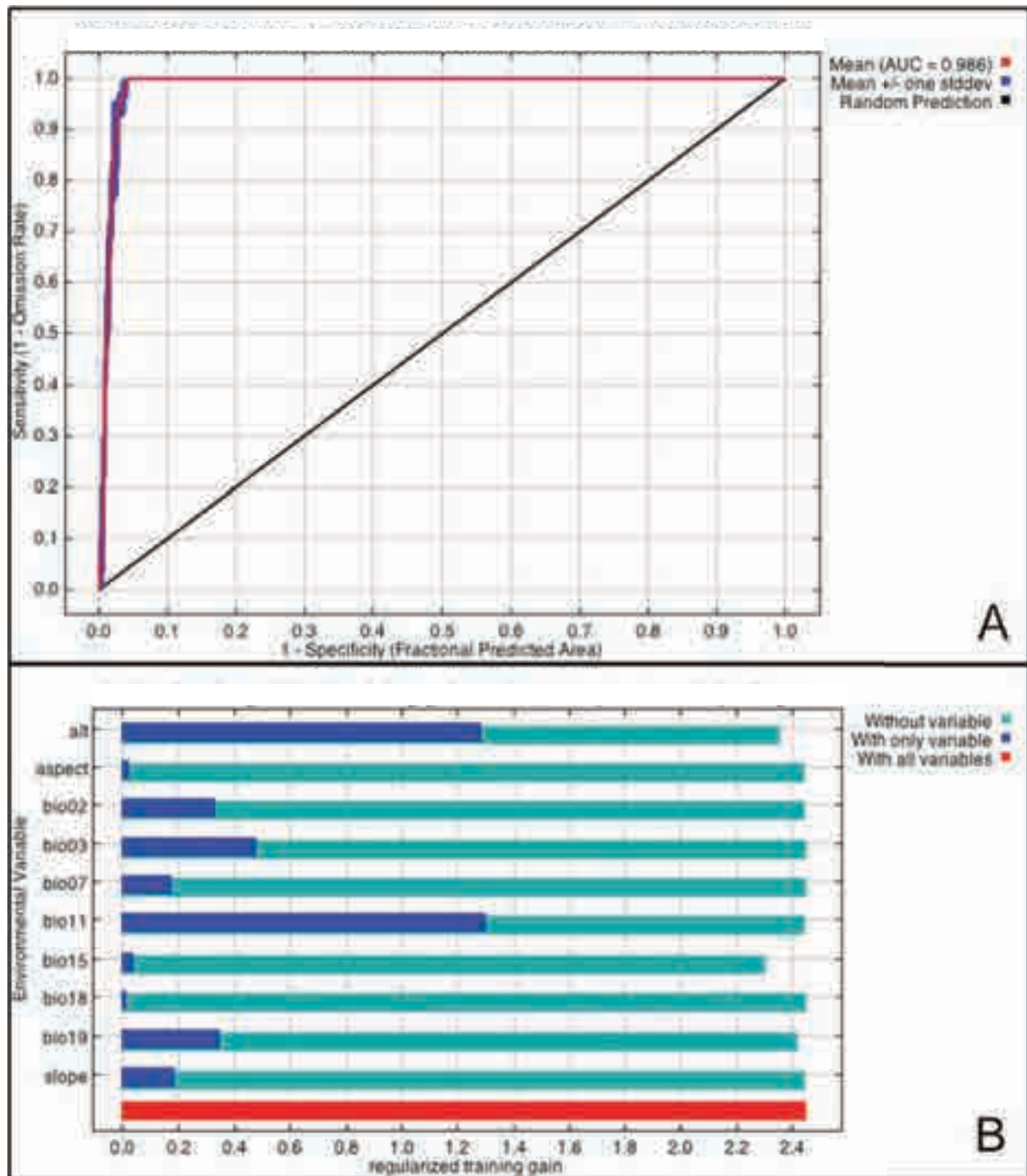


Figure 4. Current climate model of *Herpetospermum darjeelingense*: A—ROC Curve | B—Jackknife of regularized training gain.

reasonable (0.7–0.8), good (0.8–0.9), or great (0.9–1) based on AUC value. Like AUC, TSS also ranges from 0–1, with a higher value indicating a better-performing model (Allouche et al. 2006). The LPT was also used to prevent an over-fitted model. In the current study, only about 13.21% of the total study area was determined

to be suitable habitat for *H. darjeelingense*. The current model was well-performing, with high AUC (0.986) & TSS (0.948) values.

The IPCC 5th assessment report (IPCC 2014) presents the projected climate in the future driven by anthropogenic carbon emissions. The report highlights

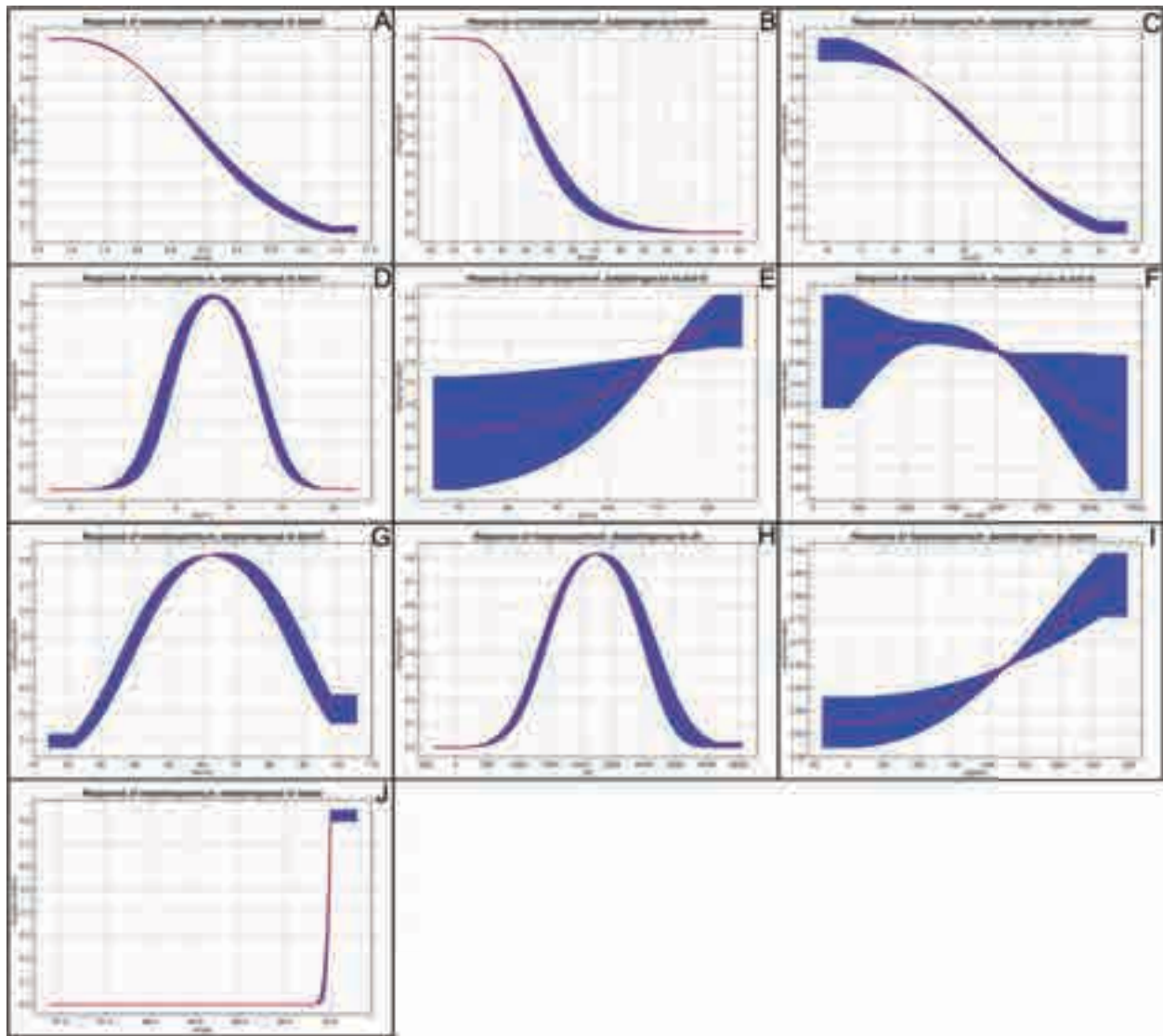


Figure 5. Response curves of *Herpetospermum darjeelingense*: A—BIO02 | B—BIO03 | C—BIO07 | D—BIO11 | E—BIO15 | F—BIO18 | G—BIO19 | H—Altitude | I—Aspect | J—Slope.

the projected scenarios based on the mitigation strategy applied. The RCPs 2.6, 4.5, and 8.5 represents scenarios where either stringent, intermediate or poor implementation of climate strategy occurred. As each GCM is published by separate research groups, it can make modeling future climate tricky. Hence, the ensemble method as per Khanum et al. (2013) was applied which reduces the ambiguity of using a single GCM. Overall, all future models created using the ensemble method, which combines three different GCMs, show the probable complete disappearance of *H. darjeelingense*. Hence, no matter the climate change mitigation strategy, it is quite possible that the species under study might disappear from the study area by 2050. In the case of endangered species, a complete

disappearance from the local environment can indicate further downstream effects on other plants. It should be noted that the results species distribution models, are based on extrapolation from available data and methods (Elith & Leathwick 2009). However, these models can provide valuable awareness of urgent future steps to be taken for the preservation of the species under study.

CONCLUSION

The present study highlights the probable suitable habitat of the cucurbit *Herpetospermum darjeelingense* in the future as well as the present day. The taxon that is often found along roadsides and hilly slopes make its

current population vulnerable to habitat destruction due to anthropogenic pressure as well as natural catastrophes. This along with climate change can result in the complete disappearance of the species. MaxEnt modeling of the present-day scenario exhibits a narrow habitat range. Furthermore, future models show that regardless of the climate mitigation strategy, the species faces local extinction. Keeping in mind the availability of limited data on distribution coordinates and population status of the taxa including the rarity of the species in the present study, the taxa should be immediately assigned to Endangered in the IUCN Red List. Furthermore, an urgent requirement to investigate active in situ and ex situ conservation strategies through botanical gardens and local nurseries is of the utmost priority at this juncture because the taxon has both traditional and pharmaceutical potential. One possible method can include the collection of seeds for storage and germination.

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Noor Fatima¹ & Ding Yang²

¹noorfatima8482@gmail.com, ²dyangcau@126.com (corresponding author)

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Author details: NOOR FATIMA is a PhD student of Prof. Ding Yang. Her research interest is the systematics of Diptera. DING YANG is a professor of systematics of Diptera and Megaloptera at the College of Plant Protection, China Agricultural University, Beijing, China.

Author contributions: NF lead author. Assisted in the study conception and design, collected, analyzed data, interpreted the results and drafted the manuscript. DY supervised this study and assisted in the analysis of data and interpretation of results. Reviewed the draft manuscript and added critically important comments.

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INTRODUCTION

Diptera is one of the largest and diverse order of class Insecta having worldwide distribution. It contains approximately 160,000 species, 10,000 genera, 150 families, 22–32 superfamilies, 8–10 infraorders, and three suborders (McAlpine & Wood 1989; Thompson 2008; Chapman 2009; Pape & Thompson 2013; Borkent et al. 2018; Evenhuis & Pape 2021). It represents more than 10–12% of animal species or 10% of planetary biota and 14% of the world's known insect fauna (Lambkin et al. 2013; Thompson & Pape 2016). According to the recent estimate, there are 23,000 species of Diptera in Oriental region, 22,000 in Nearctic region, 20,000 in the Afrotropical region, 19,000 in the Europe, and Australasian region (Pape et al. 2015).

Dipterans occur in almost all terrestrial and freshwater habitats where they display a wide range of life histories and feeding habits. From parasites to predators, leaf miners and filter feeders, flies have diversified to exploit almost all organic substrates for their development (Marshall 2012; Courtney et al. 2017). True flies having economic and ecological importance have an impact on human life much greater than any other group of insects (Mayhew 2007). The dipterous fauna of Pakistan is under studied and has not been compiled previously. Previously, the fauna of Azad Jammu & Kashmir has been primarily updated with 153 species of flies.

MATERIALS AND METHODS

Data sources

In this paper, we followed the world's catalogue and recent regional published papers for nomenclature: Calliphoridae (Kurahashi & Afzal 2002; Hassan et al. 2018; Yan et al. 2021), Chloropidae (Nartshuk 2012), Dryomyzidae (Mathis & Sueyoshi 2011), Fanniidae (Nishida 1989), Rhinophoridae (Cerretti et al. 2020), Sarcophagidae (Sugiyama 1989), Sepsidae (Pont & Meier 2002; Ozerov 2005), Sphaeroceridae (Marshall et al. 2011), Stratiomyidae (Woodley 2001; Rozkošný & Kovac 2003; Rozkošný & Hauser 2009), Syrphidae (Mengual et al. 2020; Steenis et al. 2021), Tabanidae (Stone & Philip 1974), Tachinidae (O'Hara & Cerretti 2016; O'Hara et al. 2021), Tephritidae (Özgür & Kütük 2003; Agarwal & Sueyoshi 2005; Wang & Chen 2002; Halder et al. 2015), Tipulidae (Alexander & Alexander 1973), Limoniidae (Bhagat 2014), and distributional records by following the book entitled "Insect Fauna of Azad Jammu & Kashmir" and other updated published literature from Pakistan/

Azad Jammu & Kashmir: Calliphoridae (Kurahashi & Afzal 2002; Hassan et al. 2018), Chamaemyiidae (Alam et al. 1969), Chloropidae (Alam et al. 1969; Kanmiya 1989), Dryomyzidae (Kurahashi 1989; Hassan et al. 2018), Fanniidae (Nishida 1989), Rhinophoridae (Cerretti et al. 2020), Sarcophagidae (Sugiyama 1989), Sepsidae (Iwasa 1989; Hassan et al. 2017, 2018, 2021; Fatima et al. 2019), Sphaeroceridae (Hayashi 1991), Stratiomyidae (Hassan et al. 2019), Syrphidae (Arif 2002; Ghorpadé & Shehzad 2013; Shehzad et al. 2017; Hassan et al. 2017, 2018, 2020, 2021), Tabanidae (Stone & Philip 1974), Tachinidae (Mohyuddin 1981), Tephritidae (Alam et al. 1969; Brake 2011; Zubair et al. 2019), Tipulidae (Alexander 1959), and Limoniidae (Bigot 1891; Alexander 1966).

Distribution area format

The present manuscript includes country wise and world wise distributions of true flies. In countrywide distribution Pakistan is followed by the Province/ State name or Territory, Division name and the exact locality. In worldwide distribution only country name is given. List of abbreviations for the regional distribution is as: prov. = province; stat. = state; terr. = territory; div. = division; Islamabad capi. = Islamabad.

Azad Jammu & Kashmir and Gilgit Baltistan as a political units.

In 1947, Indian sub-continent was divided into two independent countries, Pakistan and India. At that time the Princely states of Indian sub-continent were given an option to join Pakistan or India by their own choice. The territory of Kashmir under the administrative region of Pakistan is Azad Jammu & Kashmir, and the Indian administered region is Jammu Kashmir. This work documented the flies of "Pakistani Administered Kashmir" known as Azad Jammu & Kashmir and Gilgit-Baltistan. Most of the work done before the partition of Indo-Pak subcontinent mentioned only Kashmir, but now it is difficult to find out exact locality either as Pakistan administrated part or Indian administrated area of Kashmir. We have tried our best to include only those species which are presented in Pakistani administrated area.

Physiography of Azad Jammu & Kashmir and Gilgit-Baltistan:

Azad Jammu & Kashmir: Azad Jammu & Kashmir lies in the east of Pakistan. It covers an area of 13,297 km². The height of area ranges 606–4545 m. It is located between mountain ranges and characterized by deep ravines, rugged, undulating terrain with valleys and

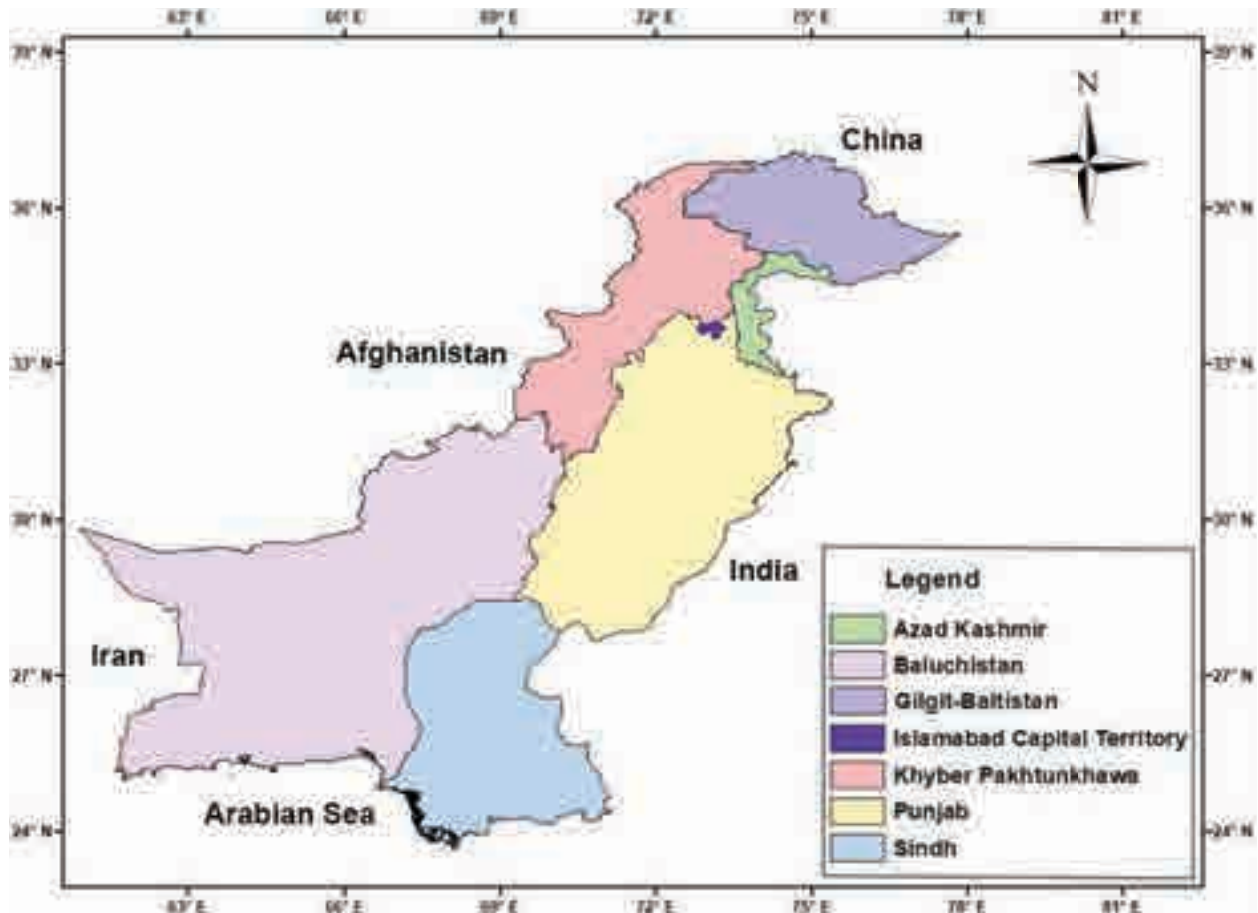


Figure 1. Outline map of Pakistan (original map based on ArcGis 10.2 boundary files).

patchy plains having diverse flora and fauna. Major rivers are Jhelum, Neelum, and Poonch. Azad Jammu & Kashmir is one-sixth of the size of Gilgit-Baltistan. Climate varies with the altitudes of the area. its snow-covered peaks, dense forests, winding rivers, turbulent foaming streams, wheat scented valleys and velvet green plateaus make it an attractive place with a diverse insect population (Behera 2007).

Gilgit-Baltistan: Gilgit-Baltistan is located in the northern Pakistan. It covers an area of over 72,971 km² and is highly mountainous. Gilgit-Baltistan is known for K2 (8,611 m) and is the second highest mountain on Earth, three of the world's longest glaciers outside the Polar Regions, four famous mountain ranges (Himalaya, Karakorum, Hindukush, and Pamirs), more than 50 peaks above 7,000 m, the longest glaciers (Siachen Glacier) and attractive landscapes (Wala 1994; Virk et al. 2003). It is also the Land of Indus River which is the largest river of Pakistan and major source of agricultural strength in Pakistan (Zain 2010). Climatic conditions, vegetation, and topography of Azad Jammu & Kashmir and Gilgit Baltistan territory are highly

suitable for dipterous fauna. This paper summarizes the current documented fauna of flies in both Azad Jammu & Kashmir and Gilgit-Baltistan. There are great chances of getting additional taxa from the high mountains of Azad Jammu & Kashmir and Gilgit-Baltistan.

RESULTS

A total of 64 genera and 153 species in 16 families are being documented in current inventory from Azad Jammu & Kashmir and Gilgit Baltistan territory.

Order Diptera Linnaeus, 1758

Suborder Nematocera Dumeril, 1805

Family Limoniidae Rondani, 1856

Subfamily Limoniinae Speiser, 1909

Genus *Limonia* Meigen, 1803

1. *Limonia neananta* Alexander, 1966

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Astore, Minimarg (Bigot 1891; Alexander 1966)); India (Bigot 1891; Alexander 1966).

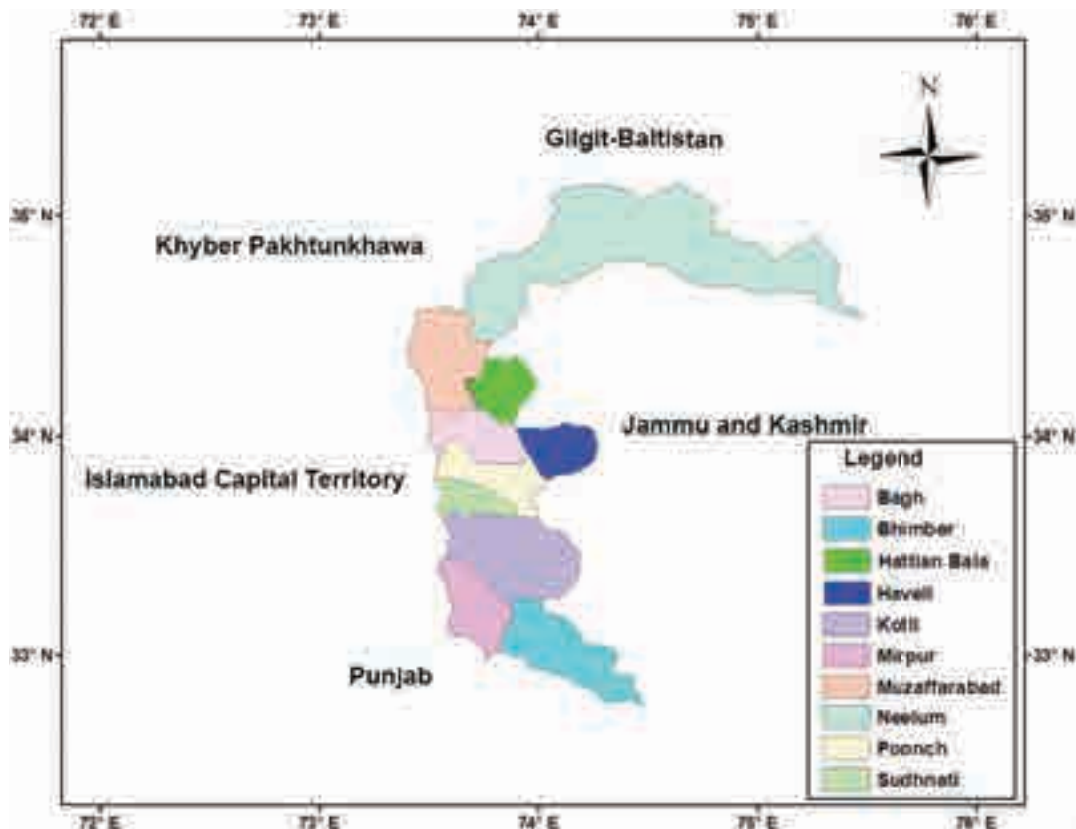


Figure 2. District boundaries of Azad Jammu & Kashmir (original map based on ArcGis 10.2 boundary files).

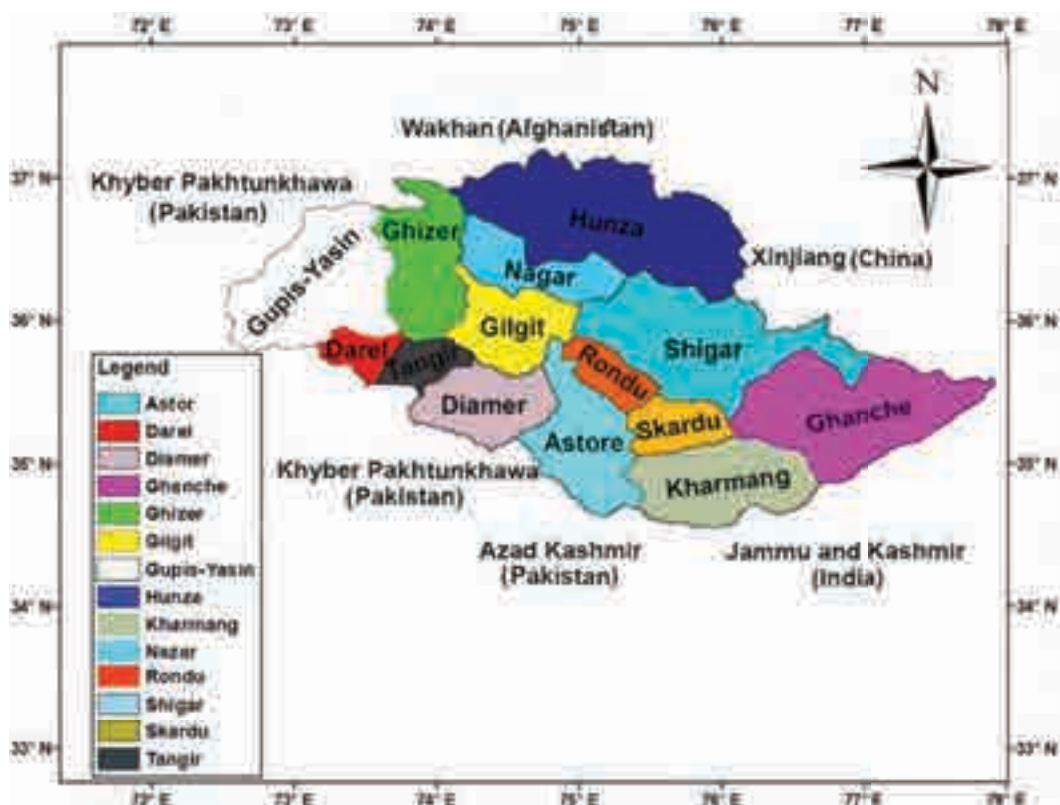


Figure 3. District boundaries of Gilgit-Baltistan (original map based on ArcGis 10.2 boundary files).

Subfamily Chioneinae Rondani, 1861**Genus *Erioptera* Meigen, 1803****2. *Erioptera palliclavata* Alexander, 1935**

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Minimarg (Bigot 1891)); India (Bhagat 2014; Banerjee et al. 2018).

Genus *Gonomyia* Meigen, 1818**3. *Gonomyia dissidens* Alexander, 1957**

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Astore (Bigot 1891)); India (Bigot 1891; Banerjee et al. 2018).

Family Tipulidae Latreille, 1802**Subfamily Chioneinae Rondani, 1861****Genus *Ctenophora* Meigen, 1803****4. *Ctenophora longisector* Alexander, 1959**

Distribution: Pakistan (Gilgit-Baltistan terr., Baltistan div., Skardu (Alexander 1959)).

Suborder Brachycera Macquart, 1834**Family Stratiomyidae Latreille, 1802****Subfamily Sarginae Leach, 1815****Genus *Ptecticus* Loew, 1855****5. *Ptecticus vulpianus* (Enderlein, 1914)**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2019)); India, Indonesia, Japan, Malaysia, Taiwan (Rozkošný & Hauser 2009).

6. *Ptecticus melanurus* (Walker, 1848)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2019)); China, India, Indonesia, Thailand, Singapore, Nepal, West and East Malaysia, Malaysia (Rozkošný & Kovac 2003).

Family Tabanidae Latreille, 1802**Subfamily Chrysopsinae Lutz, 1905****Genus *Haematopota* Meigen, 1803****7. *Haematopota kashmirensis* Stone and Philip, 1974**

Distribution: Pakistan (Gilgit-Baltistan terr., Baltistan div., Skardu; Khyber Pakhtunkhwa prov., Hazara div., Lalazar, Naran Valley (Stone & Philip 1974)); India (Stone & Philip 1974; Banerjee et al. 2018).

Family Syrphidae Latreille, 1802**Subfamily Eristalinae Newman, 1834****Genus *Mallota* Meigen, 1822****8. *Mallota rufipes* Brunetti, 1913**

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div.,

Goharaabad, Khyber Pakhtunkhwa prov., Dera Ismail Khan div., Dera Ismail Khan city (Arif 2001; Ghorpadé & Shehzad 2013; Ghorpadé 2015)); India (Ghorpadé 2015; Banerjee et al. 2018).

Genus *Mesembrius* Rondani, 1857**9. *Mesembrius quadrivittatus* (Wiedemann, 1819)**

Distribution: Pakistan (Gilgit-Baltistan terr., Skardu div., Khaplo, Dagboni; Punjab prov., Gujranwala div., Shakargarh (Hassan et al. 2017; Shehzad et al. 2017)); India, Nepal (Brunetti 1923; Thapa 2000; Ghorpadé 2015).

Genus *Ceriana* Fabricius, 1794**10. *Ceriana dimidiatipennis* (Brunetti, 1923)**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake; Gilgit-Baltistan terr., Gilgit div., Gorikot; Balochistan prov., Quetta div., Quetta; Khyber Pakhtunkhwa prov., Hazara div., Abbottabad city, Kohat div., Hangu, Malakand div., Swat city (Brunetti 1923; Alam et al. 1969; Knutson et al. 1975; Aslamkhan et al. 1997; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Hassan et al. 2018)); India (Brunetti 1923; Ghorpadé 2015).

11. *Ceriana brevis* (Brunetti, 1923)

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Brunetti 1923; Aslamkhan et al. 1997; Ghorpadé & Shehzad 2013; Ghorpadé 2015)); India (Brunetti 1923; Ghorpadé & Shehzad 2013; Ghorpadé 2015).

Genus *Eristalis* Latreille, 1804**Subgenus *Eoseristalis* Kanervo, 1938****12. *Eristalis (Eoseristalis) albibasis* Bigot, 1880**

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Karimabad (Shehzad et al. 2017)); India (Brunetti 1923; Ghorpadé 2015; Thapa 2000; Banerjee et al. 2018).

13. *Eristalis (Eoseristalis) arbustorum* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley, Singolla, Jandala; Gilgit-Baltistan terr., Gilgit div., Gohrabad.; Khyber Pakhtunkhwa prov., Malakand div., Dir, Dorosh; Balochistan prov., Sibi div., Ziarat; Punjab prov., Multan div., Multan, Rawalpindi div., Murree (Aslamkhan et al. 1997; Arif 2001; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Hassan et al. 2018)); Afghanistan, India (Brunetti 1923; Ghorpadé 2015; Banerjee et al. 2018).

14. *Eristalis (Eoseristalis) cerealis* Fabricius, 1805

Distribution: Pakistan (Azad Jammu & Kashmir terr.,

Poonch div., Banjosa lake, Datote, Jandala, Sangolla; Khyber Pakhtunkhwa prov., Malakand div., Meena Khor, Lawari Tunnel, Hazara div., Punjab prov., Rawalpindi div., Murree, Dana (Shehzad et al. 2017; Hassan et al. 2018)); India, Myanmar, Nepal (Ghorpadé 2015; Thapa 2000; Banerjee et al. 2018).

15. *Eristalis (Eoseristalis) himalayensis* Brunetti, 1908

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake (Hassan et al. 2018)); India, Nepal (Thapa 2000; Ghorpadé 2015; Banerjee et al. 2018).

Genus *Eristalis* Latreille, 1804

Subgenus *Eristalis* Latreille, 1804

16. *Eristalis tenax* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Jandala, Sangolla, Hajira; Balochistan prov., Quetta div., Quetta, Sibi div., Sibi, Ziarat; Gilgit-Baltistan terr., Baltistan div., Gorikot, Karimabad; Khyber Pakhtunkhwa prov., Hazara div., Hazara, Peshawar div., Peshawar city; Punjab prov., Rawalpindi div., Murree, Ghora Gali, Ali Pur, Faisalabad div., Faisalabad, Multan div., Khanewal, Multan, Lahore div., Lahore, Gujranwala div., Shakargarh (Rahman 1942; Aslamkhan et al. 1997; Arif 2001; Saleem et al. 2001; Sajjad & Saeed 2010; Sajjad et al. 2010; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Hassan et al. 2017, 2018; Shehzad et al. 2017; Banerjee et al. 2018)); Widely distributed throughout the world, in all zoogeographical regions (van Veen 2010; Ghorpadé 2015; Banerjee et al. 2018).

Genus *Eristalinus* Rondani, 1845

Subgenus *Eristalinus* Rondani, 1845

17. *Eristalinus (Eristalinus) aeneus* (Scopoli, 1763)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake; Balochistan prov., Quetta div., Quetta, Sibi div., Ziarat, Makran div., Panjgur; Khyber Pakhtunkhwa prov., Peshawar div., Peshawar city, Malakand div., Chitral, Garam Chashma; Punjab prov., Dera Ghazi Khan div., Chit Dagar, Jatoi, Multan div., Multan, Khanewal, Rawalpindi div., Alipur, Jhelum, Faisalabad div., Faisalabad, Lahore div., Lahore, Bahawalpur div., Bahawalpur, Zahir Peer, Gujranwala div., Shakargarh; Sindh prov., Karachi div., Karachi (Rahman 1942; Aslamkhan et al. 1997; Arif 2001; Saleem et al. 2001; Arif et al. 2002; Saeed et al. 2008; Sajjad et al. 2008, 2010; Sajjad & Saeed 2010; Ali et al. 2011; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); Afghanistan, India (Ghorpadé 2015).

18. *Eristalinus (Eristalinus) arvorum* (Fabricius, 1787)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake Islamabad capi., Chak Shehzad; Punjab prov., Dera Ghazi Khan div., Chit Dagar, Rawalpindi div., Murree, Ghora Gali, Multan div., Multan, Khanewal, Lahore div., Lahore, Gujranwala div., Shakargarh, Noor kot; Sindh prov., Karachi div., Karachi (Aslamkhan et al. 1997; Saeed et al. 2008; Sajjad & Saeed 2010; Sajjad et al. 2010; Ali et al. 2011; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); India, Nepal, Malaysia (Ghorpadé 2015; Thapa 2000; Heo et al. 2020).

19. *Eristalinus (Eristalinus) megacephalus* (Rossi, 1794)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake; Punjab prov., Gujranwala div., Shakargarh, Noor kot (Hassan et al. 2017, 2018)); Afghanistan, India, Nepal (Ghorpadé 2015).

20. *Eristalinus (Eristalinus) obliquus* (Wiedemann, 1824)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake; Punjab prov., Gujranwala div., Shakargarh (Hassan et al. 2017, 2018)); India, Nepal, Sri Lanka (Ghorpadé 2015).

21. *Eristalinus (Eristalinus) sepulchralis* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley; Balochistan prov., Quetta div., Quetta, Makran div., Panjgur; Islamabad capi., Chak Shehzad; Khyber Pakhtunkhwa prov., Peshawar div., Peshawar city; Punjab prov., Rawalpindi div., Jhelum (Aslamkhan et al. 1997; Arif 2001; Saleem et al. 2001; Arif et al. 2002; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, China, India (Ghorpadé 2015).

22. *Eristalinus (Eristalinus) tarsalis* (Macquart, 1855)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake, Hajira; Punjab prov., Gujranwala div., Shakargarh (Hassan et al. 2017, 2018)); Nepal, India (Thapa 2000; Ghorpadé 2015).

Subgenus *Eristalodes* Mik, 1897

23. *Eristalinus (Eristalodes) taeniops* (Wiedemann, 1818)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Jandala, Sangolla, Hajira, Hussainkot; Balochistan prov., Quetta div., Quetta,

Pishin; Khyber Pakhtunkhwa prov., Malakand div., Lawari Tunnel; Punjab prov., Dera Ghazi Khan div., Muzaffargarh, Multan, Khanewal, Faisalabad div., Faisalabad, Jhang, Rawalpindi div., Murree, Ghora Gali, Ali Pur, Bahawalpur div., Zahir Peer, Gujranwala div., Shakargarh (Rahman 1942; Aslamkhan et al. 1997; Arif 2001; Sajjad & Saeed 2009, 2010; Sajjad et al. 2010; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, Nepal, India (Thapa 2000; Ghorpadé 2015).

Genus *Syrirta* Lepeletier & Serville, 1828

24. *Syrirta orientalis* Macquart, 1842

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley; Punjab prov., Dera Ghazi Khan div., Muzaffargarh, Lahore div., Lahore, Gujranwala div., Shakargarh (Alam et al. 1969; Aslamkhan et al. 1997; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); India, Nepal (Ghorpadé 2015).

25. *Syrirta pipiens* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, Hajira Khyber Pakhtunkhwa prov., Banu div., Banu, Hazara div., Abbottabad city, Chattr Plan, Kohat div., Hangu, Kaghan, Narran, Malakand div., Punjab prov., Dera Ghazi Khan div., Muzaffargarh, Multan div., Multan, Khanewal, Rawalpindi div., Murree, Gujranwala div., Shakargarh (Alam et al. 1969; Sajjad & Saeed 2010; Sajjad et al. 2010; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); Africa, Asia, Europe, India, Nepal, North America (Thapa 2000; Ghorpadé 2015).

Genus *Rhingia* Scopoli, 1763

26. *Rhingia angusticincta* Brunetti, 1908

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa (Hassan et al. 2018)); India (Ghorpadé 2015).

27. *Rhingia siwalikensis* Nayar, 1968

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa (Hassan et al. 2018)); India, Nepal (Ghorpadé 2015)).

Genus *Xylota* Meigen, 1822

28. *Xylota coquillettii* Hervé-Bazin, 1914

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot (Hassan et al. 2021a)); Russia, Japan, China, South Korea (Jeong & Han 2019).

29. *Xylota nursei* Brunetti, 1923

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot; Khyber Pakhtunkhwa province: Peshawar div., Hayatabad, Nowai Kalai, Pawaki (Hassan et al. 2021a; Ghorpadé & Shehzad 2013; Shehzad et al. 2017)); India (Brunetti 1923; Ghorpadé 2015).

Subfamily Syrphinae Samouelle, 1819

Genus *Baccha* Fabricius, 1805

30. *Baccha maculata* Walker, 1852

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa; Punjab prov., Rawalpindi div., Murree, Numb Behra Mall, Kuldana (Hassan et al. 2018)); India, Nepal (Thapa 2000; Ghorpadé 2015; Banerjee et al. 2018).

Genus *Platycheirus* Lepeletier and Serville, 1828

31. *Platycheirus albimanus* (Fabricius, 1781)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); India, Nepal (Thapa 2000; Ghorpadé 2015; Banerjee et al. 2018).

32. *Platycheirus ambiguus* (Fallén, 1817)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); Afghanistan, India (Ghorpadé 2015; Banerjee et al. 2018).

Genus *Volucella* Geoffroy, 1762

33. *Volucella peleterii* Macquart, 1834

Distribution: Pakistan (Azad Jammu & Kashmir terr., Muzaffarabad div., Guldana; Gilgit-Baltistan terr., Gilgit div., Gilgit (Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2020)); India (Ghorpadé 2015).

34. *Eristalinus ruficauda* Brunetti, 1907

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Doesai (Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2020)); India (Ghorpadé 2015).

Genus *Paragus* Latreille, 1804

Subgenus *Pandasyopthalmus* Stuckenberg, 1954

35. *Paragus (Pandasyopthalmus) annandalei* Ghorpadé, 1992

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); India (Ghorpadé 2015).

36. *Paragus (Pandasyopthalmus) politus* Wiedemann, 1830

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley Jandala, Hajira; Islamabad capital, Shakarparia; Khyber Pakhtunkhwa: Hazara div., Balakot, Malakand div., Swat, Kohat div., Kohat city; Punjab prov., Rawalpindi div., Murree (Ghorpadé & Shehzad 2013; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, China, India, Indonesia, Malaysia, Nepal, Philippines, Thailand, West Bengal (Ghorpadé 2015).

37. *Paragus (Pandasyopthalmus) haemorrhous* Meigen, 1822

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, Jandala; Balochistan prov., Quetta div., Quetta; Islamabad capi., Shakarparia (Turk et al. 2014; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, China, Iran, Ireland, Israel, Italy, Kazakhstan, Malta, Mongolia, North Africa, North America, Norway, Russia, Tajikistan, Turkey, Turkmenistan (Ghorpadé 2015).

Subgenus *Paragus* Latreille, 1804

38. *Paragus (Paragus) quadrifasciatus* Meigen, 1822

Distribution: Pakistan (Gilgit-Baltistan terr., Baltistan div., Skardu (Ghorpadé & Shehzad 2013; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, China, India, Iran, Kazakhstan, Kirghizia, Russia, Tajikistan, Turkmenistan (Sorokina 2009; Ghorpadé & Shehzad 2013; Ghorpadé 2015).

39. *Paragus (Paragus) compeditus* Wiedemann, 1830

Distribution: Pakistan (Gilgit-Baltistan terr., Baltistan div., Skardu; Khyber Pakhtunkhwa prov., Peshawar div., Peshawar city (Saleem et al. 2001; Ghorpadé & Shehzad 2013; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, Iran (Khaghaninia & Hosseini 2013; Ghorpadé 2015).

Genus *Asarkina* Macquart, 1834

40. *Asarkina incisuralis* (Macquart, 1855)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa (Hassan et al. 2018)); India, Nepal, Bangladesh, Sri Lanka (Ghorpadé 2015).

Genus *Betasyrphus* Matsumura, 1917

41. *Betasyrphus aeneifrons* (Brunetti, 1913)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); India, Nepal (Shah et al. 2014; Ghorpadé 2015; Mitra

et al. 2015).

42. *Betasyrphus isaaci* (Bhatia, 1933)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa; Islamabad capi., Shakarparia; Khyber Pakhtunkhwa prov., Hazara div., Aamgah; Punjab prov., Rawalpindi div., Murree, Gujranwala div., Shakargarh (Shehzad et al. 2017; Hassan et al. 2018)); India, Myanmar, Nepal (Shah et al. 2014; Ghorpadé 2015; Mitra et al. 2015).

Genus *Chrysotoxum* Meigen, 1803

43. *Chrysotoxum baphyrum* Walker, 1849

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley; Muzafarabad div., Panjkot; Islamabad capital, Chak shehzad; Khyber Pakhtunkhwa prov., Peshawar div., Peshawar city (Alam et al. 1969; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2018)); India, Myanmar, Sri Lanka, Nepal (Thapa 2000; Ghorpadé 2015).

44. *Chrysotoxum intermedium* Meigen, 1822

Distribution: Pakistan (Gilgit-Baltistan terr., Baltistan div., Skardu, Deosai; Khyber Pakhtunkhwa prov., Hazara div., Hazara, Naran Valley Malakand div., Miandam; Malakand Div., Chitral, Hazara div., Balakot (Alam et al. 1969; Aslamkhan et al. 1997; Ghorpadé & Shehzad 2013; Ghorpadé 2015)); India (Brunetti 1923; Shannon 1926; Bańkowska 1969; Alam et al. 1969; Violovitsh 1974; Knutson et al. 1975; Peck 1988; Ghorpadé 1981, 1994, 2012, 2014; Aslamkhan et al. 1997; Ghorpadé & Shehzad 2013; Shah et al. 2014; Mitra et al. 2015).

Genus *Episyrphus* Matsumura and Adachi 1917

45. *Episyrphus balteatus* (De Geer, 1776)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley, Sangolla, Muzafarabad div., Panjkot; Islamabad capital, Chak shehzad; Balochistan prov., Makran div., Gwadar city; Gilgit-Baltistan terr., Gilgit div., Gohrabad; Khyber Pakhtunkhwa prov., Malakand div., Miandam, Dargai, Timurgarh; Hazara div., Abbottabad city, Mardan city, Batrasi, Ghari Habibullah, Kaghan, Narran, Peshawar div., Charsadda, Nowshera city, Kohat div., Hangu, Kohat city; Punjab prov., Rawalpindi div., Bhurban, Ghora gali, Murree, Jhelum, Faisalabad div., Faisalabad; Multan div., Khanewal city, Lahore div., Lahore; Dera Ghazi khan div., Muzaffargarh, Gujrawala div., Sailkot city, Gujranwala div., Shakargarh; Sindh prov., Hyderabad div., Tando jam (Rahman 1940, 1942; Alam

et al. 1969; Hamid 1984; Talpur et al. 1995; Aslamkhan et al. 1997; Arif 2001; Saleem et al. 2001; Irshad 2008; Saeed et al. 2008; Sajjad et al. 2008, 2010; Sajjad & Saeed 2010; Ali et al. 2011; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); Widespread species - Afghanistan, India, Nepal (Thapa 2000; Ghorpadé 2015; Banerjee et al. 2018).

46. *Episyrphus viridaureus* (Wiedemann, 1824)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley, Jandala, Sangolla; Punjab prov., Gujranwala div., Shakargarh (Hassan et al. 2017, 2018)); India, Bangladesh, Sri Lanka, Java, Nepal (Ghorpadé 2015; Banerjee et al. 2018).

Genus *Ischiodon* Sack, 1913

47. *Ischiodon scutellaris* (Fabricius, 1805)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley, Jandala, Hajira; Islamabad capi., terri., Chak shehzad; Khyber Pakhtunkhwa: Hazara div., Balakot, Malakand div., Swat, Butkhela, Dir, Peshawar div., Peshawar city; Punjab prov., Multan div., Multan, Khanewal, Rawalpindi div., Murree, Ghora gali, Gujranwala div., Shakargarh (Alam et al. 1969; Sajjad & Saeed 2010; Sajjad et al. 2010; Ali et al. 2011; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); India, Bangladesh, Afghanistan, Myanmar, Sri Lanka, Nepal (Ghorpadé 2015; Banerjee et al. 2018).

Genus *Eupeodes* Osten Sacken, 1877

48. *Eupeodes bucculatus* (Rondani, 1857)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley, Jandala, Sangolla, Punjab prov., Gujranwala div., Shakargarh (Hassan et al. 2017, 2018)); Afghanistan, Bhutan, India, Nepal (Ghorpadé 2015; Banerjee et al. 2018).

49. *Eupeodes corollae* (Fabricius, 1794)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley, Hajira; Punjab prov., Multan div., Multan, Khanewal, Rawalpindi div., Murree, Ghora gali, Lahore div., Lahore, Dera Ghazi Khan div., Muzaffargarh, Gujranwala div., Shakargarh; Khyber Pakhtunkhwa prov., Malakand div., Dir, Kohat div., Hangu, Peshawar div., Peshawar city (Aslamkhan et al. 1997; Irshad 2008; Saeed et al. 2008; Sajjad et al. 2008, 2010; Sajjad & Saeed 2010; Ali et al. 2011; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad

et al. 2017; Hassan et al. 2017, 2018)); Afghanistan, Bhutan, India, Nepal (Ghorpadé 2015; Banerjee et al. 2018).

50. *Eupeodes latifasciatus* (Macquart, 1829)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley; Balochistan prov., Makran div., Gwadar city; Punjab prov., Multan div., Khanewal, Lahore div., Lahore, Gujranwala div., Shakargarh (Aslamkhan et al. 1997; Arif 2001; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); Afghanistan, India, Nepal (Ghorpadé 2015; Banerjee et al. 2018).

Genus *Scaeva* Fabricius, 1805

51. *Scaeva latimaculata* (Brunetti, 1923)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, Banjosa; Gilgit-Baltistan terr., Gilgit div., Goharabad, Miskqat; Astore, Gilgit (Naltar), Skardu; Khyber Pakhtunkhwa prov., Hazara div., Abbottabad city, Peshawar div., Peshwar city; Punjab prov., Multan div., Multan, Khanewal, Dera Ghazi Khan div., Muzaffargarh, Gujranwala div., Shakargarh; Sindh prov., Sukkur div., Sukkur (Aslamkhan et al. 1997; Sajjad et al. 2010; Sajjad & Saeed 2010; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); Afghanistan, India, Nepal (Thapa 2000; Banerjee et al. 2018; Hassan et al. 2018).

52. *Scaeva pyrastris* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa, Rawalakot valley; Khyber Pakhtunkhwa prov., Hazara div., Abbottabad city, Malakand div., Swat, Dargai, Dir, Peshawar div., Peshawar city, Punjab prov., Rawalpindi div., Rawalpindi, Murree, Ghora gali, Dalha, Rawat (Alam et al. 1969; Irshad 2008; Sajjad & Saeed 2010; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Hassan et al. 2018)); Afghanistan, India (Ghorpadé 2015).

Genus *Sphaerophoria* Lepeletier and Serville, 1828

53. *Sphaerophoria bengalensis* Macquart, 1842

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, Sangola, Hajira (Hassan et al. 2018)); Afghanistan, India, Nepal (Ghorpadé 2015; Thapa 2000; Banerjee et al. 2018).

54. *Sphaerophoria indiana* Bigot, 1884

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, banjosa, Sangola;

Khyber Pakhtunkhwa prov., Malakand div., Swat, Dir, Peshawar div., Peshawar city; Punjab prov., Rawalpindi div., Bhurban, Ghora gali, Murree, Rawat (Alam et al. 1969; Irshad 2008; Sajjad & Saeed 2010; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2018)); Afghanistan, Bhutan, India, Myanmar, Nepal (Ghorpadé 2015; Banerjee et al. 2018; Thapa 2000).

55. *Sphaerophoria scripta* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, Banjosa, Hajira; Khyber Pakhtunkhwa prov., Malakand div., Dir, Kohat div., Hangu, Peshawar div., Peshawar city, Malakand div., Swat; Punjab prov., Multan div., Multan, Gujranwala div., Shakargarh (Arif 2001; Arif et al. 2001; Saeed et al. 2008; Sajjad et al. 2008; Ghorpadé & Shehzad 2013; Ghorpadé 2015; Shehzad et al. 2017; Hassan et al. 2017, 2018)); Afghanistan, India, Nepal (Thapa 2000; Ghorpadé 2015).

Genus *Syrphus* Fabricius, 1775

56. *Syrphus dalhousiae* Ghorpadé, 1994

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); India, Nepal (Ghorpadé 2015).

57. *Syrphus fulvifacies* Brunetti, 1913

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); India, Nepal (Ghorpadé 2015).

58. *Syrphus torvus* (Osten Sacken, 1875)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley (Hassan et al. 2018)); India, Nepal (Ghorpadé 2015; Thapa 2000).

59. *Syrphus vitripennis* Meigen, 1822

Distribution: Pakistan (Gilgit–Baltistan terr., Gilgit div., Gilgit, Naltar; Punjab prov., Rawalpindi div., Rawalpindi, Murree, Sunny (Shehzad et al. 2017)); India (Ghorpadé 2009; Shehzad et al. 2017).

Genus *Xanthogramma* Schiner, 1860

60. *Xanthogramma pedissequum* (Harris, 1776)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley; Khyber Pakhtunkhwa prov., Malakand Div., Bumburiate; Miandum (Aslamkhan et al. 1997; Ghorpadé 2015; Shehzad et al. 2017)); India (Ghorpadé 2015).

Family Calliphoridae Brauer & Bergenstamm, 1889

Subfamily Calliphorinae Brauer & Bergenstamm, 1889

Genus *Calliphora* Robineau-Desvoidy, 1830

61. *Calliphora chinghaiensis* Van et Ma, 1978

Distribution: Pakistan (Gilgit–Baltistan terr., Gilgit div., Khunjerab Pas (Kurahashi & Afzal 2002)); China, Nepal (Thapa 2000; Kurahashi & Afzal 2002).

62. *Calliphora himalayana* Kurahashi, 1994

Distribution: Pakistan (Gilgit–Baltistan terr., Gilgit div., Khunjerab Pas, Astore div., Babusar, Chilas (Kurahashi & Afzal 2002)); Nepal (Thapa 2000; Kurahashi & Afzal 2002).

63. *Calliphora loewi* Enderlein, 1903

Distribution: Pakistan (Azad Jammu & Kashmir terr.; Poonch div., Banjosa lake; Khyber Pakhtunkhwa prov., Hazara div., Natiagali, Dunga Gali, Ayubia Nat. Park, Thandani, Saiful–Malook Lake, Naran, Lalazar, Kagan, Malakand div., Swat, Kalam (Hassan et al. 2018)); Austria, Canada, Ukraine, Nepal, Netherland, Switzerland, Sweden, Poland, Romania, Russia, Italy, Hungary, Germany, Finland (Thapa 2000; Prado et al. 2016).

64. *Calliphora uralensis* Villeneuve, 1922

Distribution: Pakistan (Gilgit–Baltistan terr., Gilgit div., Khunjerab Pas, Astore div., Babusar, Chilas (Kurahashi & Afzal 2002)); China, Russia (Kurahashi & Afzal 2002).

65. *Calliphora vicina* Robineau–Desvoidy, 1830

Distribution: Pakistan (Balochistan prov., Sibi div., Ziarat; Gilgit–Baltistan terr., Gilgit div., Khunjerab Pas, Gakuch, Astore div., Chitral, Chilas, Murtazabad, Naltar, Kalash valley, Gulmit; Khyber Pakhtunkhwa prov., Hazara div., Natiagali, Ayubia Nat. Park, Thandani, Saiful–Malook Lake, Naran, Kagan, Malakand div., Swat (Kurahashi & Afzal 2002; Hassan et al. 2018)); China, Nepal, India, Australia, New Zealand, Russia, Mauritius, South Africa, Argentina, Cosmopolitan (Thapa 2000; Kurahashi & Afzal 2002; Rognes 2007).

66. *Calliphora vomitoria* (Linnaeus, 1758)

Distribution: Pakistan (Azad Jammu & Kashmir terr.; Poonch div., Banjosa lake, Gilgit–Baltistan terr., Gilgit div., Khunjerab Pas, Naltar; Khyber Pakhtunkhwa prov., Hazara div., Natiagali, Dunga Gali, Ayubia Nat. Park, Thandani, Saiful–Malook Lake, Naran, Lalazar, Kagan, Malakand div., Swat, Kalam; Punjab prov., Rawalpindi div., Murree, Dunga Gali (Kurahashi & Afzal 2002; Hassan et al. 2018)); China, Thailand, Nepal,

India, Afghanistan, Japan, Korea, Taiwan, Philippines, Europe, Morocco, Canary Is., N, America, Hawaiian Is. (Thapa 2000; Kurahashi & Afzal 2002).

Genus *Cynomya* Robineau-Desvoidy, 1830

67. *Cynomya mortuorum* (Linnaeus, 1761)

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Khunjerab Pas (Kurahashi & Afzal 2002)); China, Mongolia, Russia (Kurahashi & Afzal 2002).

Genus *Onesia* Robineau-Desvoidy, 1830

68. *Onesia menechmoides* (Chen, 1979)

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Khunjerab Pas, Khyber Pakhtunkhwa prov., Malakand div., Mingora; Hazara div., Kalam Valley, Miandam (Kurahashi & Afzal 2002)); China, Japan (Kurahashi & Afzal 2002).

69. *Onesia pamirica* Rohdendorf, 1962

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Khunjerab Pas (Kurahashi & Afzal 2002)); China, Kirgizatan, Tadzhikistan (Kurahashi & Afzal 2002).

Genus *Melinda* Robineau-Desvoidy, 1830

70. *Melinda scutellata* (Senior-White, 1923)

Distribution: Pakistan (Azad Jammu & Kashmir terr.; Khyber Pakhtun khwa prov., Malakand div., Swat, Kalam, Shangla pass, Daroa, Hazara div., Abbottabad, Natiagali, Ayubia National Park; Gilgit-Baltistan terr., Gilgit div., Naltar, Kalash valley; Punjab prov., Rawalpindi div., Murree, Miandarn (Kurahashi & Afzal 2002)); Malaysia, Myanmar, Nepal (Thapa 2000; Kurahashi & Afzal 2002).

Subfamily Luciliinae Shannon, 1923

Genus *Lucilia* Robineau-Desvoidy, 1830

71. *Lucilia porphyria* (Walker, 1856)

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Naltar, Kalash valley; Khyber Pakhtunkhwa prov., Malakand div., Swat, Kalam, Miandarn, Shangla pass, Daroa; Buttgram, Hazara div., Natiagali, Ayubia, Saiful-Malook Lake, Naran, Kagan, Balakot, Punjab prov., Rawalpindi div., Murree (Kurahashi & Afzal 2002; Hassan et al. 2018)); China, Thailand, Malaysia, India, Bangladesh, Sri Lanka, Nepal, Japan, Taiwan, Korea, Philippines, Indonesia, Papua New Guinea, Australia, East Palaearctic Regions (Kurahashi & Afzal 2002).

72. *Lucilia sericata* (Meigen, 1826)

Distribution: Pakistan (Balochistan prov., Quetta div., Quetta, Sibi div., Ziarat; Gilgit-Baltistan terr., Gilgit div.,

Naltar, Kalash valley, Gulmit, Inaoka, Ultar Glacier, Gakuch; Khyber Pakhtunkhwa prov., Malakand div., Chitral, Kalam, Shangla pass, Daroa, Buttgram, Hazara div., Natiagali, Naran, Kagan; Punjab prov., Rawalpindi div., Murree, Dera Ghazi Khan div., Dera Ghazi Khan., Fort Munro (Kurahashi & Afzal 2002)); Nearctic Region (Whitworth 2010), wide distribution in all countries of the Middle East. Japan, Korea, Taiwan, Philippines, China, Sri Lanka, Europe (Kurahashi & Afzal 2002; Akbarzadeh et al. 2015).

Subfamily Polleniinae Brauer and Bergenstamm, 1889

Genus *Pollenia* Robineau-Desvoidy, 1830

73. *Pollenia pediculate* Macquart, 1834

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Gilgit, Astore div., Murtazabad; Balochistan prov., Quetta div., Quetta; Khyber Pakhtunkhwa prov., Hazara div., Natiagali, Dunga Gali, Ayubia National Park (Kurahashi & Afzal 2002)).

74. *Pollenia rudis* (Fabricius 1794)

Distribution: Pakistan (Balochistan prov., Sibi div., Ziarat; Gilgit-Baltistan terr., Gilgit div., Caryga village, Gilgit, town, Naltar, Astore div., Murtazabad; Khyber Pakhtun khwa prov., Malakand div., Swat, Miandum, Kalam, Hazara div., Natiagali; Punjab prov., Rawalpindi div., Murree (Thapa 2000; Kurahashi & Afzal 2002)); Widely distributed in the Palaearctic, Nearctic Regions (Kurahashi & Afzal 2002).

Subfamily Chrysomyinae Shannon, 1923

Genus *Chrysomya* Robineau-Desvoidy, 1830

75. *Chrysomya phaonis* Ségué, 1928

Distribution: Pakistan (Balochistan prov., Sibi div., Ziarat; Gilgit-Baltistan terr., Gilgit div., Gilgit, Caryga village, Gilgit, town, Naltar, Gulmit, Gakuch Astore div., Murtazabad, Chitral, Babusar; Khyber Pakhtunkhwa prov., Malakand div., Swat, Miandum, Kalam, Hazara div., Natiagali, Ayubia, Naran, Kagan, Saiful-Malook Lake (Kurahashi & Afzal 2002)); China, Nepal, India, Afghanistan (Thapa 2000; Kurahashi & Afzal 2002).

Genus *Protocalliphora* Hough, 1899

76. *Protocalliphora azurea* (Fallén, 1817)

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Khunjerab Pas, Khyber Pakhtunkhwa prov., Hazara div., Natiagali, Thandani; Punjab prov., Rawalpindi div., Murree (Kurahashi & Afzal 2002)); Widely distributed in the temperate Asia, Palaearctic Region, North America, Europe (Sabrosky et al. 1989).

77. *Protocalliphora terraenovae* (Robineau Desvoidy, 1830)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Khunjerab Pas (Kurahashi & Afzal 2002)); widely distributed in the Palaearctic, Nearctic Region (Kurahashi & Afzal 2002).

Genus *Isomyia* Walker, 1860

78. *Isomyia electa* (Villeneuve, 1927)

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., Poonch div., Rawalakot; Gilgit-Baltistan terr., Baltistan div., Skardu (Hassan et al. 2018)); India, Thailand (Kurahashi & Afzal 2002).

Subfamily Rhiniinae Brauer & Bergenstamm, 1889

Genus *Rhinia* Robineau-Desvoidy, 1830

79. *Rhinia apicalis* (Wiedemann, 1830)

Distribution: **Pakistan** (Jammu & Kashmir terr., Poonch div., Banjosa Lake, Hajira (Hassan et al. 2018)); India, Saudi Arabia, Thailand (Hassan et al. 2018).

Genus *Stomorphina* Rondani, 1861

80. *Stomorphina discolor* (Fabricius, 1794)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Astore div., Astore; Chilas, Khyber Pakhtunkhwa prov., Hazara div., Abbottabad, Natiagali, Ayubia National Park, Kohat div., Manshera, Malakand div., Swat, Kalam; Punjab prov., Rawalpindi div., Murree (Hassan et al. 2018)); Taiwan, Philippines, China, Vietnam, Laos, Thailand, Malaysia, Indonesia, Australia, India, Sri Lanka and Fiji. Widely distributed in the Indo-Australian Region (Kurahashi & Afzal 2002).

81. *Stomorphina xanthogaster* (Wiedemann, 1820)

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., Poonch div., Hajira (Hassan et al. 2018)); China, India, Indonesia, Malaysia, Nepal, Sri Lanka (Thapa 2000; Hassan et al. 2018).

Family Fanniidae Schnabl, 1911

Subfamily Fanninae Schnabl, 1911

Genus *Fannia* Robineau-Desvoidy, 1830

82. *Fannia canicularis* (Linnaeus, 1761)

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available; Gilgit-Baltistan terr., Gilgit div., Gulmit; Khyber Pakhtunkhwa prov., Hazara div., Naran Valley, Kagan Valley, Ayubia National Park, Malakand div., Kalash Valley, Kalam Valley (Nishida 1989)); China, India, Nicobar Islands (Nishida 1989).

83. *Fannia dupla* Nishida, 1974

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available; Khyber Pakhtunkhwa prov., Hazara div., Ayubia National Park (Nishida 1989)); Nepal, Taiwan, Japan (Nishida 1994; Thapa 2000).

84. *Fannia indica* Chillcott, 1961

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available; Gilgit-Baltistan terr., Gilgit div., Gilgit; Khyber Pakhtunkhwa prov., Hazara div., Ayubia National Park (Nishida 1989)); India, Nepal (Nishida 1994; Thapa 2000).

85. *Fannia manicata* (Meigen, 1826)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Gilgit, Bagrote Valley, Babusar Pass; Khyber Pakhtunkhwa prov., Hazara div., Gulmit Gali, Naran Valley, Saif-ul-Muluk Lake; Punjab prov., Rawalpindi div., Murree (Nishida 1989)); Nepal, Taiwan, Europe, China, Japan North Africa, Nearctic (Nishida 1994; Thapa 2000).

86. *Fannia scalaris* (Fabricius, 1794)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Hunza Valley, Ultar Glacier, Gulmit; Khyber Pakhtunkhwa prov., Hazara div., Nathia Gali, Naran Valley, Malakand div., Kalam Valley; Punjab prov., Murree (Nishida 1989)); China, Taiwan, India, Holarctic, Nearctic, Ethiopian (Nishida 1989).

Family Rhinophoridae Robineau-Desvoidy, 1863

Subfamily Rhinophorinae Robineau-Desvoidy, 1863

Genus *Tromodesia* Rondani, 1856

87. *Tromodesia setiventris* (Rohdendorf, 1935)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Baltistan div., Skardu (Cerretti et al. 2020)); Turkmenia (Cerretti et al. 2020).

Family Sarcophagidae Macquart, 1834

Subfamily Sarcophaginae Macquart, 1834

Genus *Ravinia* Robineau-Desvoidy, 1863

88. *Ravinia pernix* Harris, 1780

Distribution: **Pakistan** (Balochistan prov., Sibi div., Ziarat; Gilgit-Baltistan terr., Baltistan div., Gilgit div., Babusar Pass; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Nathia Gali, Malakand div., Kalash Valley, Kalam Valley, Mingora, D.I. Khan div., Fort Munro; Punjab prov., Rawalpindi div., Murree (Sugiyama 1989)); Afghanistan, China, Europe, India, Japan, Korea, Middle East, Mongolia, Nepal, North Africa, USSR (Sugiyama 1989).

Genus *Sarcophaga* Meigen, 1826**89. *Sarcophaga aegyptica* (Salem, 1935)**

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Naltar Valley, Hunza Valley, Ultar Glacier, Gulmit; Khyber Pakhtunkhwa prov., Malakand div., Kalash Valley (Sugiyama 1989)); Egypt, Ethiopia, Europe, Iran, Israel, North Africa, Northwest China, USSR (Sugiyama 1989).

90. *Sarcophaga albiceps* Meigen, 1826

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Hunza Valley, Ultar Glacier; Khyber Pakhtunkhwa prov., Malakand div., Kalash Valley, Hazara div., Nathia Gali, Tandani, Balakot, Kot Gali, Kawai-Dasu, Miandam, Mingora; Punjab prov., Lahore div., Changa Manga (Sugiyama 1989)); Bismarck Arch, Borneo, China, Europe, Hawaii, India, Israel, Japan, Korea, Malaysia, Nepal, New Guinea, Philippines, Singapore, Solomon Is., Taiwan, Thailand, Turkey (Sugiyama 1989; Thapa 2000).

91. *Sarcophaga altitudinis* Sugiyama, 1964

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Khunjerab Pass (Sugiyama 1989)); USSR (Sugiyama 1989).

92. *Sarcophaga brevicornis* Ho, 1934

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Bagroth; Khyber Pakhtunkhwa prov., Malakand div., Kalam Valley, Miandam (Sugiyama 1989)); Borneo, China, Hainan Is., Japan, Korea, Malaysia, Nepal, Philippines, Singapore, Taiwan (Sugiyama 1989; Thapa 2000).

93. *Sarcophaga calicifera* Boettcher, 1912

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Chilas; Khyber Pakhtunkhwa prov., Malakand div., Tandani, Mingora; Sindh prov., Hyderabad div., Hyderabad, Karachi div., Karachi (Sugiyama 1989)); China, India, Nepal, Nigeria, Philippines, Ryukyu Is., Sri Lanka, Taiwan, Uganda, Zaire (Sugiyama 1989; Thapa 2000).

94. *Sarcophaga cruentata* Meigen, 1826

Distribution: **Pakistan** (Balochistan div., Sibi div., Ziarat; Gilgit-Baltistan terr., Gilgit div., Chilas; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Nathia Gali, Saif-ul-Muluk Lake; Malakand div., Kalash Valley, Kalam Valley, Kawai-Dasu; Punjab prov., Dera Ghazi Khan div., Fort Munro, Rawalpindi div., Murree (Sugiyama 1989)); Africa, Europe, Hawaii, India, Nepal,

North and South Americas (Sugiyama 1989; Thapa 2000).

95. *Sarcophaga doleschalii* Johnston et Tiegs, 1921

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Tandani, Nathia Gali; Punjab prov., Rawalpindi div., Murree (Sugiyama 1989)); Java, Moluccas, Nepal, Thailand, Vietnam (Sugiyama 1989; Thapa 2000).

96. *Sarcophaga flagellifera* Grunin, 1964

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Chilas (Sugiyama 1989)); Afghanistan, USSR (Sugiyama 1989).

97. *Sarcophaga gorodkovi* (Grunin, 1964)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Gilgit, Khunjerab Pass (Sugiyama 1989)); Mongolia, USSR (Sugiyama 1989).

98. *Sarcophaga hirtipes* Wiedemann, 1830

Distribution: **Pakistan** (Balochistan prov., Kalat div., Quetta div., Quetta, Sibi div., Sibi; Gilgit-Baltistan terr., Gilgit div., Chilas; Khyber Pakhtunkhwa prov., Bannu div., Bannu, Kohat div., Kohat, D.I. Khan div., D.I. Khan, Fort Munro; Sindh prov., Hyderabad div., Hyderabad, Karachi div., Karachi (Sugiyama 1989)); Afghanistan, Afrotropical region, Algeria, China, Egypt, India, Iran, Iraq, Israel, Jordan, Lebanon, Morocco, Saudi Arabia, Syria, Turkey, USSR (Sugiyama 1989).

99. *Sarcophaga idmais* Séguéy, 1934

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available; Gilgit-Baltistan terr., Gilgit div., Naltar Valley; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Nathia Gali, Lalazar, Naran Valley, Saif-ul-Muluk Lake, Malakand div., Kalam Valley, Miandam, Mingora, Shangla Pass; Punjab prov., Rawalpindi div., Murree (Sugiyama 1989)); China, Nepal, Taiwan, Thailand (Sugiyama 1989; Thapa 2000).

100. *Sarcophaga kentejana* (Rohdendorf, 1937)

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available; Gilgit-Baltistan terr., Gilgit div., Naltar Valley; Khyber Pakhtunkhwa prov., Hazara div., Naran Valley, Saif-ul-Muluk Lake (Sugiyama 1989)); China, Europe, Mongolia, USSR (Sugiyama 1989).

101. *Sarcophaga nathani* (Lopes, 1961)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div.,



Gilgit.; hyber Pakhtunkhwa prov., Hazara div., Kagan Valley, Balakot, Malakand div., Kalash Valley, Kalam Valley, Marghazar, Kawai-Dasu (Sugiyama 1989)); India, Indonesia, Malaysia, Nepal, Thailand (Sugiyama 1989; Thapa 2000).

102. *Sarcophaga peshelicis* Senior-White, 1930

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available; Gilgi-Baltistan terr.; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Nathia Gali, Lalazar, Naran Valley, Malakand div., Kalam Valley, Ushu; Punjab prov., Rawalpindi div., Murree (Sugiyama 1989)); India, Nepal (Sugiyama 1989; Thapa 2000).

103. *Sarcophaga portschinskyi* (Rohdendorf, 1937)

Distribution: Pakistan (Balochistan div., Sibi div., Ziarat; Gilgit-Baltistan terr., Gilgit div., Bagroth; Khyber Pakhtunkhwa prov., Hazara div., Lalazar, Naran Valley, Malakand div., Kalash Valley, Kalam Valley, Gabral, Ushu (Sugiyama 1989)); China, Europe, Mongolia, Turkey, USSR (Sugiyama 1989).

104. *Sarcophaga shresthai* Kano et Shinonaga, 1969

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available; Khyber Pakhtunkhwa prov., Hazara div., Kot Gali, Balakot, Kagan Valley, Darora, Marghazar, Miandam, Malakand div., Ayubia Gali, Nathia Gali (Sugiyama 1989)); Nepal (Sugiyama 1989).

105. *Sarcophaga tubersoa* Pandellé 1896

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Hunza Valley, Ultar Glacier (Sugiyama 1989)); China, Europe, Japan, Korea, Mongolia, North America, USSR (Sugiyama 1989).

106. *Sarcophaga yunnanensis* Fan, 1964

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available; Khyber Pakhtunkhwa prov., Hazara div., Kot Gali, Balakot, Malakand div., Ayubia Gali, Nathia Gali (Sugiyama 1989)); China, Thailand (Sugiyama 1989).

Family Tachinidae Bigot, 1853

Subfamily Phasiinae Robineau-Desvoidy, 1830

Genus *Cylindromyia* Meigen, 1803

107. *Cylindromyia evibrissata* Townsend, 1927

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Mohyuddin 1981)); China, Taiwan, India, Indonesia (O'Hara et al. 2021).

Subfamily Exoristinae Robineau-Desvoidy, 1863

Genus *Elodia* Robineau-Desvoidy, 1863

108. *Elodia morio* (Fallén, 1820)

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Mohyuddin 1981; O'Hara et al. 2021)); China, Europe, Japan, Mongolia, Russia (O'Hara et al. 2021).

Subfamily Dexiinae Macquart, 1834

Genus *Euthera* Loew, 1866

109. *Euthera tuckeri* Bezzi, 1925

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Cheema et al. 1973)); Botswana, Ghana, Kenya, Malawi, Mozambique; South Africa, Sudan, United Arab Emirates, Uganda, Zambia, Japan (O'Hara et al. 2021; O'Hara & Cerretti 2016).

Genus *Torocca* Walker, 1859

110. *Torocca munda* (Walker, 1856)

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Alam et al. 1969)); China, Palaearctic: Japan, India, Indonesia, Malaysia, Thailand, Vietnam (O'Hara et al. 2021).

Family Tephritidae Newman, 1834

Subfamily Dacinae Loew, 1862

Genus *Bactrocera* Macquart, 1835

Subgenus *Bactrocera* Guérin-Meneville, 1838

111. *Bactrocera (Bactrocera) dorsalis* (Hendel, 1794)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Hajira, Jandali, Banbhake (Zubair et al. 2019)); Taiwan, Singapore, Indian subcontinent and in South East Asia, Bangladesh, Bhutan, Cambodia, China, Jammu and Kashmir, Japan, Nepal, Pakistan, Sri Lanka, Taiwan, Thailand, United Arab Emirates, America, Angola, Botswana, Congo, Ethiopia, South Africa, Sudan, Australia, Guam and New Zealand (Halder et al. 2015).

112. *Bactrocera (Bactrocera) zonata* (Saunders, 1841)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Hajira, Jandali, Banbhake (Zubair et al. 2019)); Bangladesh, Bhutan, India, Jammu & Kashmir, Iran, Israel, Laos, Myanmar, Nepal, Saudi Arabia, Sri Lanka, Thailand, United Arab Emirates, Vietnam, Yemen, Egypt, Libya, Sudan, USA, New Zealand (Halder et al. 2015).

113. *Bactrocera (Bactrocera) correcta* (Bezzi, 1916)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Mandol (Zubair et al. 2019)); India, Sri

Lanka, Nepal, Thailand, Southern china, Bhutan, Japan, Myanmar, Taiwan, USA (Agarwal & Sueyoshi 2005).

114. *Bactrocera (Bactrocera) nigrofemoralis* Tsuruta & White, 2001

Distribution: Pakistan (Azad Jammu & Kashmir terr., Hajira, Jandali, Banbhake (Zubair et al. 2019)); China, Sri Lanka, India, Thailand, Nepal, Bhutan (Agarwal & Sueyoshi 2005).

Subgenus *Zeugodacus* Hendel, 1927

115. *Bactrocera (Zeugodacus) cucurbitae* (Coquillett, 1899)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Hajira, Jandali, Banbhake (Alam et al. 1969; Zubair et al. 2019)); Afghanistan, Bangladesh, Bhutan, Cambodia, China, Hong Kong, Japan, Nepal, Oman, India, Jammu and Kashmir, Iran, Taiwan, Israel, Laos, Myanmar, Nepal, Saudi Arabia, Sri Lanka, Thailand, United Arab Emirates, Vietnam, Yemen, Egypt, Kenya, Libya, Sudan, USA, Australia, New Zealand (Halder et al. 2015).

116. *Bactrocera (Zeugodacus) scutellaris* Bezzi, 1913

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Hajira, Jandali, Banbhake (Zubair et al. 2019)), Sri Lanka, India, Vietnam, Malaysia, Thailand, Myanmar, Bhutan, Nepal (Agarwal & Sueyoshi 2005).

117. *Bactrocera (Zeugodacus) tau* (Walker, 1849)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Hajira, Jandali, Banbhake (Zubair et al. 2019)); Bangladesh, Bhutan, Cambodia, Fujian, Hong Kong, Indonesia, Malaysia, Taiwan, Singapore, China, Sri Lanka, India, Vietnam, Malaysia, Thailand, Nepal (Halder et al. 2015).

Genus *Dacus* Fabricius, 1805

Subgenus *Callantra* Walker, 1860

118. *Dacus (Callantra) sphaerodalis* (Bezzi, 1916)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Mandol (Zubair et al. 2019)); China, Sri Lanka, India, Thailand, Nepal, Bhutan (Wang & Chen 2002; Zubair et al. 2019).

119. *Dacus (Callantra) longicornis* (Wiedemann, 1830)

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Mandol (Zubair et al. 2019)); China, Sri Lanka, India, Thailand, Nepal (Wang & Chen 2002; Zubair et al. 2019).

Genus *Tephritis* Latreille, 1804

120. *Tephritis frauenfeldi* Hendel, 1927

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot valley, Rawalakot (Baloch et al. 1971)); Albania, Austria, Estonia, Greek mainland, Hungary, Italian mainland, Italy, Latvia, Lithuania, Moldova, Near East, Romania, Russia, Slovakia, Switzerland, Turkey, Ukraine (Foote 1984; Merz 1994; Özgür & Kütük 2003).

121. *Tephritis hyoscyami* Linnaeus, 1758

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Alam et al. 1969)); Albania, Armenia, Austria, Azerbaijan, Belgium, China, Finland, French mainland, Georgia, Germany, Hungary, Ireland, Italian mainland, Latvia, Lithuania, Moldova, Near East, Netherlands, northern and central Europe, Norwegian mainland, Poland, Portuguese mainland, Romania, Russia, Slovakia, Sweden, Switzerland, Turkey, Ukraine (Foote 1984; Merz 1994; Kütük & Özgür, 2003).

Genus *Terellia* Robineau-Desvoidy, 1830

122. *Terellia serratulae* (Linnaeus, 1758)

Distribution: Pakistan: (Azad Jammu & Kashmir terr., exact location not available; Khyber Pakhtunkhwa prov., Malakand div., Hindu Kush, Shekhnayak, Tirich valley (Alam et al. 1969; Brake 2011)); Afghanistan, Tajikistan (Brake 2011).

Family Chamaemyiidae Hendel, 1916

Subfamily Chamaemyiinae Hendel, 1910

Genus *Leucopis* Meigen, 1830

123. *Leucopis* sp.

Distribution: Pakistan (Azad Jammu & Kashmir terr., exact location not available (Alam et al. 1969)).

Family Dryomyzidae Schiner, 1862

Subfamily Dryomyzinae Schiner, 1862

Genus *Dryomyza* Fallén, 1820

124. *Dryomyza pakistana* Kurahashi, 1989

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Banjosa Lake; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Dunga Gali, Nathia Gali; Punjab prov., Rawalpindi div., Murree (Kurahashi 1989; Hassan et al. 2018)); China, India (Mathis & Sueyoshi 2011; Wachkoo et al. 2018).

Family Sepsidae Walker, 1833**Subfamily Sepsinae Walker, 1833****Genus *Saltella* Robineau-Desvoidy, 1830****125. *Saltella setigera* Brunetti, 1910**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Bagh city; Islamabad Terr., Shahdara; Punjab prov., Gujranwala div., Shakargarh (Hassan et al. 2017, 2021b)); Bangladesh, India, Nepal (Ozero 2005).

Genus *Meroplius* Rondani, 1874**126. *Meroplius minutus* Wiedemann, 1830**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley, Thandi Kasi (Fatima et al. 2019)); Canada, USA, New York, North Carolina, Pennsylvania, Utah, Virginia, Washington. China, Nepal, Japan Korea, Republic of Georgia, Russia, Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Hungary, Italy, Latvia, Lithuania, Netherlands, Norway, Romania, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, former Yugoslavia, Egypt (Ozero 2005).

Genus *Australosepsis* Malloch, 1925**127. *Australosepsis frontalis* Malloch, 1925**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley; Khyber Pakhtunkhwa prov., Malakand div., Miandam (Iwasa 1989)); Australia, New Caledonia, Bangladesh, China, India, Indonesia, Japan, Malaysia, Nepal, Philippines, Singapore, Sri Lanka, Thailand, Vietnam, Asia (Ozero 2005; Thapa 2015).

Genus *Decachaetophora* Duda, 1926**128. *Decachaetophora aeneips* (de Meijere, 1911)**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Gilgit-Baltistan terr., Gilgit div., Bagrote Valley, Naltar Valley, Baltistan div., Skardu, Hussain Abad; Khyber Pakhtunkhwa prov., Hazara div., Lalazar, Naran Valley, Kagan Valley, Malakand div., Miandam, Swat, Besham (Iwasa 1989; Hassan et al. 2017, 2018)); China, India, Nepal, Sri Lanka, Afghanistan, China, Japan, Korea, Mongolia, Russia, America (Thapa 2000; Ozero 2005).

Genus *Dicranosepsis* Duda, 1926**129. *Dicranosepsis bicolor* (Wiedemann, 1830)**

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley, Goi Nala; Khyber Pakhtunkhwa prov., D.I. Khan div., D.I. Khan., Hazara div., Ayubia Gali, Dunga Gali, Nathia Gali, Balakot, Kagan Valley, Malakand div., Marghazar; Punjab prov.,

Gujranwala div., Bola Bajwa, Rawalpindi div., Murree (Iwasa 1989; Hassan et al. 2017)); Bangladesh, China, India, Nepal, Sri Lanka, Thailand, Vietnam (Iwasa et al. 1991; Thapa 2000; Ozero 2005).

130. *Dicranosepsis olfactoria* Iwasa, 1984

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley, Goi Nala; Baluchistan prov., Quetta div., Quetta; Punjab prov., Gujranwala div., Bola Bajwa, Rawalpindi div., Murree; Khyber Pakhtunkhwa prov., D.I. Khan div., D.I. Khan, Hazara div., Dunga Gali, Nathia Gali, Kagan Valley, Kawai, Malakand div., Shangla Pass, Besham, Kalam Valley, Marghazar, Miandam, Ushu (Iwasa 1989; Hassan et al. 2017)); Nepal, Vietnam (Iwasa 1989; Thapa 2000; Ozero 2005).

131. *Dicranosepsis parva* Iwasa, 1984

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Poonch dis., Rawalakot Valley, Goi Nala, Banjosa Lake (Hassan et al. 2017)); Nepal (Thapa 2000; Ozero 2005).

132. *Dicranosepsis quadrigemina* Iwasa 1989

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley, Goi Nala Gilgit-Baltistan terr., Gilgit div., Babusar Pass; Punjab prov., Rawalpindi div., Murree; Khyber Pakhtunkhwa prov., Hazara div., Ayubia Gali, Dunga Gali, Nathia Gali, Kagan Valley, Lalazar, Naran Valley, Malakand div., Shangla Pass, Besham, Marghazar, Miandam (Iwasa 1989; Hassan et al. 2018)); India, Nepal, Thailand (Iwasa 1989; Ozero 2005).

Genus *Sepsis* Fallén, 1810**133. *Sepsis barbata* Becker, 1907**

Distribution: Pakistan (Balochistan prov., Quetta div., Quetta; Gilgit-Baltistan terr., Baltistan div., Chutron, Wazirpur, Gulapur, Hussain Abad, Gilgit div., Bagroth, Gulmit, Passu (Iwasa 1989; Hassan et al. 2017)); Yemen, China, Afghanistan, Armenia, China, Iran, Israel, Kazakhstan, Russia, Syria, Turkey, Turkmenistan, Uzbekistan, Austria, Czech Republic, France, Greece, Italy, Kazakhstan, Romania, Russia, Slovakia, Spain, Turkey, Ukraine, Canary Is. (Ozero 2005).

134. *Sepsis dissimilis* Brunetti, 1910

Distribution: Pakistan (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley; Gilgit-Baltistan terr., Baltistan div., Hussain Abad (Hassan et al. 2018)); Democratic Republic of the Congo, Ethiopia, Kenya,

Madagascar, Namibia, Nigeria, Republic of South Africa, Seychelles, Swaziland, Uganda, Zimbabwe. Australia, New Fiji, Hebrides, Papua New Guinea. China, India, Indonesia, Japan, Malaysia, Nepal, Philippines, Thailand, Vietnam, Japan (Thapa 2000; Ozerov 2005).

135. *Sepsis fissa* Becker, 1903

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley; Balochistan prov., Kalat div., Quetta div., Quetta; Punjab prov., Dera Ghazi Khan div., Fort Munro (Iwasa 1989)); Iran, Israel, Kazakhstan, Lebanon, Tadzhikistan, Turkmenistan, Italy, Malta, Romania, Slovakia (Ozerov 2005).

136. *Sepsis lateralis* Wiedemann, 1830

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Bagroth, Chilas; Khyber Pakhtunkhwa prov., Malakand div., Kalam Valley Miandam, Marghazar, Hazara div., Ushu, Balakot, Kagan Valley (Iwasa 1989; Hassan et al. 2018)); Angola, Botswana, Cameroon, Democratic Republic of the Congo, Ethiopia, Ghana, Kenya, Madagascar, Malawi, Mauritius, Namibia, Nigeria, Republic of South Africa, Republic of the Congo, Réunion, Seychelles, Sierra Leone, Swaziland, Tanzania, Uganda, Zambia, Zimbabwe, Yemen, USA, Papua New Guinea, Afghanistan, China, Iraq, Israel, Japan, Syria, Cyprus, Greece, Italy, Malta, Spain, Turkey, North Africa, Bangladesh, China, India, Japan, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand (Ozerov 2005).

137. *Sepsis mediana* Iwasa, 1989

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Gilgit; Khyber Pakhtunkhwa prov., Hazara div., Lalazar, Naran Valley, Saif-ul-Muluk Lake, Malakand div., Kalam Valley, Mingora (Iwasa 1989; Hassan et al. 2018)); India, Kazakhstan, Turkmenistan (Ozerov 2005).

138. *Sepsis neocynipsea* Melander & Spuler, 1917

Distribution: **Pakistan** (Gilgit div., Naltar Valley (Iwasa 1989; Hassan et al. 2018)); Bermuda Is, Canada, Mexico, America, Nepal, Mexico Afghanistan, Armenia, China, Japan, Kazakhstan, Kyrgyzstan, Mongolia, Russia, Tadzhikistan, Uzbekistan, Austria, Czech Republic, Germany, Great Britain, Ireland, Italy, Kazakhstan, Russia, Slovakia, Spain, Switzerland (Thapa 2000; Ozerov 2005).

139. *Sepsis nitens* Wiedemann, 1824

Distribution: **Pakistan** (Azad Jammu & Kashmir terr.,

Poonch div., Rawalakot Valley; Balochistan prov., Kalat div., Kalat; Khyber Pakhtunkhwa prov., Bannu div., Peshawar div; Punjab prov., Dera Ghazi Khan div., Fort Munro (Iwasa 1989)); Australia, Papua New Guinea, Bangladesh, China, India, Indonesia, Japan, Nepal, Pakistan, Philippines, Sri Lanka, Thailand (Thapa 2000; Ozerov 2005).

140. *Sepsis orthocnemis* Frey, 1908

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Babusar, Chilas; Punjab prov., Rawalpindi div., Murree; Khyber Pakhtunkhwa prov., Hazara div., Dunga Gali, Nathia Gali, Lalazar, Naran Valley, Saif-ul-Muluk Lake, Malakand div., Marghazar, Miandam, Mingora, Ushu (Iwasa 1989; Hassan et al. 2018)); Afghanistan, Armenia, Azerbaijan, Israel, Kazakhstan, Kyrgyzstan, Mongolia, Republic of Georgia, Russia, Tadzhikistan, Turkmenistan, Uzbekistan, Austria, Belarus, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Macedonia, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Turkey, Ukraine, Sweden, Switzerland, former Yugoslavia, Algeria (Ozerov 2005).

141. *Sepsis punctum* (Fabricius, 1794)

Distribution: **Pakistan** (Gilgit-Baltistan terr., Gilgit div., Bagroth, Chilas, Baltistan div., Hussain Abad; Khyber Pakhtunkhwa prov., Malakand div., Swat (Iwasa 1989; Hassan et al. 2017, 2018)); Bermuda Is., Canada, Mexico, America, Mexico, India, Myanmar, Nepal, Afghanistan, Armenia, Azerbaijan, China, Israel, Jordan, Kazakhstan, Kyrgyzstan, Lebanon, Mongolia, Republic of Georgia, Russia, Syria, Tadzhikistan, Turkey, Turkmenistan, Uzbekistan, Austria, Belarus, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Kazakhstan, Latvia, Liechtenstein, Lithuania, Luxembourg, Macedonia, Moldova, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, former Yugoslavia; North Africa: Algeria, Canary Is, Egypt, Libya, Madeira Is, Morocco, Tunisia (Ozerov 2005).

142. *Sepsis thoracica* (Robineau-Desvoidy, 1830)

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., Poonch div., Rawalakot Valley; Gilgit-Baltistan terr., Baltistan div, Hussain Abad, Chutron (Hassan et al. 2017, 2018)); Democratic Republic of the Congo, Ethiopia, Kenya, Namibia, Nigeria, Republic of South



Africa, Swaziland, Uganda, Yemen, Zambia, Zimbabwe, America, Cambodia, China, India, Japan, Nepal, Sri Lanka, Turkey, Afghanistan, Armenia, Azerbaijan, China, Iran, Iraq, Israel, Japan, Jordan, Kazakhstan, Korea, Kyrgyzstan, Lebanon, Mongolia, Republic of Georgia, Russia, Syria, Tadjikistan, Turkey, Turkmenistan, Uzbekistan, Albania, Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Italy, Macedonia, Netherlands, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, former Yugoslavia, Algeria, Azores, Canary Is, Egypt, Madeira Is, Morocco, Tunisia (Thapa 2000; Ozerov 2005).

143. *Sepsis violacea* Meigen, 1826

Distribution: **Pakistan** (Gilgit–Baltistan terr., Gilgit div., Bagroth, Babusar, Chilas, Naltar Valley, Baltistan div., Hussain Abad; Khyber Pakhtunkhwa prov., Malakand div., Miandam (Iwasa 1989; Hassan et al. 2018)); Afghanistan, Armenia, Azerbaijan, Iran, Israel, Japan, Kazakhstan, Kyrgyzstan, Lebanon, Mongolia, Republic of Georgia, Russia, Tadjikistan, Turkey, Turkmenistan, Uzbekistan, Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Kazakhstan, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, former Yugoslavia, Morocco, Tunisia (Ozerov 2005).

Genus *Themira* Robineau-Desvoidy, 1830

144. *Themira minor* (Haliday, 1833)

Distribution: **Pakistan** (Gilgit–Baltistan terr., Gilgit div., Bagroth, Gakuch; Khyber Pakhtunkhwa prov., Hazara div., Naran Valley, Kagan Valley, Saif-ul-Muluk Lake (Iwasa 1989; Hassan et al. 2018)); Canada, America, Armenia, Japan, Jordan, Kazakhstan, Kyrgyzstan, Mongolia, Pakistan, Russia, Syria, Tadjikistan, Turkey, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Italy, Latvia, Macedonia, Moldova, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Turkey, Ukraine, former Yugoslavia, Algeria, Madeira Is, Morocco, Tunisia (Ozerov 2005).

Family Chloropidae Verrall, 1888

Subfamily Oscinellinae Becker, 1910

Genus *Polyodaspis* Duda, 1933

145. *Polyodaspis* sp.

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available (Alam et al. 1969)).

Genus *Siphunculina* Rondani, 1856

146. *Siphunculina carinata* Kanmiya, 1989

Distribution: **Pakistan** (Gilgit–Baltistan terr., Gilgit div., Gilgit (Kanmiya 1989)).

Subfamily Chloropinae Loew, 1862

Genus *Thaumatomyia* Zenker, 1833

147. *Thaumatomyia notata* (Meigen, 1930)

Distribution: **Pakistan** (Azad Jammu & Kashmir terr., exact location not available (Alam et al. 1969)); England, Finland, Palearctic region, Nearctic region (Sabrosky 1940).

Family Sphaeroceridae Macquart, 1835

Subfamily Limosininae Frey, 1921

Genus *Coproica* Rondani, 1861

148. *Coproica acutangula* (Zetterstedt, 1847)

Distribution: **Pakistan** (Gilgit–Baltistan terr., Gilgit div., Babusar Pass, Gakuch (Hayashi 1991)); Bermuda, Colombia, Dominican Republic, Guatemala, Jamaica, Mexico, Puerto Rico, Venezuela, Japan, Taiwan, Afghanistan, Algeria, Andorra, Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Georgia, Great Britain, Hungary, Italy, Kazakhstan, Kirghizia, Latvia, Macedonia, Mongolia, Montenegro, Netherlands, Norway, Poland, Romania, Russia, Slovakia, Spain, Sweden, Switzerland, Tadjikistan, Uzbekistan (Marshall et al. 2011).

149. *Coproica digitata* (Duda, 1918)

Distribution: **Pakistan** (Gilgit–Baltistan terr., Gilgit div., Babusar Pass, Gakuch, Gulmit; Khyber Pakhtunkhwa prov., Hazara div., Ayubia, Nathia Gali, Tandani, Balakot, Malakand div., Shangla Pass, Kalam Valley, Ushu (Hayashi 1991)); Afghanistan, Bulgaria, Canary Is., Czech Republic, Egypt, France, Hungary, Israel, Italy, Kirghizia, Lebanon, Macedonia, Morocco, Romania, Serbia, Slovakia, Slovenia, Spain, Tunisia, Turkmenistan, Uzbekistan (Marshall et al. 2011).

150. *Coproica hirtula* (Rondani, 1880)

Distribution: **Pakistan** (Gilgit–Baltistan terr., Gilgit div., Bagrote Valley, Hunza; Khyber Pakhtunkhwa

prov., Hazara div., Kalam Valley, Miandam, Malakand div., Shangla Pass, Balakot (Hayashi 1991)); Congo, Ethiopia, Ghana, Kenya, Madagascar, Nigeria, Seychelles, South Africa, Yemen, Zaire, Japan, Guam, Hawaii, Kiribati, Marshall Is., Micronesia, New Zealand, Northern Mariana Is., Papua New Guinea, Palau, Pitcairn Is., Canada, Chile, Argentina, Bermuda, Bolivia, Galápagos Is., Mexico, China, India, Japan, Malaysia, Nepal, Sri Lanka, Taiwan, Vietnam, Afghanistan, Andorra, Austria, Azores, Belgium, Bulgaria, Canary Is., Cyprus, Czech Republic, Egypt, Finland, Germany, Great Britain, Greece, Hungary, Italy, Israel, Japan, Latvia, Madeira, Malta, Montenegro, Netherlands, North Korea, Norway, Poland, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland, Tunisia, United Arab Emirates, Uzbekistan, Saint Helena (Marshall et al. 2011).

151. *Coproica lugubris* (Haliday, 1835)

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Bagrote Valley; Babusar Pass, Gakuch, Gulmit; Khyber Pakhtunkhwa prov., Hazara div., Nathia Gali, Tandani, Kalam Valley, Miandam, Ushu, Balakot, Lalazar; Punjab prov., Rawalpindi div., Murree, Taxila; Sindh prov., Hyderabad div., Hyderabad (Hayashi 1991)); Papua New Guinea, China, India, Taiwan, Afghanistan, Andorra, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Great Britain, Greece, Hungary, Ireland, Israel, Italy, Japan, Kazakhstan, Kirghizia, Latvia, Lebanon, Lithuania, Macedonia, Madeira, Morocco, Netherlands, North Korea, Norway, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland, Tajikistan, Tunisia (Marshall et al. 2011).

152. *Coproica pusio* (Zetterstedt, 1847)

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Bagrote Valley; Khunjerab Pass; Babusar Pass (Hayashi 1991)); Afghanistan, Andorra, Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, Germany, Great Britain, Hungary, Japan, Italy, Latvia, Lithuania, Norway, Mongolia, Russia, Serbia, Slovakia, Spain, Sweden, Switzerland (Marshall et al. 2011).

153. *Coproica rufifrons* Hayashi, 1991

Distribution: Pakistan (Gilgit-Baltistan terr., Gilgit div., Chilas; Khyber Pakhtunkhwa prov., Hazara div., Ayubia, Nathia Gali, Tandani, Battagram, Thakot, Malakand div., Kalam Valley, Marghazar, Mingora, Shangla Pass, Ushu, Balakot, Naran Valley, Kagan valley; Punjab prov., Rawalpindi div., Murree, Taxila; Islamabad Terr.;

Sindh prov., Hyderabad div., Hyderabad (Hayashi 1991)); China, Japan, Taiwan, Yemen, American Samoa, Australia, Cook Is., Fiji, French Polynesia, Kiribati, Marshall Is., Micronesia, Palau, Papua New Guinea, Solomon Is., Tonga, Vanuatu, Western Samoa, America, Argentina, Bermuda, Brazil, Bolivia, Ecuador, Galápagos Is., Grenada, Mexico, Afghanistan, Canary Is., China, Croatia, Hungary, Germany, Greece, Italy, Japan, Madeira, Malta, Saudi Arabia, Tunisia, United Arab Emirates (Marshall et al. 2011).

CONCLUSION

An updated inventory of true flies (Insecta: Diptera) from Azad Jammu & Kashmir and Gilgit-Baltistan are summed up based on the available information shown in Table 1. & Table 2, respectively. It comprises of 64 genera and 153 species in 16 families. It shows that exceptionally less area of Azad Jammu & Kashmir and Gilgit Baltistan are explored for dipteran fauna. Additionally, if we compare the total area of Azad Jammu & Kashmir and Gilgit Baltistan, the area of Gilgit Baltistan is much larger than Azad Jammu & Kashmir. However, we analyzed the species recorded, which revealed that Azad Jammu & Kashmir has 94 species and 75 species of Diptera recorded from Gilgit Baltistan.

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Table 1. List of species present/absent in Azad Jammu & Kashmir (AK) and Gilgit Baltistan (GB).

	Family	Genus	Species	AK	GB
1	Limoniidae	<i>Limonia</i> Meigen, 1803	<i>Limonia neananta</i> Alexander, 1966	-	+
2		<i>Erioptera</i> Meigen, 1803	<i>Erioptera palliclavata</i> Alexander, 1935	-	+
3		<i>Gonomyia</i> Meigen, 1818	<i>Gonomyia dissidens</i> Alexander, 1957	-	+
4		<i>Ctenophora</i> Meigen, 1803	<i>Ctenophora longisector</i> Alexander, 1959	-	+
5	Stratiomyidae	<i>Ptecticus</i> Loew, 1855	<i>Ptecticus vulpianus</i> (Enderlein, 1914)	+	-
6			<i>Ptecticus melanurus</i> (Walker, 1848)	+	-
7	Tabanidae	<i>Haematopota</i> Meigen, 1803	<i>Haematopota kashmirensis</i> Stone & Philip, 1974	-	+
8	Syrphidae	<i>Mallota</i> Meigen, 1822	<i>Mallota rufipes</i> Brunetti, 1913	-	+
9		<i>Mesembrius</i> Rondani, 1857	<i>Mesembrius quadrivittatus</i> (Wiedemann, 1819)	-	+
10		<i>Ceriana</i> Fabricius, 1794	<i>Ceriana dimidiatipennis</i> (Brunetti, 1923)	+	+
11			<i>Ceriana brevis</i> (Brunetti, 1923)	+	-
12		<i>Eristalis</i> Latreille, 1804	<i>Eristalis (Eoseristalis) albibasis</i> Bigot, 1880	-	+
13			<i>Eoseristalis (Eoseristalis) arbustorum</i> (Linnaeus, 1758)	+	+
14			<i>Eristalis (Eoseristalis) cerealis</i> Fabricius, 1805	+	-
15			<i>Eristalis (Eoseristalis) himalayensis</i> (Brunetti, 1908)	+	-
16			<i>Eristalis tenax</i> (Linnaeus, 1758)	+	+
17		<i>Eristalinus</i> Rondani, 1845	<i>Eristalinus (Eristalinus) aeneus</i> (Scopoli, 1763)	+	-
18			<i>Eristalinus (Eristalinus) arvorum</i> (Fabricius, 1787)	+	-
19			<i>Eristalinus (Eristalinus) megacephalus</i> (Rossi, 1794)	+	-
20			<i>Eristalinus (Eristalinus) obliquus</i> (Wiedemann, 1824)	+	-
21			<i>Eristalinus (Eristalinus) sepulchralis</i> (Linnaeus, 1758)	+	-
22			<i>Eristalinus (Eristalinus) tarsalis</i> (Macquart, 1855)	+	-
23			<i>Eristalinus (Eristalodes) taeniops</i> (Wiedemann, 1818)	+	-
24		<i>Syrirta</i> Lepeletier and Serville, 1828	<i>Syrirta orientalis</i> Macquart, 1842	+	-
25			<i>Syrirta pipiens</i> (Linnaeus, 1758)	+	-
26		<i>Rhingia</i> Scopoli, 1763	<i>Rhingia angusticincta</i> Brunetti, 1908	+	-
27			<i>Rhingia siwalikensis</i> Nayar, 1968	+	-
28		<i>Xylota</i> Meigen, 1822	<i>Xylota coquillettii</i> Hervé-Bazin, 1914	+	-
29			<i>Xylota nursi</i> Brunetti, 1923	+	-
30		<i>Baccha</i> Fabricius, 1805	<i>Baccha maculata</i> Walker, 1852	+	-
31		<i>Platycheirus</i> Lepeletier & Serville, 1828	<i>Platycheirus albimanus</i> (Fabricius, 1781)	+	-
32			<i>Platycheirus ambiguus</i> (Fallén, 1817)	+	-
33		<i>Volucella</i> Geoffroy, 1762	<i>Volucella peleterii</i> Macquart, 1834	+	+
34			<i>Volucella ruficauda</i> Brunetti, 1907	-	+
35		<i>Paragus</i> Latreille, 1804	<i>Paragus (Pandasyopthalmus) annandalei</i> Ghorpadé, 1992	+	-
36			<i>Paragus (Pandasyopthalmus) politus</i> Wiedemann, 1830	+	-
37			<i>Paragus (Pandasyopthalmus) haemorrhous</i> Meigen, 1822	+	-
38			<i>Paragus (Paragus) quadrifasciatus</i> Meigen, 1822	-	+
39			<i>Paragus (Paragus) compeditus</i> Wiedemann, 1830	-	+
40		<i>Asarkina</i> Macquart, 1834	<i>Asarkina incisuralis</i> (Macquart, 1855)	+	-
41		<i>Betasyrphus</i> Matsumura, 1917	<i>Betasyrphus aeneifrons</i> (Brunetti, 1913)	+	-
42			<i>Betasyrphus isaaci</i> (Bhatia, 1933)	+	-
43		<i>Chrysotoxum</i> Meigen, 1803	<i>Chrysotoxum baphyrum</i> Walker, 1849	+	-
44			<i>Chrysotoxum intermedium</i> Meigen, 1822	-	+

	Family	Genus	Species	AK	GB
45		<i>Episyrphus</i> Matsumura & Adachi 1917	<i>Episyrphus balteatus</i> (De Geer, 1776)	+	+
46			<i>Episyrphus viridaureus</i> (Wiedemann, 1824)	+	-
47		<i>Ischiodon</i> Sack, 1913	<i>Ischiodon scutellaris</i> (Fabricius, 1805)	+	-
48		<i>Eupeodes</i> Osten Sacken, 1877	<i>Eupeodes bucculatus</i> (Rondani, 1857)	+	-
49			<i>Eupeodes corollae</i> (Fabricius, 1794)	+	-
50			<i>Eupeodes latifasciatus</i> (Macquart, 1829)	+	-
51		<i>Scaeva</i> Fabricius, 1805	<i>Scaeva latimaculata</i> (Brunetti 1923)	+	+
52			<i>Scaeva pyrastris</i> (Linnaeus, 1758)	+	-
53		<i>Sphaerophoria</i> Lepeletier & Serville, 1828	<i>Sphaerophoria bengalensis</i> Macquart, 1842	+	-
54			<i>Sphaerophoria Indiana</i> Bigot, 1884	+	-
55			<i>Sphaerophoria scripta</i> (Linnaeus, 1758)	+	-
56		<i>Syrphus</i> Fabricius, 1775	<i>Syrphus dalhousiae</i> Ghorpadé 1994	+	-
57			<i>Syrphus fulvifacies</i> Brunetti, 1913	+	-
58			<i>Syrphus torvus</i> (Osten Sacken, 1875)	+	-
59			<i>Syrphus vitripennis</i> Meigen, 1822	-	+
60		<i>Xanthogramma</i> Schiner, 1860	<i>Xanthogramma pedissequum</i> (Harris, 1776)	+	-
61	Calliphoridae	<i>Calliphora</i> Robineau–Desvoidy, 1830	<i>Calliphora chinghaiensis</i> Van et Ma, 19784	-	+
62			<i>Calliphora himalayana</i> Kurahashi, 1994	-	+
63			<i>Calliphora loewi</i> Enderlein, 1903	+	-
64			<i>Calliphora uralensis</i> Villeneuve, 1922	-	+
65			<i>Calliphora vicina</i> Robineau–Desvoidy, 1830	-	+
66			<i>Calliphora vomitoria</i> (Linnaeus, 1758)	+	+
67		<i>Cynomya</i> Robineau–Desvoidy, 1830	<i>Cynomya mortuorum</i> (Linnaeus, 1761)	-	+
68		<i>Onesia</i> Robineau–Desvoidy, 1830	<i>Onesia menechmoides</i> (Chen, 1979)	-	+
69			<i>Onesia pamirica</i> Rohdendorf, 1962	-	+
70		<i>Melinda</i> Robineau–Desvoidy, 1830	<i>Melinda scutellata</i> (Senior–White, 1923)	+	-
71		<i>Lucilia</i> Robineau–Desvoidy, 1830	<i>Lucilia porphyria</i> (Walker, 1856)	-	+
72			<i>Lucilia sericata</i> (Meigen, 1826)	-	+
73		<i>Pollenia</i> Robineau–Desvoidy, 1830	<i>Pollenia pediculata</i> Macquart, 1834	-	+
74			<i>Pollenia rudis</i> (Fabricius, 1794)	-	+
75		<i>Chrysomya</i> Robineau–Desvoidy, 1830	<i>Chrysomya phaonis</i> Séguy, 1928	-	+
76		<i>Protocalliphora</i> Hough, 1899	<i>Protocalliphora azurea</i> (Fallén, 1817)	-	+
77			<i>Protocalliphora terraenovae</i> (Robineau Desvoidy, 1830)	-	+
78		<i>Isomyia</i> Walker, 1860	<i>Isomyia electa</i> (Villeneuve, 1927)	+	-
79		<i>Rhinia</i> Robineau–Desvoidy, 1830	<i>Rhinia apicalis</i> (Wiedemann, 1830)	+	-
80		<i>Stomorphina</i> Rondani, 1861	<i>Stomorphina discolor</i> (Fabricius, 1794)	-	+
81			<i>Stomorphina xanthogaster</i> (Wiedemann, 1820)	+	-
82	Fanniidae	<i>Fannia</i> Robineau–Desvoidy, 1830	<i>Fannia canicularis</i> (Linnaeus, 1761)	+	+
83			<i>Fannia dupla</i> Nishida, 1974	+	-
84			<i>Fannia indica</i> Chillcott, 1961	+	+
85			<i>Fanniamanicata</i> (Meigen, 1826)	-	+
86			<i>Fannia scalaris</i> (Fabricius, 1794)	-	+
87	Rhinophoridae	<i>Tromodesia</i> Rondani, 1856	<i>Tromodesia setiventris</i> (Rohdendorf, 1935)	-	+
88	Sarcophagidae	<i>Ravinia</i> Robineau–Desvoidy, 1863	<i>Ravinia pernix</i> Harris, 1780	-	+
89		<i>Sarcophaga</i> Meigen, 1826	<i>Sarcophaga aegyptica</i> (Salem, 1935)	-	+
90			<i>Sarcophaga albiceps</i> Meigen, 1826	-	+

	Family	Genus	Species	AK	GB
91	Sarcophagidae	<i>Sarcophaga</i> Meigen, 1826	<i>Sarcophaga altitudinis</i> Sugiyama, 1964	-	+
92			<i>Sarcophaga brevicornis</i> Ho, 1934	-	+
93			<i>Sarcophaga calicifera</i> Boettcher, 1912	-	+
94			<i>Sarcophaga cruentata</i> Meigen, 1826	-	+
95			<i>Sarcophaga doleschalii</i> Johnston et Tiegs, 1921	+	-
96			<i>Sarcophaga flagellifera</i> Grunin, 1964	-	+
97			<i>Sarcophaga gorodkovi</i> (Grunin, 1964)	-	+
98			<i>Sarcophaga hirtipes</i> Wiedemann, 1830	-	+
99			<i>Sarcophaga idmais</i> Ségué, 1934	+	+
100			<i>Sarcophaga kentejana</i> (Rohdendorf, 1937)	+	+
101			<i>Sarcophaga nathani</i> (Lopes, 1961)	-	+
102			<i>Sarcophaga peshelicis</i> Senior-White, 1930	+	+
103			<i>Sarcophaga portschinskyi</i> (Rohdendorf, 1937)	-	+
104			<i>Sarcophaga shresthai</i> Kano et Shinonaga, 1969	+	-
105			<i>Sarcophaga tubersoa</i> Pandellé, 1896	-	+
106			<i>Sarcophaga yunnanensis</i> Fan, 1964	+	-
107	Tachinidae	<i>Cylindromyia</i> Meigen, 1803	<i>Cylindromyia evibrissata</i> Townsend, 1927	+	-
108		<i>Euthera</i> Loew, 1854	<i>Euthera tuckeri</i> Bezzi, 1925	+	-
109		<i>Elodia</i> Robineau-Desvoidy, 1863	<i>Elodia morio</i> (Fallén, 1820)	+	-
110		<i>Torocca</i> Walker, 1859	<i>Torocca munda</i> (Walker, 1856)	+	-
111	Tephritidae	<i>Bactrocera</i> Macquart, 1835	<i>Bactrocera (Bactrocera) dorsalis</i> (Hendel, 1912)	+	-
112			<i>Bactrocera (Bactrocera) zonata</i> (Saunders, 1841)	+	-
113			<i>Bactrocera (Bactrocera) correcta</i> (Bezzi, 1916)	+	-
114			<i>Bactrocera (Bactrocera) nigrofemoralis</i> Tsuruta and white 2001	+	-
115			<i>Bactrocera (Zeugodacus) cucurbitae</i> (Coquillett, 1899)	+	-
116			<i>Bactrocera (Zeugodacus) scutellaris</i> Bezzi, 1913	+	-
117			<i>Bactrocera (Zeugodacus) tau</i> (Walker, 1849)	+	-
118		<i>Dacus</i> Fabricius, 1805	<i>Dacus (Callantra) sphaerodalis</i> (Bezzi, 1916)	+	-
119			<i>Dacus (Callantra) longicornis</i> (Wiedemann, 1830)	+	-
120		<i>Tephritis</i> Latreille, 1804	<i>Tephritis frauenfeldi</i> Hendel, 1927	+	-
121			<i>Tephritis hyoscyami</i> Linnaeus, 1758	+	-
122		<i>Terellia</i> Robineau-Desvoidy, 1830	<i>Terellia serratulae</i> (Linnaeus, 1758)	+	-
123	Chamaemyiidae	<i>Leucopis</i> Meigen, 1830	<i>Leucopis</i> sp.	+	-
124	Dryomyzidae	<i>Dryomyza</i> Fallén, 1820	<i>Dryomyza pakistana</i> Kurahashi, 1989	+	-
125	Sepsidae	<i>Saltella</i> Robineau-Desvoidy, 1830	<i>Saltella setigera</i> Brunetti, 1910	+	-
126		<i>Meroplus</i> Rondani, 1874	<i>Meroplus minutus</i> Wiedemann, 1830	+	-
127		<i>Australosepsis</i> Malloch, 1925	<i>Australosepsis frontalis</i> Malloch, 1925	+	-
128		<i>Decachaetophora</i> Duda, 1926	<i>Decachaetophora aeneips</i> (de Meijere, 1911)	+	+
129		<i>Dicranosepsis</i> Duda, 1926	<i>Dicranosepsis bicolor</i> (Wiedemann, 1830)	+	-
130			<i>Dicranosepsis olfactoria</i> Iwasa, 1984	+	-
131			<i>Dicranosepsis parva</i> Iwasa, 1984	+	-
132			<i>Dicranosepsis quadrigemina</i> Iwasa 1989	+	-

	Family	Genus	Species	AK	GB
133		<i>Sepsis</i> Fallén, 1810	<i>Sepsis barbata</i> Becker, 1907	-	+
134			<i>Sepsis dissimilis</i> Brunetti, 1910	+	+
135			<i>Sepsis fissa</i> Becker, 1903	+	-
136			<i>Sepsis lateralis</i> Wiedemann, 1830	-	+
137			<i>Sepsis mediana</i> Iwasa, 1989	-	+
138			<i>Sepsis neocynipsea</i> Melander et Spuler, 1917	-	+
139			<i>Sepsis nitens</i> Wiedemann, 1824	+	-
140			<i>Sepsis orthocnemis</i> Frey, 1908	-	+
141			<i>Sepsis punctum</i> (Fabricius 1974)	-	+
142			<i>Sepsis thoracica</i> (Robineau–Desvoidy, 1830)	+	+
143			<i>Sepsis violacea</i> Meigen, 1826	-	+
144		<i>Themira</i> Robineau–Desvoidy, 1830	<i>Themira minor</i> (Haliday, 1833)	-	+
145	Chloropidae	<i>Polyodaspis</i> Duda, 1933	<i>Polyodaspis</i> sp.	+	-
146		<i>Siphunculina</i> Rondani, 1856	<i>Siphunculina carinata</i> Kanmiya, 1989	-	+
147		<i>Thaumatomyia</i> Zenker, 1833	<i>Thaumatomyia notate</i> (Meigen, 1930)	+	-
148	Sphaeroceridae	<i>Coproica</i> Rondani, 1861	<i>Coproica acutangula</i> (Zetterstedt, 1847)	-	+
149			<i>Coproica digitata</i> (Duda, 1918)	-	+
150			<i>Coproica hirtula</i> (Rondani, 1880)	-	+
151			<i>Coproica lugubris</i> (Haliday, 1835)	-	+
152			<i>Coproica pusio</i> (Zetterstedt, 1847)	-	+
153			<i>Coproica rufifrons</i> Hayashi, 1991	-	+

Table 2. Total Number of species recorded in Azad Jammu & Kashmir (AK) and Gilgit Baltistan (GB).

Family	Genus	Total Species	AK	GB
Limoniidae	<i>Limonia</i> Meigen, 1803	1	0	1
	<i>Erioptera</i> Meigen, 1803	1	0	1
	<i>Gonomyia</i> Meigen, 1818	1	0	1
	<i>Ctenophora</i> Meigen, 1803	1	0	1
Stratiomyidae	<i>Ptecticus</i> Loew, 1855	2	2	0
Tabanidae	<i>Haematopota</i> Meigen, 1803	1	0	1
Syrphidae	<i>Mallota</i> Meigen, 1822	1	0	1
	<i>Mesembrius</i> Rondani, 1857	1	0	1
	<i>Ceriana</i> Fabricius, 1794	2	2	1
	<i>Eristalis</i> Latreille, 1804	5	4	3
	<i>Eristalinus</i> Rondani, 1845	7	7	0
	<i>Syritta</i> Lepeletier and Serville, 1828	2	2	0
	<i>Rhingia</i> Scopoli, 1763	2	2	0
	<i>Xylota</i> Meigen, 1822	2	2	0
	<i>Baccha</i> Fabricius, 1805	1	1	0
	<i>Platycheirus</i> Lepeletier & Serville, 1828	2	2	0
	<i>Volucella</i> Geoffroy, 1762	2	1	2
	<i>Paragus</i> Latreille, 1804	5	3	2
	<i>Asarkina</i> Macquart, 1834	1	1	0
	<i>Betasyrphus</i> Matsumura, 1917	2	2	0

Family	Genus	Total Species	AK	GB
	<i>Chrysotoxum</i> Meigen, 1803	2	1	1
	<i>Episyrphus</i> Matsumura & Adachi 1917	2	2	1
	<i>Ischiodon</i> Sack, 1913	1	1	0
	<i>Eupeodes</i> Osten Sacken, 1877	3	3	0
	<i>Scaeva</i> Fabricius, 1805	2	2	1
	<i>Sphaerophoria</i> Lepeletier & Serville, 1828	3	3	0
	<i>Syrphus</i> Fabricius, 1775	4	3	1
	<i>Xanthogramma</i> Schiner, 1860	1	1	0
Calliphoridae	<i>Calliphora</i> Robineau-Desvoidy, 1830	6	2	5
	<i>Cynomya</i> Robineau-Desvoidy, 1830	1	0	1
	<i>Onesia</i> Robineau-Desvoidy, 1830	2	0	2
	<i>Melinda</i> Robineau-Desvoidy, 1830	1	1	0
	<i>Lucilia</i> Robineau-Desvoidy, 1830	2	0	2
	<i>Pollenia</i> Robineau-Desvoidy, 1830	2	0	2
	<i>Chrysomya</i> Robineau-Desvoidy, 1830	1	0	1
	<i>Protocalliphora</i> Hough, 1899	2	0	2
	<i>Isomyia</i> Walker, 1860	1	1	1
	<i>Rhinia</i> Robineau-Desvoidy, 1830	1	1	0
	<i>Stomorphina</i> Rondani, 1861	2	1	1
Fanniidae	<i>Fannia</i> Robineau-Desvoidy, 1830	5	3	4
Rhinophoridae	<i>Tromodesia</i> Rondani, 1856	1	0	1
Sarcophagidae	<i>Ravinia</i> Robineau-Desvoidy, 1863	1	0	1
	<i>Sarcophaga</i> Meigen, 1826	18	6	15
	<i>Cylindromyia</i> Meigen, 1803	1	1	0
Tachinidae	<i>Euthera</i> Loew, 1854	1	1	0
	<i>Elodia</i> Robineau-Desvoidy, 1863	1	1	0
	<i>Torocca</i> Walker, 1859	1	1	0
Tephritidae	<i>Bactrocera</i> Macquart, 1835	7	7	0
	<i>Dacus</i> Fabricius, 1805	2	2	0
	<i>Tephritis</i> Latreille, 1804	2	2	0
	<i>Terellia</i> Latreille, 1758	1	1	0
Chamaemyiidae	<i>Leucopis</i> Meigen, 1830	1	1	0
Dryomyzidae	<i>Dryomyza</i> Fallén, 1820	1	1	0
Sepsidae	<i>Saltella</i> Robineau-Desvoidy, 1830	1	1	0
	<i>Meroplius</i> Rondani, 1874	1	1	0
	<i>Australosepsis</i> Malloch, 1925	1	1	0
	<i>Decachaetophora</i> Duda, 1926	1	1	1
	<i>Dicranosepsis</i> Duda, 1926	4	4	0
	<i>Sepsis</i> Fallén, 1810	11	4	9
	<i>Themira</i> Robineau-Desvoidy, 1830	1	0	1
Chloropidae	<i>Polyodaspis</i> Duda, 1933	1	1	0
	<i>Siphunculina</i> Rondani, 1856	1	0	1
	<i>Thaumatomyia</i> Zenker, 1833	1	1	0
Sphaeroceridae	<i>Coproica</i> Rondani, 1861	6	0	6
Total		153	94	75

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INTRODUCTION

The Thar Desert or the Great Indian Desert is a subtropical hot desert that stretches between the Aravalli Mountains and the Indus River in the northwestern part of the Indian sub-continent with an area of over 4,000 km² (Sivaperuman et al. 2009; Dhir & Singhvi 2012). It is an extension of the Sahara-Arabian and southern Iranian subtropical desert regions and forms an important biogeographical region of India which has unique habitat types of desert grasslands, rocky expanses and sand dunes (Sømme 1995; Sivaperuman et al. 2009) and is the only subtropical desert present in the Oriental realm. The Indian stretch of the Thar Desert is located entirely in the western part of Rajasthan (Image 1). A few invertebrate groups of the region (dung beetles, darkling beetles, spiders, and ants) have been documented (Sewak 2009; Sivaperuman & Rathore 2009; Tak 2009). Except for the report of three carabid species (*Calosoma orientalis* (Hope, 1833) erroneously termed as *Carabus orientalis*; *Anthia sexguttata* (Fabricius, 1775) erroneously termed as *Anthia sexmaculata*; and *Calosoma imbricatum* Klug, 1832 erroneously identified as *Calosoma maderae*) from a regional study (Kazmi & Ramamurthy 2004), no data on the Carabidae fauna of the Thar Desert, exists in contrast to the detailed report of Carabidae from the adjoining Sahara-Arabian and southern Iranian subtropical desert regions (Abdel-Dayem 2012; Assmann et al. 2015; Azadbakhsh & Nozari 2015; Abdel-Dayem et al. 2018, 2019). Desert carabids have to be well adapted to high temperatures and lack of water (Andersen et al. 1986). Carabidae inhabiting the desert are usually of larger size as relative water loss decreases with increasing body size (Andersen et al. 1986; Sømme 1995; Zachariassen 1996). The present effort provides data on the Carabidae of the Thar Desert, which includes the list of species, distribution pattern, images, and a key to the species.

MATERIALS AND METHODS

Collections of Carabidae available in the Zoological Survey of India, Desert Regional Center (ZSI DRC), Jodhpur (Collected between 1962 to 2001) have been identified. Specimens were identified till subfamily and tribe level with the modified Keys prepared from Andrewes (1929, 1935) by the first author. Generic and species level identification were carried out using keys in Chanu & Swaminathan (2017), Akhil (2019), Akhil & Sabu (2019), and Akhil et al. (2020). Identification and

imaging were done with the help of a Leica M205C stereo zoom microscope fitted with a Leica MC 170 HD camera and Leica Application Suite (LAS V4.12) software having auto montage feature. All specimens were identified to species level by S.V. Akhil.

RESULTS

Checklist of Carabidae from Thar Desert

(* first records from Rajasthan state)

Subfamily Anthiinae Bonelli, 1813

Tribe Anthiini Bonelli, 1813

Genus *Anthia* Weber, 1801

Anthia sexguttata (Fabricius, 1775)

Image 2A

Specimen examined: 1 ex., male, India: Rajasthan: Jodhpur, 30.xi.1963, coll. R.N. Bhargava

Distribution: India (Himalaya; Rajasthan: Jodhpur; Gujarat: Surat; Maharashtra: Pune; Karnataka: Bangalore; Tamil Nadu: Kalayar kovil, Edaikazhinadu (Gangathakuppam), Kattupakkam, Nemili, Kunnathu pond (Villupuram dt.), Vedanthangal, Karkodai (Theni dt.), Vedur Reservoir (Tindivanam), Palavakal, Thiruvannamalai, Mudumalai, Pachaimalai hills, Manchavadi, Tharangambadi; Pondicherry), Turkmenistan, Iran, Pakistan, Afghanistan, Kazakhstan, Uzbekistan, and Nepal.

Tribe Heliinae Hope, 1838

Genus *Omphra* Dejean, 1825

Omphra complanata Reiche, 1843 *

Image 2B

Specimen examined: 1 ex. female, '304/4', India: Rajasthan: Jodhpur: Ratanada, 20.viii.1984, coll. N.S. Rathore

Distribution: India (Himachal Pradesh: Shimla; Rajasthan: Jodhpur (Ratanada); Odisha: Chilika lake; Maharashtra: Nagpur, Mumbai, Nasik, Sangli, Ratnagiri; Karnataka: Belagavi; Tamil Nadu: Madurai; Pondicherry), Nepal (Janakpur).

Subfamily Brachininae Bonelli, 1810

Tribe Brachinini Bonelli, 1810

Genus *Brachinus* Weber, 1801

Brachinus pictus (Hope, 1833) *

Image 2C

Specimens examined: 6 exs.; 1 male, 1 female, '218/13', India: Rajasthan: Pali Dist.: Hemawas dam, 02.xi.1974, coll. T.G.Vazirani; 1 male, India: Rajasthan:



Image 1. A & B: Location of Thar Desert. (Major Collection Sites: Jodhpur, Bikaner, Jaipur and Udaipur marked). Image Courtesy: Google Earth.

Jodhpur, 25.vii.1972, coll. R.C. Sharma; 1 female, 1 sex undetermined '6928/3', India: Rajasthan: Amar Sagar, 20.vii.1978, coll. N.S. Rathore; 1 sex undetermined, India: Rajasthan: Jodhpur: Bijolai, 30.ix.1962, coll. R.C. Sharma.

Distribution: India (Delhi; Haryana: Kalka; Rajasthan: Pali Dt.: Hemawas dam, Jodhpur (Bijolai), Amar Sagar;

Siwaliks; Bengal; Jharkhand: Medininagar; Maharashtra: Pune, Nagpur; Karnataka: Belgavi, Bengaluru; Tamil Nadu: Chennai; Kerala: Thrissur), Sri Lanka (Hambantota), Iran, and Pakistan.

Genus *Pheropsophus* Solier, 1833***Pheropsophus lissoderus* Chaudoir, 1850 ***

Image 2D

Specimen examined: 1 ex., female, India: Rajasthan: Jodhpur, 06.vi.1963, coll. R.C. Sharma.

Distribution: India (Jammu & Kashmir; Himachal Pradesh; Rajasthan: Jodhpur; Uttarakhand; Sikkim; Arunachal Pradesh; Tamil Nadu: Coimbatore; Kerala: Kalpetta), Sri Lanka (Kandy and Peradeniya), Bhutan, China (Tibet), and Pakistan (Islamabad, Khyber Pakhtunkhwa and Muzaffarabad).

***Pheropsophus sobrinus* (Dejean, 1826) ***

Image 2E

Specimen examined: 1 ex., sex undetermined, India: Rajasthan: Jodhpur: Sardar Samand, 15.i.1963, coll. Motilal.

Distribution: India (Jammu and Kashmir; Himachal Pradesh; Rajasthan: Jodhpur (Sardar Samand); Uttarakhand; Bengal: Kolkata; Sikkim; Arunachal Pradesh; Tamil Nadu: Coimbatore, Tharangambadi, Anaimalai Hills; Puducherry: Karaikal; Kerala: Palakkad), Sri Lanka, Nepal, Bhutan, Taiwan, Pakistan (Rawalpindi, Chakwal, Poonch), and Yemen.

Subfamily Carabinae**Tribe Carabini****Genus *Calosoma* Weber, 1801*****Calosoma imbricatum imbricatum* Klug, 1832**

Image 2F

Specimens examined: 2 exs. 1 male, '1/873', India: Rajasthan: Jodhpur, 25.ix.1964, coll. R.N. Bhargava; 1 female, '3046', India: Rajasthan: Jodhpur, 10.ix.1969, coll. R.N. Bhargava.

Distribution: India (Rajasthan: Jaipur, Mount Abu, Jodhpur, Thar Desert), Pakistan, Afghanistan, Iran, Iraq, Kazakhstan, Turkmenistan, Uzbekistan, Mongolia, Russia, Syria, Saudi Arabia, Qatar, Kuwait, Oman, United Arab Emirates, Yemen, Algeria, Burkina Faso, Cabo Verde, Canary Islands, Djibouti, Egypt, Eritrea, Ethiopia, Kenya, Libya, Mali, Niger, Senegal, Somalia, Sudan, Chad, Namibia, and South Africa.

***Calosoma orientale* (Hope, 1834)**

Image 2G

Specimen examined: 1 ex., male, '8880/5', India: Rajasthan: Jodhpur: ZSI campus, 17.viii.2001, coll. R. Sewak.

Distribution: India (West Bengal; Bihar: Chapra; Rajasthan: Jodhpur (ZSI Campus); Gujarat: Bhavnagar, Godhra; Madhya Pradesh: Khandwa; Maharashtra: Pune,

Nasik; Karnataka: Bengaluru, Chikamagaluru; Tamil Nadu: Coimbatore, Kodaikanal, Madurai, Manaparai), Sri Lanka, China? (Häckel 2017), and Pakistan? (Häckel 2017).

Subfamily Harpalinae Bonelli, 1810**Tribe Anisodactylini Lacordaire, 1854****Genus *Pseudognathaphanus* Schaubberger, 1932*****Pseudognathaphanus punctilabris* (W.S. Macleay, 1825)***

Image 2H

Specimens examined: 4 exs., sex undetermined, '304/4', India: Rajasthan: Jodhpur: Ratanada, 20.viii.1984, coll. N.S. Rathore.

Distribution: India (Himachal Pradesh: Kulu; Rajasthan: Jodhpur (Ratanada); Assam: Kohora; Odisha: Ganjam (Surada); Tamil Nadu: Anaimalai Hills; Puduchery; Andaman and Nicobar Islands), Sri Lanka, Myanmar, Thailand, Indonesia (Java, Sumatra, Sulawesi), Vietnam, Nepal, Philippines, and China.

Subfamily Licininae, Bonelli, 1810**Tribe Chlaenini Brulle, 1834****Genus *Chlaenius* Bonelli, 1810*****Chlaenius germanus* Chaudoir, 1876**

Image 2I

Specimens examined: 2 exs., 1 sex undetermined, India: Rajasthan: Jodhpur: Mandore, 05.v.1965, coll. V.C. Agarwal; 1 female, India: Rajasthan: Jodhpur: Kailana, 12.ix.1979, coll. K.V. Rama Rao.

Distribution: India (Rajasthan: Jaipur (Durgapura), Jodhpur (Kailana, Mandore); Uttarakhand: Bhatkot, Kumaon; Karnataka: Kerwadi; West Bengal: Kolkata), Myanmar, Bangladesh, and Laos.

***Chlaenius laevioplaga frater* Chaudoir, 1876 ***

Image 3A

Specimens examined: 3 exs., 1 male, '3152', India: Rajasthan: Jodhpur: Kailana, 24.ix.1964, coll. K.V.S Rao; 1 male, '8260/3', India: Rajasthan: Jodhpur: Kailana, 21.ix.1979, coll. N.S. Rathore; 1 female, '4128', India: Rajasthan: Jodhpur, 15.iv.1965, coll. V.C. Agarwal.

Distribution: India (Rajasthan: Jodhpur (Kailana); Gujarat: Kathiawar: Sasan; Bihar: Pusa; Jharkhand: Singhbhum; Madhya Pradesh: Mhow, Hoshangabad, Motinala; Maharashtra: Nagpur, Pune; Tamil Nadu: Teppukadu, Chennai; Kerala: Malabar), Pakistan, and China.

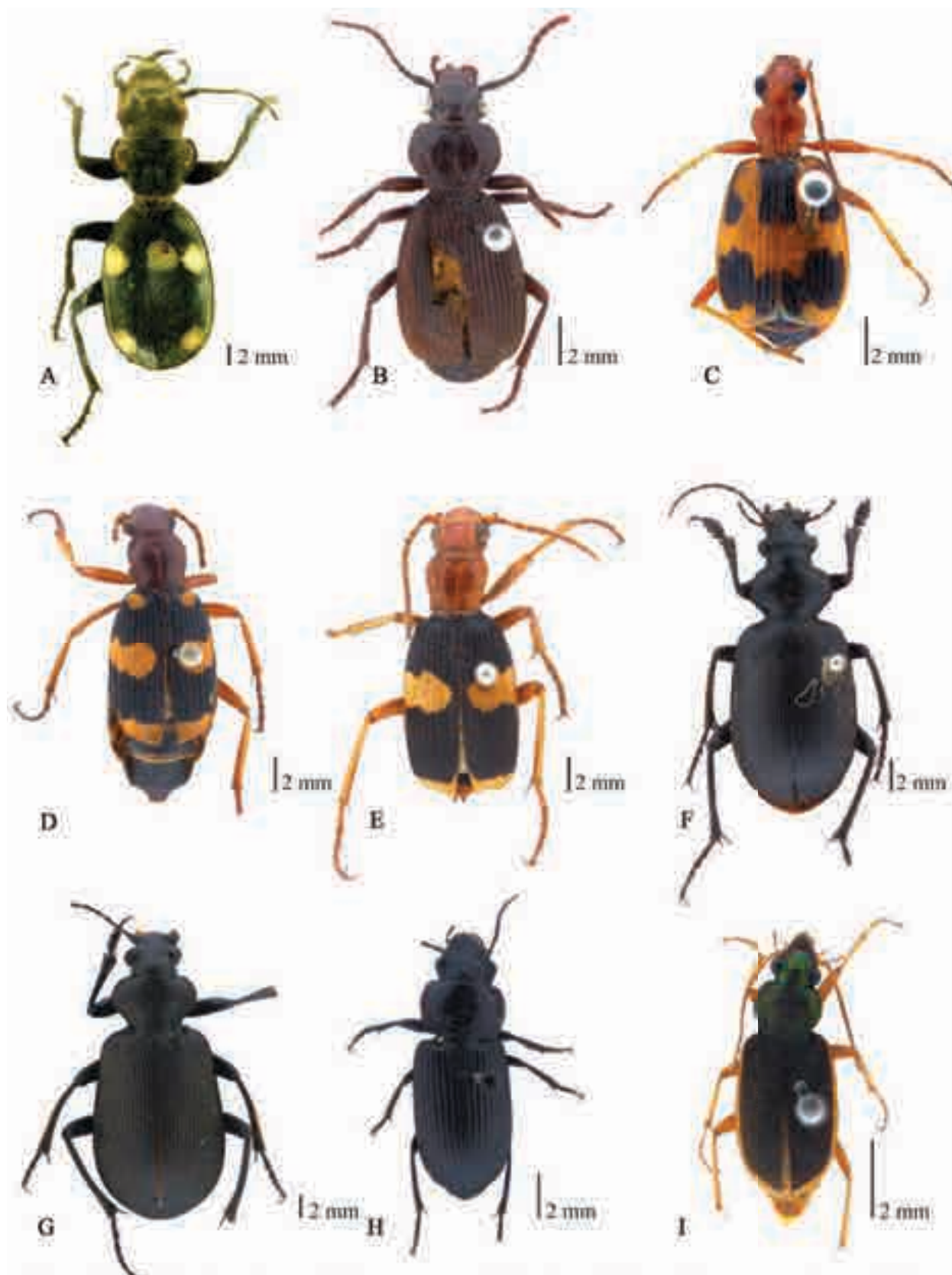


Image 2. Dorsal habitus of: A—*Anthia sexguttata* (Fabricius, 1775) | B—*Omphra complanata* Reiche, 1843 | C—*Brachinus pictus* Hope, 1833 | D—*Pheropsophus lissoderus* Chaudoir, 1850 | E— *Pheropsophus sobrinus* (Dejean, 1826) | F—*Calosoma imbricatum imbricatum* Klug, 1832 | G— *Calosoma orientale* Hope, 1834 | H—*Pseudognathaphanus punctilabris* (W.S.Macleay, 1825) | I—*Chlaenius germanus* Chaudoir, 1876.

Key to Carabidae of Thar Desert

(Modified from Andrewes 1929, 1935; Chanu & Swaminathan 2017; Akhil 2019)

1. Venter with six visible segments 4
 - Venter with seven or eight visible segments (mandibles with setae in the scrobe, elytra truncate and with a narrow membranous border at apex) 2 (Tribe Brachiniini)
2. Mandibular scrobe unisetose *Brachinus pictus* (Genus *Brachinus*)
 - Mandibular scrobe plurisetose 3 (Genus *Pheropsophus*)
3. Head entirely reddish yellow, or reddish brown with frons reddish yellow; pronotum with sides of disc convex anteriorly and straight posteriorly; elytral humeral spot if present very small *Pheropsophus sobrinus*
 - Head entirely reddish brown; pronotum with sides of disc almost straight throughout; elytra with large humeral spot *Pheropsophus lissoderus*
4. Head with two supraorbital seta on each side 5
 - Head with one supraorbital seta on each side 6
5. Antennae inserted immediately beneath the preocular ridges *Omphra complanata* (Tribe Helluonini)
 - Antennae inserted far below the preocular ridges, level with the lower margin of the eyes *Anthia sexguttata* (Tribe Anthiini)
6. Mesocoxal cavities not entirely enclosed by sterna, mesepimera reaching the coxae 7 (Tribe Carabini)
 - Mesocoxal cavities entirely enclosed by sterna, mesepimera not reaching the coxae 8
7. Lateral margins of pronotum bisetose *Calosoma imbricatum imbricatum* (Subgenera *Caminara*)
 - Lateral margins of pronotum unisetose *Calosoma orientale* (Subgenera *Ctenosta*)
8. Epipleura with preapical plica. Antennae with first three antennomeres glabrous 9 (Tribe Chlaeniini)
 - Epipleura without preapical plica. Antennae with first two antennomeres glabrous *Pseudognathaphanus punctilabris*
9. Elytra pubescent 10 (Genus *Chlaenius*)
 - Elytra glabrous *Harpaglossus opacus* (Genus *Harpaglossus*)
10. Elytra with distinct pale lateral longitudinal band from base to apex, or with fascia or spots 11
 - Elytra without distinct pale longitudinal band or fascia or spots *Chlaenius pretiosus*
11. Elytra with distinct pale longitudinal band but without spots or fascia 12
 - Elytra without distinct pale longitudinal band but with spots or fascia 16
12. Pronotum coarsely punctate and pubescent *Chlaenius germanus*
 - Pronotum sparsely punctate and pubescent 13
13. Elytral lateral longitudinal band very narrow, with or without broad apical region 14
 - Elytral lateral longitudinal band broad, without broad apical region 15
14. Elytral longitudinal band broadening at apex forming an apical band *Chlaenius laeviplaga frater*
 - Elytral longitudinal band not broadening at apex *Chlaenius velocipes*
15. Form large; elytral intervals coarse with dense punctures *Chlaenius propinquus*
 - Form small; elytral intervals smooth without punctures *Chlaenius nitidicollis*
16. Elytra with distinct inverted comma like fascia near the apex *Chlaenius virgulifer*
 - Elytra with two distinct rounded spots near the apex *Chlaenius posticus*

***Chlaenius nitidicollis* Dejean, 1826**

Image 3B

Specimen examined: 1 ex., male, '3152', India: Rajasthan: Jodhpur: Kailana, 24.ix.1964, coll. K.V.S Rao.

Distribution: India (Haryana: Kalka; Rajasthan: Jodhpur (Kailana), Udaipur, Durgapura, Ajmer, Bhilwara; West Bengal; Maharashtra: Pune), Myanmar, and Pakistan.

***Chlaenius posticus* (Fabricius, 1798)**

Image 3C

Specimen examined: 1 ex., male, India: Rajasthan: Jodhpur: Mandore, 09.ix.1964, coll. V.C. Agarwal.

Distribution: India (Rajasthan: Mandore, Udaipur, Kota; Uttarakhand: Dehra Dun, Kalsi; Bengal: Kolkata; Assam: Brahmaputra river above Jorhat; Bihar: Pusa; Odisha: Puri; Maharashtra: Bhandara, Pune, Sangli; Karnataka: Gundelpet; Kerala: Tholpetty, Muthanga, Silent Valley, Nilambur), Pakistan (Jhelum), Bangladesh (Dhaka), Myanmar (Rangoon, Teinzo), Nepal, Vietnam (Annam), Indonesia (Java, Sumatra), and China.

***Chlaenius pretiosus* Chaudoir, 1856**

Image 3D

Specimens examined: 5 exs., 1 male, India: Rajasthan: Jodhpur: Kailana, 12.ix.1979, coll. K.V. Rama Rao; 1 female, '1/720', India: Rajasthan: Jodhpur: Kailana, 04.ix.1964, coll. V.C. Agarwal; 1 female, '1/728', India: Rajasthan: Jodhpur: Thakat Sagar, coll. V.C. Agarwal; 1 female, '3152', India: Rajasthan: Jodhpur: Kailana, 24.ix.1964, coll. K.V.S. Rao; 1 female, '304/4', India: Rajasthan: Jodhpur: Ratanada, 20.viii.1984, coll. N.S. Rathore.

Distribution: India (Delhi; Rajasthan: Jaipur, Ajmer, Jodhpur (Kailana, Thakat Sagar, Ratanada); Uttar Pradesh: Sitapur, Mughal Sarai, Lucknow; Uttarakhand: Dehra Dun, Almora), Pakistan, and Sri Lanka.

***Chlaenius propinquus* Csiki, 1931 ***

Image 3E

Specimens examined: 2 exs., 1 female, '8884/5', India: Rajasthan: Jodhpur; ZSI Campus, 24.viii.2001, coll. R. Sewak; 1 sex undetermined, India: Rajasthan: Jodhpur: Kailana, 10.iv.1964, coll. V.C. Agarwal.

Distribution: India (Rajasthan: Jodhpur (ZSI campus, Kailana); Gujarat) and Bangladesh.

***Chlaenius velocipes* Chaudoir, 1876**

Image 3F

Specimen examined: 1 ex. female, '3/443', India: Rajasthan: Jodhpur: Tiwari village, 05.i.1963, coll. K.C.

Kansal.

Distribution: India (Himachal Pradesh: Kangra; Rajasthan: Jaipur (Durgapura), Jodhpur, Udaipur (Udai Sagar, RCA Campus), Bhilwara, Banswara; Uttarakhand: Someshwar, Nainital, Almora, Bhimtal, Haldwani; Bengal: Purulia; Manipur; Maharashtra: Kasara; Tamil Nadu: Kodaikanal, Nilgiri Hills; Kerala: Cardamom hills, Periyar Lake), Sri Lanka (Dikoya), and Nepal.

***Chlaenius virgulifer* Chaudoir, 1876 ***

Image 3G

Specimens examined: 2 exs., 1 male, '8260/3', India: Rajasthan: Jodhpur: Kailana, 21.ix.1979, coll. N.S. Rathore; 1 male, '3/283', India: Rajasthan: Jodhpur: Bijolai tank, date unknown, coll. R.C. Sharma.

Distribution: India (Rajasthan: Jodhpur (Kailana, Bijolai tank); Maharashtra: Pune, Koyna Wildlife Sanctuary, Satara), China, Japan, North Korea, and South Korea.

Genus *Harpaglossus* Motschulsky, 1858***Harpaglossus opacus* Chaudoir, 1857**

Image 3H

Specimens examined: 46 exs., 1 sex undetermined, '1335', India: Rajasthan: Jodhpur: Paota, 19.ix.1963, coll. R.N. Bhargava; 9 males, 5 females, '1/608', India: Rajasthan: Jodhpur: Kailana, 22.vii.1964, coll. V.C. Agarwal; 1 female, '1/635', India: Rajasthan: Jodhpur: Agolai village, 28.vii.1964, coll. V.C. Agarwal; 2 females, '3048', India: Rajasthan: Jodhpur, 10.ix.1964, coll. R.N. Bhargava; 1 female, '1/805', India: Rajasthan: Jodhpur: Agolai Tank, 18.ix.1964, coll. R.N. Bhargava; 1 sex undetermined, India: Rajasthan: Jodhpur: Paota, 29.vii.1961, coll. K.C. Kansal; 2 females, '12142', India: Rajasthan: Jodhpur: Agolai, 19.vii.1965, coll. P.D. Gupta; 1 male, 2 females, '3039', India: Rajasthan: Jodhpur: Mandore, 09.ix.1964, coll. V.C. Agarwal; 1 female, '1/660', India: Rajasthan: Jodhpur: Paota (Patodi House), 15.viii.1964, coll. R.N. Bhargava; 2 males, 9 females, '8877/5', India: Rajasthan: Jodhpur: ZSI Campus, 11.viii.2001, coll. R. Sewak; 3 females, '12170', India: Rajasthan: Jodhpur: Bariganga, 03.viii.1965, coll. V.C. Agarwal; 2 males, '1/871', India: Rajasthan: Jodhpur: Paota, 25.ix.1964, coll. K. V. S. Rao; 1 male, India: Rajasthan: Jodhpur: Khandia tank, 13.ii.1963, coll. K.C. Kansal; 1 male, '1/841', India: Rajasthan: Jodhpur: Danjur, 22.ix.1964, coll. K.K.S. Rao; 1 male, '1419', India: Rajasthan: Jodhpur: Mandore, 04.x.1963, coll. K.C. Kansal; 1 male, '1/1775', India: Rajasthan: Jodhpur: Mandore, 09.ix.1964, coll. V.C. Agarwal,

Distribution: India (Rajasthan: Jodhpur (Paota,

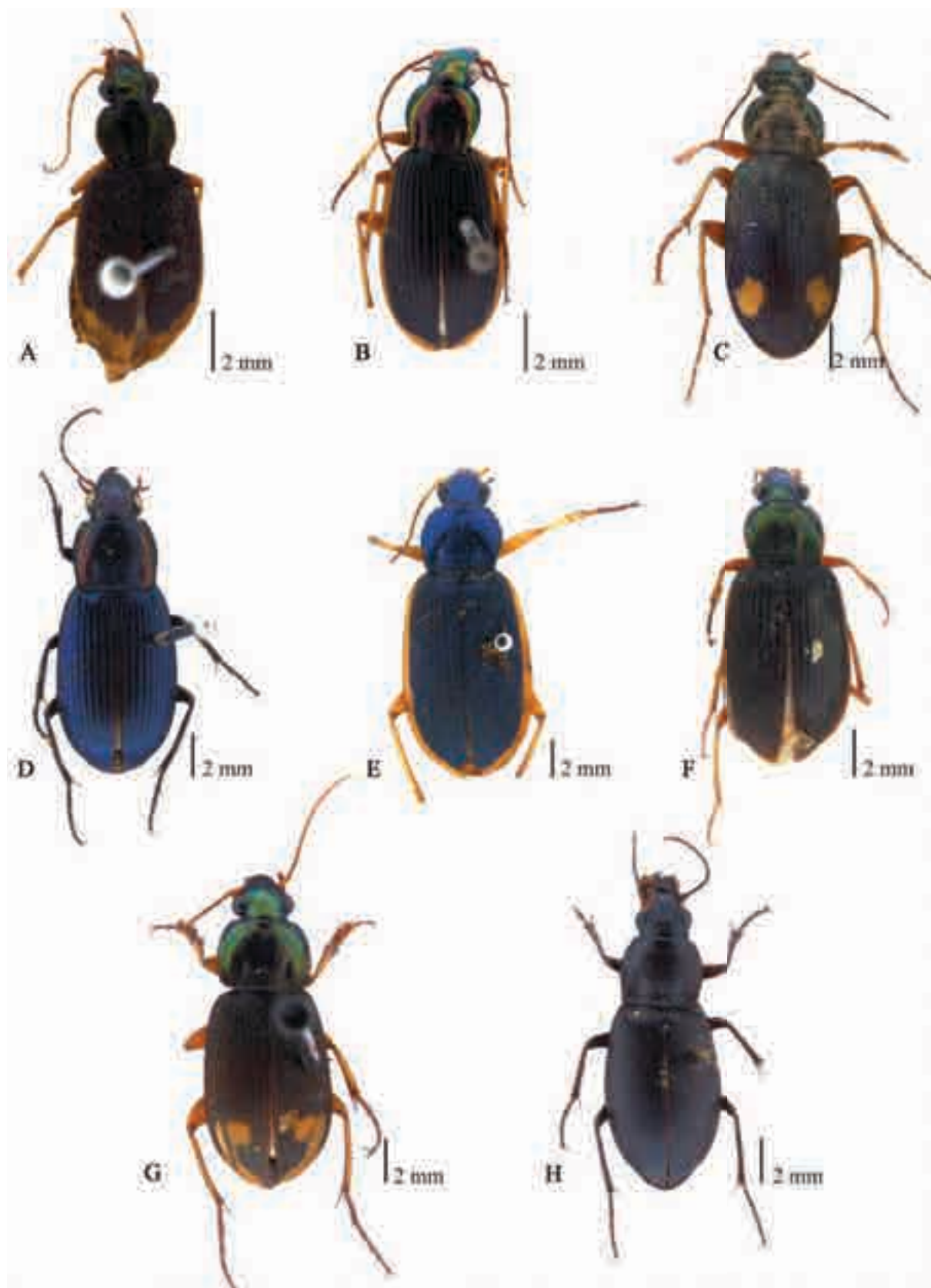


Image 3. Dorsal habitus of: A—*Chlaenius laevi-plaga frater* Chaudoir, 1876 | B—*Chlaenius nitidicollis* Dejean, 1826 | C—*Chlaenius posticus* (Fabricius, 1798) | D—*Chlaenius pretiosus* Chaudoir, 1856 | E—*Chlaenius propinquus* Csiki, 1931 | F—*Chlaenius velocipes* Chaudoir, 1876 | G—*Chlaenius virgulifer* Chaudoir, 1876 | H—*Harpaglossus opacus* (Chaudoir, 1857).

Kailana, Agolai Village, Mandore, ZSI campus, Khandia tank, Bariganga), Ajmer; Gujarat: Kathiawar, Ghogha; West Bengal; Uttar Pradesh: Sitapur; Tamil Nadu: Thiruchirapally) and Sri Lanka.

DISCUSSION

Seventeen species of Carabidae belonging to five subfamilies (Anthiinae, Brachininae, Carabinae, Harpalinae, and Licininae) were recorded from Thar Desert in contrast to the record of 32 species belonging to 10 subfamilies (Brachininae, Carabinae, Dryptinae, Harpalinae, Lebiinae, Licininae, Platyninae, Pterostichinae, Scaritinae, and Trechinae) (Ghahari et al. 2012; Azadbakhsh & Nozari 2015) from southern Iran subtropical desert region to which Thar Desert is connected. Eight species (*Brachinus pictus* (Hope, 1833); *Chlaenius laevipectus* Chaudoir, 1876; *C. propinquus* Csiki, 1931; *C. virgulifer* Chaudoir, 1876; *Omphra complanata* Reiche, 1843; *Pheropsophus lissoderus* Chaudoir, 1850; *P. sobrinus* (Dejean, 1826) and *Pseudognathaphanus punctilabris* W.S. Macleay, 1825) are first records from Rajasthan.

Among the two species of *Calosoma* (*C. imbricatum* Klug, 1832; *C. orientale* Hope, 1834) recorded from the Thar Desert, *C. imbricatum* is a desert specialist showing a distinct distributional pattern along the Saharo-Arabian desert belt. Globally, seven subspecies of *Calosoma imbricatum* (*C. imbricatum andrewesi* Breuning, 1928; *C. imbricatum augustasi* Obydov, 2005; *C. imbricatum deserticola* Semenov, 1897; *C. imbricatum hottentottum* Chaudoir, 1852; *C. imbricatum imbricatum* Klug, 1832; *C. imbricatum linnavuorii* Mandl, 1968; *C. imbricatum loeffleri* Mandl, 1953) were recorded (Mandl 1970; Lorenz 2020) so far, with only one subspecies, *C. imbricatum andrewesi* Breuning, 1928 with distribution outside a desert environment (recorded from Assam; and north of old Bengal Presidency which could be part of current Rajasthan state) (Breuning 1928; Andrewes 1929). *Calosoma imbricatum loeffleri* Mandl, 1953 was synonymised with *Calosoma imbricatum imbricatum* Klug, 1832 by Bruschi (2013).

Of the 17 species recorded from the Thar Desert, only two species (*Anthia sexguttata* and *Calosoma imbricatum*) had desert adaptations like large size and flattened body (fused elytra is an additional desert adaptation in *Anthia sexguttata*) which help in reducing the respiratory water loss (Cloudsley-Thompson 1964; Ahearn & Hadley 1969; Andersen et al. 1986). *Calosoma imbricatum* does not have fused

elytra but have strong flight ability (Farkač & Häckel 2012) which help them to avoid low humidity and dry air (Andersen et al. 1986). These two species are recorded only from arid and semi-arid regions at global level. They are widely present and are large non-subterranean/surface dwelling carabid species in the Thar Desert habitat. Thus, these two species should be taken as the flagship predatory Carabidae of the Indian Thar Desert region.

Of the 17 species recorded, nine species are of subfamily Licininae (eight species of *Chlaenius* and one species of *Harpaglossus*). While analysing the collections and labels of Licininae from the desert region, it was observed that each species was collected in multiple numbers from a single locality, which points towards its previous reports (Bonacci et al. 2004) of showing aggregation behaviour. Members of both *Chlaenius* and *Harpaglossus* show aggregation behaviour, which is a desert adaptation, by which the relative humidity of the habitat could be increased thus decreasing the collective cuticular transpirational water loss (Andersen et al. 1986; Bonacci et al. 2004). Also, most *Chlaenius* are seen near available water bodies in deserts (Bonacci et al. 2004; Kataev pers. comm. 2021), as observed during the present study also. It is apparent from the distribution that other than the two large species – *Anthia sexguttata* and *Calosoma imbricatum* – most species are widely distributed in India and do not have any specific adaptation for desert habitat.

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INTRODUCTION

Coral reefs provide several ecosystem services such as fisheries, tourism and protection to the coastal habitats of tropical and subtropical countries (Yap 2012). Despite of their global importance, the ecological, economic and social integrity of this ecosystem is degrading at an alarming rate due to several natural and anthropogenic disturbances (Barbier 2017; Cox et al. 2017; Woodhead et al. 2019). Therefore, we need to prioritize the conservation of coral reef habitats and the protection of marine fisheries from natural and anthropogenic threats (Simon et al. 2011; Burta et al. 2013). In recent times, coral restoration is emerging as a potential management strategy to protect the degraded reefs along with its associated biota (Edwards & Gomez 2007; Lirman & Schopmeyer 2016). Therefore, the presence of submerged artificial reef structures in the marine ecosystems have proven to play a key role in providing suitable habitat for the enrichment of fish diversity, while serving as a breeding and nursery ground for many fish assemblages (Campbell et al. 2011; Rybicki & Hanski 2013). Reef fishes specifically rely on living corals and the structural complexity provided by the reef environment (Coker et al. 2014). Few experimental studies have documented that fish numbers and diversity are greater in restored coral reefs rather than control or natural environments within a week of the transplantation either on single substrate or multiple substrate which demonstrate the fast rate of fish recolonization (Clynick et al. 2008; Burta et al. 2013; Opel et al. 2017). Reef associated fishes are also important in benthic cover dynamics as they help in the growth and survival of corals by feeding on unwanted macroalgae that grow on live corals (Hughes et al. 2007; Seraphim et al. 2020).

In India, successful coral restoration stories are rare, few completed studies on coral restoration can be found in Gulf of Kutch and the Lakshadweep archipelago (Babu & Sureshkumar 2016; Kumar et al. 2017). Previous studies have used artificial frames and slabs used to transplant corals and make artificial reef structures enabling the restoration of the reef ecosystem (Maragos 1974; Quinn & Kojis 2006; Ferse et al. 2021). Hence, to protect and improve the health and cover of the tropical coral reef and to restore the structure and function of reef ecosystem, a research team from the National Centre for Coastal Research (NCCR) implemented coral reef restoration and submerged artificial reef formation concept in Gulf of Mannar and Palk Bay regions of southeastern coast of India. The present study highlights

the growth and survival of transplanted corals and provides a preliminary report on the fish assemblages in the region which grouped into seven families.

MATERIALS AND METHODS

In February 2021, a reef restoration programme was initiated by the NCCR research team in two selected sites of Palk bay (Site1: Munaikadu, 9.2893°N, 79.1325°E; Site2: Thonithurai, 9.2847°N, 79.1745°E). The waters of Palk Bay joins the Bay of Bengal from the northeast and joins the Gulf of Mannar in the south. The Palk Strait is just 35 km long and is narrower than the English Channel (Azeez et al. 2016). It separates the northern coast of Sri Lanka and southeastern coast of India. It is well known for its rich seagrass ecosystem and its associated biota, it is also an important habitat for endangered marine mammals like Dugongs (Azeez et al. 2016). However, coral reefs in this region are under developed. More than 344 animals from different taxa have been reported by various studies, 186 species of birds, 16 species of mangroves, and nine seagrass species (Bhatt et al. 2012). A traditional method of coral transplantation technique (Ramesh et al. 2020) was used to build the submerged artificial reefs. Iron frames and cement slabs were deployed underwater at a depth of 2.5 m and the deployment was done at a distance of 40 m away from the Low Tide Line (LTL) of seashore in Palk Bay. Frames were installed near to the seagrass bed and few outgrowths of *Padina gymnospora* were observed. The iron frames and cement slabs of the artificial reefs were designed in such a way to reduce sediment deposition on the coral fragments and break the high waves near the sea shore, thus restricting beach erosion (Figure 1). Iron frames are placed in 45° angle with respect to the land and coral fragments (5.00–6.00 cm in size) are tied to the cement slabs with plastic tags and placed on the iron frames. Each frame contains 40 slabs with coral fragments. A total of 6 frames and 240 coral fragments were initially installed underwater at a depth of 2.5 m on 05 February 2021. The location of the restoration site was marked with handheld GPS etrex30 device. Sampling and monitoring was done every month to assess the growth and survival of coral fragments used in coral restoration. During regular monitoring and underwater marine biodiversity surveys in Palk Bay on 09 June 2021, excellent fish assemblages were documented and photographed, by using the NIKON underwater W300 camera. Furthermore, samples were collected and described. Most of fish identification was

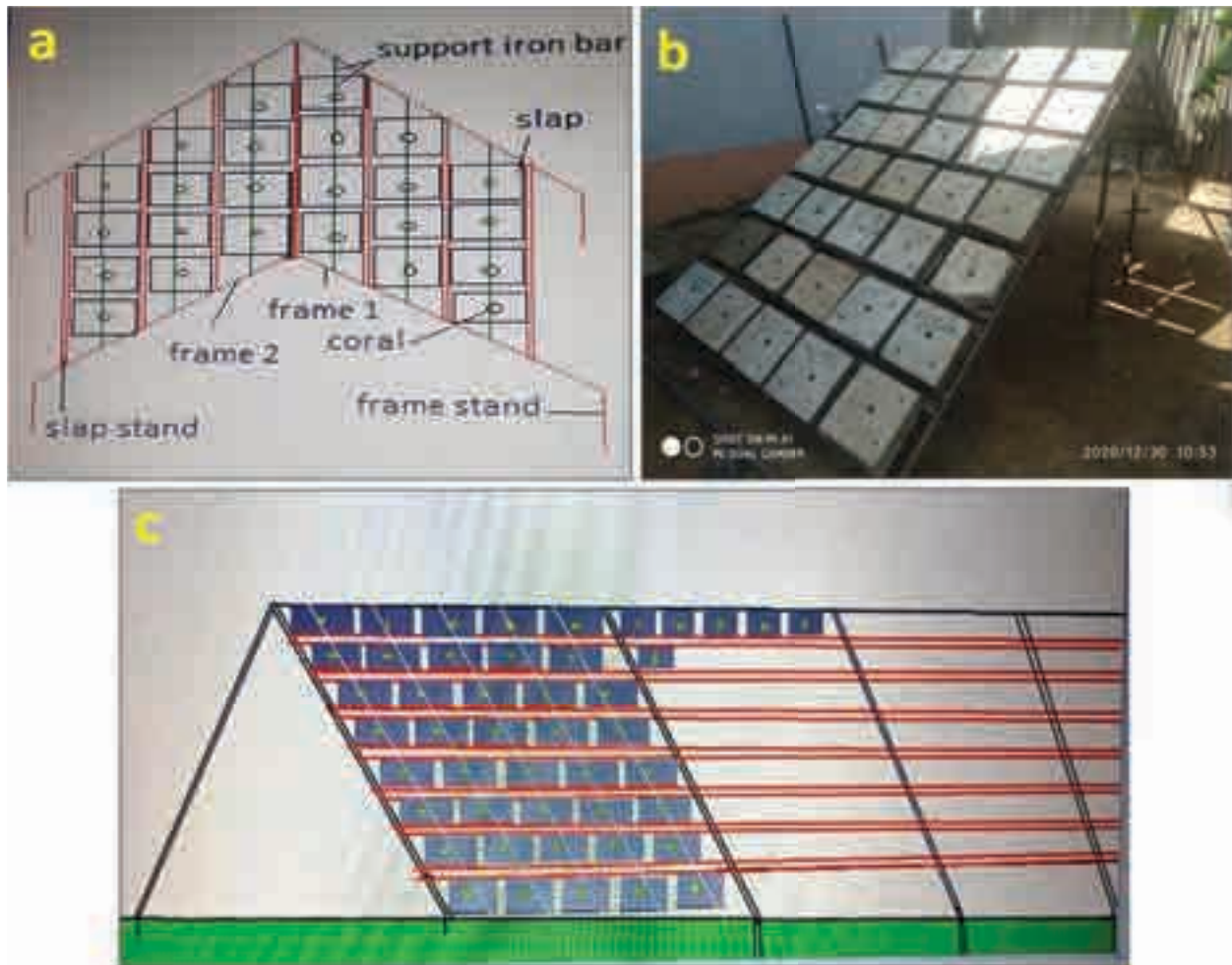


Figure 1. Design of the artificial substrate used for coral transplantation in Palk Bay | a—Top View | b—Front view of the structure | c— Front view of the design.

done up to the family level whereas three fishes were identified up to the species level (Allen & Steene 1998).

RESULTS

The present study analyzed the growth and survival rate of restored corals. A total of 240 scleractinian coral fragments were used for making the artificial reef structure. *Acropora* sp. and *Montipora* sp. were installed on the cement slabs. During installation in February 2021, the initial size of the coral fragments ranged from 6.00 ± 0.05 cm (Figure 2). After five months of coral transplantation, the *Acropora* corals attained the size of 10.19 ± 0.53 cm in site 1 (Munaikadu) and 9.48 ± 0.61 cm in site 2 (Thonithurai), whereas *Montipora* corals attained a size of 8.52 ± 0.30 cm in Munaikadu and 8.10 ± 0.58 cm in Thonithurai (Figure 2). The average monthly growth rate of *Acropora* sp. (0.94 cm/month) was higher

than *Montipora* sp. (0.56 cm/month) in a combined assessment of both sites (Figure 2). The survival rate of *Acropora* sp. was (65.0%) higher than *Montipora* sp. (50%) (Figure.3). Based on field observations, it was found that regular bleaching during the month of April and May causes high mortality to the *Montipora* fragments. In the present study, a total of 173 individuals of seven families of fishes have been found on the restoration site. School of fishes was also recorded during the survey that was conducted on 9 June 2021. Within five months of restoration in Palk Bay, observations revealed a high abundance of Scaridae (Parrot fish), Terapontidae (Grunter fish), Acanthuridae (Surgeonfish), Siganidae (Rabbitfish), Pempheridae (Sweepers), Pomacentridae (Damsel fish), Lethrinidae (Sea bream) near the restoration site (Image1). Three fish species, named as *Terapon jarbua* (Grunter fish), *Pomacentrus trilineatus* (Three line Damsel fish), and *Siganus javus* (Rabbit fish) were found more frequently near the artificial substrate

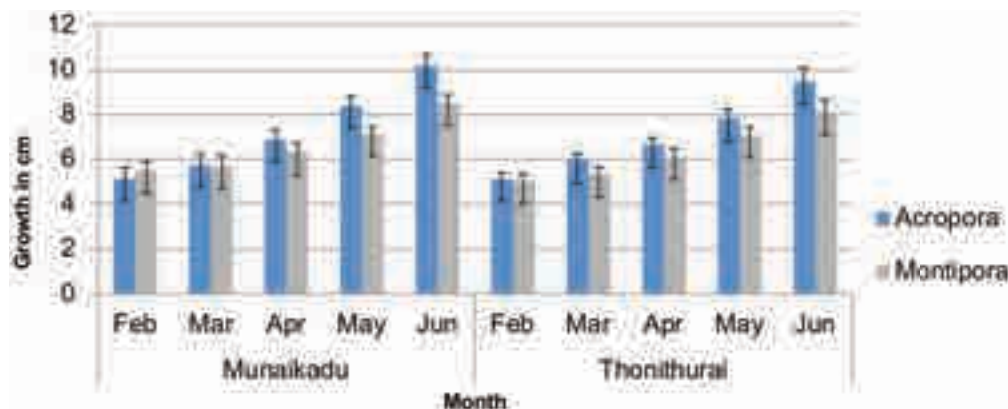


Figure 2. Growth rate of restored corals in Palk Bay during the study.

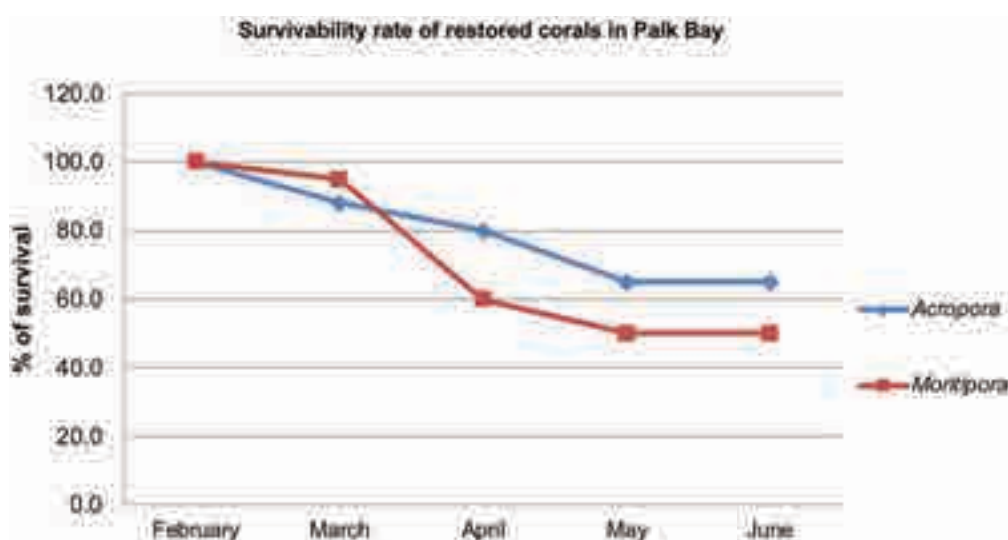


Figure 3. Survival rate of coral species used in restoration activities during the study.

used for coral transplantation (Image 1). Similar observations were made as large assemblages of *T. jarbua* fish were documented on the artificial substrates after three months of deployment (Balaji et al. 2019). Among these three fish species, *S. javus* is commercially important fish species found to be abundant near to the coral transplantation area, whereas *T. jarbua* is used as fish bait by small scale fishers. Seasonal variation in the fish abundance was not studied.

The Palk Bay contains diversified and productive ecosystems such as estuaries, salt marshes, coral beds, seagrass beds and mangroves that are sensitive to human activities (Azeez et al. 2016). However, over the past few decades, this region is highly disturbed by anthropogenic activities such as fishing and aquaculture (Sathiadhas et al. 2014). Over-exploitation and destructive fishing activities are one of the major threats to the coral reefs in India leading to the patchy nature

of coral cover. Hence a significant fish assemblage has been found on the coral restoration site as compared to the natural reef site (authors' personal observation). The growth and survivability of transplanted corals (0.94 cm/month for *Acropora* sp. and 0.56 cm/month for *Montipora* sp.) shows a promising sign for a healthy artificial reef structure similar to the earlier studies of corals transplanted in other parts of the world (> 0.39–0.68 cm) (Xin et al. 2016). Habitat plays a critical role in regulating fish community structure (Zhenhua 2015). The observations made by present study revealed that the frames used to set up the artificial reef can act as a substrate for organisms and can additionally create suitable habitats for the fish to take shelter and forage organisms attached to the frame (Image 1). Fish and invertebrates use both natural and artificial structures for shelter, feeding, spawning, energy economy and orientation (Osenberg et al. 2002; Ropicki et al. 2006).

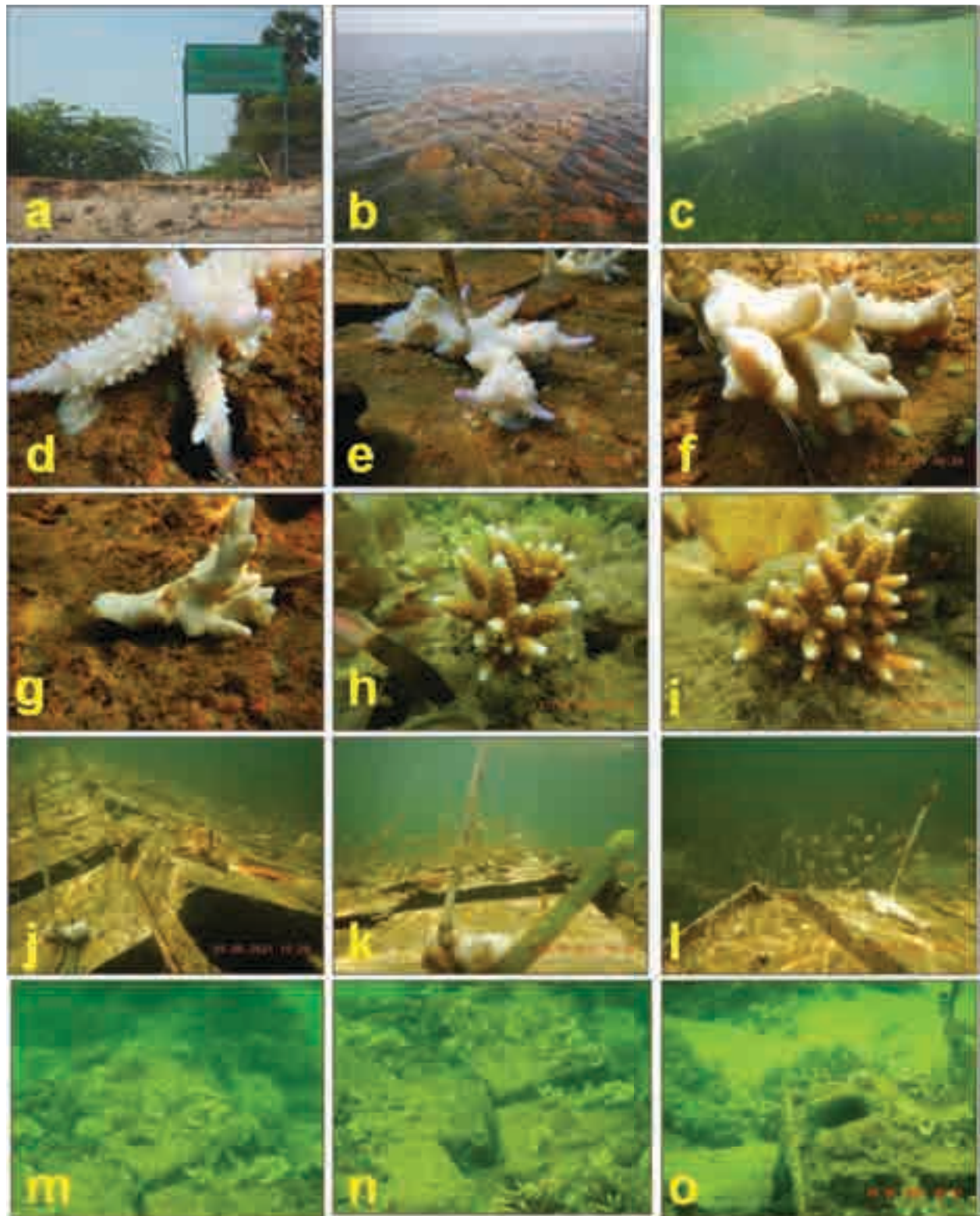


Image 1. Artificial reef structure and assemblage of fishes: a–c—Deployment of underwater submerged artificial substrate for coral restoration | d–f—Restored corals bleached during summer (April) | g—*Montipora* sp. | h–i—*Acropora* sp. | j–o—Assemblage of different fishes in artificial reef structure | j–l—grunter fish *Terapon jarbua* | m–n—Rabbit fish *Siganus javus* | o—Damsel fish *Pomacentrus trilineatus*.
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The larvae of small invertebrates, zooplankton and phytoplankton aggregate in the reef which provides sufficient food and nutrition to fishes. Present study observed the accumulation of many fishes to the artificial structure used for coral restoration. In India, Kasim et al. (2013) studied the income of fishery from artificial reef and non-artificial reef sites by gillnet and hook during 2007–08 from 11 fishing villages in six coastal districts of Tamil Nadu. As per the studies, the artificial reef site offered economic benefit from fisheries (net income INR 1,242 by gill net & INR 4,650 by hook & line) which was higher by INR 1,705.9 per net unit compared to natural site (INR 449 by gill net & INR 1,919 by hook & line) (Kasim et al. 2013). Therefore, development of artificial reef in a degraded site or selected no reef zone site could improve the abundance of marine bio-resources and provide income generation to the local communities in Palk Bay. Recently, establishment of artificial reef concept was also carried out in Sethubhavachattiram, a fish landing center in northern Palk Bay, India which revealed that artificial reefs provide better sheltering ground for fishes (Balaji et al. 2019). However, in the present study, the NCCR team is developing an artificial reef aimed to increase the reef building coral cover in Palk Bay and as well as provide artificial structure to improve the marine resources available especially fishes, molluscs, and echinoderms. Therefore, the present study aims to develop a coral nursery garden and provide the marine habitat for fishery resources. It also provides a hope for successful coral restoration practice to be done in Palk Bay for the first time. Regular monitoring of coral growth, survivability and seasonal fish abundance near the restoration site is under the progress and detail report on the current investigation will be delivered in future.

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Systematics of the enigmatic and narrowly endemic toad genus *Bufo*ides Pillai & Yazdani, 1973: rediscovery of *Bufo*ides *kemp*i (Boulenger, 1919) and expanded description of *Bufo*ides *meghalayanus* (Yazdani & Chanda, 1971) (Amphibia: Anura: Bufonidae) with notes on natural history and distribution

R.S. Naveen¹, S.R. Chandramouli², Gautam Kadam³, S. Babu⁴, P.V. Karunakaran⁵,
H.N. Kumara⁶ & N. Parthasarathy⁷

^{1,3,4,5,6} Sálím Ali Centre for Ornithology and Natural History, Anaikatty, Coimbatore, Tamil Nadu 641108, India.

^{1,2,7} Department of Ecology and Environmental Sciences, School of Life Sciences, Pondicherry University, Puducherry 605014, India.

¹ naveen89240@gmail.com, ² findthesnakeman@gmail.com (corresponding author), ³ gautamkadam7wild@gmail.com,

⁴ sanbabs@gmail.com (corresponding author), ⁵ karunakaran.pv@gmail.com, ⁶ honnavallik@gmail.com, ⁷ nparthasarathypu@gmail.com

Abstract: *Bufo*ides *kemp*i (Boulenger, 1919) known only from the two historical syntype specimens until now was rediscovered after more than a century from near its type locality in the Garo Hills, Meghalaya, northeastern India. Analysis of mitochondrial 16S rRNA gene reveals congenericity between *B. kemp*i and *B. meghalayanus* with an inter-specific genetic divergence of 4.67%. Description of *B. kemp*i is expanded based on the six male and two female specimens collected during this study. We provide the first description of calls for this genus, notes on their breeding biology and larval morphology. Additional specimens of *B. meghalayanus* collected during this study are described to supplement its characterization.

Keywords: Amphibians, breeding biology, calls, Garo Hills, Khasi Hills, larval morphology, new records, syntype specimens.

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Author details: R.S. NAVEEN works on herpetofauna & ichthyofauna and obtained his MSc in ecology from Pondicherry University. S.R. CHANDRAMOULI obtained his PhD in ecology and environmental sciences from Pondicherry University works on systematics, ecology, biogeography and conservation of herpetofauna of India, with special focus on the Andaman and Nicobar Islands. GAUTAM KADAM is interested in the study of spiders and herpetofauna and holds a MSc in biodiversity and wildlife conservation from Mumbai University. S. BABU is a senior scientist at SACON, Tamil Nadu and his research focuses on the conservation of birds, landscape ecology and remote sensing & GIS. HONNAVALI N. KUMARA is a principal scientist in SACON. His research focuses on various aspects of conservation. DR. P.V. KARUNAKARAN is a senior principal scientist at SACON, conducting biodiversity and natural resource based studies and strategic planning in different landscapes of India. PROF. N. PARTHASARATHY is a faculty at the department of Ecology and Environmental Sciences, engaging in teaching of ecology of terrestrial biome, forest botany, medicinal plant resources, functional ecology, and dynamics of tropical forests across the forests of Western and Eastern Ghats, Andamans and Coromandel coast of India.

Author contributions: SRC and SB conceived and designed the study, RSN & GK conducted field studies, SRC, RSN, & SB generated and analysed the data, SRC, RSN, & SB wrote the manuscript, SB, PVK, & HNK procured funds, SB, PVK, HNK, & NP supervised the study, reviewed, & edited the manuscript draft, and all authors approved the final draft.

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INTRODUCTION

The cosmopolitan anuran family Bufonidae Gray, 1825 is represented in India by nine genera comprising 33 species spread across several biogeographic regions (Frost 2022). One of the most poorly-known among them is the genus *Bufoides* Pillai & Yazdani (1973) which comprises two species namely *B. meghalayanus* (Yazdani & Chanda 1971) and *B. kempī* (Boulenger 1919) (after Chandramouli & Amarasinghe 2016). Among them, the type species, *B. meghalayanus* is fairly better-known in terms of its distribution, biology, ecology, and natural history (Yazdani & Chanda 1971; Pillai & Yazdani 1973; Das et al. 2009; Deuti et al. 2012). On the other hand, there has been no information on any of the above aspects for the previously-described species *B. kempī*. *B. kempī* was originally described as *Nectophryne kempī* from ‘above Tura, 2,500 ft’ based on two specimens, an adult and a subadult (ZSI 18481a,b), from which the species is known till date (Boulenger 1919). It was later transferred to the genus *Pedostibes* Günther, 1875 by Barbour (1938) and subsequently transferred to *Bufoides* by Chandramouli & Amarasinghe (2016) based on morphological characters. As a part of an on-going project documenting faunal diversity in community reserves of Meghalaya, we rediscovered *B. kempī* from near its type locality and located additional specimens of *B. meghalayanus* whose descriptions are expanded based on new data.

MATERIALS AND METHODS

The study was conducted in the northeastern Indian state of Meghalaya. Surveys were conducted in ten different locations spread across the Garo, Khasi, and Jaintia Hills of Meghalaya. Specifically, the type localities of the two known species viz. “above Tura, Garo Hills” (Boulenger 1919) for *B. kempī* and “Mawblang plateau” (Yazdani & Chanda 1971) for *B. meghalayanus* and the vicinity of these localities were surveyed intensively, in addition to the other sites to locate the target species. Additional sites within Meghalaya (Deuti et al. 2012), apart from these two localities from where *Bufoides* is known until now were also surveyed. Field sampling was carried out from March–May & October 2021. A total of seven specimens of *B. kempī*, comprising six adult males and one adult female along with a subadult female were collected from Eman Asakgre (25.37° N, 90.55° E, 100–250 m). Likewise, three adult specimens of *Bufoides meghalayanus* were collected from a hill stream in the

Khasi Hills (25.23° N, 91.73° E, 1,100–1,250 m). Other locations of *B. kempī* and *B. meghalayanus* were marked with a GPS. Encounter rates of these species, expressed as the number of individuals encountered over an hour’s duration of field sampling, is presented as an index of their abundance.

The following measurements were recorded to the nearest 0.02 mm from the specimens with a dial caliper: snout–vent length (SVL, from the tip of the snout to the anterior margin of the cloaca), axilla–groin distance (AG, from the posterior margin of the forelimb at its insertion point on the body to the anterior margin of the hind limb at its insertion point on the body), head length (HL, from the posterior edge of the mandible to the tip of the snout), head width (HW, the maximum width of the head at the angle of the jaws), head depth (HD, the maximum depth of the head), body width (BW, the maximum width of the body at the trunk), eye diameter (ED, the greatest horizontal diameter of the orbit), eye–nostril distance (EN, from the anterior border of the orbit to the middle of the nostril), eye–snout distance (ES, from the anterior border of the orbit to the tip of the snout), upper eyelid width (UEW, the maximum width of the upper eyelid), interorbital distance (IO, distance between the upper eyelids), internarial distance (IN, distance between the nostrils), upper arm length (UAL, from the axilla to elbow), lower arm length (LAL, from the posterior margin of the elbow to the base of the outer metacarpal tubercle), palm length (PAL, from the posterior border of the outer metacarpal tubercle to tip of the 3rd finger), femur length (FEL, from the cloaca to the knee), tibia length (TBL, from knee to heel), foot length (FOL, from inner metatarsal tubercle to the tip of the 4th toe). Webbing formulae follows Savage & Heyer (1997). A principal component analysis was conducted based on 18 morphometric measurements (standardised to their SVL; Table 1) of the two *Bufoides* species to examine their morphometric distinction from each other. Calls were recorded with the camera as videos and the audio (as .mp4 at an audio sampling rate of 48 kHz) was extracted and analysed with Adobe Audition 6 and Adobe Soundbooth CS3. Two specimens, SACON VA 157 (female) and VA 159 (male) were radiographed to study osteological characters of *B. kempī*. A brief description of its osteology is provided following the terminologies of Noble (1931).

Eggs of *B. kempī* observed in tree holes were collected and reared for 11 days, and the growth of the larvae was monitored with preservation of samples across various developmental stages. The following measurements of the tadpoles: HBL— head-body length; HBW— head-

body width; HBD— head-body depth; TOT— total length; TAIL— tail length; IO— inter-orbital distance; and TH— tail-fin height were recorded with a stereo microscope following Chandramouli & Kalaimani (2014). Staging of tadpoles follow Gosner (1960) and terminologies follow McDiarmid & Altig (1999). Labial tooth-row formula for the larvae follow Rödel (2000).

Total genomic DNA was extracted from one specimen of *B. kemp* (SACON VA 180) with a DNA extraction and purification kit, following the manufacturer's protocols. 16S rRNA gene was amplified using the primers 16sAR-L (5'-CGCCTGTTTATCAAAAACAT-3') and 16sB R-H (5'-CCGGTCTGAACCTCAGATCACGT 3'), respectively (Kocher et al. 1989). Amplifications were performed in a Applied Bio Systems Veriti 96 well thermal cycler: 20 µl reactions with 4 µl of 5X Phusion HF buffer, 0.4 µl of 10 mM dNTP, 0.2 µl of Phusion DNA Polymerase, 0.1 µl each of forward and reverse primers, 2.0 µl of DNA template and 13.2 µl of nuclease free water with the following procedure: initial denaturation of DNA at 95 °C for 5 min, 35 cycles of: denaturation at 95 °C for 1 m, annealing at 55 °C for 1 min, extension at 72 °C for 1 m and at last, final extension at 72 °C for 10 min. The amplicon was checked by running it through an agarose gel electrophoresis for a clear band of the desired region in the amplified PCR product. The amplified PCR product was purified and sequenced commercially (National Centre for Biological Sciences, Bengaluru). The sequence thus obtained (NCBI voucher no: OP920605) was aligned along with ten other taxa from Bufonidae, comprising the genera *Adenomus*, *Beduka*, *Blythophryne*, *Bufoides*, *Bufo*, *Duttaphrynus*, and *Pedostibes* with *Hyla arborea* as the outgroup taxon. The sequences were aligned with MUSCLE (Edgar 2004) in MEGA 6.0 (Tamura et al. 2013). This alignment of 491 bp was exported in FASTA and MEGA formats, and was then used to determine uncorrected pairwise genetic distances between the samples with MEGA 6. The FASTA alignment was converted to PHYLIP format in the Alignment Transformation Environment (ALTER) website (www.sing.ei.uvigo.es/ALTER) and was subjected to a maximum likelihood (ML) analysis in RAXML GUI v. 1.3 (Stamatakis 2006) using the general time reversible model, GTR GAMMA, (as RAXML uses only the general time reversible (GTR) model of sequence evolution) with 500 bootstrap replicates. Likewise, for the Bayesian analysis, the FASTA alignment was converted to NEXUS format and analysed in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003) by running it for three million MCMC iterations initially until the standard deviation of the split frequencies reached a value of ≤ 0.001 . Else, the analysis was continued for another 10000–100000 generations

until the standard deviation of ≤ 0.001 was obtained for the split frequencies. Initial 20% of the trees were discarded as 'burn-in'. The tree files generated were then visualized using Fig Tree v. 1.4.0.

RESULTS

Our analyses of molecular data (both maximum likelihood - ML and Bayesian - BI) recovered the two species allocated to the genus *Bufoides* to form a monophyletic group; with the two species *B. meghalayanus* and *B. kemp* showing a congeneric, sister relationship to each other with high support (87 & 1.0 in ML and BI, respectively). The ML and BI analyses recovered the genera *Blythophryne* Chandramouli et al., 2016 & *Beduka* Dubois et al., 2021 to be close to the genus *Bufoides*, as assessed earlier (Chandramouli et al. 2016) although with low support (36 & 0.63 in ML & BI, respectively). Pairwise genetic divergence between *B. meghalayanus* and *B. kemp* was found to be moderate (4.67 % at 16s rRNA) supporting their specific distinction from each other (Figures 1a,b). The PCA conducted based on 18 morphometric variables clearly separates the two species into two discrete clusters (Figure 2, Table 1).

SYSTEMATICS

Bufoides kemp (Boulenger, 1919)

Nectophryne kemp Boulenger, 1919

Pedostibes kemp – Barbour, 1938

Bufoides kemp – Chandramouli & Amarasinghe, 2016

Syntypes: Two specimens; an adult (29.8 mm SVL) and a subadult (17.4 mm) (ZSI 18481 a&b, respectively)

Other material studied: SACON VA 157 (an adult female) and, VA 181(a subadult female), and SACON VA156; VA 158 –160; VA 164 & VA 180 six adult males collected from Eman Asakgre (25.37°N, 90.54°E, 200 m asl.), Garo Hills, Meghalaya (Image 1).

Diagnosis: (after Chandramouli & Amarasinghe 2016)

A semi-arboreal to rupicolous *Bufoides* from the Garo Hills diagnosed by: small to medium body size (SVL 24.1–32.36 mm); presence of irregular, non-keratinized cranial ridges (pre and post orbital); short, ovoid parotoid glands; absence of an externally visible tympanum; moderate degree of webbing between toes (two phalanges of toe IV free); partial webbing between fingers, and the presence of small, slightly dilated, rounded terminal digital discs at the tips of both fingers and toes. Dorsum black with mossy green shade along the flanks in males, females predominantly green with black reticulations; a

Table 1. Eigenvalues and the proportion of variance explained by each of the principal component.

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
AG	0.13	-0.05	0.66	-0.25	-0.26	-0.16	0.19	0.13
BW	0.73	-0.38	-0.01	0.25	-0.25	0.11	-0.02	0.09
HL	0.24	0.32	-0.13	-0.46	-0.29	-0.10	0.05	-0.07
HW	0.51	0.20	-0.02	-0.02	0.31	0.01	0.04	0.10
HD	0.11	0.09	-0.19	0.08	0.02	-0.24	-0.15	-0.25
ED	0.16	0.08	0.06	0.10	-0.02	0.01	-0.36	0.23
EN	0.08	0.26	0.09	0.13	-0.16	0.22	-0.38	-0.49
ES	0.04	0.20	-0.22	0.16	-0.20	0.32	0.39	-0.18
UEW	0.04	0.08	-0.12	-0.09	0.16	0.13	-0.22	0.14
IO	-0.06	0.22	-0.12	0.16	-0.05	0.19	-0.03	0.33
IN	-0.07	0.27	0.03	0.08	-0.17	0.34	-0.15	0.54
UAL	-0.06	0.18	0.53	0.54	0.05	-0.08	-0.14	-0.14
LAL	-0.01	0.14	0.01	0.22	-0.13	-0.29	0.48	0.12
PAL	0.11	0.06	-0.17	0.38	0.35	-0.13	0.26	0.01
FEL	0.14	0.54	0.01	-0.03	-0.15	-0.10	0.09	-0.17
TBL	-0.03	0.29	-0.01	0.04	0.09	-0.40	-0.10	0.31
TAR	0.06	0.14	0.33	-0.18	0.45	0.51	0.28	-0.07
FOL	0.19	0.07	0.05	-0.22	0.44	-0.21	-0.14	-0.04
Eigenvalue	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
% variance	46.40	24.50	11.91	6.90	5.37	2.64	1.47	0.81

pale white venter; eggs partially pigmented and laid in strings within water-filled tree holes (phytotelmata).

Description and variation: (based on the newly collected material) Table 2

Female (SVL 32.36 mm) slightly larger than males (mean SVL 26.38 mm $\pm$ 0.88, n = 6). Head flat, fairly large, and distinct, (HL:SVL 0.31), broader than long, slightly more wider in the female (HL:HW 0.82) than in males (HL:HW 0.91), with an obtusely pointed to rounded snout tip. Trunk short (AG:SVL 0.4) and slightly gracile in males (AG:BW 1.45) than in females (AG:BW 1.81). Eyes fairly large (ED:HL 0.33) their diameter shorter than the snout length (ED:ES 0.71). Nostrils situated closer to the snout tip than to the eyes (EN:ES 0.74). Upper eyelids wide, (mean UEW 2.92 $\pm$ 0.12) rugose with keratinized pustules, narrower than the interorbital space (IO:UEW 1.79). Inter-orbital space broader than inter-narial space (IO:IN 1.59). Upper arms short (UAL:SVL 0.23), nearly as long as the lower arms (UAL:LAL 1.02); palm slightly shorter than the upper arms (UAL:PAL 0.88). Fingers partially webbed, webbing formula $I_{0-1} II_{2-3} III_{3-2} IV_{2-1} V$; relative length of fingers $III > IV > II > I$. Outer metacarpal tubercle large and evident. Finger tips with slightly expanded rounded discs. Femur relatively short (FEL:SVL 0.38), tibia slightly longer than femur (FEL:TBL 0.91); foot about as long as the femur

(FEL:FOL 0.99). Toes moderately webbed, webbing formula: $I_{0-0} II_{0-0.5} III_{0.5-2} IV_{2-1} V$, a relatively large inner and a slightly smaller ovoid metatarsal tubercle at the base of the foot. Toe tips with discs as broad as the toes; tarsal ridge not discernible. Vocal sac not discernibly distinct in males. Skin rugose in texture with keratinized granules.

Colouration in life

Males were generally dark grey in colour with traces of mossy green along the flanks and yellow patches near the axilla, belly and groin on the ventro-lateral region. Females are predominantly mossy green with an irregular black hour-glass pattern on the dorsum. Limbs visibly barred with black. Venter pale and much lighter than the dorsum (Image 2).

Osteology

Skull large and triangular, with an obtusely pointed snout tip. Pre and post-orbital ridges discernible. Fronto-parietals fairly broad and hexagonal in shape. Nasal bones of the skull short, nearly as long as broad. Vertebral column with eight procoelous presacral vertebrae; the first four relatively larger than the following. Sacral diapophyses broad, flattened, and expanded laterally. Urostyle cylindrical, about half the length of the presacral

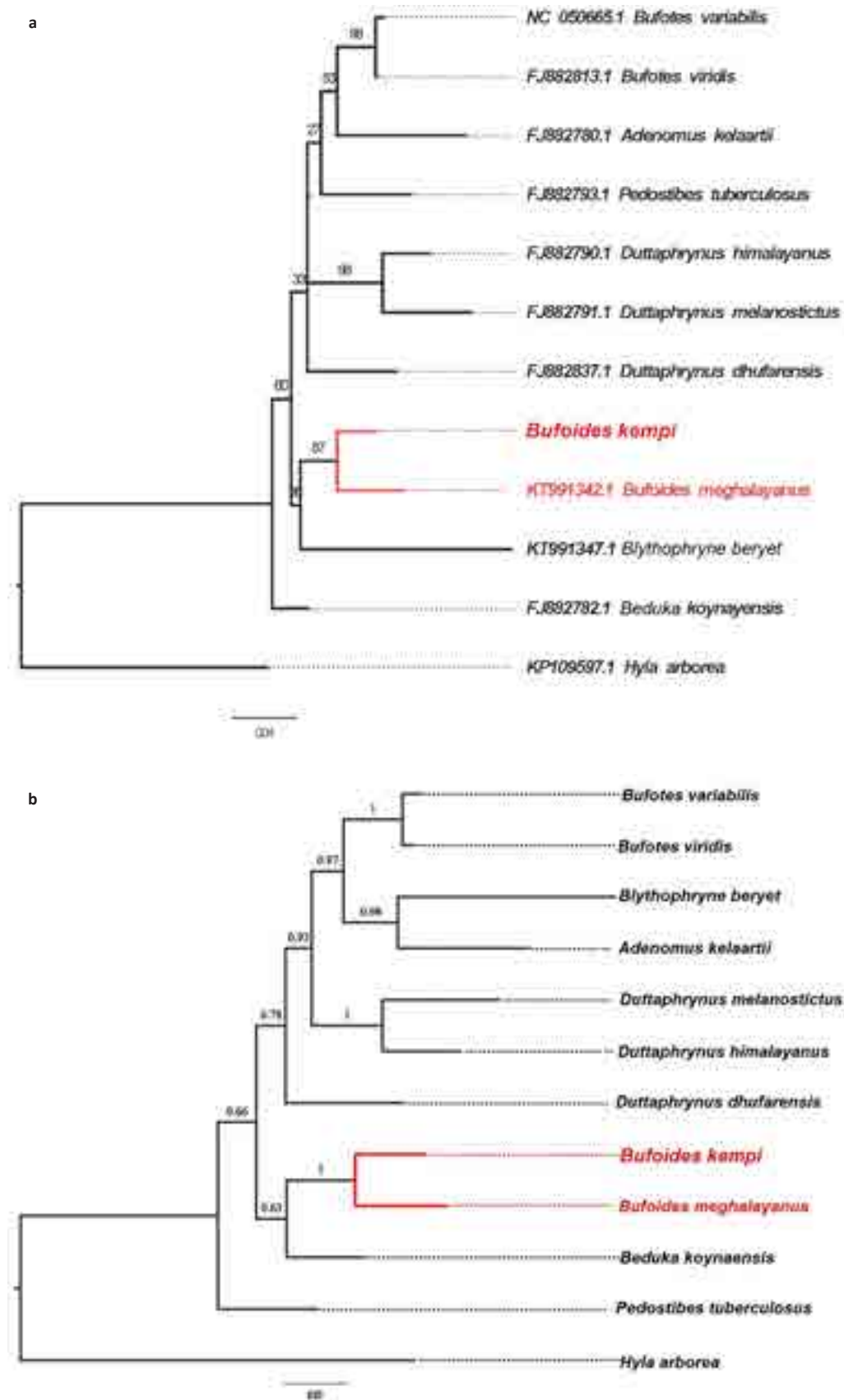


Figure 1. a— Maximum likelihood | b— Bayesian phylogenetic trees of Bufonids, showing the distinction of *Bufo* from other genera and sister relationship between *B. meghalayanus* and *B. kemp*.

Table 2. Morphometric measurements of *Bufoides kempī* and *B. meghalayanus* at SACON.

Species	<i>Bufoides kempī</i>								
Voucher no:	VA 164	VA 159	VA 158	VA 160	VA 180	VA 156	MEAN	±SD	VA 157
Sex	M	M	M	M	M	M			F
SVL	25.3	29.3	26.8	24.1	32.7	26.3	27.4	3.1	32.4
AG	8.5	11.3	10.9	7.8	13.9	10.6	10.5	2.2	13.8
BW	6.2	9.5	7.3	5.3	12.5	5.5	7.7	2.8	7.6
HL	7.7	9.5	8.06	8.7	10.4	8.4	8.8	1.0	9.9
HW	8.8	10.6	8.8	9.2	13.9	9.2	10.1	2.0	12.1
HD	4.1	4.0	4	4.2	5.1	3.6	4.2	0.5	5.5
ED	2.8	3.1	2.9	2.3	3.8	2.5	2.9	0.5	3.3
EN	2.6	2.9	3.4	3.1	3.3	2.2	2.9	0.4	3.5
ES	4.1	4.7	3.7	4.6	4.9	2.9	4.1	0.8	4.5
UEW	2.6	2.9	2.2	2.7	2.8	2.7	2.7	0.3	3.2
IO	4.9	4.9	4.3	4.7	4.9	4.0	4.6	0.4	5.9
IN	2.9	2.9	2.4	2.6	2.2	2.2	2.5	0.3	4.1
UAL	6.5	6.2	7.7	5.4	8.9	6.2	6.8	1.3	7.2
LAL	6.3	6.2	6.1	5.8	8.1	5.6	6.4	0.9	7.4
PAL	7.2	6.9	6.3	6.7	9.4	6.2	7.1	1.2	8.9
FEL	9.6	10.1	9.7	10.2	12.2	9.4	10.2	1.0	12.3
TBL	11.2	11.1	10.9	10.6	13.3	11.2	11.4	1.0	13.1
TAR	5.4	7.6	6.2	6.2	8.9	7.3	6.9	1.3	7.5
FOL	8.8	10.2	9.3	9	12.7	10.4	10.1	1.4	13.0
F1	1.7	2.3	1.2	1.4	2.3	1.6	1.8	0.5	2.5
F2	2.8	2.6	2.4	1.7	3.1	2.4	2.5	0.5	4.9
F3	5.4	4.4	4.2	3.6	5.9	3.5	4.5	1.0	7.1
F4	3.7	4.0	2.9	2.6	4.9	2.8	3.5	0.9	5.5
T1	1.3	1.4	1.2	0.9	1.5	1.3	1.3	0.2	2.1
T2	2.5	4.1	1.8	2.7	2.7	2.1	2.7	0.8	2.9
T3	4.9	3.8	2.6	4.1	4.0	3.3	3.8	0.8	3.9
T4	7.4	6.0	4.7	5.9	6.6	4.8	5.9	1.0	7.7
T5	4.0	2.4	2.7	4.2	3.9	2.9	3.3	0.8	4.9

Species	<i>Bufoides meghalayanus</i>				
Voucher no:	VA 215	VA 251	VA 252	MEAN	±SD
Sex	M	M	M		
SVL	31.3	33.5	31.2	31.9	1.3
AG	10.7	13.2	12.1	12.3	1.4
BW	12.0	14.1	10.9	12.3	1.6
HL	9.7	12.9	12.7	11.8	1.8
HW	12.0	16.8	12.9	13.9	2.5
HD	5.4	6.2	5.4	5.7	0.5
ED	3.2	5.3	3.8	4.1	1.1
EN	2.1	4.9	3.6	3.5	1.4
ES	4.2	5.4	5.1	4.9	0.6
UEW	3.2	4.1	3.1	3.5	0.6

Species	<i>Bufoides meghalayanus</i>				
Voucher no:	VA 215	VA 251	VA 252	MEAN	±SD
IO	4.3	5.7	5.1	5.0	0.7
IN	1.1	3.2	2.9	2.4	1.2
UAL	5.3	8.2	6.4	6.6	1.5
LAL	6.5	7.4	7.6	7.1	0.6
PAL	8.6	9.7	7.5	8.6	1.1
FEL	9.6	14.4	13.2	12.4	2.5
TBL	11.9	14.4	13.4	13.2	1.3
TAR	6.4	9.3	6.9	7.6	1.5
FOL	11.9	14.3	11.8	12.7	1.4
F1	2.1	2.0	1.8	1.9	0.2
F2	3.2	3.2	3.1	3.2	0.1
F3	5.4	4.7	4.4	4.8	0.5
F4	4.3	4.4	3.9	4.2	0.3
T1	2.1	2.4	1.6	2.0	0.4
T2	3.2	2.9	3.2	3.1	0.2
T3	5.4	4.5	3.7	4.5	0.8
T4	9.7	6.6	5.9	7.4	2.0
T5	6.5	5.0	3.8	5.1	1.3

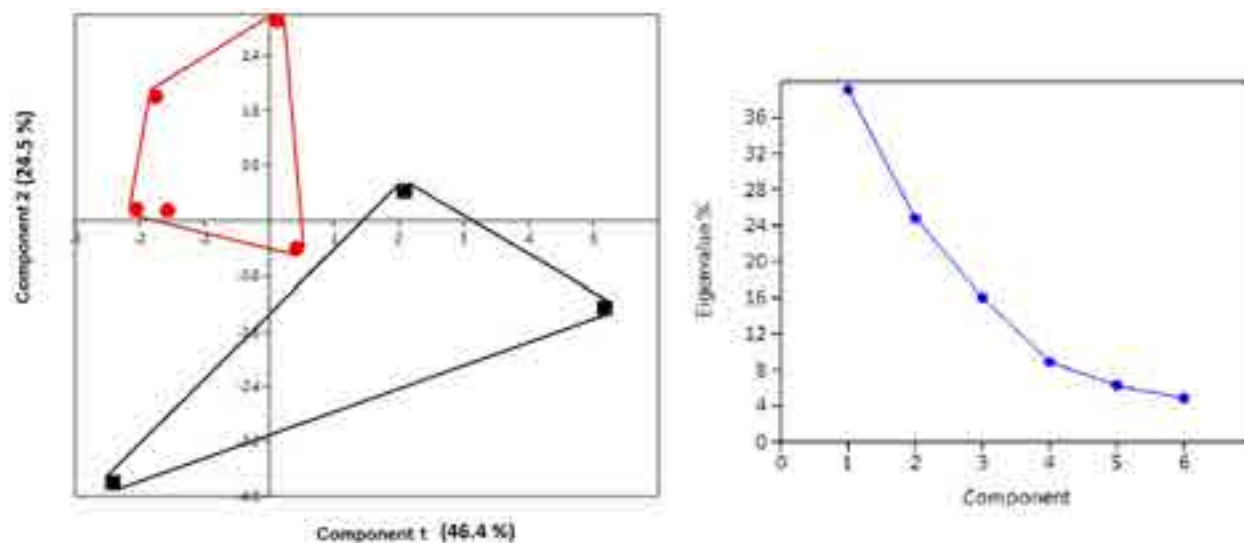


Figure 2. Left— Plot of PCA showing morphometric distinction between *B. meghalayanus* (black squares) and *B. kempī* (red dots) | Right— Scree plot showing the eigenvalues of each principal components.

vertebral column, lacking lateral expansions. Ilii curved laterally, as long as the urostyle. Ischium protruding posteriorly. Pectoral girdle arciferous. Humerus longer than radio-ulna. Phalangeal formula of the fingers: 2-2-3-3. Femur long, nearly as long as the tibiofibula; tarsus about $\frac{3}{4}$ th the length of tibiofibula. Phalangeal formula of the toes 2-2-3-4-3 (Image 3).

Breeding biology and natural history

A total of 17 individuals were seen in the following precise locations surrounding Eman Asakgre community reserve, South Garo Hills, the details of which are mentioned below (Image 4).

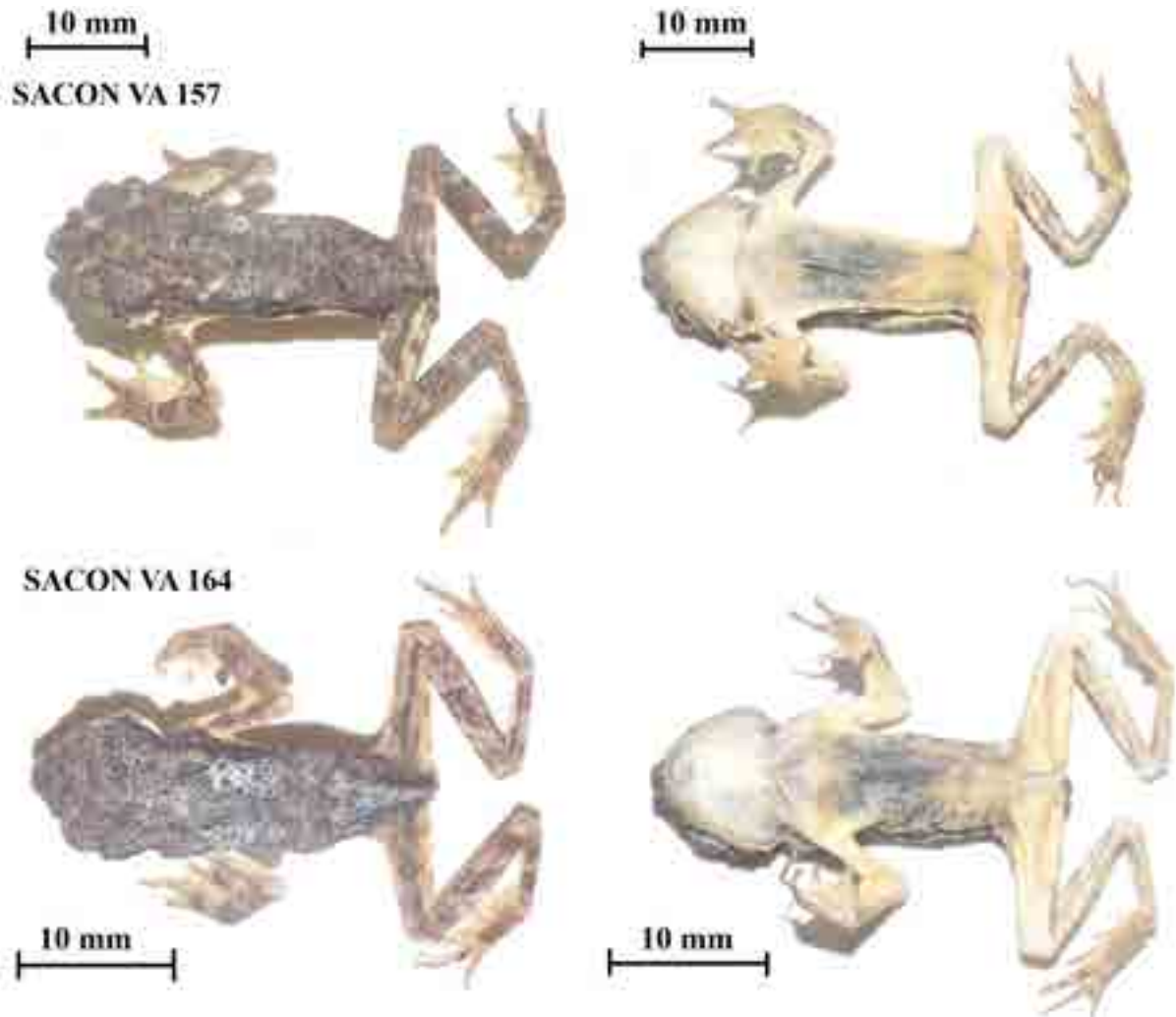


Image 1. *Bufo*ides *kemp*i in preservation—adult female (above: SACON VA 157) and an adult male (below: SACON VA 164) collected during this study.

Chibanda cave (25.36° N, 90.53° E, 122 m)

The cave was about 6 m below the ground level, surrounded by moist evergreen forest. A small creek was flowing into the cave and the surface of the rocks and boulders in the area was covered with moss and were wet. The canopy cover provided about 70% shade to the ground. The first individual, a subadult male was found under a boulder near the mouth of the cave. Second individual was seen inside the cave in a deep narrow Horizontal crevice of a limestone rock. *Odorrana chloronota*, *Amolops assamensis*, *Limnonectes khasianus*, and *Ingerana borealis* were some of the anuran species that were observed in sympatry with *B. kemp*i at this location. During the night surveys in the subsequent months, seven more individuals were spotted in total. Some individuals were observed on leaves of shrubs without exhibiting any specific

behaviour between 1900–2200 h. Later in October, an adult male was sighted in a tree hole filled with rain water at a height of about 2 m above the ground and a subadult female was recorded on low lying shrubs at about one foot above the ground.

Dhangit cave (25.36° N, 90.52° E, 220 m)

This cave, surveyed in May was at a depth of about 12 m, surrounded by moist evergreen forest; the terrain was rocky and filled with boulders covered with moss. No individuals were found inside the cave, however two males were observed near the cave about 150 m away which were calling actively from a cavity filled with rain water (5 cm deep, water temperature 21° C) measuring 10 cm in diameter at about 1.2 m off the ground at around 2200 h possibly trying to attract the attention of a receptive female nearby. Two males were observed



Image 2. *Bufoides kemp*i (male—left & middle and female—right) in life from Eman Asakge, Garo Hills. © left and middle: S.R. Chandramouli, right: R.S. Naveen

Table 3. Larval measurements of *Bufoides kemp*i.

Stage	20	20	20	Mean	±SD	21	30	31	31	Mean
HBL	5.4	6.14	5.8	5.77	0.37	5.3	5.34	5.28	5.5	5.39
TOT	10.1	9.56	9.58	9.75	0.31	10.6	11.64	12.2	14.36	13.28
TAIL	4.7	3.42	3.78	3.98	0.66	5.3	6.3	6.92	8.86	7.89
HBW	1.7	2.3	1.74	1.91	0.34	2.76	2.36	2.72	2.8	2.76
TH	1.6	2.44	1.7	1.91	0.46	2.34	1.88	2.44	2.2	2.32
IO	0.3	0.3	0.3	0.00	0.00	0.4	1.46	2.64	2.6	2.62
HBH	2.82	2.6	1.9	2.44	0.48	2.0	2.24	2.24	2.42	2.33

to show aggression by kicking each other while calling and one of them was seen kicking the other with its hind feet repeatedly. Upon further search in the region, a tree cavity with about 30–40 eggs laid in strings was found. Eggs from this cavity were collected and maintained in a plastic jar with water from the same cavity for the next 11 days.

Cehise Stream (25.34° N, 90.51° E, 250 m)

The area surrounding a small stream flowing near the village of Eman Dura Banda was surveyed in May. This area had a rocky terrain and was covered with moist evergreen forest, with a tall canopy. An adult male was found resting under a boulder.

Eman Asakgre Community Reserve (25.37° N, 90.54° E, 108 m)

Congregation of four males was seen along with a female in a buttress root cavity at a height of 0.6 m above the ground, measuring about 15 cm in diameter, filled with 10 cm of rain water. The water temperature here was 23.5° C, the humidity of the location was 80%. The group was first spotted at about 1730 h in amplexus with four males and a female. One of the four males was seen mounted ventrally while the other three

males were mounted dorsally and laterally. Amplexus was axillary. The episode lasted till about 2100 h by the time the female laid egg strings with 30–40 partially pigmented eggs that measured about 2 mm diameter. Once the eggs were laid both males and the female started leaving the cavity and no further attendance was observed.

Description of calls

The call of *B. kemp*i described here was composed of syllables of 'treek.. treek...' that lasted for a duration of 2.1 s and was composed of three distinct notes, each of which comprise seven–ten pulses. The mean duration of each note was 6.33 ms, with a mean interval of 1.05 s in between. Maximum amplitude of the call was -1 dB with a dominant frequency of 2.5 kHz (Image 5).

Larval description: (Table 3)

On day three, 39 larvae emerged which were reared subsequently for the next eight days during which their development was documented. Stage-wise descriptions of the larvae are presented in detail below.

Stage 20: (n = 3)

The larvae reached stage 20 on day two after

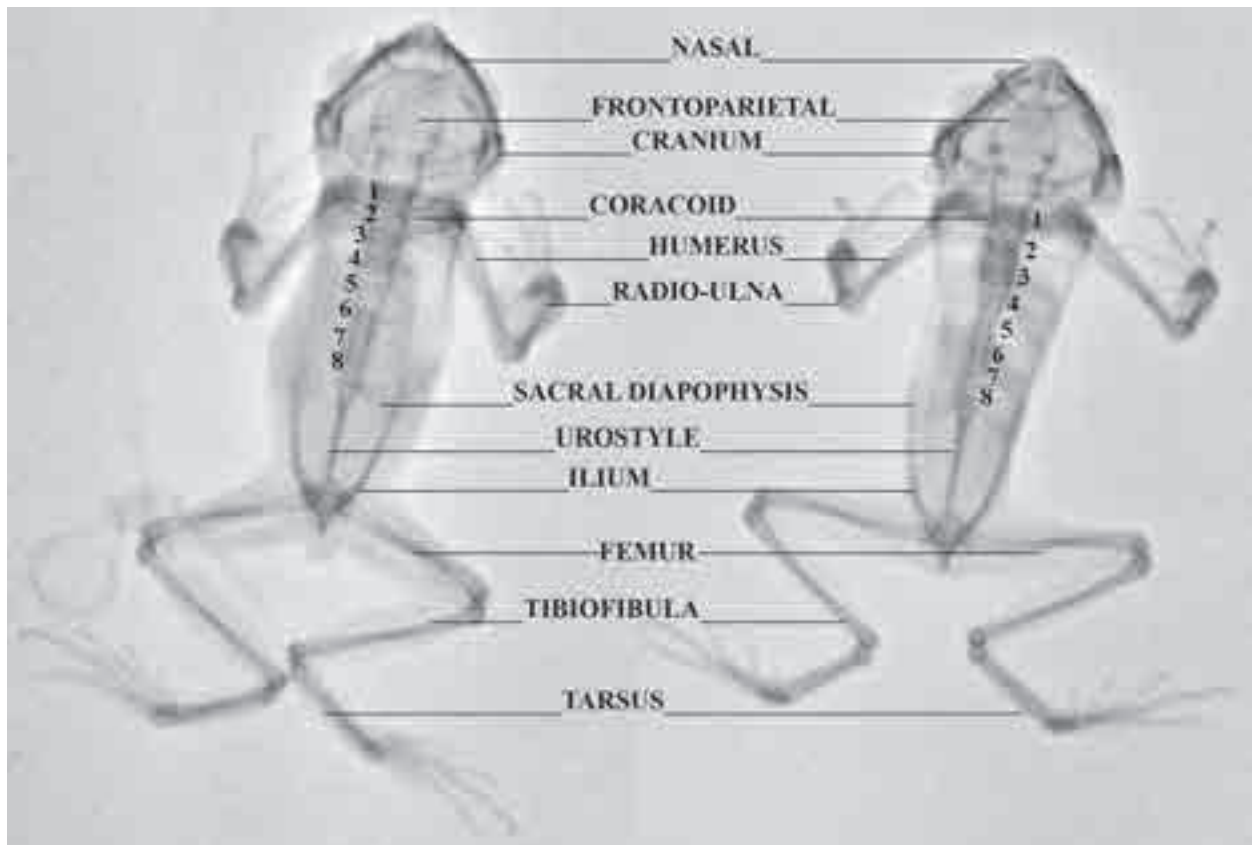


Image 3. Osteology of *B. kempī* based on an adult male and female vouchers SACON VA 157 and VA 159, respectively.



Image 4. Axillary amplexus between one female and three males of *B. kempī* observed in a phytotelmata at Eman Asakgre, Garo Hills.

emerging from the eggs. At this stage, they measured $9.75 \text{ mm} \pm 0.31$ in total length, with a head-body length of $5.77 \text{ mm} \pm 0.37$; streamlined and narrow in form (HBW $1.91 \text{ mm} \pm 0.3$); slightly higher than broad (HBH $2.44 \text{ mm} \pm 0.48$); tail a little shorter than the head-body ($3.98 \text{ mm} \pm 0.66$); and with an average tail height of $1.91 \text{ mm} \pm 0.46$. Eyes and mouthparts not discernible at this stage.

Stage 22: (n = 1)

The larvae reached stage 22 on day four after emergence from the eggs. At this stage, the body & tail elongated a little more, with the larva measuring 10.6 mm in total length, with a head-body length of 5.3 mm and a relatively longer tail (5.3 mm) which equalled the HBL. Head-body oval & narrow (HBW 2.76 mm; HBH 2.0 mm). Tail-fin relatively well developed than in the earlier stage, longer and broader, with a height of 2.34 mm. Eyes dorsal in position and traces of gills discernible at this stage.

Stage 30: (n = 2)

At this stage, the larvae grew a little longer, measuring

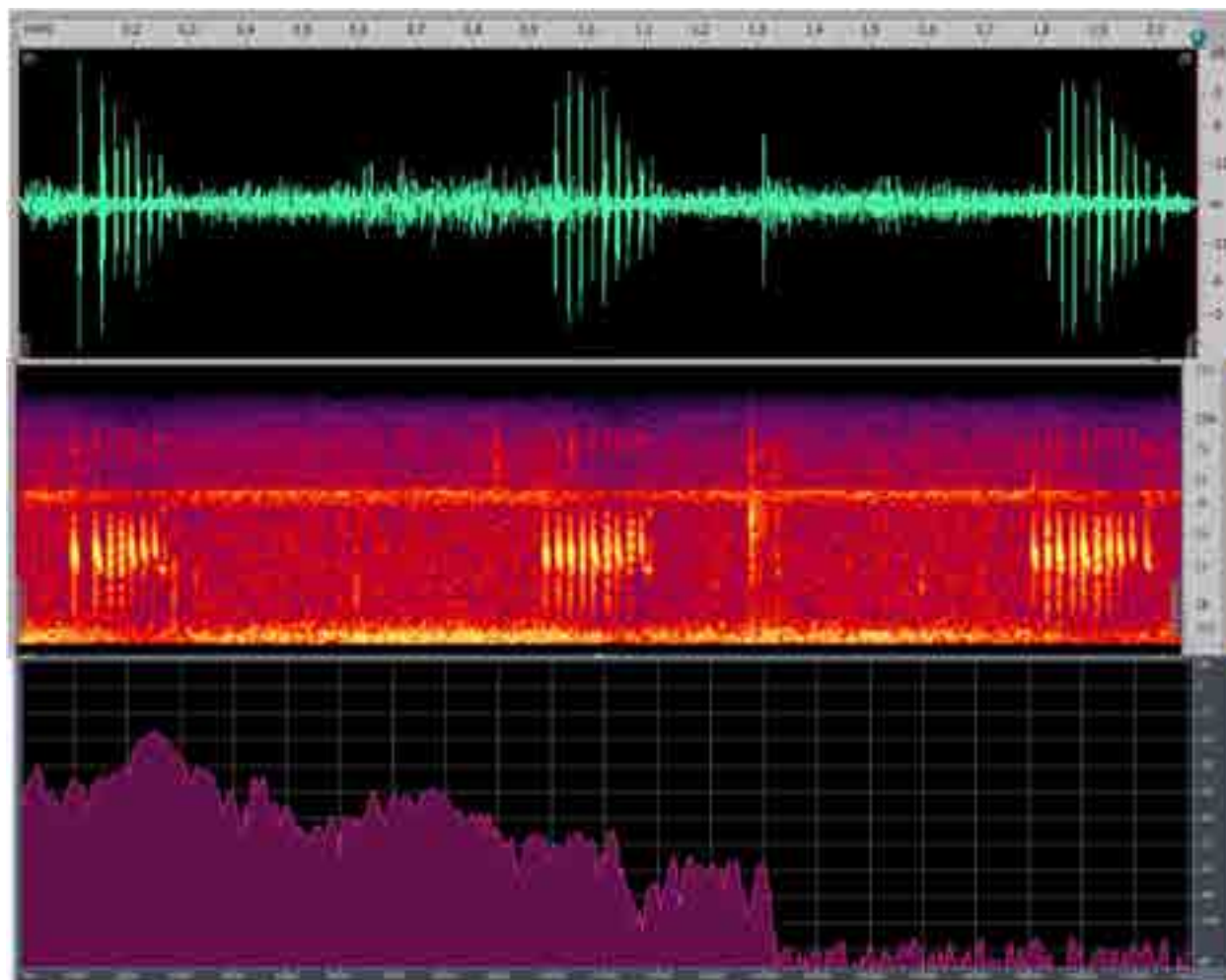


Image 5. Oscillogram, spectrogram and power spectrum of the call of *B. kemp*i.



Image 6. Stage 30 larva of *B. kemp*i: Mouthpart and ventral view.

13.28 mm in length, with a 7.9 mm long tail and 5.39 mm long head-body. Head-body ovoid, broader (HBW 2.76 mm) than deep (HBH 2.33 mm). Oral disc discernible at this stage with keratodont and keratinized jaw sheaths; oral tooth-row formula 1/1+1//1/1. Tail fins transparent

and high 2.32 mm. Rudimentary hindlimb buds visible at the posterior end of the head-body. Eyes well developed than in the earlier stage and in lateral position, with an inter-orbital space of 2.62 mm (Image 6).

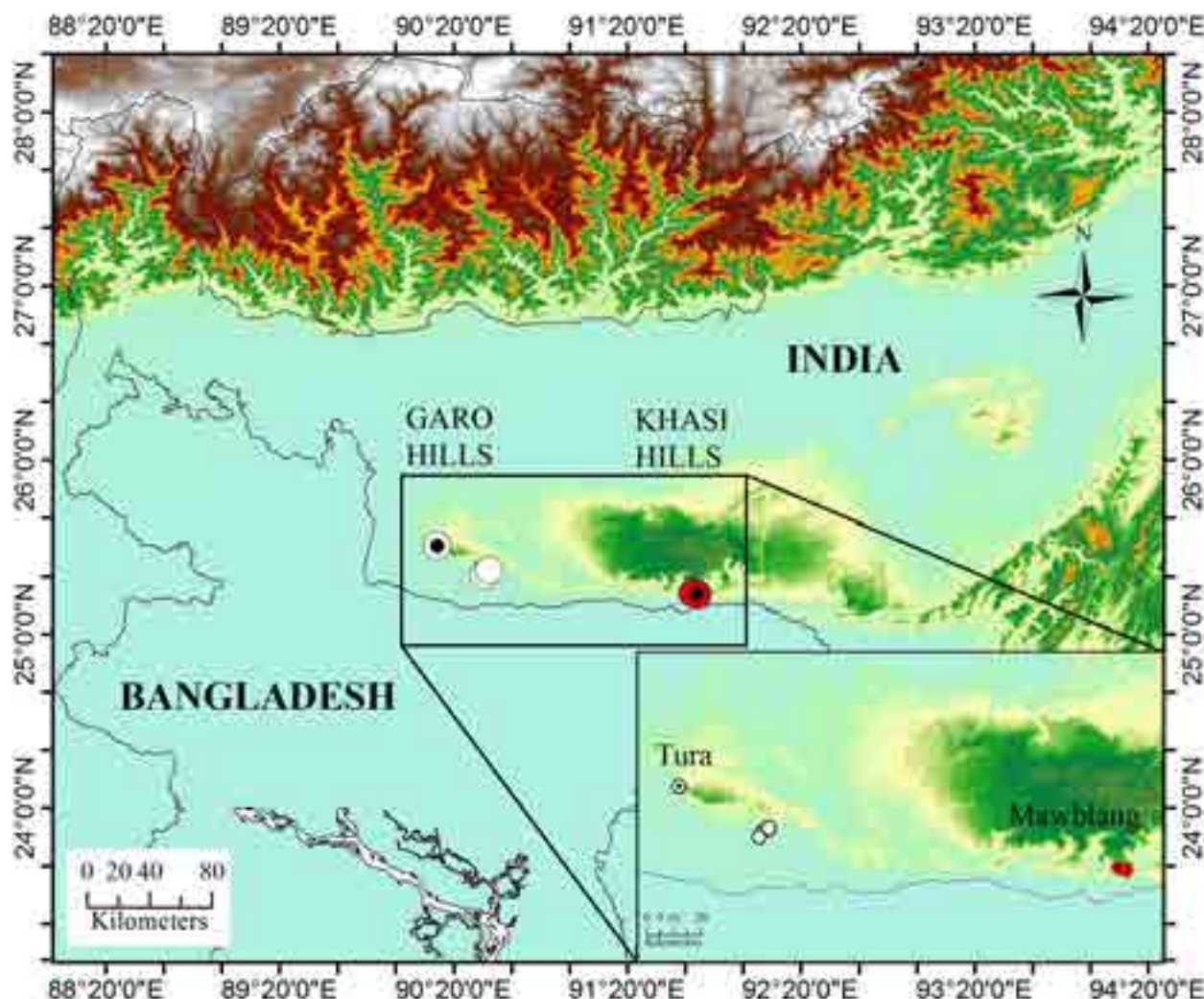


Figure 3. Map showing the distribution of *B. kemp*i (white circles; Garo Hills) and *B. meghalayanus* (red circles; Khasi Hills; type localities with a dot in the middle).

Distribution

During the present study, *B. kemp*i was recorded from the above four locations, of which, two are quite close-by and the type locality, Tura, lies at about 64 km northwest of the present study sites. However, our surveys at locations north of the Garo ridge at a higher elevation such as Mandalgre (25.50 °N, 90.37 °E, 1,019 m) and Daribokgre (25.48 °N, 90.31 °E, 1,123 m) could not locate this species. Further surveys at the vicinity of the current study sites and locations to the north of the Garo ridge in the lower reaches are necessary to determine whether the species occurs in those areas as well (Figure 3).

Abundance

The rate of encounter of *B. kemp*i was 0.53/hour, or two hours of effort to locate one individual in this region.

The encounter rate across the survey duration of 57 hours ranged from 0.17–2.0 (Table 4).

*Bufo*ides *meghalayanus* (Yazdani & Chanda, 1971)

Ansonia meghalayana Yazdani & Chanda, 1971

Holotype: ZSIC A 6969, an adult from Mawblang, Cherrapunji, Khasi Hills, Meghalaya.

Material studied: SACON VA 215, SACON VA 251 and SACON VA 252 three adult males from a hill stream in the Khasi Hills, Meghalaya (Image 7).

Diagnosis and comparison: A semi-arboreal to rupicolous *Bufo*ides from the Khasi Hills diagnosed by: small–medium body size (SVL 31. –33.5 mm); presence of irregular, non-keratinized cranial ridges (pre and post orbital); short, elongated parotoid glands (vs. ovoid in *B. kemp*i); absence of an externally visible tympanum; well-developed webbing between toes, with only half a



phalange free of webbing on toe IV (vs. relatively poor, with two phalanges free of webbing on toe IV in *B. kempī*); partial, but better developed webbing between fingers than *B. kempī* and the presence of small, slightly dilated, rounded terminal digital discs at the tips of both fingers and toes. Dorsum black with mossy green along the flanks in males, females predominantly green with black reticulations; a dark grey venter with small white spots (vs. pale white in *B. kempī*).

Description and Variation: (based on the newly collected material) Table 2

Body small (31.99 mm $\pm$ 0.76), trunk relatively short (AG:SVL 0.38) and stout (AG:BW 1.0). Head large (HL:SVL 0.37); slightly broader than long (HL:HW 0.85); and half as deep as long (HL:HD 2.08); snout tip obtusely pointed in dorsal view. Eyes large (ED:HL 0.35); snout slightly longer than eye (ED:ES 0.84); interorbital space about one and half times the width of the upper eyelid (IO:UEW 1.46) and nearly twice the internarial distance (IO:IN 2.09). Tympanum absent. Upper arm short (UAL:SVL 0.21); lower arm slightly longer than upper arm (UAL:LAL 0.93); palm a little longer (UAL:PAL 0.77). Fingers partially webbed, webbing formula $I_{0-1} II_{0-2} III_{1-1} IV$; relative length of fingers $III > IV > II > I$. Outer metacarpal tubercle large & evident. Thigh relatively short (FEL:SVL 0.39); tibia a little longer than the thigh (FEL:TBL 0.94); foot nearly as long as the thigh (FEL:FOL 0.98). Toes partially webbed, webbing formula: $I_{0-0} II_{0-0} III_{0-0.5} V_{0.5-0} V$, a relatively large inner and a slightly smaller ovoid metatarsal tubercles at the base of the foot. Toe tips with discs as broad as the toes; tarsal ridge not discernible. Finger and toe-tips bearing slightly expanded terminal discs lacking circum-marginal grooves. Dorsal colouration uniform black, with irregular feeble yellow markings; venter grey with fine white spots.

Description of calls

The call of *B. meghalayanus* recorded during this study comprised of a series of high pitched syllables of 'ti-tuk' that lasted for a duration of 20 s and was composed of eight separated notes, each of which comprise two pulses. The mean duration of each note was 0.28 ms, with a mean interval of 2.45 s in between. Maximum amplitude of the call was -7 dB with a dominant frequency of 1.0 kHz (Image 8).

Distribution: (Table 4)

During this study, *B. meghalayanus* was recorded from a few locations in the Khasi hills within an altitudinal range of 1060–1240 m that are mapped in Figure 3. Additional localities were provided by Deuti et al. (2012).

Abundance

The rate of encounter of *B. meghalayanus* was 0.875 / hour, or about an hour of effort to locate one individual in this region. The encounter rate across the survey duration of 38 h ranged from 0.67–5.33 (Table 4).

DISCUSSION

B. kempī was described by Boulenger in 1919 based on the two specimens collected by S.W. Kemp, and presented to him by Nelson Annandale from 'above Tura, 2,500 ft' in the Garo Hills. Since its description, no further records or observations of this species have been made until now. Studies conducted in this region have uncovered several new and noteworthy species but *B. kempī* remained elusive to scientists until now (Datta-Roy et al. 2013; Deuti et al. 2012; Biju et al. 2016; Giri et al. 2019). Although Das et al. (2009) mentioned a specimen (MFA 10134) of *Bufoideis* collected from Tura, Garo Hills, no taxonomic assessment of this specimen has been made until now, which still remains unidentified. Therefore, with the results of the present study, we announce the authentic rediscovery of *B. kempī* after a period of more than a century (1919–2022) from near the type locality, Garo Hills. First ever field observations on its ecology, behaviour, breeding biology, and natural history have been presented here. Observation on their breeding in phytotelmata and multiple males participating in amplexus with a single female have been made for the first time. Das & Dutta (2007) noted the absence of any larval descriptions for *B. kempī*, which has now been provided for the first time, across three developmental stages. Based on our field observations, the distribution of *B. kempī* mapped here shows that it is restricted to the lower reaches of a small hillock in the western part of Meghalaya, south of the Garo Hills, across an elevation range of 100–250 m. above which *B. kempī* was not detected despite intensive surveys. In comparison, *B. meghalayanus* was found to be a strictly montane species occurring only on the hilltops between elevations of 1,000–1,240 m. Further surveys north of the Garo ridge could possibly uncover additional locations characterized by lowland evergreen forests with perennial streams and rock boulders, that could potentially be occupied by *B. kempī* and we recommend additional surveys in such localities in the future.

Unlike *B. kempī*, for *B. meghalayanus*, the original descriptions of the species as well as the genus were comprehensive in terms of both morphology & natural history (Yazdani & Chanda 1971; Pillai & Yazdani 1973).



Image 7. Adult male *Bufoides meghalayanus* in life (dorsal and ventral views). © S.R. Chandramouli.

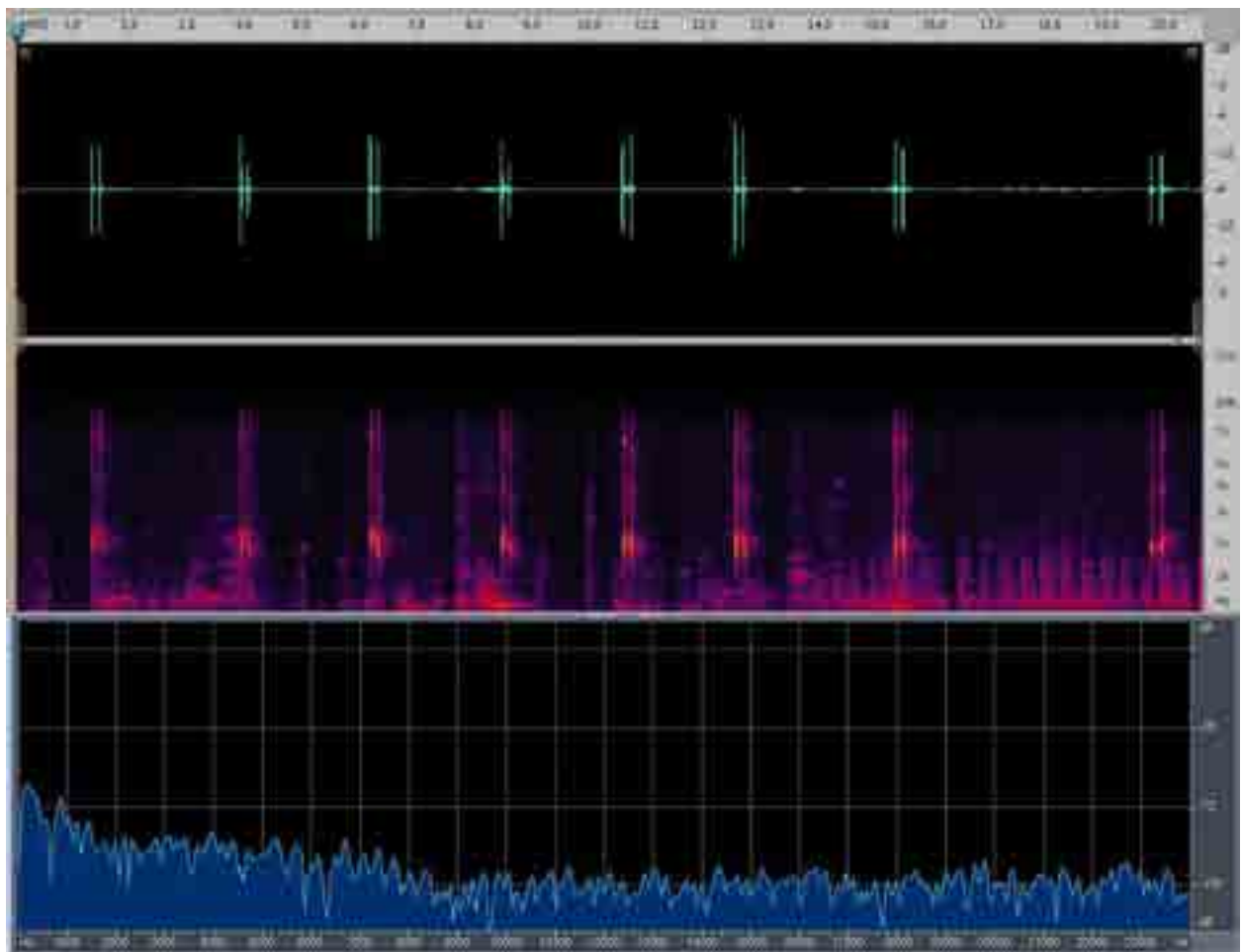


Image 8. Oscillogram, spectrogram and power spectrum of the call of *B. meghalayanus*.

Table 4. Abundance estimates of *B. kempī* and *B. meghalayanus*.

<i>Bufoides kempī</i>												
Site	Lat. (°N)	Long. (°E)	Elevation (m asl.)	time	Duration (h)	No. of ind.	ER	Microhabitat	Forest type	Habitat	Canopy cover	Ambient temperature (°C)
Eman Asakgre	25.40	90.54	225	day	3	1	0.33	Under boulder	Evergreen	Dry stream	90	26
Eman Asakgre	25.36	90.53	122	day	6	1	0.17	Under boulder	Evergreen	Stream	90	27
Eman Asakgre	25.37	90.54	202	day	6	5	0.83	Tree hole	Evergreen	Forest	90	26
Eman Asakgre	25.36	90.53	122	night	6	2	0.33	Tree hole	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	122	night	5	1	0.20	on leaf	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	220	night	6	2	0.33	Tree hole	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	220	night	6	2	0.33	on leaf	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	220	night	6	4	0.67	on leaf/under boulder	Evergreen	Forest	90	23
Eman Asakgre	25.34	90.51	250	night	6	1	0.17	Under boulder	Evergreen	Forest	90	23
Eman Asakgre	25.37	90.54	202	night	6	3	0.50	on leaf	Evergreen	Forest	90	23
Eman Asakgre	25.36	90.53	122	night	1	2	2.00	Tree hole/on leaf	Evergreen	Forest	90	23
					57	24	0.53					
<i>Bufoides meghalayanus</i>												
Stream behind Mawsmi cave	25.25	91.72	1200	Day/ Night	1	0	0		Montane	Stream	70	21
Stream behind Mawsmi cave	25.25	91.72	1200	Day/ Night	1	0	0		Montane	Stream	70	21
Stream 1 behind Mablang village	25.24	91.74	1200	Day/ Night	1	0	0		Montane	Stream	80	21
Stream 1 behind Mablang village	25.23	91.74	1200	Day/ Night	2	1	0.67	Rock Crevice	Montane	Stream	80	21
Stream behind Mawsmi cave	25.25	91.72	1200	Night	1	0	0		Montane	Stream	70	20
Stream 1 behind Mablang village	25.23	91.74	1200	Day/ Night	4	0	0		Montane	Stream	80	20
Stream behind Mawsmi cave	25.25	91.72	1200	Day/ Night	5	0	0		Montane	Stream	70	20
Stream 2 behind Mablang village	25.23	91.74	1200	Day/ Night	4	0	0		Montane	Stream	80	20
Stream 2 behind Mablang village	25.23	91.74	1200	Day	4	0	0		Montane	Stream	80	20
Stream 1 on-route to Thangkarank park	25.239	91.73	1200	Day	6	4	1.33	Rock Crevice	Montane	Dry stream	90	18
Stream 2 on-route to Thangkarank park	25.23	91.74	1200	Day/ Night	6	32	10.67	Rock Crevice / Pandanus tree	Montane	Dry stream	95	21
Stream 1 on-route to Thangkarank park	25.23	91.73	1200	Day	6	19	3.17	Rock Crevice	Montane	Dry stream	90	22
					38	56	0.875					

Subsequent studies have supplemented information on its morphology (Das et al. 2009), osteology (Chandramouli & Amarasinghe 2016); ecology and distribution (Deuti et al. 2012). Das et al. (2009) after examining the specimen reported by Pawar & Birand (2001) from Mizoram, opined that it is not conspecific with *B. meghalyanus*. Hence, we do not include that record within the range of *B. meghalyanus*. Therefore, the Mizoram population of *Bufo*ides reported by Das et al. (2009) still needs a proper taxonomic assessment.

Availability of Data

Specimens collected and studied are deposited in the collections of SACON. DNA sequence generated in this study has been deposited in the genbank under the NCBI voucher number OP920605.

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Avifaunal diversity in Indian Institute of Technology Guwahati Campus, Assam, India

Umang H. Rathod¹ & Rupam Bhaduri²

¹Department of Mechanical Engineering, ²Centre for the Environment,
Indian Institute of Technology Guwahati, North Guwahati, Assam 781039, India.
¹umang174351003@iitg.ac.in (corresponding author), ²bhadurirupam@gmail.com

Abstract: Indian Institute of Technology - Guwahati (IITG), Assam, is an ecologically rich campus hosting different species of birds, butterflies and mammals. It accommodates several migratory and resident species of birds across different seasons. However, information is scanty on avian diversity with respect to the different habitats of the campus. Therefore, the present study attempts to gain insight into avian diversity with respect to habitat heterogeneity by considering the species presence-absence dataset collected for three years (2017–2020). A multivariate Beta (β) diversity analysis is carried out for the IITG campus constituted of five primary habitats, viz., secondary growth, eco-forest, water bodies, swampy-marshy area, and constructions. Of 152 bird species observed in the IITG campus, the highest number is reported from secondary growth, followed by eco-forest. The multivariate analysis shows that the average β -diversity for the IITG campus is approximately equal to 79%, which is in accordance with another published study. These observations are examined in light of hypotheses and phenomena documented in the literature, such as habitat heterogeneity hypothesis, niche-based hypothesis and anthropogenic impact on habitats. The study also establishes that the IITG is among the educational institutes and campuses that host many migratory bird species. Lastly, based on the outcomes of β -diversity analysis, it is suggested that the conservation effort for avian species in the campus should be directed towards individual habitats uniformly.

Keywords: Campus avian diversity, habitat heterogeneity, presence-absence dataset, multivariate β – diversity.

Abbreviations: 2D—Two dimensional | α —Number of species shared between two habitats | A_R —Richness agreement | $b_{c,c}$ —Number of species present in one habitat but absent in another | CBC—Campus Bird Count | D_R —Richness difference | IBA—Important bird and biodiversity area | IITG—Indian Institute of Technology Guwahati | N_s —Species nestedness | PAST—Paleontological Statistics software package for education and data analysis | R_s —Species replacement | S_j —Jaccard's similarity index | $\bar{x}$ —Mean index | α —Alpha diversity, diversity of individual habitat | β —Beta diversity | β_a —Additive beta diversity | β_x —Multiplicative beta diversity | β_j —Jaccard's dissimilarity index or Beta diversity | $\bar{\beta}_j$ —Mean of all multivariate beta diversity values | $\beta_{j,avg}$ —Average of multivariate beta diversity of one habitat | β_s —Sorensen's index | γ —Gamma diversity, the total number of species in an area encircling all the habitats.

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Author details: UMANG H. RATHOD is a PhD student of Mechanical Engineering at Indian Institute of Technology Guwahati (IITG), besides being a member of Assam Bird Monitoring Network (ABMN). His research interests cover bio-inspired engineering designs, wind turbines, soft computing, and avifauna; and has authored several research articles in these topics. RUPAM BHADURI is a PhD student from Centre for the Environment, Indian Institute of Technology Guwahati, besides being a member of Assam Bird Monitoring Network. His research interests cover analyzing climate dynamics in infrastructure decision making process with a lens of political ecology. He is an avid birder with large interest in understanding avifauna, their behavior and impact of climate change on avian migration.

Author contributions: Documentation, data analysis, structure and overall preparation of the manuscript, and communication are done by UHR. RB has contributed in terms of documentation, data analysis, and manuscript structure.

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INTRODUCTION

The state of Assam, situated in the northeastern part of India, is home to over 700 avian species (BirdLife-International 2022). The bird and biodiversity hotspots of Assam include 55 IBAs (Important bird and biodiversity areas), which also cover the state's National Parks and Wildlife Sanctuaries (Rahmani et al. 2016). However, the presence of avian species is not limited to the aforementioned designated sites. It extends to lesser-known birding areas such as a city, a remote wetland, a college campus or even an individual's backyard. Among them, the university campuses are distinctive because they can possess heterogeneous habitats along with continuous anthropogenic influences. Moreover, Liu et al. (2021) reviewed the campus biodiversity surveys of at least 300 universities and colleges worldwide since 1940. They found that each campus contains an average of 66 bird species, including threatened species, offering a major refuge for birds in nearby urban areas. It was then proposed that the campuses with high diversity should be protected for research, conservation, and biodiversity education. Further, to implement more bio-diversity-friendly designs, the suggested primary step is to monitor and investigate the biodiversity of university campuses. Similarly, from the perspective of the Indian academic campuses, Guthula et al. (2022) found an average of 88 bird species per campus based on the survey conducted on total of 335 Indian academic campuses. These observations and suggestions motivated the present authors to study the avian diversity of the Indian Institute of Technology, Guwahati (IITG) campus.

The campus of IITG is beautifully manicured in the proximity of many IBAs located nearby Guwahati city viz, 'Dadara-Pasara-Singimari', 'Deepor Beel' (Assamese: lake) bird sanctuary, 'Amchang' hills, 'Chandubi' lake, and adjoining areas, 'Jengdia Beel-Satgaon' and 'Pabitora' wildlife sanctuary (Rahmani et al. 2016). The campus is composed of diverse habitats such as forest patches, hillocks, wetlands, bushes, and a few lakes, making it perfectly suitable for accommodating a wide range of bird species. The diverse vegetation found in such habitat heterogeneous sites decides the overall rich avifaunal composition of the area (MacArthur & MacArthur 1961). The campus with diverse habitats hosts not only resident birds but also many migratory species. However, no scientific documentation of the avifauna inside the IITG campus was conducted in the past. Thus, a study addressing the avian diversity within the IITG campus was deemed necessary. This investigation is an attempt

to document the avian species of the IITG campus for three years (2017–2020) and to perform a diversity analysis of the bird species among different habitats of the campus using multivariate Beta (β) diversity analysis. This has been one of the most prevalent techniques to compare the diversities of different species assemblages (Anderson et al. 2011; Schmera et al. 2020), especially where the field data are collected only as the presence or absence of species. This study is particularly important to highlight the richness of avian species on the campus besides quantitative comparison of diversity among the different campus habitats. In the literature, there are many documented campus-based avian studies, but only as species checklists (Gupta et al. 2009; Surasinghe & Alwis 2010; Ali et al. 2013; Kabir et al. 2017; Manohar et al. 2017; Sailo et al. 2019), without thorough and quantitative habitat-wise diversity analysis. On the other hand, Chakdar et al. (2016) & Trivedi & Vaghela (2020) conducted a diversity & abundance analysis based on the dataset of species-wise number of individual birds. The overall trend suggests that most campus-based diversity analyses are checklists or abundance-based and are not based on a presence-absence dataset. To address this skewness, the present study is aimed to carry out a diversity analysis based on the presence-absence dataset. Moreover, this technique can emphasize the individual identity of the species rather than its abundance (Anderson et al. 2011). This technique is elaborated in the Methodology section, followed by results, discussion, and a brief conclusion emphasizing the threats and conservation measures.

METHODS

Study Area

The present study has been carried out in the IITG campus located at 26.185°N and 91.688°E, nearby Guwahati, Assam. The campus spanning over 2.8 km² of area is situated on the northern banks of the river Brahmaputra. The campus was established in 1995, and since then, the habitat has been significantly changed due to infrastructural development. The climate of the campus area is warm and humid, with an average annual rainfall of 1,752 mm. The temperature of the site ranges between maximum and minimum temperatures of 32.6° (August) and 11.0° (January) (Govt. of India 2021). The campus is surrounded by marshy areas to the east and north, human settlements to the west, and the 'Brahmaputra' river and sandy riverbanks in the south. Moreover, in the proximity of the campus, the hilly areas

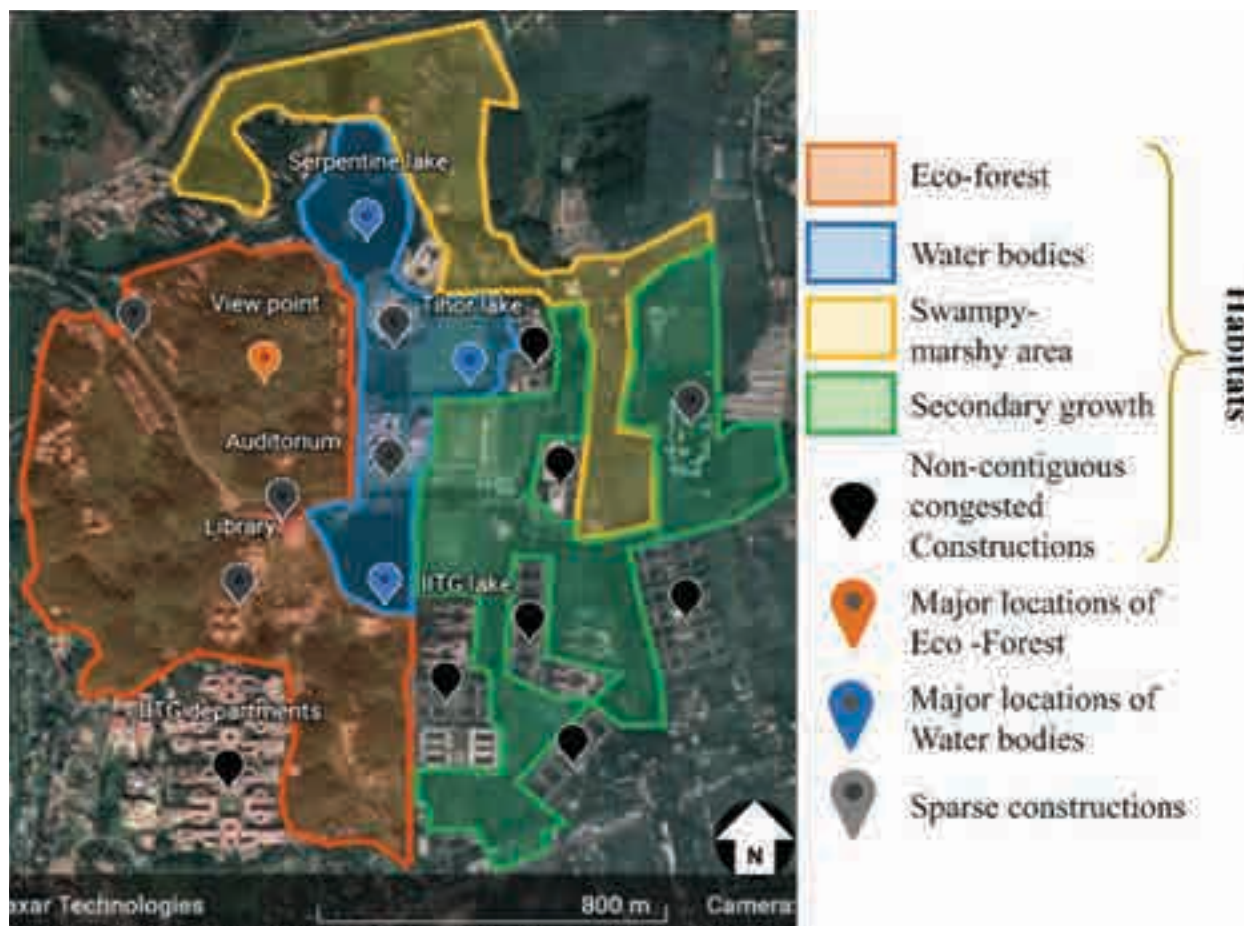


Image 1. Habitats of the Indian Institute of Technology, Guwahati campus.

‘Kali Pahar’ (Assamese: hill) in the north and ‘Nilanchal’ hills in the south are located.

The campus area is divided into multiple habitat types, as depicted in Image 1 in the form of a map based on their topology and vegetation type. The approximate area of each habitat type is estimated by Google Earth and listed in Table 1 in ascending order. The eco-forest habitat, spread mainly over a hilly and uneven campus area, is the remains of the wooded forest that was present before the establishment of the campus. The highest peak in the eco-forest habitat is the ‘view-point’, with the lowest human disturbance compared to other habitats. The dominant tree species and other plants in this habitat are *Tectona grandis*, *Dipterocarpus* sp., *Eucalyptus maculata*, *Acacia auriculiformis*, *Bombax ceiba*, *Erythrina stricta*, *Butea monosperma*, *Ficus hispida*, *Ficus racemosa*, *Artocarpus heterophyllus*, *Ailanthus excelsa*, *Neolamarckia cadamba*, *Aegle marmelos*, *Aglaia spectabilis*, *Toona ciliata*, *Holmskioldia sanguinea*, *Aporosa octandra*, *Nyctanthes arbor-tristis*, *Costus speciosus*, and *Areca catechu* among others (Kar

Table 1. Area of different habitats.

Habitat type	Area (km ²)
Water bodies	0.235
Swampy-marshy	0.307
Secondary growth	0.456
Eco-forest	0.783
Constructions	1.019
Total	2.8

et al. 2012). The aquatic habitat of the campus is of two major categories: water bodies & swampy-marshy habitats. The water bodies are a combination of large lakes & ponds, viz., ‘Tihor’, Serpentine, and IITG lakes, as delineated in the form of blue location icons in Image 1. These lakes were present before the establishment of the campus and are not yet landfilled. Among the lakes, Serpentine contains island-type small patches, providing safe shelter to the aquatic birds. The water bodies are surrounded by trees such as *Roystonea* sp., *Cassia*

javanica, *Delonix regia*, *Lagerstroemia speciosa*, and *Michelia champaca* for campus beautification. Most of the patches of the swampy-marshy habitat are a result of rainwater accumulation over the sites from which the vegetation was removed and then abandoned with no construction. Some of them have been present before the establishment of the campus. The aquatic species include *Canna indica*, *Colocasia esculenta*, *Nymphaea rubra*, *Eichhornia crassipes*, *Hymenachne* sp., and some species of ferns are abundant in this habitat. The scattered distribution of tree species such as *Cocos nucifera*, *Ziziphus jujuba*, *Syzygium cumini*, *Ailanthus integrifolia*, *Dillenia indica*, *Mimusops elengi*, *Ficus religiosa*, *Lantana* sp., and *Bambusa* sp. can be observed on the fringes of the Swampy-marshy habitat. The secondary growth habitat consists of shrubs, bushes, grassy meadows, and sparsely distributed trees. This area usually remains disturbed by construction activities, transportation, and human activities. Additionally, the playgrounds having grass/lawns are also included in this habitat. This habitat is dominated by tree species such as *Alstonia scholaris*, *D. regia*, *Dalbergia sissoo*, *Acacia farnesiana*, *Eucalyptus hybrida*, *Albizia lebbbeck*, *Gmelina arborea*, *Psidium guajava*, *Terminalia bellirica*, *Samanea saman*, *Monoon longifolium*, *Terminalia arjuna*, *Phyllanthus emblica*, *Mangifera indica*, *Polyalthia longifolia*, *Cassia fistula*, *Azadirachta indica*, *M. elengi*, *Ficus benghalensis*, and others. Lastly, the habitat type named 'Constructions' (their locations marked by black icons in Image 1) is the only habitat which is non-contiguous and dispersed within the range of other aforementioned habitats. This area is scarcely populated with tree species such as *D. sissoo*, *A. lebbbeck*, *M. longifolium*, *M. elengi*, *N. cadamba*, *A. scholaris* and *P. longifolia*, along with other floral species planted for campus beautification. It is important to note that sparse construction sites (their locations marked by gray icons in Image 1) are still present in all other habitats; however, they are not as congested as the construction habitat.

Data Collection

To collect species presence-absence datasets for the diversity analysis, methodologies described in Hill et al. (2005) are implemented, as discussed in this section. As this task involves mobile species, the line/strip transect survey method is preferred, in which the surveyor walks along the line and records the presence/absence of individual species. The line transect method has been widely implemented in many avian surveys (Surasinghe & Alwis 2010; Devi et al. 2012; Kottawa-Arachchi & Gamage 2015; Chakdar et al. 2016; Pragasan & Madesh

2018; Singh et al. 2020; Trivedi & Vaghela 2020). Other attributes of this survey method, such as the number of individuals and their perpendicular distances from the line, are omitted here since the aim of the present survey does not include density and detectability parameters. Additionally, some of the merits of the line transect method are the ability to cover a large distance, address the common, and elusive species, low bias, versatility, and efficiency (Hill et al. 2005). Considering this, the line transect method is applied especially over the well-defined fixed routes, trails, bridle paths, and roads in the IITG habitats and boundaries around the habitats, water bodies, and swampy-marshy areas. Other documented studies have also adopted a similar methodology (Gupta et al. 2009; Ali et al. 2013; Kabir et al. 2017).

To standardize this technique, timed search type method is intertwined with the same, especially while surveying for the presence-absence of the species. Therefore, the line transect surveys were usually made in the early morning (06:00–09:00) and sometimes at night for nocturnal species such as owls (Ali et al. 2013). Such surveys were conducted weekly for three years (2017–2020) in all the seasons of a year (viz, winter, summer, and monsoon), and the data were tabulated habitat-wise (Appendix 1). Sometimes, the point counts method and opportunistic sightings of the birds were also used along with line transects for the habitats (Pragasan & Madesh 2018). It is important to clarify that birds in flight are included in the dataset only when the particular species is found using the particular habitat; for example, any raptor hovering or soaring in search of prey, the swifts or swallows hawking in proximity to the habitat or transects.

Instruments such as cameras (Nikon Coolpix P510 and Canon Powershot Sx50 hs) and field binoculars (Solognac 500 dpi, 8 × 40) were used to record the observations. Audio records were also used to identify the bird species by listening to the call on the spot or recording it in an audio recorder (Zoom H4n) and later analyzing it. Every identified species was cross-checked with the help of bird guides and handbooks (Grimmett et al. 2016), besides referring to the eBird database (ebird 2021). The abundance code is qualitative; for example, if an individual of a species is found slightly less than 10 times out of 10 different visits for birding, it is assigned as C—common, and, similarly, species were assigned as U—uncommon and R—rare, if recorded roughly for five times & 1–2 times out of 10 visits, respectively. These abundance codes, along with residency status & migratory status for each species, are provided in Appendix 1.

Mathematical Formulation for Data Analysis

As mentioned in the data collection section, the data of avian species in the aforementioned habitats are collected in the form of a presence-absence matrix. In this method, the presence & absence of a given species for each habitat are recorded in binary values 1 & 0 (Appendix 1), respectively. Usually, this approach is preferred when the difference/variations of species numbers/identities among assemblages, communities, habitats, and along spatial or temporal gradients are emphasized (Magurran 1988). Moreover, a focus on the identities of species (especially the role of rare species) rather than their abundance (individual numbers) is necessary for conservation and biodiversity studies (Anderson et al. 2011). Since the present investigation opted for a comparison of diversity between habitats of the IITG campus, the presence-absence dataset is sufficient.

As per the literature, the β – diversity is one of the most prevalent techniques used to compare the diversities of different assemblages whenever the species presence-absence data is available (Koleff et al. 2003; Anderson et al. 2011). Historically, the concept of β – diversity and its mathematical formulation in the form of β – diversity indices were proposed by R.H. Whittaker in 1960, and thereafter, ecologists have derived many indices for different applications. Some of these indices can even facilitate the use of abundance and presence-absence data. Basically, the β – diversity quantifies the dissimilarity or variation between habitats and assemblages in terms of varieties of species. Ecologists have classified the broad range of β – diversity indices into two major classical categories, viz., multiplicative & additive indices, as expressed in Equations 1 & 2, respectively.

$$\text{Multiplicative } \beta\text{-diversity, } \beta_{\text{mult}} = \frac{\gamma}{\bar{\alpha}} \quad (1)$$

$$\text{Additive } \beta\text{-diversity, } \beta_{\text{add}} = \gamma - \bar{\alpha} \quad (2)$$

Where, γ = total numbers of species in area circling all the habitats
 $\bar{\alpha}$ = average of number of species found in each habitats, or average diversity

The latter is more popular since it has the same dimension and unit as its independent variables (γ , α); hence, they can be directly compared. Therefore, the additive approach of β – diversity is chosen for the present investigation. It is important to note that the present study uses the measure of multivariate additive β – diversity instead of classical additive β – diversity. This approach facilitates the comparison of β – diversity of a given assemblage/habitat with all of the other habitats

available in the given area in the form of their pairs which is not possible in the classical approach (Anderson et al. 2011). Moreover, the value of β – diversity depends on the value of γ ; therefore, it should be normalized by the value of γ as per equation 3 (Ricotta & Pavoine 2015).

$$\text{Normalized } \beta\text{-diversity, } \beta_{\text{norm}} = \frac{\beta}{\gamma} \quad (3)$$

One common usage of β – diversity is to study the change of species diversity along an environmental gradient (i.e., elevation, latitude, longitude, temperature, upstream to downstream of a river, and others) (Legendre & Legendre 2012). On the other hand, the same index can also be used to compare species diversity & highlight dissimilarity in species compositions of different assemblages or habitats (Magurran 1988). As the multivariate β – diversity analysis deals with the dissimilarity between two assemblages (mentioned in the last paragraph), it is necessary to define an index which can quantify the same. In the literature on numerical ecology, more than 24 types of different types of β – diversity indices are available for the purpose (Koleff et al. 2003). Among them, Jaccard's dissimilarity index is mathematically less vigorous yet intuitive. To understand the index, the notions of shared and unshared species between two assemblages/habitats have to be clarified, as shown in Figure 1. The species shared between both the assemblages/habitats are marked as 'a'. The species present in Habitat—1 but not in Habitat—2 are marked as 'b'. Similarly, the species present in Habitat—2 but not in Habitat—1 are defined as 'c'. The summation of these quantities gives γ – diversity. The species absent from both the habitats, but present in other habitats, are excluded while calculating multivariate indices, i.e., exclusion of joint absences is implemented in multivariate analysis (Anderson et al. 2011). Using the definitions of a, b and c, the Jaccard's similarity & dissimilarity (β – diversity) in the normalized form can be calculated using Equations 4 & 5. β , emphasizes species b & c, which are not shared by both habitats, clearly quantifying the dissimilarity between the two habitats. The summation of β , & S_j , results in unity.

$$\text{Jaccard's similarity index, } S_j = \frac{a}{a+b+c} = \frac{a}{\gamma} \quad (4)$$

$$\text{Jaccard's dissimilarity index, } \beta_j = \text{Beta diversity} = \text{Species variation} =$$

$$\frac{b+c}{a+b+c} = 1 - S_j \quad (5)$$

The dissimilarity (β_j) between two habitats can be divided into two parts, namely the species replacement (R_s) & richness difference (D_R), as depicted in Figure 1. When a particular number of species in focal Habitat – 1 is replaced by different but the same number of species in Habitat – 2, then the phenomenon is known as species replacement (R_s) and the number of species participated is known as replaced species (Podani & Schmera 2011; Legendre 2014). It is important to clarify that the term ‘replacement’ or ‘variation’ is used for heterogeneous habitats-based studies, while the alternative term ‘turnover’ is more prevalent for gradient-based studies (Anderson et al. 2011). The number of dissimilar species not part of the replacement phenomenon is marked as the difference in richness (D_R). Both these quantities are defined in Equations 6 & 9, respectively. The (1 – component) of both the quantities are known as species nestedness (N_s) and richness agreement (A_R) as expressed by Equations 7 & 8, respectively. Whenever the species of Habitat – 1 is a subset of Habitat – 2, it can be stated that both habitats have pure nestedness between them. It is also observed that the higher the value of β_j , the higher the anti-nested characteristics for the artificial presence-absence dataset (Podani & Schmera 2011). The species nestedness (N_s) & species agreement (A_R) can clearly be visualized in Figure 2.

$$\text{Species nestedness index, } N_s = \frac{a+b-c}{a+b+c} = 1 - R_s \quad (6)$$

$$\text{Richness agreement index, } A_R = \frac{a + (2 \times \min(b, c))}{a+b+c} \quad (7)$$

$$= S_j + \left(2 \times \frac{\min(b, c)}{a+b+c} \right) \quad (8)$$

$$= S_j + (2 \times \text{William's index}) \quad (9)$$

$$\text{Richness difference index, } D_R = \frac{b+c}{a+b+c} = 1 - A_R \quad (10)$$

$$S_j + R_s + D_R = 1 \quad (11)$$

The above indices can be calculated using PAST (Paleontological Statistics software package for education and data analysis) software (Hammer et al. 2001). It is important to note that all the indices cannot be calculated directly by PAST software; however, William's index (Koleff et al. 2003) & Jaccard's similarity index can be estimated directly by the software. Using the estimated values of both indices, the remaining indices are calculated by equations 4 through 10. It is important to note that the normalization using the denominator ($2a+b+c$) can also be implemented in the form of Sorenson's index (β_j). Nevertheless, the Jaccard's index (β_j) is chosen since it gives an amplified

value because of the lower value of ($a+b+c$), i.e., $\beta_j > \beta_s$.

To visualize the numerical values of indices intuitively, the simplex approach of visualization is implemented since the summation of S_j , R_s and D_R result in a value equal to 1 as per equation 10 (Podani & Schmera 2011). A graphical depiction of the 2D (two-dimensional) simplex approach in the form of a Ternary plot is shown in Figure 3. The apices of the equilateral triangle in the ternary plot represent 100% values of R_s , S_j & D_R . Their values decrease along their respective simplices and result into 100% values of their (1 – component), creating apices of the inner equilateral triangle. The apices of this inner triangle represent 100% values of N_s , β_j and A_R . The dotted sides of the inner triangle denote 50% values of R_s , S_j and D_R . Any point inside a ternary plot possesses values of R_s , S_j and D_R corresponding to a pair of dissimilar habitats/assemblages. Thus, the 2D simplex approach in the form of a ternary graph is used to represent indices for present investigations.

In multivariate analysis, the aforementioned indices are calculated for different pairs of habitats; therefore, if there are m number of habitats in a given area, the total number of such pairs would be ${}_m C_2$ as per equation 11. Hence, the average value of these indices from these pair values can be calculated using Equation 12.

$$\text{Number of total habitat-wise pairs} = {}_m C_2 = \frac{m!}{2!(m-2)!} = \frac{m(m-1)}{2} \quad (12)$$

Where, m = Number of total habitats or assemblages

$$\text{Average index, } \bar{x} = \frac{2 \times \sum_{i=1}^m x_i}{(m^2 - m)} \quad (13)$$

RESULTS AND DISCUSSION

Species Richness

In total, 152 species of birds belonging to 108 genera, 50 families and 14 orders were recorded on the IITG campus (Appendix 1). Among them, 35 species are winter migrants (including altitudinal migrants), four summer migrants, and others are resident and local migrants (Choudhury 2000; Grimmett et al. 2016). The highest number of species is found in secondary growth (83 species), followed by eco-forest (68 species), swampy-marshy area (57 species), constructions (38 species), and water bodies (33 species), as shown in Figure 3. In the case of species that are specific to a habitat type, the highest numbers are recorded in eco-forest followed by swampy-marshy areas, secondary growth, water bodies and, constructions. The highest difference between the aforementioned numbers (total

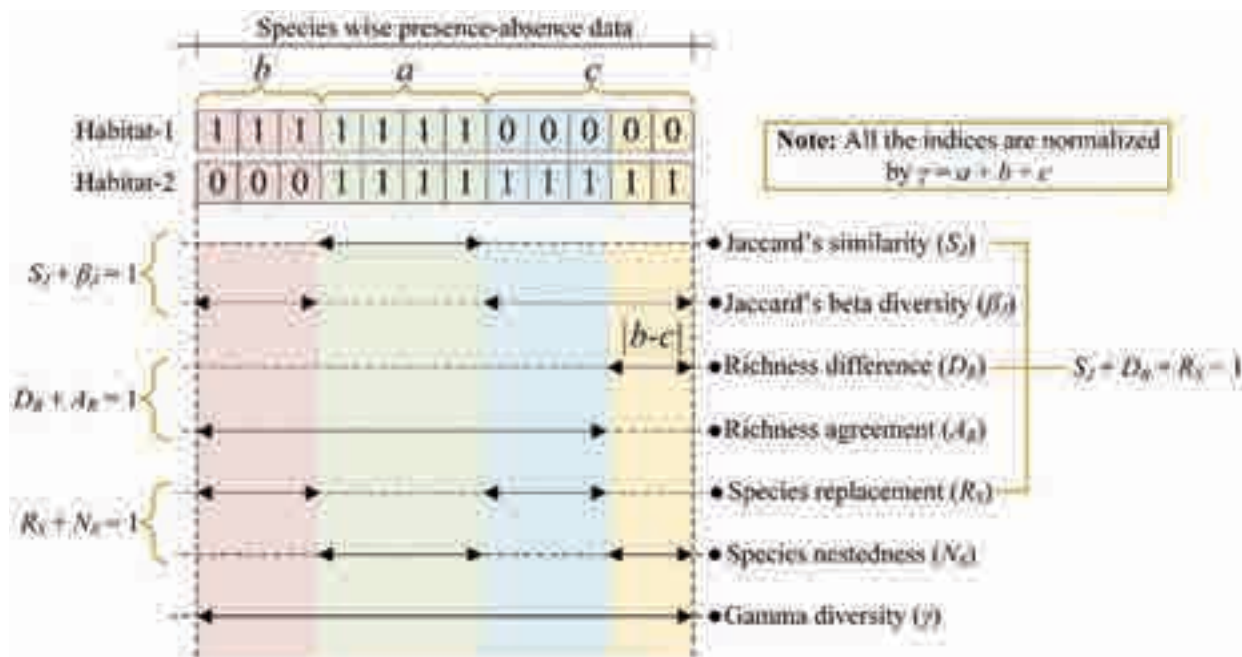


Figure 1. Derivation of different additive diversity indices.

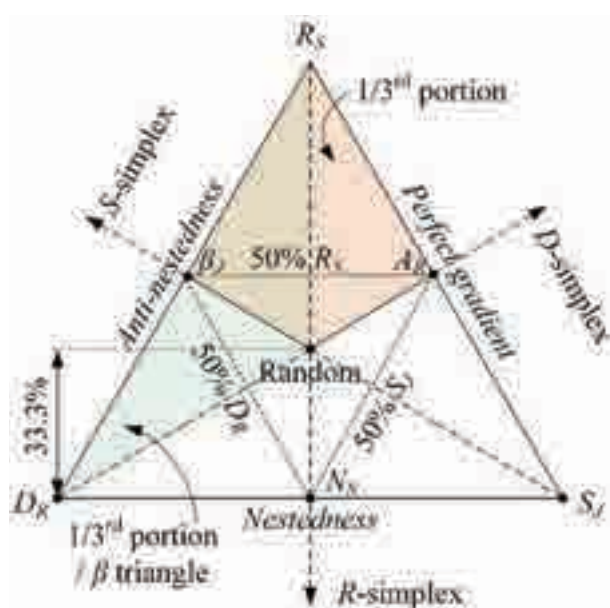


Figure 2. 2D simplex approach by ternary plot.

number of species found in a habitat and the number of species that can only be found in the same habitat) is found in secondary growth, which clearly indicates that most of the species are generalists. The lowest difference is found for water bodies indicating a major share of specialist species. Approximately 36%, 35%, and 33% of the total species are specialists in species composition of water bodies, swampy-marshy areas, and eco-forest

habitats, respectively. On the other hand, the values are 17% & 13% (approximately $1/3^{\text{rd}}$ of previous values) for habitats like secondary growth & construction habitats, which clearly indicate that the percentage of specialist species decreases due to construction work & associated disturbances. These results are also supported by similar findings for the Assam University Campus (Chakdar et al. 2016).

Approximately 49% of species belong to only one habitat type, i.e., nearly half of the total species are specialists (Table 2). Five species are found in all of the five habitats; Black Kite *Milvus migrans*, Asian Barred Owlet *Glaucidium cuculoides*, Spotted Owlet *Athene brama*, Black Drongo *Dicrurus macrocerus*, and Red-vented Bulbul *Pycnonotus cafer*. Similarly, species namely the Spotted Dove *Streptopelia chinensis*, Cattle Egret *Bubulcus ibis*, Shikra *Accipiter badius*, Taiga Flycatcher *Ficedula albicilla*, and White Wagtail *Motacilla alba* are recorded in four habitats (different habitats for each species) out of the total five habitats. The qualitative abundance of each species is tabulated in Appendix 1.

Variation in Species Compositions Among Different Habitats of IITG Campus

Following the methodology discussed in the section on mathematical formulation for data analysis, multivariate values of Jaccard's similarity index (S_j) & William's index are estimated (Table 3). After that, other indices such as β_j , R_s , D_R , N_s , and A_R are calculated. As per

Table 4, all of the multivariate β_j values are more than 50%, clearly showing high β – diversity of all the habitats in the IITG campus. The high β – diversity values can be explained by the habitat heterogeneity hypothesis, which states that an increase in the number of distinct habitats leads to an increase in β – diversity and hence the overall diversity in a landscape (MacArthur & MacArthur 1961). Because of habitat heterogeneity, a successful adaptation of a particular species to one habitat leads to its inferior competitiveness for another habitat. As a tradeoff between both, distinct habitats in an area may be distinct in terms of species composition, resulting in higher β – diversity among them (Cramer & Willig 2005; Soininen et al. 2007). Additionally, the number of partitionable niche dimensions is expanded due to habitat heterogeneity. The maximum value of $\beta_j = 94.8\%$ is obtained between eco-forest & water bodies habitats. Although both the habitats are contiguous, these habitat types have very contrasting characteristics, i.e., the former is a hilly wooded forest and the latter is aquatic. A similar trend is reported for contrasting habitats even in a gradient-based study (Goettsch & Hernández 2006). The lowest value of $\beta_j = 57.1\%$ is found between eco-forest & swampy-marshy areas. Average β – diversities ($\beta_{j, avg}$) (e.g., for habitat – 1 of the present case, β_j for pairs 12, 13, 14, and 15 are averaged) of each habitat is more than 70%. The overall $\bar{\beta}_j$ calculated using Equation 12 is approximately 79%, showing very high β – diversity for the overall IITG campus area.

The authors of the present paper implemented the current approach of β – diversity analysis in another documented research article (Surasinghe & Alwis 2010) to gain more insight into the species variation in different habitats of college campuses besides the present study. The study recorded 145 species distributed into seven different habitats of the ‘Sabargamuwa’ university campus (area $\approx 0.5 \text{ km}^2$, established in 1990); however, β – diversity analysis and species variation along habitats were not analyzed. Authors of the present paper calculated $\bar{\beta}_j \approx 82\%$ for ‘Sabargamuwa’ university campus, which is close to the $\bar{\beta}_j \approx 79\%$ of the IITG campus area (area $\approx 2.8 \text{ km}^2$, established in 1995).

The results of the multivariate analysis are presented in a graphical ternary plot (Figures 5,6) using the values of R_s , D_R , and S_j listed in Tables 3 & 5. As discussed, the ternary plot provides a better understanding of the relative composition of richness difference (D_R) & species replacement (R_s) constituting β_j . Figure 4 shows that most of the multivariate data points are enclosed by β – triangle (depicted in Figure 2) and are leaning towards the left side of the equilateral triangle, indicating high β_j

Table 2. Number of species found in the given number of habitats.

Number of total habitats	Number of species
1	74
2	46
3	22
4	5
5	5

Table 3. Values for Jaccard's similarity index- S_j (upper triangle) and William's index (lower triangle).

Habitats*	1	2	3	4	5
1	—	0.052	0.096	0.428	0.235
2	0.291	—	0.304	0.105	0.111
3	0.403	0.173	—	0.241	0.160
4	0.219	0.211	0.267	—	0.367
5	0.200	0.412	0.296	0.057	—

*Habitats are tagged as: 1—Eco-forest | 2—Water bodies | 3—Swampy-marshy area | 4—Secondary growth | 5—Constructions.

values. A similar type of trend is also observed in Figure 5. Further, the majority of the points (circular & solid red markers) are congregated in the top 1/3rd portion of a quadrilateral (depicted in Figure 2) with a propensity towards replacement (R_s apex) rather than the richness difference (D_R apex). Therefore, species replacement is dominating factor behind the high β – diversity of IITG habitats. The reason might be that the specialist species of one habitat are replaced by those of another habitat without much relative difference between them in terms of species numbers. This can also be explained by the niche-based hypothesis, which states that the difference in habitat compositions drives species turnover between different locations along a gradient or species variation through replacement among different habitats in a given area (Anderson et al. 2011; Lorenzón et al. 2016).

On the other hand, the points are equally dispersed towards R_s apex (circular markers) & D_R apex (solid circular markers) for Figure 5. Hence, the species replacement and richness difference are equally responsible for the high β – diversity of ‘Sabargamuwa’ university campus. Graphically, the points of Figure 4 are distributed along R – simplex, while they are along S – simplex for Fig. 6 while maintaining inclination towards high β_j values. The habitat pair of secondary growth and constructions yields the highest value of nestedness ($N_s \approx 88.5\%$) among IITG habitats, indicating a subset relationship between them. The dispersed and non-contiguous nature of the constructions habitat inside the

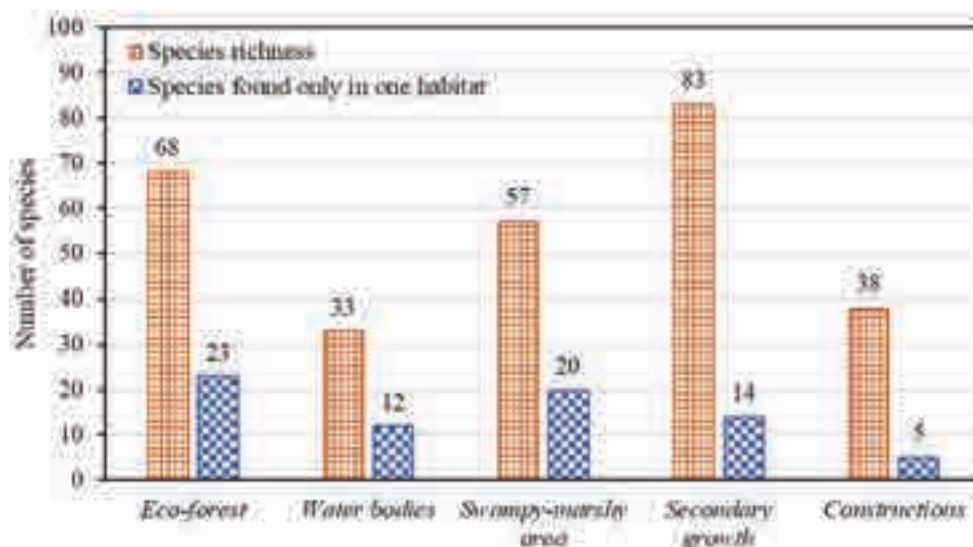


Figure 3. Habitat-wise species richness.

Table 4. Values for Jaccard's dissimilarity index (θ_j) (upper triangle) and Nestedness index (N_j) (lower triangle).

Habitats*	1	2	3	4	5	$\theta_{i, avg}$	θ_j
1	—	0.947	0.903	0.571	0.764	0.796	
2	0.416	—	0.695	0.894	0.888	0.856	
3	0.192	0.652	—	0.758	0.835	0.799	0.789
4	0.561	0.576	0.464	—	0.632	0.714	
5	0.600	0.174	0.407	0.885	—	0.781	

* Habitats are tagged as: 1—Eco-forest | 2—Water bodies | 3—Swampy-marshy area | 4—Secondary growth | 5—Constructions.

secondary growth habitat might be the reason for such species composition. A corresponding multivariate point (red square marker) is also located towards the triangle's lower side, clearly showing a prominent nestedness behavior. Likewise, the nestedness is observed between dry-mixed semi-evergreen forest and residential habitat in Figure 5 (red solid square marker at point 45). A similar trend of high β – diversity is observed for the Colorado fish dataset (Smith 1978), involving six different sites and 26 fish species in the ternary plot (Podani & Schmera 2011). The high β – diversity was constituted by D_R as a major factor and R_S as a minor factor. The reason behind this trend was believed to be many extinctions and a few successful colonization.

Therefore, it can be concluded that the habitats in IITG habitats proclaim high β – diversity due to habitat heterogeneity. The main factor behind high β – diversity is species replacement rather than species richness differences. Most importantly, habitat heterogeneity is also a result of anthropogenic impacts. The β – diversity is observed to increase during the initial stage

of the anthropogenic impacts due to the extinction of rarer specialist species and the establishment of invasive generalist species (considering the campus a biogeographic island) (Socolar et al. 2016). Gradually, the invasive generalist species become more dominant while eradicating native specialist species. Hence, the entire process gives a momentary increment in β -diversity followed by a simultaneous drop in β -diversity and overall species richness. Therefore, the high β -diversity of the IITG campus indirectly indicates the initial phase of anthropogenic impact.

It is noteworthy to clarify that the present analysis only emphasizes presence-absence data, not the abundance data, providing equal weightage to both rare and abundant species. Nevertheless, the species list with qualitative abundance code is provided in Appendix 1 for further insights.

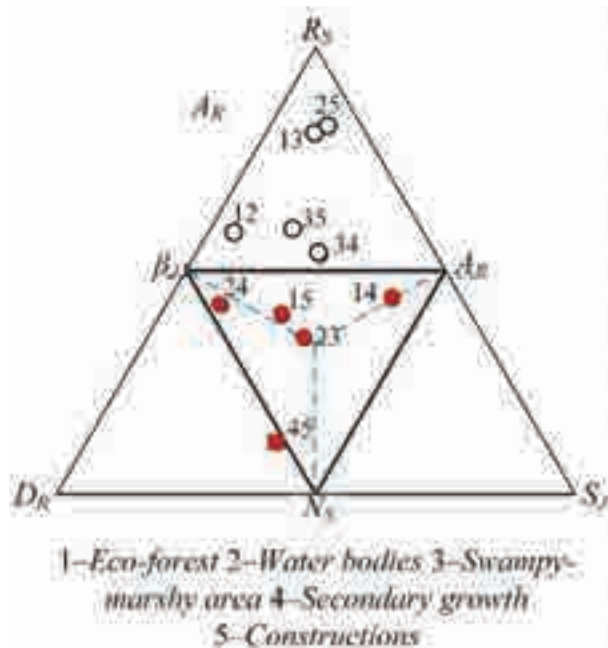


Figure 4. Ternary plot for IITG habitats.

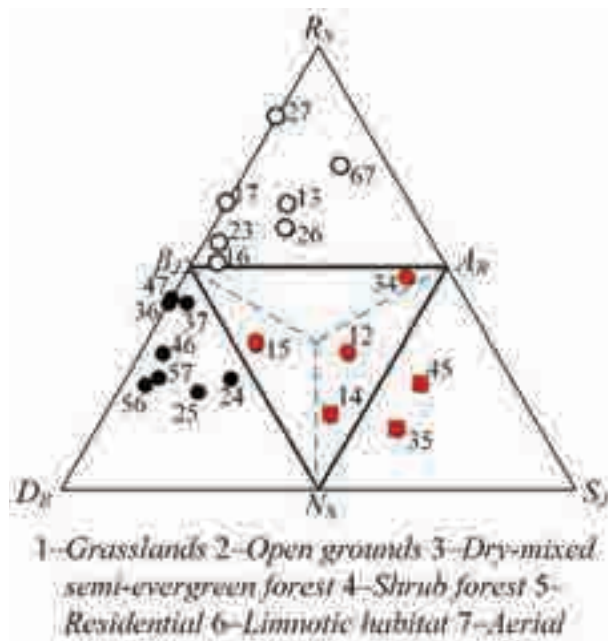


Figure 5. Ternary plot for reported data of Surasinghe & Alwis (2010).

THREATS AND CONSERVATION MEASURES

Not much past documented data are available in the literature about the avian diversity of the IITG campus; nevertheless, a checklist from a web source is available from July 2000–February 2002 (Praveen 2002). The documentation was done during that time of the year

Table 5. Values for Species replacement index (R_s) (upper triangle) and Richness difference index (D_R) (lower triangle).

Habitats*	1	2	3	4	5
1	—	0.583	0.807	0.438	0.400
2	0.364	—	0.347	0.423	0.825
3	0.096	0.347	—	0.535	0.592
4	0.133	0.471	0.223	—	0.114
5	0.364	0.063	0.246	0.517	—

* Habitats are tagged as: 1—Eco-forest | 2—Water bodies | 3—Swampy-marshy area | 4—Secondary growth | 5—Constructions.

Table 6. List of recorded species under the IUCN Red List.

IUCN Red List status	Species name	Taxonomic name
Critically Endangered	Slender-Billed Vulture	<i>Gyps tenuirostris</i>
Endangered	Greater Adjutant	<i>Leptoptilos dubius</i>
Vulnerable	Common Pochard	<i>Aythya ferina</i>
	Lesser Adjutant	<i>Leptoptilos javanicus</i>
Near Threatened	Ferruginous Duck	<i>Aythya nyroca</i>
	Himalayan Griffon	<i>Gyps himalayensis</i>
	Red-Breasted Parakeet	<i>Psittacula alexandri</i>
	Oriental Darter	<i>Anhinga melanogaster</i>

when most of the area within the campus was a part of the wetland on which the autonomous institute was built. As eBird was launched only in 2014 in India, the earlier historical records of species within campus could not be found in the portal. Hence, the authors had to rely on the website on which the aforementioned documentation had been uploaded. The checklist listed 120 species, most of which had been observed during the period of the present study (2017–2020). The exceptions are Eurasian Wryneck *Jynx torquilla*, Little-ringed Plover *Charadrius dubius*, Osprey *Pandion haliaetus*, Eurasian Marsh Harrier *Circus aeruginosus*, and Common Kestrel *Falco tinnunculus* that were not observed during the period. These species are common in nearby wetlands and water bodies. The reason behind their disappearance from the campus could be the deterioration of water bodies and marshy areas besides the peripheral vegetation that came up due to construction activities.

During the 2017–2020 timeframe, one critically endangered, one endangered, two vulnerable and three near threatened species were recorded as per IUCN Red List norms as enlisted in Table 6. Both the migratory aquatic species, viz., Common Pochard *Aythya ferina* and Ferruginous Duck *Aythya nyroca* can be observed in the water bodies of the campus during winter in small

numbers (10–20); however, their presence have become less frequent with each winter as per the observations of the authors. Another important observation by the authors is that both the species, besides other duck & pochard species, are mostly found in Serpentine and ‘Tihor’ lakes, and not in the IITG lake. The reason can be the small island type patches and bushes on the fringes of the lakes, except that of the IITG lake, which provides safe roosting places for the aforementioned species (as the majority of them are nocturnal feeders) (Ali & Ripley 1978) away from the reach of feral cats, dogs, and Indian Jackal *Canis aureus indicus*. Over time, vegetation on the fringes of the IITG lake has been removed due to constant construction work, fencing, and campus beautification by planting Bottle palm tree species. This would be one of the probable reasons behind their less frequent presence. Preservation of the small island patches and vegetation on peripheral fringes can be an important step to maintain the Water bodies undegraded for the critically endangered and near threatened aquatic species besides other species.

The Red-breasted Parakeet usually prefers forest and wooded habitats. Therefore, it is recorded in eco-forest and wooded areas of secondary growth. It nests in the cavities of trees and is mainly frugivorous. Therefore, it is advisable to conserve already present teak wood patches and other trees along with fruit-bearing ones like Gular Tree *Ficus racemosa*.

As mentioned in the ‘Results and Discussion’ section, the IITG campus has a high value of β – diversity, with species replacement as a dominating factor. It is reported in the literature that the replacement across multiple habitats in a given area (or turnover for gradient-based study) implies the focus to be on conservation efforts over multiple habitats rather than any single habitat (Socolar et al. 2016). Hence, the conservation effort for the avian community of the IITG campus should be directed towards each habitat uniformly. Moreover, the species richness of the campus is 152 species, which is way over the average species richness (by considering the dataset of 300 plus campuses), equal to 66, as per the review conducted by (Liu et al. 2021). For such avian (or overall) diversity-rich campuses, different key steps were suggested (Kobori & Primack 2003; Colding & Barthel 2017; Liu et al. 2021). It is recommended that a—certain parts of the campus should be protected with minimal scraping and disturbance | b—diversity of university campuses should be monitored thoroughly to plan more diversity-friendly designs, | c—provide nature-based education and awareness to campus residents, especially the students as they are the next generation of potential

birders/naturalists | d—restoration of biodiversity in the surrounding area with biodiversity protected in campus | e—implement primary biodiversity educational courses. In this direction, different activities are being carried out in the IITG campus, as narrated in the following paragraph.

Awareness of the avifauna within the IITG campus was restricted only to the birders with experience. Therefore, the authors, with support from the IITG population (refer to acknowledgement), tried to spread the message of the presence of birds within the campus by organizing ‘Bird Walk’ events frequently. During these events, participants were provided with the necessary support to identify and understand the importance of birds. These events have been organized as a part of the ‘Campus Bird Count (CBC)’ and ‘Bihu Bird Count’ projects every year since 2017 & 2020, respectively. The CBC, conducted under the banner of ‘Great Backyard Bird Count’ by Bird Count India (<https://birdcount.in/about/>), has further accelerated the process of counting the species and the number of birds in a given time frame within various campuses across the country. Other Campuses within Assam have also participated in CBC since its inception in 2014, with IITG recording one of the highest numbers of species yearly. ‘Bihu Bird Count’ is a regional citizen science project hosted by Assam Bird Monitoring network and Bird Count India, integrating with the celebration of ‘Bihu’ festivals (celebrated three times a year) with documentation of avifauna since its initiation in the year 2020. Especially for water bodies and swampy-marshy habitats, the ‘Asian Waterbird Census’ (by Bird Count India and International Waterbird census – IWC) has also been organized in the IITG campus to record migratory waterbirds. Plantation drives are also being organized from time to time in the eco-forest, secondary growth and periphery of the water bodies, which will be beneficial, especially for IITG lake, to address the concerns mentioned earlier. Further, a pictorial guide on birds in the form of a coffee table book (Bhaduri et al. 2020) is also launched by the IITG to inform visitors and students about avian diversity.

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Appendix 1. Checklist of avian species recorded in IITG during 2017–2020.

	Name	Scientific name	Presence-Absence data for different habitats					Abundance code	Residency code	IUCN Status
			Eco-forest	Water bodies	Swampy -marshy area	Secondary growth	Constructions			
1	Common Pochard	<i>Aythya ferina</i>	0	1	0	0	0	U	WM	VU
2	Cotton Pygmy-Goose	<i>Nettapus coromandelianus</i>	0	1	0	0	0	R	R	LC
3	Eurasian Wigeon	<i>Anas penelope</i>	0	1	0	0	0	R	WM	LC
4	Ferruginous Duck	<i>Aythya nyroca</i>	0	1	0	0	0	U	WM	NT
5	Fulvous Whistling-Duck	<i>Dendrocygna bicolor</i>	0	1	1	0	0	U	WM	LC
6	Gadwall	<i>Mareca strepera</i>	0	1	0	0	0	U	WM	LC
7	Green-Winged Teal	<i>Anas crecca</i>	0	1	0	0	0	U	WM	LC
8	Lesser Whistling-Duck	<i>Dendrocygna javanica</i>	0	1	1	0	0	C	R	LC
9	Tufted Duck	<i>Aythya fuligula</i>	0	1	0	0	0	U	WM	LC
10	Yellow-Footed Green-Pigeon	<i>Treron phoenicopterus</i>	1	0	0	1	0	C	R	LC
11	Spotted Dove	<i>Streptopelia chinensis</i>	1	0	1	1	1	C	R	LC
12	Red Collared-Dove	<i>Streptopelia tranquebarica</i>	0	0	0	1	0	U	R	LC
13	Eurasian Collared-Dove	<i>Streptopelia decaocto</i>	1	0	0	1	0	C	R	LC
14	Rock Pigeon	<i>Columba livia</i>	0	0	0	1	0	C	R	LC
15	Asian Koel	<i>Eudynamis scolopaceus</i>	1	0	0	1	1	C	R	LC
16	Common Hawk-Cuckoo	<i>Hierococcyx varius</i>	1	0	0	1	0	C	R	LC
17	Banded Bay-Cuckoo	<i>Cacomantis sonneratii</i>	1	0	0	0	0	R	R	LC
18	Greater Coucal	<i>Centropus sinensis</i>	1	0	1	1	0	C	R	LC
19	Lesser Coucal	<i>Centropus bengalensis</i>	0	0	1	1	0	C	R	LC
20	Green-Billed Malkoha	<i>Phaenicophaeus tristis</i>	1	0	0	0	0	U	R	LC
21	Plaintive Cuckoo	<i>Cacomantis merulinus</i>	1	0	0	0	0	R	R	LC
22	Pied Cuckoo	<i>Clamator jacobinus</i>	1	0	0	0	0	U	SM	LC
23	Indian Cuckoo	<i>Cuculus micropterus</i>	1	0	0	1	0	C	SM	LC
24	Asian Palm-Swift	<i>Cypsiurus balaisensis</i>	1	0	0	1	0	C	R	LC
25	House Swift	<i>Apus nipalensis</i>	0	0	0	0	1	C	R	LC
26	Brown-Cheeked Rail	<i>Rallus indicus</i>	0	0	1	0	0	R	WM	LC
27	Slaty-Breasted Rail	<i>Lewinia striata</i>	0	0	1	0	0	R	R	LC
28	Eurasian Moorhen	<i>Gallinula chloropus</i>	0	1	1	0	0	C	R	LC
29	White-Breasted Waterhen	<i>Amaurornis phoenicurus</i>	0	1	1	1	0	C	R	LC
30	Eurasian Coot	<i>Fulica atra</i>	0	1	0	0	0	C	R	LC
31	Grey-Headed Swampphen	<i>Porphyrio poliocephalus</i>	0	0	1	0	0	R	R	LC
32	Red-Wattled Lapwing	<i>Vanellus indicus</i>	0	0	1	1	1	C	R	LC
33	Bronze-Winged Jacana	<i>Metopidius indicus</i>	0	1	1	0	0	C	R	LC
34	Pheasant-Tailed Jacana	<i>Hydrophasianus chirurgus</i>	0	1	0	0	0	R	R	LC
35	Asian Openbill	<i>Anastomus oscitans</i>	0	1	1	0	0	C	R	LC
36	Greater Adjutant	<i>Leptoptilos dubius</i>	0	0	1	0	0	U	R	EN
37	Lesser Adjutant	<i>Leptoptilos javanicus</i>	0	0	1	0	0	U	R	VU
38	Oriental Darter	<i>Anhinga melanogaster</i>	0	0	1	0	0	R	R	NT

	Name	Scientific name	Presence-Absence data for different habitats					Abundance code	Residency code	IUCN Status
			Eco-forest	Water bodies	Swampy -marshy area	Secondary growth	Constructions			
39	Little Cormorant	<i>Microcarbo niger</i>	0	1	1	0	0	C	R	LC
40	Great Cormorant	<i>Phalacrocorax carbo</i>	0	1	0	0	0	R	R	LC
41	Cinnamon Bittern	<i>Ixobrychus cinnamomeus</i>	0	0	1	1	0	C	R	LC
42	Indian Pond-Heron	<i>Ardeola grayii</i>	0	1	1	0	0	C	R	LC
43	Black-Crowned Night-Heron	<i>Nycticorax nycticorax</i>	0	0	1	0	0	U	R	LC
44	Purple Heron	<i>Ardea purpurea</i>	0	1	1	0	0	U	R	LC
45	Striated Heron	<i>Butorides striata</i>	0	0	1	0	0	U	R	LC
46	Cattle Egret	<i>Bubulcus ibis</i>	0	1	1	1	1	C	R	LC
47	Little Egret	<i>Egretta garzetta</i>	0	1	1	1	0	C	R	LC
48	Intermediate Egret	<i>Ardea intermedia</i>	0	0	1	0	0	U	R	LC
49	Yellow Bittern	<i>Ixobrychus sinensis</i>	0	0	1	0	0	R	R	LC
50	Booted Eagle	<i>Hieraaetus pennatus</i>	1	0	0	0	0	U	WM	LC
51	Crested Serpent-Eagle	<i>Spilornis cheela</i>	1	0	0	0	0	R	R	LC
52	Short-Toed Snake-Eagle	<i>Circaetus gallicus</i>	0	0	0	1	0	R	R	LC
53	Oriental Honey-Buzzard	<i>Pernis ptilorhynchus</i>	1	0	0	0	0	R	R	LC
54	Black Kite	<i>Milvus migrans</i>	1	1	1	1	1	C	R	LC
55	Black-Winged Kite	<i>Elanus caeruleus</i>	0	0	0	1	0	R	R	LC
56	Shikra	<i>Accipiter badius</i>	1	0	1	1	1	C	R	LC
57	Himalayan Griffon	<i>Gyps himalayensis</i>	1	0	0	0	0	R	WM	NT
58	Slender-Billed Vulture	<i>Gyps tenuirostris</i>	0	0	0	1	0	R	R	CR
59	Asian Barred Owlet	<i>Glaucidium cuculoides</i>	1	1	1	1	1	C	R	LC
60	Spotted Owlet	<i>Athene brama</i>	1	1	1	1	1	C	R	LC
61	Barn Owl	<i>Tyto alba</i>	0	0	0	1	1	U	R	LC
62	Brown Hawk-Owl	<i>Ninox scutulata</i>	0	0	1	1	1	C	R	LC
63	Oriental Scops Owl	<i>Otus sunia</i>	1	0	0	0	0	R	R	LC
64	Eurasian Hoopoe	<i>Upupa epops</i>	0	0	0	1	0	C	R	LC
65	Oriental Pied Hornbill	<i>Anthraceroceros albirostris</i>	1	0	0	0	0	R	R	LC
66	Stork-Billed Kingfisher	<i>Pelargopsis capensis</i>	0	0	1	0	0	U	R	LC
67	White-Throated Kingfisher	<i>Halcyon smyrnensis</i>	0	1	1	1	0	C	R	LC
68	Common Kingfisher	<i>Alcedo atthis</i>	0	1	1	0	0	R	R	LC
69	Pied Kingfisher	<i>Ceryle rudis</i>	0	1	0	0	0	R	R	LC
70	Green Bee-Eater	<i>Merops orientalis</i>	1	0	0	1	0	C	R	LC
71	Chestnut-Headed Bee-Eater	<i>Merops leschenaulti</i>	1	0	0	0	0	U	SM	LC
72	Blue-Tailed Bee-Eater	<i>Merops philippinus</i>	0	0	0	1	0	U	SM	LC
73	Indo-Chinese Roller	<i>Coracias affinis</i>	0	0	0	1	1	C	R	LC
74	Coppersmith Barbet	<i>Psilopogon haemacephalus</i>	1	0	0	1	0	U	R	LC
75	Blue-Throated Barbet	<i>Psilopogon asiaticus</i>	1	0	0	1	0	C	R	LC
76	Lineated Barbet	<i>Psilopogon lineatus</i>	1	0	0	1	0	C	R	LC
77	Fulvous-Breasted Woodpecker	<i>Dendrocopos macei</i>	1	0	0	1	0	C	R	LC

	Name	Scientific name	Presence-Absence data for different habitats					Abundance code	Residency code	IUCN Status
			Eco-forest	Water bodies	Swampy -marshy area	Secondary growth	Constructions			
78	Black-Rumped Flameback	<i>Dinopium benghalense</i>	1	0	0	1	0	C	R	LC
79	Greater Flameback	<i>Chrysocolaptes guttacristatus</i>	1	0	0	1	0	C	R	LC
80	Rufous Woodpecker	<i>Micropternus brachyurus</i>	1	0	0	0	0	R	R	LC
81	Peregrine Falcon	<i>Falco peregrinus</i>	0	0	0	1	0	R	WM	LC
82	Rose-Ringed Parakeet	<i>Psittacula krameri</i>	0	0	0	1	0	U	R	LC
83	Red-Breasted Parakeet	<i>Psittacula alexandri</i>	1	0	0	1	0	U	R	NT
84	Large Cuckooshrike	<i>Coracina macei</i>	1	0	0	1	1	C	R	LC
85	Black-Hooded Oriole	<i>Oriolus xanthornus</i>	1	0	0	1	1	C	R	LC
86	Ashy Woodswallow	<i>Artamus fuscus</i>	0	0	0	1	0	C	R	LC
87	Common Iora	<i>Aegithina tiphia</i>	1	0	1	1	0	C	R	LC
88	White-Throated Fantail	<i>Rhipidura albicollis</i>	0	0	0	1	0	U	R	LC
89	Black Drongo	<i>Dicrurus macrocercus</i>	1	1	1	1	1	C	R	LC
90	Ashy Drongo	<i>Dicrurus leucophaeus</i>	1	0	0	0	0	U	WM	LC
91	Hair-Crested Drongo	<i>Dicrurus hottentottus</i>	1	0	0	1	0	C	R	LC
92	Greater Racket-Tailed Drongo	<i>Dicrurus paradiseus</i>	1	0	0	0	0	R	R	LC
93	Black-Naped Monarch	<i>Hypothymis azurea</i>	1	0	0	0	0	U	R	LC
94	Long-Tailed Shrike	<i>Lanius schach</i>	0	0	0	1	1	C	WM	LC
95	Brown Shrike	<i>Lanius cristatus</i>	1	0	0	1	1	C	WM	LC
96	Grey-Backed Shrike	<i>Lanius tephronotus</i>	1	0	0	1	1	C	WM	LC
97	House Crow	<i>Corvus splendens</i>	0	0	0	1	1	C	R	LC
98	Large-Billed Crow	<i>Corvus macrorhynchos</i>	0	0	0	1	1	U	R	LC
99	Rufous Treepie	<i>Dendrocitta vagabunda</i>	1	0	0	1	0	C	R	LC
100	Grey-Headed Canary-Flycatcher	<i>Culicicapa ceylonensis</i>	1	0	0	0	0	U	WM	LC
101	Cinereous Tit	<i>Parus cinereus</i>	1	0	0	1	1	C	R	LC
102	Gray breasted Prinia	<i>Prinia hodgsonii</i>	1	0	1	1	0	C	R	LC
103	Plain Prinia	<i>Prinia inornata</i>	0	0	1	1	0	U	R	LC
104	Common Tailorbird	<i>Orthotomus sutorius</i>	1	0	0	1	1	C	R	LC
105	Thick-Billed Warbler	<i>Arundinax aedon</i>	0	0	1	1	0	U	WM	LC
106	Clamorous Reed Warbler	<i>Acrocephalus stentoreus</i>	0	0	1	1	0	U	WM	LC
107	Zitting Cisticola	<i>Cisticola juncidis</i>	0	0	0	1	0	U	R	LC
108	Paddyfield Warbler	<i>Acrocephalus agricola</i>	0	0	1	0	0	U	WM	LC
109	Blyth's Reed Warbler	<i>Acrocephalus dumetorum</i>	0	0	1	0	0	U	WM	LC
110	Rusty Rumped Warbler	<i>Locustella certhiola</i>	0	0	1	0	0	R	WM	LC
111	Striated Grassbird	<i>Megalurus palustris</i>	0	0	0	1	0	U	R	LC
112	Barn Swallow	<i>Hirundo rustica</i>	0	0	1	0	0	C	R	LC
113	Red-Rumped Swallow	<i>Cecropis daurica</i>	0	0	1	0	0	U	WM	LC
114	Striated Swallow	<i>Cecropis striolata</i>	0	0	1	0	0	U	R	LC
115	Bengal bush lark	<i>Mirafrassa assamica</i>	0	0	1	0	0	R	R	LC
116	Red-Vented Bulbul	<i>Pycnonotus cafer</i>	1	1	1	1	1	C	R	LC

	Name	Scientific name	Presence-Absence data for different habitats					Abundance code	Residency code	IUCN Status
			Eco-forest	Water bodies	Swampy -marshy area	Secondary growth	Constructions			
117	Black-Crested Bulbul	<i>Rubigula flaviventris</i>	1	0	0	0	0	R	R	LC
118	Red-Whiskered Bulbul	<i>Pycnonotus jocosus</i>	1	0	0	1	0	C	R	LC
119	Tickell's Leaf Warbler	<i>Phylloscopus affinis</i>	1	0	0	0	0	U	WM	LC
120	Dusky Warbler	<i>Phylloscopus fuscatus</i>	0	1	1	1	0	C	WM	LC
121	Greenish Warbler	<i>Phylloscopus trochiloides</i>	1	0	0	0	0	C	WM	LC
122	Blyth's Leaf Warbler	<i>Phylloscopus reguloides</i>	1	0	0	0	0	R	WM	LC
123	Oriental White-Eye	<i>Zosterops palpebrosus</i>	1	0	0	1	0	C	R	LC
124	Pin-Striped Tit-Babbler	<i>Mixornis gularis</i>	1	0	0	0	0	R	R	LC
125	Puff Throated Babbler	<i>Pellorneum ruficeps</i>	1	0	0	0	0	R	R	LC
126	Jungle Babbler	<i>Turdoides striata</i>	1	0	0	1	1	C	R	LC
127	Asian Pied Starling	<i>Gracupica contra</i>	0	0	0	1	1	C	R	LC
128	Chestnut-Tailed Starling	<i>Sturnia malabarica</i>	1	0	0	1	0	C	R	LC
129	Common Myna	<i>Acridotheres tristis</i>	0	0	0	1	1	C	R	LC
130	Jungle Myna	<i>Acridotheres fuscus</i>	1	0	0	1	0	U	R	LC
131	Great Myna	<i>Acridotheres grandis</i>	0	0	1	0	0	U	R	LC
132	Common Hill Myna	<i>Gracula religiosa</i>	0	0	0	1	0	R	R	LC
133	Taiga Flycatcher	<i>Ficedula albicilla</i>	1	0	1	1	1	C	WM	LC
134	Oriental Magpie-Robin	<i>Copsychus saularis</i>	1	0	0	1	1	C	R	LC
135	White-Rumped Shama	<i>Copsychus malabaricus</i>	1	0	0	0	0	U	R	LC
136	Blue Whistling-Thrush	<i>Myophonus caeruleus</i>	0	0	0	0	1	U	R	LC
137	Blue Rock-Thrush	<i>Myophonus caeruleus</i>	0	0	0	0	1	R	WM	LC
138	Siberian Rubythroat	<i>Calliope calliope</i>	0	0	1	0	0	R	WM	LC
139	Black Redstart	<i>Phoenicurus ochruros</i>	1	0	0	1	0	U	WM	LC
140	Siberian Stonechat	<i>Saxicola maurus</i>	0	0	1	1	0	C	WM	LC
141	Crimson Sunbird	<i>Aethopyga siparaja</i>	1	0	0	1	1	C	R	LC
142	Purple Sunbird	<i>Cinnyris asiaticus</i>	1	0	0	1	1	C	R	LC
143	Baya Weaver	<i>Ploceus philippinus</i>	0	0	1	1	0	C	R	LC
144	Chestnut Munia	<i>Lonchura atricapilla</i>	1	0	0	1	0	U	R	LC
145	Scaly-Breasted Munia	<i>Lonchura punctulata</i>	1	0	0	1	1	C	R	LC
146	House Sparrow	<i>Passer domesticus</i>	0	0	0	0	1	C	R	LC
147	Eurasian Tree Sparrow	<i>Passer montanus</i>	0	0	0	0	1	C	R	LC
148	White-Browed Wagtail	<i>Motacilla maderaspatensis</i>	0	1	1	0	0	U	WM	LC
149	White Wagtail	<i>Motacilla alba</i>	0	1	1	1	1	C	WM	LC
150	Paddyfield Pipit	<i>Anthus rufulus</i>	0	0	1	1	1	C	R	LC
151	Citrine Wagtail	<i>Motacilla citreola</i>	0	1	0	0	0	U	WM	LC
152	Olive-Backed Pipit	<i>Anthus hodgsoni</i>	1	0	0	1	0	U	WM	LC
Abundance code: C—common U—uncommon R—rare Residency status: R—resident SM—summer migrant WM—winter migrant IUCN Red List status: LC—Least Concern NT—Near Threatened VU—Vulnerable EN—Endangered CR—Critically Endangered										



Threatened flora of Uttarakhand: an update

D.S. Rawat¹ , Satish Chandra² & Preeti Chaturvedi³

^{1,3}Department of Biological Sciences, College of Basic Sciences & Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, Uttarakhand 263145, India.

²Department of Botany, Government Degree College, Tiuni, Dehradun, Uttarakhand 248199, India.

¹drds_rawat@yahoo.com (corresponding author), ²satishchandrasemwal07@gmail.com, ³an_priti@yahoo.co.in

Abstract: Encompassing 1.69% land area of India, Uttarakhand State sustains more than 25% species of flowering plants of India reflecting richness of flora. Large numbers of species in the state are threatened and several sources have come up with their own lists of threatened species using different threat categories leading to ambiguity. This communication attempts to compile a complete list of threatened Angiosperm species from eleven authentic sources with updated nomenclature, systematic position, original sources, threat assessment, elevational and global distribution. A total of 290 species belonging to 176 genera, 63 families, and 29 orders are listed which represent about 6% of the total flora. Elevational distribution of species shows that the 2–3 km elevation zone harbors more than half of the threatened flora (52.14%) and more than 44% endemic species despite the fact that maximum species richness is known in the 1–2 km elevation zone. Perusal of literature shows that selection of species for micropropagation is skewed towards medicinal plants rather than only threat status of a species. A disparity exists in two important sources (IUCN Red List 2020–21 and Indian Red Data Book) listing threatened taxa with only six species common to both. Eight additional species in IUCN Red List 2020–21 and 49 additional species in Indian Red Data Book are not included and vice versa. 267 species listed as threatened in various sources are not even evaluated by recent IUCN Redlist guidelines and thus warrant their immediate assessment to understand their correct present status in nature.

Keywords: Angiosperms, assessment, Indian Red Data Book, IUCN Red List, micropropagation, plants.

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Author details: DR D.S. RAWAT has been working on different aspects of flora of Uttarakhand since 1987 and has trekked extensively in Uttarakhand for collection and photographing the plants. He has described four new species, several new records to India and western Himalaya, together with many rediscoveries. DR SATISH CHANDRA is working as assistant professor in the Department of Botany, Government Degree College Tyuni, Dehadun, Uttarakhand, India. He completed his PhD in plant taxonomy by working on the family Caryophyllaceae and his research interests include plant nomenclature, biodiversity and ethnobotany. DR P. CHATURVEDI is professor & head, Department of Biological Sciences, G.B. Pant University of Agriculture & Technology, Pantnagar. In vitro morphogenesis, conservation biology of threatened medicinal plants, plant biodiversity and bioprospecting of bryophytes are key research areas of her interest. She has successfully developed micropropagation protocols of several medicinal plants viz., *Centella asiatica*, *Aconitum balfourii*, *Picrorrhiza kurroa*, *Polygonatum verticillatum* and *Rheum emodii*.

Author contributions: Concept of work and compilation of data: DSR; writing article: DSR, SC, PC.

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INTRODUCTION

The actual number of all extant living species on Earth is yet not exactly known but we are now beginning to understand this enormous diversity of life on Earth (Wilson 1999). Though, the estimates range from 8.6 million to 15 millions of eukaryotes and trillions of prokaryotes (bacteria and archaea) living on Earth (Mora et al. 2011; Hinchliff et al. 2015; Locey & Lenon 2016; Larsen et al. 2017), only about 1.8 million are named and listed in Catalogue of Life 2020 (Roskov et al. 2020). The Earth's biosphere has already entered into the sixth mass extinction, majorly because of human impact. With a 1,000 fold increase in the natural rate of extinction of species (Pimm et al. 2014; De Vos et al. 2015) it is no exaggeration to state that a large number of species will disappear from Earth without even getting any name. Among the estimated described 21,37,939 species 31,030 species are already facing the threat of extinction owing to various natural and anthropogenic factors (IUCN Redlist 2020). Following the IUCN data, perhaps about 20% of all existing species might become extinct within the next few decades and 40% or more by the end of the present century (Pimm et al. 2014; Kew 2016; Pimm & Raven 2017).

Green Plants (Viridiplantae) are among the better known groups of organisms and dominated by more than 0.36 million Angiosperms (flowering plants) of which 38,445 species have been assessed for threat categories. The results show that 148 are already extinct from the wild, 15,624 are threatened and 2,594 do not have adequate data to assess threats to them as per IUCN Red List 2020–21 (<https://www.iucnredlist.org/search>). India is one of the top 10 species-rich nations of the world and 18,666 species of flowering plants are known within its territory (Mao & Dash 2019). Till date, 2020 species of flowering plants of India have been assessed as per the IUCN Redlist criteria according to which six species are extinct, two are extinct from the wild, 411 are threatened (84 Critically Endangered, 180 Endangered, 147 Vulnerable), 1601 are not threatened while 93 do not have adequate data today to assess threat (<https://www.iucnredlist.org/search>).

Uttarakhand is a small (53,483 km²), mountain dominated state of India, located in the Himalayan global biodiversity hot spot and constitutes the easternmost part of the western Himalayan phytogeographical province of India (Balakrishnan 1996). Following Takhtajan (1986) and Welk (2016) Uttarakhand embraces two floristic kingdoms- 'Holarctic' (above 1–1.5 km elevation) and 'Paleotropical' (below 1km elevation) and

surrounded by western Tibetan provinces in the north and the Gangetic province in the south. Three major floristic regions represented in Uttarakhand are western Himalayan province at the higher elevation, eastern Himalayan province in mid-elevations, and the Gangetic province at lower elevations and plains, while the arid western Tibetan province also finger-in at the head of anterior valleys (Welk 2016).

Uttarakhand is enriched with 24,303 km² of forests covering 45.44% of its total geographical area and about 4800 wild taxa of seed plants within 1,400 genera of 215 families (Uniyal et al. 2007; Pusalkar & Srivastava 2018; India State of Forest 2019). Representation of different phytogeographical elements, extensive elevation gradient (ca. 200–7,817 m), mountain dominated terrain, and enormous diversity in microclimatic conditions have resulted in a high diversity of angiosperm flora which accounts for nearly 25% of total Indian flora in only 1.69% geographical area of the country. Owing to its high species richness of Angiosperms, the flora of Uttarakhand can also be assumed to having many threatened species (Images 1–24). In addition to these species, 107 species, endemic to Uttarakhand (Singh et al. 2015; Pusalkar & Srivastava 2018) are also important for conservation due to their restricted distribution in the nature.

It has already been pointed out by Pimm et al. (2014) and reiterated by Raven & Wackernagel (2020) that the species most likely to become extinct are by definition the rare ones, and most undescribed species are relatively rare. Obviously, the first step towards conservation is to know which species are rare ones (threatened species). Considering the risk of extinction of the species IUCN has prepared categories and criteria for classification of species under different threat categories (IUCN 2012). It played a pivotal role in prioritizing the threatened species and in the drafting of their conservation plans. IUCN Redlist of Threatened Species is revised and updated thrice in each calendar year and country-wise lists are available in it.

Biodiversity of India is confronting various threats due to climate change, global temperature rise, habitat destruction, poor land use practices, invasive alien species, over-exploitation of the resources and environmental pollutions (Barik et al. 2018) and flora of Uttarakhand is no exception to this (Pusalkar and Srivastava 2018). Red Data Book of Indian Plants (Nayar & Sastry 1987–90) is an incomplete document wherein data on some threatened vascular plants were provided on the basis of herbarium history of these species. Later, Rao et al. (2003) listed 1,255 species of

threatened Indian vascular plants on the basis of the 1997 IUCN Red List of Threatened Plants. While these lists or data are available at country level, an updated list of threatened and endemic species of the species-rich state of Uttarakhand is yet to be compiled. Various scientific publications (Singh et al. 2010; Balakrishna et al. 2012; Bisht et al. 2013) often mention different species as threatened without correctly justifying their threat categories by appropriate source references. The present work is an attempt to provide a recent and complete list of threatened Angiosperm species with updated nomenclature and systematic position with original sources.

MATERIALS AND METHODS

The earliest holistic endeavour of publishing available data on threatened vascular species of India was attempted by the Botanical Survey of India, Ministry of Environment and Forests, Gol and published as 'Indian Red Data Books volume-1-3' (Nayar & Sastry 1987-90) wherein important data on 602 species were published. The species listed in these volumes and occurring in Uttarakhand are included in our list of threatened species (Table 1, column 1) with threat status (indicated by superscript '1' with threat status in column 2 of Table 1; e.g., R¹). Similarly, distribution of all 1,215 angiosperm species listed in Rao et al. (2003) was studied carefully and all species known in Uttarakhand were included in table-1 and shown by superscript '2' (e.g., E²). The species listed in recent IUCN Red List 2020-21 (<https://www.iucnredlist.org/>) of threatened species for India known to be occurring in Uttarakhand are included and status listed in IUCN Red List is shown by superscript '3' (e.g., CR³). Data Deficient species (DD) in this red list are also included here on account of their rarity due to which adequate data is not available for their assessment. The recent version of IUCN Red List (IUCN Red List 2020) now has the facility to search threatened species of a particular state of India and the species found in this list are also included with their status shown by superscript '3A' (e.g., CR^{3A}). Though, IUCN Red List for India and IUCN Red List of Uttarakhand are obtained from the same data source, they show a few differences due to which these two search results are shown differently. Ved et al. (2003) have published threatened medicinal plant species of Indian western Himalaya after threat assessment based on IUCN criteria. Those species which are listed in it and known in Uttarakhand are included in table-1 and threat assessment is shown by superscript '4' (e.g.,

EN⁴). Internationally, appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) play an important role in regulated trade of threatened species. All the species listed in CITES appendices (2019) and known in Uttarakhand are also included in Table 1. Since no specific threat status is mentioned in CITES appendices, in column 2 status is shown as '1A' (for species listed in Appendix 1) or '2A' (species listed in appendix-2) with superscript '5' (e.g., 2A⁵).

The first volume of Flora of Uttarakhand (Pusalkar & Srivastava 2018) has also provided a list of threatened species and endemic species, separately, with threat status following IUCN Red List, and these are also included in Table 1. Endemic species are included in our list with 'VU' status, following Pusalkar & Srivastava (2018), based on their small area of occurrence in the world. Uniyal et al. (2007) also listed threatened species of Uttarakhand with checklist of seed plants of Uttarakhand and these species are included and shown by the superscript '7' (e.g., R⁷). Similarly, the species indicated as threatened in Uttarakhand by National Biodiversity Authority at <http://nbaindia.org/uploaded/pdf/notification/4.4%20%20Uttarakhand.pdf> (shown as VoE⁸, VoE= verge of extinction), Uttarakhand Biodiversity Board at https://sbb.uk.gov.in/files/act/4.4_Uttarakhand.pdf (shown as HT⁹, HT= high threat), ebook by Uttarakhand State Biodiversity Board at <https://sbb.uk.gov.in/pages/display/88-books> (edited by Shah; shown as HT¹⁰), and ENVIS Centre, Botanical Survey of India at http://www.bsienvic.nic.in/Database/E_3942.aspx (shown by superscript '11' with threat status in column-2, e.g., R¹¹) are also included in Table 1. All the sources have not followed IUCN criteria so the statuses mentioned are not comparable. Species endemic to Uttarakhand are also marked by '*'. Species names are given in bold case and synonyms are in italicized normal case. If names used in original sources have changed these are given as synonyms. Names of all species are mainly checked in Plants of the World Online POWO (2019), Singh et al. (2019) and Catalogue of Life 2020 (Roskov et al. 2020) for nomenclatural updates. Synonyms, basionym wherever required (considering use in regional or national flora) are also given. After the scientific name, habit of the plant is given in column-1. In the second column threat status as given in original documents is mentioned. In the third column distribution of species in India/Himalayas and global distribution based on different sources is given. In the fourth column, elevational distribution of species compiled from various authentic sources is given. Wherever information is not available



Figure 1. Location map of photographed species.

it is indicated by ‘?’.

All the threatened species listed in Table 1 are arranged order and family wise following arrangement and circumscription of families given in Angiosperm Phylogeny Group classification (APG IV 2016). Abbreviations used in Table 1 for different geographical areas (Indian states, Himalayan areas, Countries) are detailed out below Table 1. Some of the species listed in original documents are dropped from Table-1 on account of various reasons elaborated in results and discussion part (Table 2).

RESULTS AND DISCUSSION

The compiled list of threatened species shows the presence of 290 threatened species (211 herbs, 43 shrubs, 24 trees, 12 climbers) belonging to 29 orders, 63 families and 176 genera. This number of species is about 6% of the total wild flora of the state. Some of the species listed as threatened in different sources are not included in it on account of clearly being synonyms of other common species, wrong identification, variety being not recognized in recent works or international databases, invasive species, or being cultivated species (Table 3). More than 100 species are endemic to the state. Source wise number of species included in Table 1 is depicted in table-2 which shows that maximum numbers of species are based on Pusalkar & Srivastava (2018) which is a

recent document on flora of Uttarakhand.

Family wise, Orchidaceae (27⁺ genera and 47⁺ spp.) contains the largest number of threatened species followed by Fabaceae (15 genera, 26 spp.), Poaceae (14 genera, 19 spp.), Apiaceae (12 genera, 16 spp.), Rosaceae (8 genera, 16 spp.), Asteraceae (8 genera, 14 spp.), Balsaminaceae (1 genus, 10 spp.), and Ranunculaceae (7 genera, 10 spp.). Rest of the families contain less than 10 threatened species. The genus *Impatiens* L. has 10 threatened species and *Berberis* L. has eight species threatened out of total 30 spp. each, known in Uttarakhand while seven species of *Spiraea* L. out of 18 total known in Uttarakhand are threatened (Uniyal et al. 2007; Pusalkar & Srivastava 2018).

Elevational distribution of 280 species compiled from different sources shows that the maximum number of threatened species (146 spp., 52.14%) are distributed in the 2.0–3.0 km elevation zone, followed by the 3.0–4.0 km zone (126 spp., 45.0%), 1.0–2.0 km zone (99 spp., 35.35%), 4.0–5.0 km zone (66 spp., 23.57%) and up to 1.0 km (58 spp., 20.71%). The lowest number of threatened species (11 spp., 3.92%) is found in the 5.0–6.0 km zone which is obvious being a species poor zone. On elevation gradient, maximum forest cover (India State of Forest Report 2019) and highest species richness across all habits was recorded in the 1–2 km zone (1.4–1.6 km) by Kharkwal et al. (2005) while Oommen & Shanker (2005) found the 1.0–2.3 km zone with the highest diversity of woody elements. Threatened species, however, are more concentrated in the 2–3 km zone and then in the 3–4 km zone, thus not directly influenced by high forest cover or species richness. Elevational distribution of 96 endemic species also shows a similar pattern with a maximum of 43 species (44.79%) in the 2–3 km zone, followed by 36 species (37.5%) in the 3–4 km zone, 28 species (29.16%) in the 1–2 km zone, 16 species (16.6%) in the 0.2–1 km zone, and 14 species (14.58%) in the 4–5 km zone. Species richness is expected to reduce with increasing elevation but in the Himalayas it is noticed highest in mid hills (1,500–2,500 m) above which it starts decreasing making a hump-shaped pattern (Grytnes & Vetaas 2002; Kharkwal et al. 2005). The species richness of threatened species as well as endemic species more or less also follows this pattern with moderate richness at lower elevations which increases to highest value in mid elevation (2–3 km elevation zone) and then starts reducing. It is apparent that species with limited elevation range (<500 m) require special attention as these are either narrow range endemics (e.g., *Eremogone curvifolia* (Majumdar) Pusalkar & D.K. Singh) or rarely collected (e.g., *Rubus*

almorensis Dunn).

Today, threat statuses accepted by IUCN only are considered correct and valid in international literature. As of the recent IUCN Red List 2020–21, only 54 species known in Uttarakhand have been evaluated, within which only 14 species are threatened. Critically Endangered (CR) species are *Aucklandia costus* Falc. (= *Saussurea costus* (Falc.) Lipsch.), *Gentiana kurroo* Royle, *Lilium polyphyllum* D. Don and *Nardostachys jatamansi* (D. Don) DC. Endangered species (EN) are *Aconitum heterophyllum* Wall. ex Royle, *Angelica glauca* Edgew., *Cypripedium elegans* Rchb.f., *C. himalaicum* Rolfe, and *Pittosporum eriocarpum* Royle. Vulnerable species (VU) are *Aconitum violaceum* Jacquem. ex Stapf, *Cypripedium cordigerum* D. Don, *Dalbergia latifolia*, *Dienia muscifera* Lindl. (= *Malaxis muscifera* (Lindl.) Kuntze), and *Ulmus wallichiana* Planch. Thirty-one species are evaluated but not found threatened and accordingly categorized as Least Concern (LC, 30 spp.) or Near Threatened (NT, 01 sp.). Nine species could not be evaluated for lack of sufficient data and categorized as Data Deficient (DD). A clear disparity can be seen in two important sources (IUCN Red List 2020–21 and Indian Red Data Book) where only six species (*Nardostachys jatamansi* (D. Don) DC, *Aucklandia costus* Falc., *Cypripedium cordigerum* D. Don, *C. elegans* Rchb.f., *C. himalaicum* Rolfe, *Pittosporum eriocarpum* Royle) are common. Eight additional species listed as threatened in IUCN Red List 2020–21 and 49 additional species listed as threatened in Indian Red Data Book are not included vice versa. IUCN Red List has also not assessed 256 species listed as threatened in various sources which warrant their immediate assessment by recent IUCN guidelines to understand their correct status in nature.

One of the important tools for conservation of plant species is micropropagation (Fay 1992). Reasons for selection of species for micropropagation may be various but one of them is the threatened status of a species. It is found in this study that out of 14 threatened species listed in IUCN Red List and known in Uttarakhand, successful tissue culture protocols have been developed for nine only. As per the list of threatened species by Indian Red Data Book, however, 55 species are known in Uttarakhand and only 10 species have been micropropagated. In all, tissue culture protocols are available for only 16 species (Grewal & Atal 1976; Lal et al. 1988; Mathur 1992; Sharma et al. 1993; Sharma & Seth 2001; Pandey et al. 2004, 2005; Jabeen et al. 2006; Pandey et al. 2011; Radha et al. 2011; Bhandari et al. 2013; Mishra- Rawat et al. 2013; Sharma et al. 2014; Kumari et al. 2015; Gondval et al. 2016; Gupta

Table 1. Threatened flora of Uttarakhand.

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
Order 1- AUSTROBAILEYALES Takht. ex Reveal Family 1- SCHIZANDRACEAE Blume			
1. <i>Schisandra grandiflora</i> (Wall.) Hook.f. & Thomson [= <i>Kadsura grandiflora</i> Wall.]; Cl	I ² , NE ³ , I ⁷ , I ¹¹	HP, UK, S; Nep, Bhu, Chi	1500-3500
2. <i>Schisandra propinqua</i> (Wall.) Baill. [= <i>Kadsura propinqua</i> Wall.]; Cl	I ² , NE ³ , I ⁷ , I ¹¹	UK, S; Nep, Bhu, Chi	1200-3000
Order 2- MAGNOLIALES Juss. ex Bercht. & J. Presl Family 2- Magnoliaceae Juss.			
3. <i>Magnolia doltsopa</i> (Buch.-Ham. ex DC.) Filger [= <i>Michelia doltsopa</i> Buch.-Ham. ex DC.]; T	DD ³	UK, WB, S, AP, MN, MG; Ba, Mya, Chi	900-2200
4. <i>Magnolia kisopa</i> (Buch.-Ham. ex DC.) Filger [= <i>Michelia kisopa</i> Buch.-Ham. ex DC.]; T	DD ³	UK, S; Nep; Tib	1500-2300
Order 3- LAURALES Juss. ex Bercht. & J. Presl Family 3- LAURACEAE Juss.			
5. <i>Alseodaphne himalayana</i> Kosterm.; Sh	NE ³ , VU ⁶	UK; Nep	?
6. <i>Cinnamomum glanduliferum</i> (Wall.) Meisn. [= <i>Laurus glandulifera</i> Wall.]; T	I ² , LC ³	UK; Nep, Ban, Bhu, Mal, Chi	1200-2150
7. <i>Cinnamomum tamala</i> T. Nees & Eberm.; T	LC ³ , VU ⁴	Himal, A; Mya, Lao, Viet	450-2150
Order 4- DIOSCOREALES R. Br. ex Mart. Family 4- DIOSCOREACEAE R. Br.			
8. <i>Dioscorea belophylla</i> (Prain) Voigt ex Haines [= <i>Dioscorea nummularia</i> var. <i>belophylla</i> Prain]; Cl	I ² , NE ³	Himal, NE India, Pen India	300-1800
9. <i>Dioscorea deltoidea</i> Wall. ex Griseb.; Cl (Image-1)	V ¹ , NE ³ , EN ⁴ , 2A ⁵ , EN ⁶	Himal, NE India	900-3500
Order 5- LILIALES Perleb Family 5- MELANTHIACEAE Batsch ex Borkh.			
10. <i>Paris polyphylla</i> Sm. [= <i>Daiswa polyphylla</i> (Sm.) Raf.]; H	NE ³ , EN ⁴ , EN ⁶ , HT ⁹	HP, UK, WB, S, AP, A, NL, MN, MG; Pak, Nep, Bhu, Mya, Chi, Jap	2000-3000
11. <i>Trillium govanianum</i> Wall. ex D. Don [= <i>Trillidium govanianum</i> (Wall. ex D. Don) Kunth]; H	NE ³ , EN ⁶ , HT ⁹	J&K, HP, UK, S, WB; Paki, Nep	2500-4000
Family 6- COLCHICACEAE DC.			
12. <i>Gloriosa superba</i> L.; H	LC ³ , VU ⁴	India; Nep, Ban, Bhu, Chi, Mya, Lao, Mal, Africa	300-1500
Family 7- SMILACEAE Vent.			
13. <i>Smilax wightii</i> A.DC.; Cl	R ¹ , R ² , NE ³	UK, E & C Himal, TN	Upto 500
Family 8- LILIACEAE Juss.			
14. <i>Fritillaria cirrhosa</i> D. Don [= <i>F. roylei</i> Hook.]; H (Image-2)	NE ³ , EN ⁴ , EN ⁶	J&K, HP, UK, S, WB; Afg, Pak, Nep, Bhu, Chi, Mya	2400-4500
15. <i>Lilium polyphyllum</i> D. Don; H	CR ^{3A} , CR ³ , CR ⁴ , CR ⁶ , HT ⁹	J&K, HP, UK; Afg, Pak, Nep	2000-4000
16. <i>Lilium wallichianum</i> Schult. & Schult.f.; H	I ² , NE ³ , EN ⁶ , I ⁷ , HT ⁹ , I ¹¹	UK, S; Nep, Bhu, Mya	1200-2400
Order 6- ASPARGALES Link Family 9- ORCHIDACEAE Juss.			
17. <i>Aphyllorchis gollanii</i> Duthie; H	E ¹ /PEX ¹ , Ex ² /E ² , NE ³ , 2A ⁵ , VU ⁶ , Ex ⁷ , Ex ¹¹	UK; China	2400-3000
18. <i>Bulbophyllum reptans</i> (Lindl.) Lindl. ex Wall. [= <i>Bulbophyllum rauai</i> Arora; <i>Tribrachia reptans</i> Lindl.]; H	I ² , NE ³ , 2A ⁵ , I ¹¹	UK, S, AP, NG, MN, MG, MZ, WB; Nep, Ban, Mya, Chi, Thai, Lao, Viet	500-1500
19. <i>Calanthe alismifolia</i> Lindl.; H	I ² , NE ³ , 2A ⁵ , I ⁷ , I ¹¹	UK, S, AP, MG, NG, WB; Bhu, Chi, Mya, Jap, Lao, Tai, Viet	1500-2000
20. <i>Calanthe alpina</i> Hook.f. ex Lindl.; H	R ¹ , R ² , NE ³ , 2A ⁵ , R ⁷ , R ¹¹	UK, S, AP, NG, WB; Nep, Bhu, Mya, Chi, Tai, Jap	2500-3500
21. <i>Calanthe davidii</i> Franch. [= <i>Calanthe pachystalix</i> Reichb.f. ex Hook.f.]; H	E ¹ , NE ³ , 2A ⁵	UK, AP; Nep, Chi, Tai, Viet, Jap	1500-2000
22. <i>Calanthe mannii</i> Hook.f.; H	R ¹ , NE ³ , 2A ⁵ , R ¹¹	UK, S, AP, MZ, MN, MG; Nep, Bhu, Mya, Chi, Viet, Jap	1300-2200
23. <i>Coelogyne cristata</i> Lindl.; H	R ² , NE ³ , 2A ⁵	HP, UK, S, AP, A, MN, MG, WB; Nep, Bhu, Ban, Chi	1000-2000
24. <i>Coelogyne flaccida</i> Lindl.; H	I ² , NE ³ , 2A ⁵	UK, S, AP, A, MN, MG, NG; Nep, Bhu, Ban, Mya, Lao, Thai	1000-2100
25. <i>Coelogyne nitida</i> (Wall. ex D. Don) Lindl. [= <i>Cymbidium nitidum</i> Wall. ex D. Don]; H	R ² , NE ³ , 2A ⁵	UK, S, AP, MN, MZ, MG, NG, WB; Nep, Bhu, Mya, Chi, Lao, Thai, Viet	1500-2300

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
26. <i>Crepidium acuminatum</i> (D.Don) Szlach. [= <i>Malaxis acuminata</i> D.Don]; H	NE ³ , 2A ⁵ , VU ⁶	HP, UK, S, AP, A, MP, MG, MZ, WB, KN, KR, TN; Nep, Bhu, Thai, Viet, Lao, Ban, Chi, Mya, Phi, Australia	600-3000
27. <i>Cymbidium eburneum</i> Lindl.; H	V ¹ , NE ³ , 2A ⁵	UK, S, AP, A, MN, MG, MN, WB; Nep, Mya, Chi, Viet	1000-1500
28. <i>Cymbidium goeringii</i> (Rchb.f.) Rchb.f. [= <i>Cymbidium mackinnonii</i> Duthie; <i>Maxillaria goeringii</i> Rchb.f.]; H	NE ³ , 2A ⁵ , VU ⁶	UK, S, AP; Bhu, Chi, Kor, Jap	1700-1800
29. <i>Cymbidium hookerianum</i> Rchb.f.; H	V ¹ , NE ³ , 2A ⁵	UK, S, AP, MG, MN, MZ; Bhu, Nep, Mya, Chi, Viet	1500-2500
30. <i>Cypripedium cordigerum</i> D.Don; H	R ¹ , VU ^{3A} , VU ³ , 2A ⁵ , EN ⁶ , HT ⁹ , R ¹¹	J&K, HP, UK, S; Pak, Nep, Bhu, Chi	2100-4000
31. <i>Cypripedium elegans</i> Rchb.f.; H	R ¹ , EN ^{3A} , EN ³ , 2A ⁵ , EN ⁶ , HT ⁹	UK, S; Nep, Bhu, Chi	2500-4000
32. <i>Cypripedium himalaicum</i> Rolfe; H (Image-3)	R ¹ , EN ³ , 2A ⁵ , EN ⁶ , HT ⁹ , R ¹¹	J&K, HP, UK, S; Nep, Bhu, Mya, Chi	2700-4300
33. <i>Dactylorhiza hatagirea</i> (D.Don) Soo [= <i>Orchis hatagirea</i> D.Don]; H	NE ³ , CR ⁴ , 2A ⁵ , EN ⁶ , HT ⁹	J&K, HP, UK, S; Pak, Nep, Bhu, Chi, Mon	2500-4400
34. <i>Dendrobium macrostachyum</i> Lindl. [= <i>Dendrobium gamblei</i> King & Pantl.]; H	I ² , LC ³ , 2A ⁵ , I ¹¹	Throughout India; Nep, Ban, Mya, Borneo, Jawa, Malaya, Sri Lanka, Sumatra, Thai, Viet	300-800
35. <i>*Dendrobium normale</i> Falc.; H	I ² , NE ³ , 2A ⁵ , I ⁷ , I ¹¹	UK	900-2700
36. <i>Dienia muscifera</i> Lindl. [= <i>Malaxis muscifera</i> (Lindl.) Kuntze, <i>Microstylis muscifera</i> (Lindl.) Ridl.]; H	VU ³ , 2A ⁵ , EN ⁴ , EN ⁶ , HT ⁹	J&K, HP, UK, S, AP, WB; Paki, Nep, Bhu, Mya, Chi	1800-4000
37. <i>Diplomeris hirsuta</i> (Lindl.) Lindl. [= <i>Diplochlilos hirsutus</i> Lindl.]; H	V ¹ , NE ³ , 2A ⁵ , EN ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰ , V ¹¹	UK, S, AP, MG, NG, WB; Nep, Chi	800-1200
38. <i>*Eria occidentalis</i> Seidenf. [= <i>Pinalia occidentalis</i> (Seidenf.) Schuit., Y.P. Ng & H.A. Pedersen]; H	R ¹ , R ² , NE ³ , 2A ⁵ , VU ⁶ , R ⁷ , R ¹¹	UK	1200-1500
39. <i>Eulophia mackinnonii</i> Duthie; H	R ¹ , R ² , NE ³ , 2A ⁵ , R ⁷ , R ¹¹	UK, UP, JR, CG, MP; Nep, Ban	300-800
40. <i>Eulophia obtusa</i> (Lindl.) Hook.f. [= <i>Cyrtopera obtusa</i> Lindl.]; H	I ² , NE ³ , 2A ⁵ , VU ⁶ , I ⁷ , I ¹¹	UK, UP; Nep	250-900
41. <i>Flickingeria hesperis</i> Seidenf. [= <i>Dendrobium hesperis</i> (Seidenf.) Schuit. & Peter B.Adams]; H	E ¹ , E ² , NE ³ , 2A ⁵ , VU ⁶ , E ⁷ , HT ⁹ , E ¹¹	UK, MN	1500-2000
42. <i>Galeola falconeri</i> Hook.f. [= <i>Cyrtosia falconeri</i> (Hook.f.) Aver.]; H	I ² , NE ³ , 2A ⁵ , I ⁷ , I ¹¹	UK, S, AP, MN, MG, MZ, WB; Nep, Bhu, Thai, Viet, Chi	1200-2000
43. <i>Galeola lindleyana</i> (Hook.f. & Thomson) Rchb.f. [= <i>Cyrtosia lindleyana</i> Hook.f. & Thomson]; H	I ² , NE ³ , 2A ⁵	HP, UK, AP, MN, MG, MZ, NG, WB; Nep, Chi, Viet, Sumatra	1200-2400
44. <i>*Gastrochilus garhwalensis</i> Z.H.Tsi; H	NE ³ , 2A ⁵ , VU ⁶	UK	1000
45. <i>Habenaria edgeworthii</i> Hook.f. ex Collett [= <i>Herminium edgeworthii</i> (Hook.f. ex Collett) X.H. Jin, Schuit., Raskoti & Lu Q. Huang]; H	NE ³ , 2A ⁵ , VU ⁶	J&K, HP, UK, S, AP, WB; Paki, Nep, Bhu, Chi	1500-3000
46. <i>Habenaria intermedia</i> D.Don; H	NE ³ , EN ⁴ , 2A ⁵ , VU ⁶	J&K, HP, UK, CG; Nep Paki, Chi	1500-3000
47. <i>*Herminium kumaunense</i> Deva & H.B.Naithani; H	NE ³ , 2A ⁵ , VU ⁶	UK	3300-3600
48. <i>Neottia acuminata</i> Schltr. [= <i>Aphyllorchis parviflora</i> King & Pantl.]; H	R ¹ , LC ³ , 2A ⁵ , R ¹¹	UK, S, AP; Nep, Chi, Rus, Mon, Kor, Jap, Tai	3300-3600
49. <i>*Neottia mackinnonii</i> Deva & H.B.Naithani; H	NE ³ , 2A ⁵ , DD ⁵ , VU ⁶	UK	1500-1800
50. <i>*Neottia microglottis</i> (Duthie) Schltr. [= <i>Archineottia microglottis</i> (Duthie) S.C.Chen; <i>Listera microglottis</i> Duthie]; H	R ¹ , R ² , NE ³ , 2A ⁵ , VU ⁶ , R ⁷ , R ¹¹	UK	1500-4000
51. <i>*Neottia nandadeviensis</i> (Hajra) Szlach. [= <i>Listera nandadeviensis</i> Hajra]; H	NE ³ , 2A ⁵ , VU ⁶	UK	2400-3500
52. <i>*Nervilia gleadowii</i> A.N.Rao; H	NE ³ , 2A ⁵ , VU ⁶	UK	1000
53. <i>Nervilia mackinnonii</i> (Duthie) Schltr. [= <i>Pogonia mackinnonii</i> Duthie]; H	I ² , NE ³ , 2A ⁵ , VU ⁶ , I ⁷ , I ¹¹	UK; Nep, Mya, Chi	1500-1800
54. <i>*Nervilia pangteyana</i> Jalal, Kumar & G.S.Rawat; H	NE ³ , 2A ⁵ , VU ⁶	UK	800-1000
55. <i>Nervilia plicata</i> (Andrews) Schltr. [= <i>Nervilia biflora</i> (Wight) Schltr.; <i>Arethusa plicata</i> Andrews]; H	I ² , NE ³ , 2A ⁵ , I ¹¹	Throughout India; Pak, Nep, Ban, Bor, Jawa, Lao, Mya, Phil, Tai, Viet, Aus	300-1500
56. <i>Oreorchis foliosa</i> (Lindl.) Lindl. var. <i>indica</i> (Lindl.) N. Pearce & P.J. Cribb [= <i>Corallorhiza indica</i> Lindl., <i>Oreorchis indica</i> (Lindl.) Hook.f.]; H	I ² , NE ³ , 2A ⁵ , I ⁷ , I ¹¹	HP, UK, S; Nep, Bhu, Chi, Jap	2000-2700
57. <i>Oreorchis micrantha</i> Lindl.; H (Image-4)	I ² , NE ³ , 2A ⁵	J&K, HP, UK, S, AP, WB; Nep, Bhu, Mya, Chi	2400-3300
58. <i>Pecteilis gigantea</i> (Sm.) Raf. [= <i>Orchis gigantea</i> Sm.]; H (Image-5)	NE ³ , 2A ⁵ , VoE ⁸ , HT ⁹ , HT ¹⁰	Throughout India; Pak, Nep, Mya, Chi	300-2000
59. <i>Peristylus elisabethae</i> (Duthie) R.K.Gupta [= <i>Peristylus kumaonensis</i> Renz]; H	NE ³ , 2A ⁵ , VU ⁶	HP, UK, S, WB; Nep, Bhu, Mya, Chi	2000-2200

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
60. <i>Phaius tankervilleae</i> (Banks) Blume [= <i>Limodorum tankervilleae</i> Banks]; H	NE ³ , 2A ⁵ , VU ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	UK, S, AP, MN, MG, MZ, WB, NG, TR, KL, OD; Nep, Bhu, Ban, Mya, Chi, Jap, Viet, Sri etc	300-500
61. <i>*Ponerorchis renzii</i> Deva & H.B. Naithani; H	NE ³ , 2A ⁵ , VU ⁶	UK	3200-3400
62. <i>Satyrium nepalense</i> D. Don; H	NE ³ , 2A ⁵ , VU ⁶	Himal, NE India, South India; Pak, Chi, Mya, Sri Lanka	1500-4000
63. <i>Tipularia cunninghamii</i> (King & Prain) S.C. Chen, S.W. Gale & P.J. Cribb [= <i>Didickea cunninghamii</i> King & Prain]; H	E ¹ , E ² , NE ³ , 2A ⁵ , E ⁷ , E ¹¹	UK, S; Tai	2000-3100
Family 10- IRIDACEAE Juss.			
64. <i>Iris milesii</i> Baker ex Foster; H	I ² , NE ³	W Himal; Chi	1600-2700
Family 11- AMARYLLIDACEAE J. St.-Hil.			
65. <i>Allium auriculatum</i> Kunth; H	E ² , NE ³	J&K, HP, UK; Nep	3300-5500
66. <i>Allium loratum</i> Baker; H	E ² , NE ³	J&K, HP, UK; Afg, Chi	2600-3700
67. <i>Allium roylei</i> Stearn; H	E ² , NT ³	J&K, UK; Afg, Pak	1900-3200
68. <i>Allium stracheyi</i> Baker; H	V ¹ , V ² , NE ³ , VU ⁴ , VU ⁶ , V ⁷ , V ¹¹	J&K, HP, UK; Pak, Nep	2000-3800
Family 12- ASPARAGACEAE Bercht. & J. Presl			
69. <i>Asparagus filicinus</i> Buch.-Ham. ex D. Don; H (Image-6)	DD ³	Himal; Ban, Mya, Chi, Tai, Viet	2100-3000
70. <i>*Dipcadi reidii</i> Deb & S. Dasgupta; H	PEX ¹ , EX ² , NE ³ , CR ⁶ , VU ⁶ , EX ⁷ , EX ¹¹	UK; Nep(?)	1500-2500
71. <i>Polygonatum cirrhifolium</i> (Wall.) Royle [= <i>Convallaria cirrhifolia</i> Wall.]; H	NE ³ , VU ⁴ , VU ⁶	J&K, HP, UK, S; Pak, Nep, Bhu, Chi	1200-4500
72. <i>Polygonatum graminifolium</i> Hook.; H (Image-7)	I ² , NE ³ , I ⁷ , I ¹¹	J&K, HP, UK; Nep, Bhu	2600-4650
73. <i>Polygonatum verticillatum</i> (L.) All. [= <i>Convallaria verticillata</i> L.]; H	VU ⁴ , VU ⁶	Himal	1500-4500
Order 7- ARECALES Bromhead Family 13- ARECACEAE Bercht. & J. Presl (PALMAE Juss.)			
74. <i>Phoenix rupicola</i> T. Anderson; T	R ¹ , V ² , NT ³	UK, NE India	Up to 800
75. <i>*Trachycarpus takil</i> Becc.; T (Image-8)	R ¹ , NE ³ , CR ⁶ , VU ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰ , R ¹¹	UK	1800-2550
Order 8- ZINGIBERALES Griseb Family 14- ZINGIBERACEAE Martinov			
76. <i>Cautleya spicata</i> (Sm.) Baker [= <i>Cautleya petiolata</i> Baker]; H	I ² , LC ³ , I ⁷ , I ¹¹	Himal; Chi, Mya	1800-2800
77. <i>Hedychium spicatum</i> Sm.; H	NE ³ , VU ⁶	Himal; Chi, Mya, Thai	1500-2800
Order 9- POALES Small Family 15- ERIOCALACEAE Martinov			
78. <i>Eriocaulon nepalense</i> J.D. Prescott ex Bong. var. <i>luzulifolium</i> (Mart.) Praj. & J. Parn. [= <i>Eriocaulon pumilio</i> Hook.f.]; H	I ² , NE ³ , DD ⁶ , I ⁷ , I ¹¹	W Himal, NE India; Nep, Chi, Thai, New Guinea	900-2000
Family 16- CYPERACEAE Juss.			
79. <i>Carex clavispica</i> S.R. Zhang [= <i>Kobresia duthiei</i> C.B. Clarke]; H	I ² , NE ³	Himal	3600-4500
80. <i>Carex esenbeckii</i> Kunth [= <i>Kobresia esenbeckii</i> (Kunth) Noltie; <i>Kobresia trinervis</i> var. <i>foliosa</i> (C.B. Clarke) Kuekenh.; H	I ² , NE ³	Himal; Bhu, Chi, Tib	3300-5000
81. <i>*Carex nandadeviensis</i> Ghildyal, U.C. Bhattach. & Hajra; H	NE ³ , VU ⁶	UK	3000-4000
Family 17- POACEAE Barnhart (GRAMINEAE Juss.)			
82. <i>Cymbopogon microstachys</i> (Hook.f.) Soenarko [= <i>Cymbopogon flexuosus</i> var. <i>microstachys</i> (Hook.f.) Bor]; H	R ² , NE ³ , R ⁷ , R ¹¹	N, E & NE India, Indian plains; Chi, Indochina	300-1000
83. <i>*Cymbopogon osmastonii</i> R. Parker; H	V ² , NE ³ , VU ⁶	UK, N India	300-500
84. <i>*Dendrocalamus somdevae</i> H.B. Naithani; Sh	NE ³ , END ¹¹	UK	600-1500
85. <i>Digitaria duthieana</i> Henrard ex Bor; H	DD ³	UK, UP, MP	300
86. <i>Elymus duthiei</i> (Melderis) G. Singh [= <i>Agropyron duthiei</i> Melderis]; H	I ² , NE ³ , I ⁷ , I ¹¹	W&E Himal	1000-2000
87. <i>*Eulalia madkotiensis</i> Kandwal, B.K. Gupta & S.K. Srivast.; H	NE ³ , VU ⁶	UK	1200-1500
88. <i>*Festuca lucida</i> Stapf; H	I ² , NE ³ , I ⁷ , I ¹¹	UK	2300-3000
89. <i>*Festuca nandadevica</i> Hajra; H	NE ³ , VU ⁶	UK	3300-3600
90. <i>*Helictotrichon uniyalii</i> Kandwal & B.K. Gupta; H	NE ³ , VU ⁶	UK	2500-3000

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
91. * <i>Microstegium falconeri</i> (Hook.f.) Clayton [= <i>Ischnochloa falconeri</i> Hook.f.]; H	I ² , NE ³ , VU ⁶ , I ⁷ , I ¹¹	NW & E Himal	1800-3000
92. <i>Piptatherum hilariiae</i> Pazij [= <i>Oryzopsis humilis</i> Bor; <i>Oryzopsis hilariiae</i> (Pazij) Uniya]; H	I ² , NE ³ , I ⁷ , I ¹¹	W Himal; Taj, Afg, Paki, Tib, Chi	2000-2500
93. * <i>Poa garhwalensis</i> D.C. Nautiyal & R.D. Gaur; H	NE ³ , VU ⁶	UK	3900-4200
94. <i>Poa pseudamoena</i> Bor; H	I ² , NE ³ , I ⁷ , I ¹¹	W Himal; Tib, China	3000-3800
95. * <i>Poa rhadina</i> Bor; H	E ² , NE ³ , VU ⁶ , E ⁷ , E ¹¹	UK	2600-4100
96. * <i>Poa royleana</i> Nees ex Steud.; H	NE ³ , END ¹¹	UK	2000-3300
97. * <i>Pseudodanthonia himalaica</i> (Hook.f.) Bor & C.E. Hubb. [= <i>Danthonia himalaica</i> Hook.f.]; H	I ² , NE ³ , VU ⁶ , VU ⁶	W Himal	2000-2300
98. <i>Puccinellia thomsonii</i> (Stapf ex Hook.f.) R.R. Stewart [= <i>Glyceria thomsonii</i> Stapf ex Hook.f.]; H	I ² , NE ³	W Himal; Tib	4000-4500
99. * <i>Sehima notatum</i> (Hack.) A. Camus [= <i>Ischaemum notatum</i> Hack.]; H	NE ³ , VU ⁶	UK	1200-2100
100. <i>Trisetum micans</i> (Hook.f.) Bor [= <i>Avena micans</i> Hook.f.]; H	I ² , NE ³ , I ⁷ , I ¹¹	W Himal, Indian plains	2400-3800
Order 10- RANUNCULALES Juss. ex Bercht. & J. Presl Family 18- PAPAVERACEAE Juss.			
101. <i>Corydalis cashmeriana</i> Royle; H	E ² , NE ³	J&K, HP, UK, S; Pak, Nep, Chi	2800-4700
102. * <i>Corydalis devendrae</i> Pusalkar; H	NE ³ , VU ⁶	UK	3800-5000
103. <i>Papaver guilemi-waldemarii</i> (Klotzsch) Christenh. & Byng [= <i>Meconopsis guilemi-waldemarii</i> Klotzsch; <i>Meconopsis aculeata</i> Royle]; H	E ² , NE ³ , EN ⁴	J&K, HP, UK, S; Pak, Nep, Bhu	3500-5200
104. * <i>Papaver robustum</i> (Hook.f. & Thomson) Christenh. & Byng [= <i>Meconopsis robusta</i> Hook.f. & Thomson]; H (Image-9)	NE ³ , VU ⁶	UK; Nep	2500-4300
Family 19- BERBERIDACEAE Juss.			
105. * <i>Berberis affinis</i> G. Don; Sh	R ¹ , R ² , NE ³ , VU ⁶ , VU ⁶ , R ⁷ , R ¹¹	UK	2200-3000
106. * <i>Berberis ahrendtii</i> R.R. Rao & Uniya; Sh	NE ³ , EN ⁶ , VU ⁶	UK	2000-3000
107. * <i>Berberis garhwalensis</i> C.K. Schneid.; Sh	NE ³ , DD ⁶ , VU ⁶	UK	3000-4000
108. * <i>Berberis jaunsarensis</i> (Ahrendt) Laferr. [= <i>Mahonia jaunsarensis</i> Ahrendt]; Sh	I ² , NE ³ , VU ⁶ , I ⁷ , I ¹¹	UK	1500-2600
109. * <i>Berberis lambertii</i> R. Parker; Sh	V ¹ /E ¹ , V ² , NE ³ , CR ⁶ , VU ⁶ , V ¹¹ /E ¹¹	UK	2650-2900
110. * <i>Berberis osmastonii</i> Dunn; Sh (Image-10)	R ¹ , R ² , NE ³ , VU ⁶ , R ⁷ , R ¹¹	UK	1700-3000
111. <i>Berberis pseudumbellata</i> R. Parker; Sh	I ² , NE ³ , I ⁷ , I ¹¹	J&K, HP, UK; Pak	2200-3800
112. * <i>Berberis rawatii</i> U.L. Tiwari & B.S. Adhikari; Sh	NE ³ , VU ⁶	UK	2200-2400
113. <i>Podophyllum hexandrum</i> Royle [= <i>Sinopodophyllum hexandrum</i> (Royle) T.S. Ying]; H	NE ³ , EN ⁴ , 2A ³ , EN ⁶	J&K, HP, UK, S, AP; Pak, Nep, Bhu, Chi	2000-4000
Family 20- RANUNCULACEAE Juss.			
114. <i>Aconitum heterophyllum</i> Wall. ex Royle; H	EN ^{3A} , EN ³ , CR ⁴ , EN ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	J&K, HP, UK; Pak, Nep	2700-4800
115. <i>Aconitum laeve</i> Royle; H	NE ³ , EN ⁶	J&K, HP, UK; Pak, Nep	2000-3500
116. <i>Aconitum lethale</i> Griff. [= <i>A. falconeri</i> Stapf var. <i>latilobum</i> Stapf; <i>A. balfourii</i> var. <i>rhombilobatum</i> Stapf; <i>A. falconeri</i> Stapf var. <i>falconeri</i>]; H (Image-11)	V ¹ , I ² , NE ³ , VU ⁴ , EN ⁶ , I ⁷ , VoE ⁸ , HT ¹⁰ , I ¹¹	UK; Nep	2800-4000
117. <i>Aconitum violaceum</i> Jacquem. ex Stapf; H (Image-12)	VU ^{3A} , VU ³ , VU ⁴ , VU ⁶ , VoE ⁸ , HT ¹⁰	J&K, HP, UK; Pak, Nep	3200-4800
118. * <i>Anemone raui</i> Goel & U.C. Bhattach.; H	NE ³ , VU ⁶	HP, UK	2500-3500
119. <i>Aquilegia nivalis</i> (Baker) Falc. ex B.D. Jacks [= <i>Aquilegia glauca</i> Lindl. var. <i>nivalis</i> ; <i>Aquilegia nivalis</i> (Baker) Bruehl]; H	E ² , NE ³	J&K, HP, UK; Pak	3200-4500
120. <i>Delphinium koelzii</i> Munz; H	I ² , NE ³	HP, UK	1600-2500
121. * <i>Oxygraphis kumaonensis</i> I.D. Rai & G.S. Rawat; H	NE ³ , VU ⁶	UK	4000-4100
122. * <i>Ranunculus uttaranchalensis</i> Pusalkar & D.K. Singh; H	NE ³ , VU ⁶	UK	4000-4350
123. <i>Trollius acaulis</i> Lindl.; H (Image-13)	E ² , NE ³	J&K, HP, UK; Ira, Pak, Nep, Chi	3200-5000
Order 11- SAXIFRAGALES Bercht. & J. Presl Family 21- SAXIFRAGACEAE Juss.			
124. <i>Bergenia ciliata</i> (Haw.) Sternb. [= <i>Megasea ciliata</i> Haw.]; H	NE ³ , VU ⁶	J&K, HP, UK; Pak, Nep, Mya	1000-4300

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
125. <i>Saxifraga jacquemontiana</i> Decne.; H	E ² , NE ³	J&K, HP, UK, S; Nep, Bhu, Chi	3900-5800
126. <i>Saxifraga meeboldii</i> Engl. & Irmsch.; H	NE ³ , DD ⁶	J&K, HP, UK; Tib	4000-4200
127. * <i>Saxifraga minutissima</i> D.S. Rawat; H	NE ³ , VU ⁶	UK	4200-4800
Family 22- CRASSULACEAE J.St.-Hil.			
128. * <i>Sedum bhattacharyae</i> R. Manik., N.B. Singh & S.K. Srivast. [= <i>Sedum pedicellatum</i> N.B.Singh & U.C.Bhattach.]; H	NE ³ , VU ⁶	UK	1500-3500
129. * <i>Sedum duthiei</i> Frod.; H	I ² , NE ³ , DD ⁶ , VU ⁶ , I ⁷ , I ¹¹	UK	4500-4700
130. <i>Sedum heterodontum</i> Hook.f. & Thomson [= <i>Rhodiola heterodonta</i> (Hook.f. & Thomson) Boriss.]; H	NE ³ , VU ⁴	J&K, HP, UK; Ira, Afg, Pak, USSR, Nep, Tib, Mon	2500-5100
131. * <i>Sedum seelemannii</i> Raym.-Hamet; H	NE ³ , DD ⁶ , VU ⁶	UK	4500-4700
Order 12- FABALES Bromhead Family 23- FABACEAE Lindl. (LEGUMINOSAE Juss.)			
132. <i>Abrus fruticulosus</i> Wall. ex Wight & Arn.; Cl	DD ^{3A} , DD ³	UK, Indian plains, NE India; Chi, Tropical Africa	?
133. <i>Astragalus langtangensis</i> Podlech; Sh	DD ^{3A} , DD ³	UK, Nep	3500-4000
134. * <i>Astragalus nainitalensis</i> L.B. Chaudhary; Sh	NE ³ , VU ⁶	UK	1700-1900
135. <i>Astragalus stewartii</i> Baker [= <i>Astragalus bakeri</i> Ali]; Sh	I ² , NE ³	J&K, UK; Paki	1500-3200
136. * <i>Astragalus uttaranchalensis</i> L.B. Chaudhary & J.H. Khan; Sh	NE ³ , VU ⁶	UK	3200-3500
137. <i>Butea peltita</i> Hook.f. ex Prain [= <i>Meizotropis peltita</i> (Hook.f. ex Prain) Sanjappa]; Sh (Image-14)	NE ³ , CR ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	UK; Nep	1400-1500
138. <i>Dalbergia lanceolaria</i> L.f.; T	NE ³ , 2A ⁵	UK, Tropical Himal, India; Sri Lanka, Mya	300-1000
139. <i>Dalbergia latifolia</i> Roxb.; T	VU ³ , 2A ⁵	UK, India; Nep, Mal	300-500
140. <i>Dalbergia sericea</i> G. Don; T	NE ³ , 2A ⁵	UK; Nep, Ban, Chi	300-1500
141. <i>Dalbergia sissoo</i> Roxb. ex DC.; T	NE ³ , 2A ⁵	J&K, HP, UK; Ira, Afg, Pak, Nep, Ban, Mya	300-1200
142. <i>Dalbergia volubilis</i> Roxb.; Cl	NE ³ , 2A ⁵	UK, E Himal, NE India, Indian plains; Nep, Ban, Mya, China, Thai, Viet, Lao	300-600
143. * <i>Derris kanjilalii</i> K.C. Sahni & H.B. Naithani; Cl	NE ³ , VU ⁶	UK; Nep	300-400
144. * <i>Desmodium garhwalensis</i> L.R. Dangwal & R.D. Gaur; Sh	NE ³ , VU ⁶	UK	700-1800
145. <i>Hedysarum astragaloides</i> Benth. ex Baker; H	R ¹ , R ² , NE ³	J&K, HP, UK; Afg, Paki	3500-4500
146. <i>Hedysarum cachemirianum</i> Benth. ex Baker; H	R ¹ /V ¹ , NE ³	J&K, UK; Paki	3700-4000
147. <i>Hedysarum microcalyx</i> Baker; H	V ¹ , NE ³	J&K, HP, UK; Paki	2700-4400
148. <i>Indigofera cedrorum</i> Dunn; Sh	I ² , NE ³ , VU ⁶	HP, UK	1200-2500
149. <i>Indigofera dosua</i> Buch.-Ham. ex D.Don var. <i>simlensis</i> (Ali) Sanjappa [= <i>Indigofera simlensis</i> Ali]; Sh	I ² , NE ³ , VU ⁶	HP, UK	600-3000
150. <i>Indigofera thothathrii</i> Sanjappa; Sh	NE ³ , VU ⁶	UK, UP, A	Up to 500
151. <i>Indopiptadenia oudhensis</i> (Brandis) Brenan [= <i>Piptadenia oudhensis</i> Brandis]; T	NE ³ , EN ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	UK, UP; Nep	300-600
152. <i>Macrotyloma sar-garhwalensis</i> R.D. Gaur & L.R. Dangwal; H	NE ³ , VU ⁶	UK	600- 1500
153. * <i>Pueraria garhwalensis</i> L.R. Dangwal & D.S. Rawat; Cl	NE ³ , VU ⁶	UK	300-600
154. * <i>Senna davidsonii</i> (V. Singh) V. Singh [= <i>Cassia davidsonii</i> V. Singh]; Sh	NE ³ , VU ⁶ , DD ⁶	UP (UK ?)	?
155. <i>Thermopsis inflata</i> Cambess.; H	I ² , NE ³	J&K, HP, UK, S; Paki, Nep, Chi	4900-5500
156. <i>Uraria picta</i> (Jacq.) Desv. ex DC. [= <i>Hedysarum pictum</i> Jacq.]; H (Image-15)	LC ³ , HT ⁹	Himal, India; Pak, Ban, Mya, Chi, Jawa, Male, Phil, Sri Lanka, Thai, Tr Africa	Up to 1500
157. <i>Vigna aconitifolia</i> (Jacq.) Marechal [= <i>Phaseolus aconitifolius</i> Jacq.]; H	DD ³	UK, Throughout India; Pak, Ban, Mya, Chi, Sri	Up to 2000
Order 13- ROSALES Bercht. & J. Presl Family 24- ROSACEAE Juss.			
158. * <i>Alchemilla pali</i> Panigrahi & Purohit; Sh	NE ³ , DD ¹¹	UK	?
159. * <i>Cotoneaster parkinsonii</i> Panigrahi & Arv. Kumar; Sh	NE ³ , VU ⁶	UK, E Himal, NE India; Nep, Mya	2400-2500

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
160. <i>Cotoneaster roseus</i> Edgew. [= <i>Cotoneaster osmastonii</i> G.Klotz]; Sh	NE ³ , VU ⁶	W Himal; Afg, Pak	2400-3300
161. <i>Cotoneaster simonsii</i> Hort. ex Baker; Sh	I ¹ , I ² , NE ³	UK, S; Bhutan	1500-3200
162. * <i>Geum aequilobatum</i> K.M. Purohit & Panigrahi; H	NE ³ , END ¹¹	UK	1000-1500
163. <i>Prunus jacquemontii</i> Hook.f. Sh	DD ³	J&K, UK; Afg, Pak	2800-3500
164. * <i>Rosa hirsuta</i> Ghora & Panigrahi; Sh	NE ³ , VU ⁶	UK	3600-3800
165. * <i>Rubus almorensis</i> Dunn; Sh	I ² , NE ³ , DD ⁶ , VU ⁶ , I ⁷ , I ¹¹	UK	2400-2700
166. * <i>Sibbaldia axilliflora</i> (Hook.f.) Chatterjee [= <i>Potentilla axilliflora</i> Hook.f.]; Sh	NE ³ , DD ⁶ , VU ⁶	UK	?
167. * <i>Spiraea diversifolia</i> Dunn; Sh	I ² , NE ³ , I ⁷ , I ¹¹	UK; Nep(?) (CoL)	2700-4400
168. * <i>Spiraea duthieana</i> Zinserl.; Sh	NE ³ , VU ⁶	UK	2400-3100
169. * <i>Spiraea hypoleuca</i> Dunn.; Sh	NE ³ , VU ⁶	UK; Nep? (CoL)	2100-3300
170. * <i>Spiraea panchanani</i> Panigrahi & K.M. Purohit; Sh	NE ³ , VU ⁶	UK	2400
171. * <i>Spiraea panigrahiana</i> K.M. Purohit.; Sh	NE ³ , VU ⁶	UK	1900
172. * <i>Spiraea parkeri</i> Panigrahi & K.M. Purohit; Sh	NE ³ , DD ⁶ , VU ⁶	UK	1900-2000
173. * <i>Spiraea raizadae</i> Panigrahi & K.M. Purohit; Sh	NE ³ , VU ⁶	UK	3200-3300
Family 25- RHAMNACEAE Juss.			
174. * <i>Sageretia devendrae</i> Pusalkar; Sh	NE ³ , VU ⁶	UK	2000-2500
Family 26- ULMACEAE Mirb.			
175. <i>Ulmus wallichiana</i> Planch.; T	E ² , VU ³ , NE ³	J&K, HP, UK; Afg, Pak, N	1500-3000
Order 14- FAGALES Engl. Family 27- BETULACEAE Gray			
176. <i>Carpinus faginea</i> Lindl.; T	DD ^{3A} , DD ³	UK; Nep	1200-2200
177. <i>Corylus jacquemontii</i> Decne.; T	DD ^{3A} , DD ³	J&K, HP, UK; Nep	2000-2700
Order 15- CUCURBITALES Juss. ex Bercht. & J. Presl Family 28- DATISCAEAE Dumort.			
178. <i>Datisca cannabina</i> L.; H	NE ³ , EN ⁴	UK; Mediterranean, Afg, Pak, Nep, Viet	700-1550
Order 16- MALPIGHIALES Juss. ex Bercht. & J. Presl Family 29- HYPERICACEAE Juss.			
179. <i>Hypericum perforatum</i> L.; H	LC ³ , VU ⁴	J&K, HP, UK; Europe, E Asia, N Africa, Chi	1000-3000
Family 30- VIOLACEAE Batsch			
180. <i>Viola kunawarensis</i> Royle; H	I ² , NE ³ , I ⁷ , I ¹¹	J&K, HP, UK, S; Afg, Pak, Nep, Tib	2800-5200
181. <i>Viola repens</i> Wall. ex Ging.; H	NE ³ , VU ⁶	Himal; Pak, Mya, Chi, Thai, Sri Lanka	500-3200
Family 31- EUPHORBIACEAE Juss.			
182. <i>Euphorbia royleana</i> Boiss.; T	NE ³ , 2A ⁵	J&K, HP, UK, NE India, E Himal; Pak, Nep, Mya, Chi, Tai	600-1800
Order 17- MYRTALES Juss. ex Bercht. & J. Presl Family 32- ONAGRACEAE Juss.			
183. <i>Epilobium glaciale</i> P.H. Raven; H	I ² , NE ³	J&K, HP, UK; Pak	3600-4400
Order 18- CROSSOSOMATALES Takht. ex Reveal Family 33- STAPHYLEACEAE Martinov			
184. <i>Staphylea cochinchinensis</i> (Lour.) Byng & Christenh. [= <i>Ticeros cochinchinensis</i> Lour.; <i>Turpinia cochinchinensis</i> (Lour.) Merr.; <i>Turpinia nepalensis</i> Wall.]; T	NE ³ , VU ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	UK, E Himal; Nep, Ban, Mya, Thai, Viet, Lao	1000-2200
Order 19- SAPINDALES Juss. ex Bercht. & J. Presl Family 34- SAPINDACEAE Juss.			
185. <i>Acer caesium</i> Wall. ex Brandis; T	V ¹ , V ² , NE ³ , VU ⁶ , V ⁷ , V ¹¹	J&K, HP, UK; Pak, Nep, Chi	2000-3350
Family 35- RUTACEAE Juss.			
186. <i>Zanthoxylum armatum</i> DC.; T	LC ³ , VU ⁴	J&K, HP, UK, MN, MG, NG, OD, AD; Pak, Nep, Mya, Chi, Jap, Kor, Phil, Tai, Viet	1000-2200

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Order 20- BRASSICALES Bromhead Family 36- BRASSICACEAE Burnett			
187. * <i>Eutrema purii</i> (D.S. Rawat, L.R. Dangwal & R.D. Gaur) Al-Shehbaz, G.Q. Hao & J. Quan Liu [= <i>Dilophia purii</i> D.S. Rawat, L.R. Dangwal & R.D. Gaur]; H (Image-16)	NE ³ , VU ⁶	UK	4500-5000
Order 21- CARYOPHYLLALES Juss. ex Bercht. & J. Presl Family 37- POLYGONACEAE Juss.			
188. * <i>Koenigia binsarii</i> (Silas & R.D. Gaur) R.D. Gaur [= <i>Polygonum binsarii</i> Silas & R.D. Gaur]; H	NE ³ , VU ⁶	UK	2400-2700
189. <i>Rheum australe</i> D. Don [= <i>Rheum emodii</i> Wall. ex Meisn.]; H	NE ³ , EN ⁴ , EN ⁶	UK, E Himal; Mya	3000-4450
190. <i>Rheum moorcroftianum</i> Royle; H	NE ³ , VU ⁶	HP, UK; Nep	3500-4800
191. <i>Rheum webbianum</i> Royle; H	NE ³ , VU ⁴ , VU ⁶	J&K, HP, UK; Pak, Nep, Tib	2400-5000
192. * <i>Rumex gangotrianus</i> Aswal & S.K. Srivast.; H	NE ³ , VU ⁶	UK	2600-3150
Family 38- CARYOPHYLLACEAE Juss.			
193. <i>Arenaria neelgherrensis</i> Wight & Arn.; H	I ² , NE ³	J&K, HP, UK, S; Paki, Nep, Maha, TN	900-3700
194. <i>Cerastium thomsonii</i> Hook.f.; H	I ² , NE ³ , I ⁷ , I ¹¹	J&K, HP, UK	2500-3650
195. * <i>Eremogone curvifolia</i> (Majumdar) Pusalkar & D.K. Singh [= <i>Arenaria curvifolia</i> Majumdar]; H (Image-17)	E ¹ , E ² , NE ³ , CR ⁶ , VU ⁶ , HT ⁹ , E ¹¹	UK	3300-3650
196. * <i>Eremogone ferruginea</i> (Duthie ex F.N. Williams) Pusalkar & D. K. Singh [= <i>A. ferruginea</i> Duthie ex F.N. Williams]; H	E ¹ , E ² , NE ³ , DD ⁶ , VU ⁶ , I ⁷ , I ¹¹	UK	2400-3050
197. <i>Odontostemma thangoense</i> (W.W. Sm.) Rabaler & W.L. Wagner [= <i>Arenaria thangoensis</i> W.W. Sm.]; H	V ¹ , V ² , NE ³	UK, S; Tib	3300-3600
198. <i>Silene kumaonensis</i> F.N. Williams; H	R ¹ , R ² , NE ³ , R ¹¹	UK; Nep	2500-3000
199. <i>Silene stracheyi</i> Edgew.	NE ³ , DD ⁶	UK, S; Nep, Bhu	2250-3030
200. <i>Stellaria depressa</i> Em. Schmid; H	I ² , NE ³	J&K, UK; Tib	4800-5000
Order 22- ERICALES Bercht. & J. Presl Family 39- BALSAMINACEAE A. Rich.			
201. * <i>Impatiens devendrae</i> Pusalkar; H	NE ³ , VU ⁶	UK	1200-3200
202. * <i>Impatiens duthiei</i> Hook.f.; H	NE ³ , VU ⁶	UK	2700m
203. * <i>Impatiens inayatii</i> Hook.f.; H	NE ³ , DD ⁶ , VU ⁶	UK	2400-2700
204. * <i>Impatiens jaeschkei</i> Hook.f.; H	NE ³ , END ¹¹	UK	2700-3000
205. * <i>Impatiens kaliensis</i> Grey-Wilson; H	NE ³ , VU ⁶	UK	2200-3250
206. * <i>Impatiens langeana</i> Hook.f.; H	NE ³ , DD ⁶ , VU ⁶	UK	?
207. * <i>Impatiens podocarpa</i> Hook.f.; H	NE ³ , END ¹¹	UK	2100-2400
208. * <i>Impatiens polysciadia</i> Hook.f.; H	NE ³ , DD ⁶ , VU ⁶	UK	?
209. * <i>Impatiens reidii</i> Hook.f.; H	NE ³ , DD ⁶ , VU ⁶	UK	1800-2600
210. * <i>Impatiens violoides</i> Edgew. ex Hook.f.; H	NE ³ , DD ⁶ , VU ⁶	UK	2400-2700
Family 40- PRIMULACEAE Batsch ex Borkh.			
211. <i>Embelia tsjeriam-cottam</i> (Roem. & Schult.) A. DC. [= <i>Ardisia tsjeriam-cottam</i> Roem. & Schult.]; T	NE ³ , VU ⁴	J&K, UK, WB; Pak, Nep, Ban, Mya, Thai, Viet, Sri Lanka	450-1800
212. * <i>Primula garhwalia</i> (Balodi & S. Singh) K.K. Khanna & An. Kumar [= <i>Androsace garhwalicum</i> Balodi & S. Singh]; H	NE ³ , VU ⁶	UK	4100-4400
213. <i>Primula drummondiana</i> Craib; H	I ² , NE ³	HP, UK; Nep	2400
214. <i>Primula minutissima</i> Jacquem. ex Duby; H	E ² , NE ³	J&K, HP, UK; Nep	3500-5450
Family 41- SYMPLOCACEAE Desf.			
215. <i>Symplocos paniculata</i> Miq.; T	NE ³ , VU ⁴	Himal; Pak, Ban, Mya, Chi, Jap, Lao, Viet	1000-2900
Family 42- ERICACEAE Juss.			
216. * <i>Rhododendron rawatii</i> I.D. Rai & B.S. Adhikari; T	NE ³ , VU ⁶	UK	3100-3350
Order 23- GENTIANALES Juss. ex Bercht. & J. Presl Family 43- RUBIACEAE Juss.			
217. <i>Clarkella nana</i> (Edgew.) Hook.f. [= <i>Ophiorrhiza nana</i> Edgew.]; H	R ¹ , R ² , NE ³ , R ⁷ , R ¹¹	UK; Mya, Chi, Thai	1200-2400
218. * <i>Leptodermis riparia</i> R. Parker; Sh	NE ³ , VU ⁶	UK	700-1600

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
219. <i>Rubia edgeworthii</i> Hook.f.; Cl	V ¹ , V ² , NE ³ , V ⁷ , V ¹¹	UK, Chi	900-1200
Family 44- GENTIANACEAE Juss.			
220. <i>Gentiana cachemirica</i> Decne.; H	E ² , NE ³	J&K, UK; Paki	2600-3900
221. <i>Gentiana crassuloides</i> Bureau & Franch.; H	R ² , NE ³ , R ⁷ , R ¹¹	UK, S, AP; Nep, Bhu, Chi	3400-5000
222. <i>Gentiana kurroo</i> Royle; H	CR ^{3A} , CR ³ , CR ⁴ , EN ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	HP, UK; Afg, Pak, Mya	1500-3000
223. * <i>Gentiana saginoides</i> Burkill; H	R ² , NE ³ , CR ⁶ , VU ⁶ , R ⁷ , R ¹¹	UK	3000-3600
224. * <i>Gentiana tetrasepala</i> Biswas; H	NE ³ , VU ⁶ , VU ⁶	UK	3800-4500
225. <i>Kuepferia infelix</i> (C.B.Clarke) Adr. Favre [= <i>Gentiana infelix</i> C.B. Clarke]; H (Image-18)	R ² , NE ³ , VU ⁶ , R ⁷ , R ¹¹	HP, UK, S; Nep, Bhu, Mya, Chi	4000-4900
226. * <i>Swertia alpina</i> U.C.Bhattach. & S.Agrawal; H	NE ³ , VU ⁶	UK	3200-4500
227. <i>Swertia chirayita</i> (Roxb.) H. Karst. [= <i>Gentiana chirayita</i> Roxb.]; H	NE ³ , EN ⁴ , EN ⁶	J&K, HP, UK, E Himal; Nep,	1200-3600
Family 45- APOCYNACEAE Juss.			
228. <i>Ceropegia angustifolia</i> Wight; Cl	V ¹ , V ² , NE ³	UK, UP, S, A, MG, WB; Ban	1000-2400
229. <i>Ceropegia bulbosa</i> Roxb.; Cl	V ² , NE ³ , EN ⁴ , VU ⁶	All over India	300-600
230. <i>Rauvolfia serpentina</i> (L.) Benth. ex Kurz; Sh	NE ³ , VU ⁴ , 2A ⁵	All over India	300-600
Order 24- BORAGINALES Juss. ex Bercht. & J. Presl			
Family 46- BORAGINACEAE Juss.			
231. <i>Arnebia benthamii</i> (Wall. ex G.Don) I.M. Johnst. [= <i>Echium benthamii</i> Wall. & G.Don]; H	E ² , NE ³ , VU ⁶ , CR ⁴ , HT ⁹	J&K, HP, UK; Paki, Nep	3000-5000
232. <i>Arnebia euchroma</i> (Royle ex Benth.) I.M. Johnst. [= <i>Lithospermum euchromum</i> Royle ex Benth.]; H	NE ³ , EN ⁴ , VU ⁶ , HT ⁹	J&K, HP, UK; Ira, Afg, Pak, Kaza, Nep, Tib, Chi	3500-4600
233. * <i>Cynoglossum jaunsarensis</i> (Kazmi) Pusalkar [= <i>Ivanjohnstonia jaunsariensis</i> Kazmi]; H	NE ³ , DD ⁶ , VU ⁶	UK	2200-2400
Order 25- SOLANALES Juss. ex Bercht. & J. Presl			
Family 47- CONVULVACEAE Juss.			
234. <i>Ipomoea laxiflora</i> H.J. Chowdhery & M.R. Debta; Cl	NE ³ , VU ⁶	UK, MH	Up to 800
Family 48- SOLANACEAE Juss.			
235. <i>Hyoscyamus niger</i> L.; H	NE ³ , VU ⁴	J&K, HP, UK, S, AP; Temperate Eurasia, NW Africa	2800-4200
Order 26- LAMIALES Bromhead			
Family 49- OLEACEAE Hoffmanns. & Link			
236. <i>Fraxinus micrantha</i> Lingelsh.; T	DD ³	W Himal; Pak, Nep	1500-2400
237. <i>Schrebera swietenoides</i> Roxb.; T	NE ³ , EN ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰		450-762
Family 50- GESNERIACEAE Rich. & Juss.			
238. <i>Didymocarpus aromaticus</i> Don; H	NE ³ , VU ⁶		1800-3000
239. <i>Didymocarpus pedicellatus</i> R.Br.; H	NE ³ , VU ⁴		500-1700
Family 51- PLANTAGINACEAE Juss.			
240. * <i>Kashmiria himalaica</i> (Hook.f.) D.Y. Hong [= <i>Falconeria himalaica</i> Hook.f.; <i>Wulfenia himalaica</i> (Hook.f.) Pennell]; H (Image-19)	NE ³ , VU ⁶ , VU ⁶	UK	2400-3800
241. <i>Neopicrorhiza scrophulariiflora</i> (Pennell) D.Y. Hong [= <i>Picrorhiza scrophulariiflora</i> Pennell]; H	NE ³ , EN ⁶	Himal; Chi	3000-4600
242. <i>Picrorhiza kurroa</i> Royle ex Benth.; H	V ¹ , NE ³ , CR ⁴ , 2A ⁵ , EN ⁶	J&K, HP, UK; Pak	3000-4600
243. * <i>Picrorhiza tungnathii</i> Pusalkar; H	NE ³ , VU ⁶	UK	3500-3800
Family 52- SCROPHULARIACEAE Juss.			
244. * <i>Scrophularia obtusa</i> Edgew. ex Hook.f.; H	NE ³ , VU ⁶	UK	1500-2100
Family 53- ACANTHACEAE Juss.			
245. * <i>Phlogacanthus lambertii</i> Raizada; Sh	NE ³ , DD ⁶ , VU ⁶	UK	800-900
Family 54- BIGNONIACEAE Juss.			
246. <i>Incarvillea emodi</i> (Royle ex Lindl) Chatterjee [= <i>Amphicome emodi</i> Royle ex Benth.]; Sh	NE ³ , VU ⁶ , HT ⁹	J&K, HP, UK; Afg, Pak, Nep	450-2500
Family 55- LENTIBULARIACEAE Rich.			

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
247. <i>Pinguicula alpina</i> L.; H (Image-20)	NE ³ , VU ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	W Himal: Europe, Siberia, Tib, Chi	3000-4400
Family 56- LAMIACEAE Martinov (LABIATAE Juss.)			
248. <i>Elsholtzia densa</i> Benth.; H	I ² , NE ³	J&K, HP, UK; Taji, Afg, Pak, Nep, Bhu, Tib, Chi	2600-3650
249. <i>Nepeta campestris</i> Benth.; H	NE ³ , I ²	J&K, HP, UK	2500-3000
250. <i>Phlomis superba</i> (Royle ex Benth.) Kamelin & Makhm. [=Eremostachys superba Royle ex Benth.]; H	NE ³ , VU ⁴ , EN ⁶ , VoE ⁸ , HT ⁹ , HT ¹⁰	J&K, HP, UK; Afg, Pak	300-700
251. <i>Roylea cinerea</i> (D.Don) Baill. [=Ballota cinerea D. Don]; Sh	NE ³ , VU ⁴	J&K, HP, UK; Nep	500-2400
Family 57- OROBANCHACEAE Vent.			
252. <i>Gleadovia ruborum</i> Gamble & Prain; H	NE ³ , EN ⁶	HP, UK; Chi	2500-3900
Order 27- ASTERALES Link Family 58- CAMPANULACEAE Juss.			
253. <i>Campanula wattiana</i> B.K. Nayar & Babu; H	R ¹ , R ² , NE ³ , R ⁷ , END, R ¹¹	HP, UK	2200-3800
254. <i>Cyananthus integer</i> Wall. ex Benth.; H	R ¹ , R ² , NE ³ , R ⁷ , END ⁶ , R ¹¹	UK; Nep	3000-4500
Family 59- ASTERACEAE Bercht. & J. Presl (COMPOSITAE Giseke)			
255. * <i>Artemisia austrohimalayaensis</i> Y.R.Ling & H.S.Puri [=Artemisia austrohimalayana Y.R. Ling & H.S.Puri]; H	NE ³ , VU ⁶	HP, UK	3500-4200
256. <i>Aucklandia costus</i> Falc. [=Saussurea costus (Falc.) Lipsch.; Saussurea lappa (DC.) Sch. Bip.]; H	E ¹ , E ² , CR ³ , 1A ⁵	J&K, HP, UK-Cultivated	2000-3800
257. * <i>Catamixis baccharoides</i> Thomson; Sh	V ¹ , V ² , NE ³ , CR ⁶ , VU ⁶ , V ¹¹	UK, HP; Nep	450-900
258. <i>Cremanthodium arnicoides</i> (DC. ex Royle) R.D. Good [=Ligularia arnicoides DC. ex Royle]; H	E ² , NE ³	J&K, HP, UK; Nep, Chi	2500-5200
259. <i>Dolomiaea macrocephala</i> DC. ex Royle [=Jurinea dolomiaea Boiss.]; H	NE ³ , EN ⁴ , EN ⁶	J&K, HP, UK; Pak, Nep, Chi	3000-4300
260. <i>Himalaiella foliosa</i> (Edgew.) Raab- Straube [=Aplotaxis foliosa Edgew.; Saussurea foliosa (Edgew.) Hook.f.]; H	I ² , NE ³ , I ¹¹	J&K, HP, UK; Nep	2400-3200
261. * <i>Melanoseris filicina</i> (Stebbins) N. Kilian [=Lactuca filicina Duthie ex Stebbins; =Cicerbita filicina (Duthie ex Stebbins) Mumgain & R.R. Rao]; H	E ¹ , E ² , NE ³ , CR ⁶ , VU ⁶ , E ¹¹	UK	1800-2500
262. <i>Saussurea atkinsonii</i> C.B. Clarke; H (Image-21)	I ² , NE ³	J&K, HP, UK	3000-4600
263. <i>Saussurea bracteata</i> Decne.; H	R ¹ , R ² , NE ³ , R ⁷ , R ¹¹	J&K, HP, UK; Pak, Chi	3500-5600
264. <i>Saussurea pterocaulon</i> Decne.[=Saussurea clarkei Hook.f.]; H	R ¹ , R ² , NE ³	J&K, UK	4000-4500
265. <i>Saussurea gossypiphora</i> D.Don; H	NE ³ , VU ⁴	Himal; Chi	3600-5600
266. <i>Saussurea obvallata</i> (DC.) Edgew. [=Aplotaxis obvallata DC.]; H	NE ³ , EN ⁴ , VU ⁶	Himal; Pak, Chi	3800-5300
267. <i>Saussurea roylei</i> (DC.) Sch. Bip. [=Aplotaxis roylei DC.]; H	I ² , NE ³	J&K, HP, UK; Nep, Chi	3300-4800
268. * <i>Saussurea sudhansui</i> Hajra; H	NE ³ , VU ⁶	UK	4500-5000
Order 28- DIPSACALES Juss. ex Bercht. & J. Presl Family 60- CAPRIFOLIACEAE Juss.			
269. <i>Nardostachys jatamansi</i> (D.Don) DC. [=Patrinia jatamansi D.Don]; H (Image-22)	CR ^{3A} , CR ³ , CR ⁴ , EN ⁶ , HT ⁹ , HT ¹⁰	UK, E Himal; Nep, Mya, Chi	2500-4800
270. <i>Valeriana jatamansi</i> Jones; H	NE ³ , VU ⁴ , VU ⁶	Himal; Mya, Chi, Thai, Viet	1500-3600
271. * <i>Valeriana mussooriensis</i> Ved Prakash, Aswal & Mehrotra; H	NE ³ , VU ⁶	UK	1500-2000
272. * <i>Valeriana roylei</i> Klotzsch; H	NE ³ , VU ⁶	UK	?
Order 29- APIALES Nakai Family 61- PITTOSPORACEAE R. Br.			
273. <i>Pittosporum eriocarpum</i> Royle; T	I ¹ , I ² , EN ³ , VU ⁶ , VU ⁶ , I ⁷ , R ¹¹	HP, UK	300-2300
Family 62- ARALIACEAE Juss.			
274. <i>Panax pseudoginseng</i> Wall.; H (Image-23)	V ¹ , NE ³ , CR ⁶	UK, NE India; Nep	2100-4300
Family 63- APIACEAE Lindl. (UMBELLIFERAE Juss.)			
275. <i>Angelica archangelica</i> L.; H	LC ³ , EN ⁶	W Himal	3000-4000
276. <i>Angelica glauca</i> Edgew.; H (Image-24)	EN ^{3A} , EN ³ , EN ⁴ , EN ⁶	W Himal	2400-4500

ORDER, FAMILY Species Name; Habit	Threat Assessment	Geographical Distribution INDIA; Outside India	Elev. Distr. (m) in UK
277. * <i>Angelica indica</i> Pimenov & Kljuykov; H	NE ³ , VU ⁶	UK	3000-3300
278. * <i>Bupleurum maddenii</i> C.B. Clarke; H	NE ³ , VU ⁶	UK	2500-3000
279. <i>Ferula jaeskeana</i> C.B. Clarke; H	NE ³ , VU ⁴	J&K, UK	2400-3600
280. <i>Heracleum candicans</i> Wall. ex DC.; H	NE ³ , VU ⁴	W Himal; Mya	1800-3600
281. <i>Heracleum jacquemontii</i> C.B. Clarke [= <i>Heracleum jacquemontii</i> C.B. Clarke ex Hook.f.]; H	I ¹ , I ² , NE ³ , I ⁷ , I ¹¹	W Himal	?
282. * <i>Hymenidium dentatum</i> (DC.) Pimenov & Kljuykov [= <i>Pleurospermum erosa</i> (DC.) P.K. Mukh.; <i>Hymenolaena dentata</i> var. <i>erosa</i> DC.]; H	NE ³ , VU ⁶	UK	2700-3900
283. * <i>Kailashia robusta</i> Pimenov & Kljuykov; H	NE ³ , VU ⁶	UK	3700-3850
284. * <i>Kedarnatha meifolia</i> Pimenov & Kljuykov; H	NE ³ , VU ⁶	UK	3300
285. * <i>Kedarnatha sanctuarii</i> P.K. Mukh. & Constance; H	NE ³ , END ¹¹	UK	?
286. * <i>Oreocome aegopodioides</i> Pimenov & Kljuykov; H	NE ³ , VU ⁶	UK	3000-3300
287. * <i>Peucedanum dehradunense</i> Babu; H	NE ³ , VU ⁶	UK	700-800
288. * <i>Pimpinella stracheyi</i> C.B. Clarke; H	NE ³ , VU ⁶	UK	2200-2300
289. <i>Pleurospermum angelicoides</i> (Wall. ex DC.) Benth. ex C.B. Clarke [= <i>Hymenolaena angelicoides</i> DC.]; H	NE ³ , VU ⁴	UK, NE India; Nep	2400-4200
290. * <i>Trachyspermum falconeri</i> (C.B. Clarke) H. Wolff. [= <i>Carum falconeri</i> C.B. Clarke]; H	NE ³ , VU ⁶	UK	1500-2700

*endemic species; H=herb, Sh=shrub, T=tree, Cl=climber;

Ex=extinct, PEx=presumed extinct, E=endangered, V=vulnerable, R=rare, I= Indeterminate; EX=extinct, EW=extinct in wild, CR=critically endangered, EN=endangered, VU=vulnerable, DD=data deficient, NE³= not evaluated for threat assessment, LC=least concerned, NT=near threatened ; 1A=listed in Appendix-1 of CITES, 2A=listed in Appendix-2 of CITES; VoE=verge of extinction; HT= highly threatened.

A=Assam, AD= Andhra Pradesh, AP=Arunachal Pradesh, Himal= Entire Himalaya from J&K to Arunachal Pradesh, HP=Himachal Pradesh, India= throughout India, J&K=Jammu & Kashmir, MG=Meghalaya, MN=Manipur, NE India= North East Indian states, NI= Nicobar Island, NL=Nagaland, OD=Odisha, Pen India= Peninsular India, S=Sikkim, TN=Tamil Nadu, UK=Uttarakhand, UP=Uttar Pradesh, WB=West Bengal.

Afg= Afganistan, Ban=Bangladesh, Bh=Bhutan, Chi=China, Ira=Iran, Kor=Korea, Kaz=Kazakhstan, La=Laos, Mal=Malesia, Mon=Mongolia, Mya=Myanmar, Nep=Nepal, Pak=Pakistan, Tib=Tibet, Viet=Vietnam

Table 2. Taxa excluded from Table 1 (Threatened flora of Uttarakhand) on account of various reasons.

Species/taxa excluded	Reason for exclusion (reference)
1. <i>Iris duthie</i> Foster (Iridaceae)	Synonym of <i>Iris kemaonensis</i> Wall. ex D. Don, a common species (POWO 2019)
2. <i>Microschoenus duthie</i> C.B. Clarke (Cyperaceae)	Synonym of <i>Juncus duthie</i> (C.B. Clarke) Noltie, a common species (POWO 2019)
3. <i>Berberis petiolaris</i> Wall. ex G. Don var. <i>garhwalana</i> Ahrendt (Berberidaceae)	Variety not recognized in recent works (Pusalkar & Srivastava 2018)
4. <i>Aconitum ferox</i> Wall. ex Ser. (Ranunculaceae)	Erroneous identification; species not known in Western Himalaya (Pusalkar & Srivastava 2018)
5. <i>Aconitum deinorrhizum</i> Stapf. (Ranunculaceae)	Erroneous identification; species not known in Western Himalaya (Pusalkar & Srivastava 2018)
6. <i>Caragana aegacanthoides</i> (R. Parker) L.B. Chaudhary & S.K. Srivast. (Fabaceae)	Not endemic to Uttarakhand (POWO 2019)
7. <i>Pueraria stracheyi</i> Baker (Fabaceae)	Synonym of <i>Apios carnea</i> (Wall.) Benth. ex Baker; a common species (POWO 2019)
8. <i>Saraca asoca</i> (Roxb.) W.J. de Wilde (Fabaceae)	Cultivated species in Uttarakhand
9. <i>Acer osmastonii</i> Gamble (Sapindaceae)	Erroneous identification (Pusalkar & Srivastava 2018)
10. <i>Acer oblongum</i> Wall. ex DC. var. <i>membranaceum</i> Banerji (Sapindaceae)	Variety not recognized in recent work (Pusalkar & Srivastava 2018)
11. <i>Santalum album</i> L. (Santalaceae)	Cultivated species in Uttarakhand
12. <i>Sagina purii</i> R.D. Gaur (Caryophyllaceae)	Synonym of <i>Sagina apetala</i> Ard. (Chandra 2015)
13. <i>Impatiens vexillaria</i> Hook.f. (Balsaminaceae)	Known by type only and described from Himachal Pradesh (Hooker 1910)
14. <i>Arnebia nandadeviensis</i> Chandra Sek. & R.S. Rawal (Boraginaceae)	Synonym of <i>Onosma bracteata</i> Wall. (Tiwari 2016)
15. <i>Ageratum haustonianum</i> Mill. (Asteraceae)	Common invasive species in Uttarakhand
16. <i>Nardostachys grandiflora</i> DC. (Valerianaceae)	Synonym of <i>N. jatamansi</i> (D. Don) DC. (POWO 2019)

Table 3. Original sources, threat status and number of species included in Table 1 (threatened flora of Uttarakhand) based on them.

Superscript number used in table-1; Original source	Threat statuses used	Threat statuses followed by original source	Number of species
¹ Nayar & Sastry (1987-90)	Ex, E, V, R, I	Lucas & Synge (1978)	55
² Rao et al. (2003)	Ex, E, V, R, I	IUCN (1994)	115
³ IUCN Red List for India	EX, EW, CR, EN, VU, DD	IUCN (new)	34
^{3A} IUCN Red List for Uttarakhand	EX, EW, CR, EN, VU, DD	IUCN (new)	12
⁴ Ved et al. (2003)	EX, EW, CR, EN, VU, I	IUCN (older)	44
⁵ CITES Appendices (2019)	None	None	12*
⁶ Pusalkar & Srivastava (2018)	EX, EW, CR, EN, VU, DD	IUCN (new?)	165*
⁷ Uniyal et al. (2007)	Ex, E, V, R, I	Lucas & Synge (1978)	55
⁸ National Biodiversity Authority list for Uttarakhand	Verge of Extinction (VoE)	None	15
⁹ Uttarakhand State Biodiversity Board, Annexure-2	Highly Threatened (HT)	None	27
¹⁰ Shah (ebook publ. by Uttarakhand State Biodiversity Board)	Highly Threatened (HT)	None	15
¹¹ Threatened Taxa list available at ENVIS Centre BSI	Ex, E, V, R, I	Lucas & Synge (1978)	67

12+= 12 listed species and all orchids in the area; 165+= 165 listed species and all orchids in the area.

et al. 2016; Panwar et al. 2015, 2016, 2017; Thakur et al. 2016). This selection of species seems more skewed towards economically important species (12 species, mainly medicinal) than on only threatened species which suggests that only threatened status is considered a meagre reason for micropropagation.

It has been emphasized that for conservation of biodiversity we have to focus on biodiversity hotspots and documentation of distribution of biodiversity has to be improved (Myers et al. 2000; Raven & Wackernagel 2020). Uttarakhand is one of the important zones of the Himalayan biodiversity hotspot with more than 45% flowering plant species, 60% genera, 92% families, thus, sustaining rich flowering plant diversity interspersed with a large number of threatened species (Rana & Rawat 2017; Pusalkar & Srivastava 2018). Threat statuses of threatened plant species in entire India, including Uttarakhand, are ambiguous and require their correct assessment using categories and criteria suggested in recent IUCN Redlist to be globally acceptable (Barik et al. 2018). The information in this communication is an attempt to provide the current situation of threatened flora of Uttarakhand as identified by various official sources. Images of 24 threatened species and locations of individuals photographed are shown in Figure 1 to further facilitate conservation studies on these species. It is now crucial to assess these proposed threatened species (barring 34 already assessed) with modern IUCN threat categories to find the most threatened species for prioritized conservation by all available means. Such an assessment will restrict the unnecessary inflation of threatened plants list consequently reducing pressure

on the resources being spent for conservation. The given list of species will also be helpful to subsequent scientific publications for correctly referring to any species threatened in Uttarakhand, however, it should be used judiciously as all species listed in it are not threatened strictly according to the IUCN Red List criteria.

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Images 1–12. Threatened flora of Uttarakhand: 1—*Dioscorea deltoidea* | 2—*Fritillaria cirrhosa* | 3—*Cypripedium himalaicum* | 4—*Oreorchis micrantha* | 5—*Pecteilis gigantea* | 6—*Asparagus filicinus* | 7—*Polygonatum graminifolium* | 8—*Trachycarpus takil* | 9—*Papaver robustum* | 10—*Berberis osmastonii* | 11—*Aconitum lethale* | 12—*Aconitum violaceum*. © D.S. Rawat & Satish Chandra.



Images 13–24. Threatened Flora of Uttarakhand: 13—*Trollius acaulis* | 14—*Butea peltata* | 15—*Uraria picta* | 16—*Eutrema purii* | 17—*Eremogone curvifolia* | 18—*Kuepferia infelix* | 19—*Kashmiria himalaica* | 20—*Pinguicula alpine* | 21—*Saussurea atkinsonii* | 22—*Nardostachys jatamansi* | 23—*Panax pseudoginseng* | 24—*Angelica glauca*. © D.S. Rawat & Satish Chandra.

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REVIEW

A systematic review on the feeding ecology of Sloth Bear *Melursus ursinus* Shaw, 1791 in its distribution range in the Indian subcontinent

Vasantkumar Rabari¹ & Nishith Dharaiya²

^{1,2} Wildlife and Conservation Biology Research Lab, Department of Life Sciences, Hemchandracharya North Gujarat University, Patan, Gujarat 384265, India.

¹rabarivasant016@gmail.com, ²nadharaiya@ngu.ac.in (corresponding author)

Abstract: The Sloth Bear being myrmecophagous is specialized to feed on ants, termites, and fleshy food; however, no discernible comparison exists on a diet, seasonal feeding pattern, and factor influence in a different habitat of an Indian sub-continent. A review of available literature suggested the dominance of plant matter in the Sloth Bear diet during the summer season, while an equal quantum of plant & animal matter was recorded in the monsoon & winter seasons. Fleshy fruits, flowers, flower buds, delicate leaves, and sometimes roots are considered plant food items in different studies, while ants, termites, honey, honey wax, and carrion feed are recorded as animal food items. Availability and accessibility of food materials in the different seasons, energy requirements, geographical variations, and human interference are notable factors influencing the feeding strategy of Sloth Bears. Cumulative data on food & feeding behavior of Sloth Bears helps to understand the pivotal role of species across various habitats. A systematic review of all the available studies to understand the diet of Sloth Bears in different seasons across its distribution range is presented in this paper, which can be a holistic approach to know the habitat selection with reference to the availability of food. A better understanding of such behavior also provides a key strategy for the management of large mammals in different geographical areas.

Keywords: Conservation, diet, Indian Bear, myrmecophagous, nutrition, scat analysis.

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Author details: VASANTKUMAR RABARI is a research scholar at the Department of Life Sciences, Hemchandracharya North Gujarat University, Patan. His research interests include behavioral aspects of mammals, especially Indian Bear. NISHITH DHARAIYA is currently positioned as an associate professor at Department of Life Sciences, HNG University, Patan, Gujarat, India. He is co-chair of the IUCN SSC Sloth Bear Expert Team and co-founder and Director of Research of WCB Research Foundation. His areas of interest include large mammals' ecology, biodiversity monitoring and human-wildlife interaction.

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INTRODUCTION

Nutrition plays an important role in the growth and development of every organism consequently need of healthy diet influences habitat selection. The family Ursidae comprises eight species of bears, widely distributed throughout northern hemisphere and partially in southern hemisphere. The food & feeding habit of bears are largely influenced by the geographical regions (Joshi et al. 1997). Out of the eight bear species, the Polar Bear is carnivorous and the Giant Panda is dependent on bamboo, while the rest of them are known to feed on a variety of foods and are termed omnivorous. Mostly they are opportunistic feeders, for growth and reproductive success they need a good amount of protein in their diet along with fat and carbohydrate for metabolism and energy fulfillment they feed on both plants and animals (Noyce et al. 1997). Varied habitat conditions majorly determine the feeding habits of bears with some similarities intact. The Sloth Bear is unique among all the bear species in being myrmecophagous in nature, feeding on ants, termites, honey, and fruits depending on availability (Joshi et al. 1997; Sukhadiya et al. 2013). Sloth Bear has a special feeding adaptation, it has highly specialized morphological features characterized to feed on insects which include a lack of upper incisor, broad palate, protrusible mobile lips, long snout, and nostrils that can be closed to create suction (Launre & Seidensticker 1997). They also possess a distinctively long shaggy coat with no underfur and reduced hair on the snout, which helps in the defense against honey bees and termite secretion during feeding. Competitive pressure and the temporal patterning of resource availability are two major factors in the evolution of Sloth Bear feeding specialization towards myrmecophagy (Launre & Seidensticker 1997).

The studies on the feeding behavior of Sloth Bears are well documented in different parts of the Indian subcontinent, but there is a need of a concrete review on diet of Sloth Bear to understand food preferences in different season as well as in different geographic regions through the Indian sub-continent. Thus, this review is aimed to compare variations in diet and dietary patterns of Sloth Bears in its distribution range along with the comparison of the methods by which it was studied.

METHODS

The distribution of Sloth Bears is constrained by the ocean to the south, desert to the north-west, and mountains to the north & east. Although, they are found

abundantly in Indian peninsula with a patchy, disturbed, and fragmented habitat due to anthropogenic pressure. Their actual distribution is confined to India, Nepal, and Sri Lanka and they have been recently extirpated from Bangladesh. In India, the Sloth Bears are patchily distributed in five different regions—northern, northeastern, central, southeastern, and southwestern (Johnsingh 2003; Yoganand et al. 2005; Dharaiya et al. 2016).

The literature survey was performed for published articles using keywords 'Sloth Bear', 'food', 'diet', '*Melursus ursinus*', 'nutrition', 'scat analysis' and fecal material', 'feeding behavior' in the search engines such as Google Scholar and Research Gate and also found from references cited in available papers. The review was conducted from the oldest literature on Sloth Bear diet in 1967 to the most recent by Schaller & Philip et al. (2021). A total of 21 literatures were used in this study relevant to Sloth Bear feeding behavior through its distribution range; out of which, 17 studies were conducted in India, three in Nepal, and one in Sri Lanka (Figure 1). To understand the dietary habit of Sloth Bears and the relative composition of plants and animal matter, we used the percentage volume of different food items in scats of Sloth Bears from all checked literature.

RESULTS AND DISCUSSION

Sampling methods used in different studies

The nocturnal foraging habits of Sloth Bears primarily do not permit adequate data to be gathered based on direct observation of their feeding behavior. Feeding ecology is mainly studied by scat analysis, one of the widely used techniques to study the diets of large carnivores and also described as one of the best available methods for studying the food habits of Sloth Bears (Dharaiya & Ratnayeke 2009; Mewada 2010). Scats of Sloth Bears can be more easily identified than scats of other mammals in the area on the basis of shape, size, and undigested food (seeds, bee wax, ant heads, and insect body parts). The scats were collected in different studies by surveying forest trails, bear dens, and resting sites. It is noted that collection of scats during the monsoon is quite difficult due to increased vegetation cover and erosion by rains where den sites are considered a prime way for scat collection during the monsoon (Bargali et al. 2004). Although direct observation is used to study foraging behavior of bears in Kumbhalgarh Wildlife Sanctuary, Rajasthan (Chhangani 2002) and in Royal Chitwan National Park (Joshi et al. 1997). Radio-collared

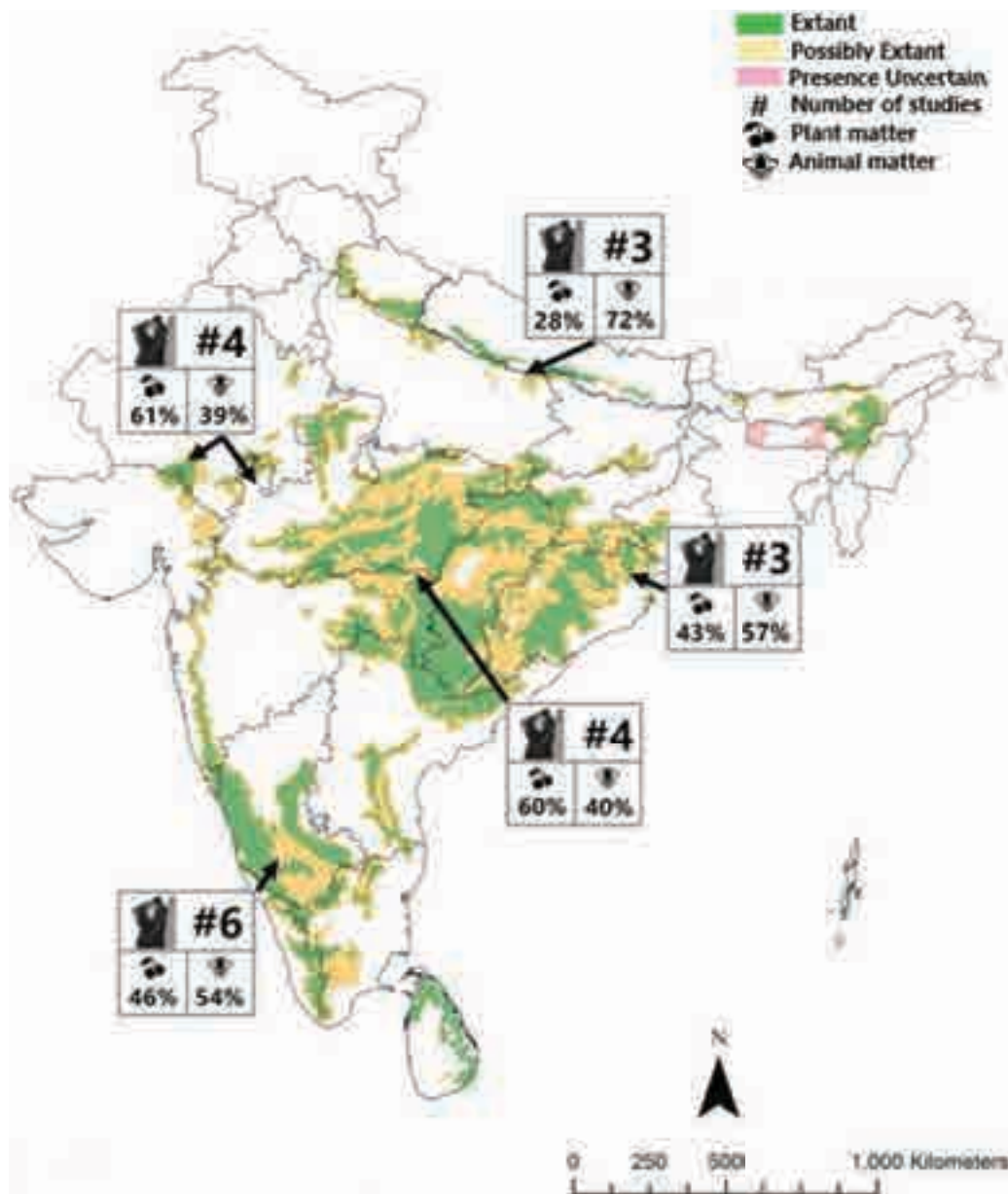


Figure 1. Distribution of Sloth Bears and locations of diet studies carried out by different researchers (West—#4 | Central—#4 | East—#3 | Northeast—#3 | South—#6) (Dharaiya et al. 2016).

Sloth Bears were monitored in Royal Chitwan National Park from the back of an elephant using binoculars at a distance of 30–50 m without disturbing their activities.

Plant and Animal-based diet

Studies on feeding behavior show that sloth bears consume both animal and plant matter in their regular diet. According to Akhtar et al. (2004) among the Ursidae, only the Sloth Bear is uniquely adapted for feeding on insects and fruit and a less amount of vegetables, mammals, fishes, and other insects. Being an opportunistic feeder, the Sloth Bear has been observed

to switch between fruits and insects depending on the availability and amount as mainly fruit content is recorded in fruiting seasons and vice versa.

Plant matter was found to be dominating the diet of Sloth Bear in comparison to animal matter due to less availability of the latter in the Kumbhalgarh Wildlife Sanctuary (Chhangani 2002). Similarly, Schaller (1967), Bargali et al. (2004), Yoganand (2005), Mewada & Dharaiya (2010), Sukhadiya et al. (2013), Mewada (2015), and Kumar & Paul (2021) found plant material in abundance than animal matter in Sloth Bear scat on the basis of dry-weight in central and western India

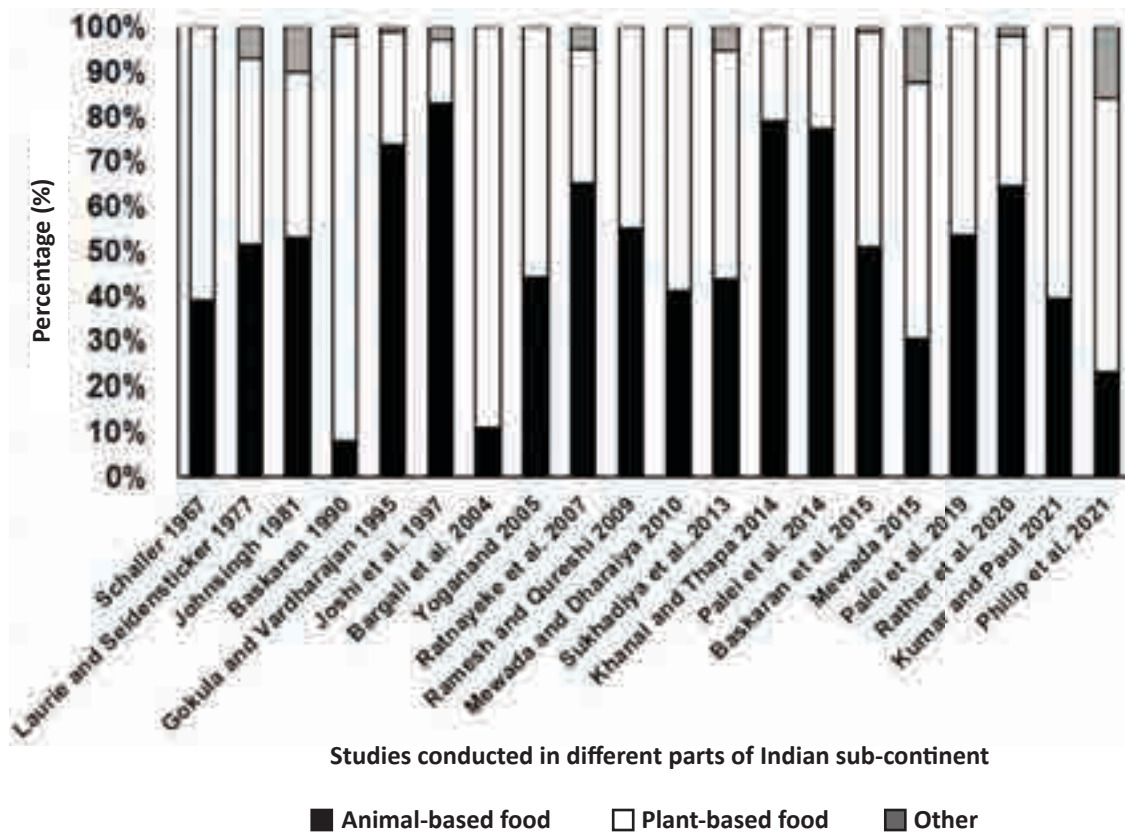


Figure 2. Diet composition of Sloth Bear based on the available literature surveyed in this review.

(Figure 2, 3). According to Chhangani (2002), 40 species have been recorded in Kumbhalgarh wildlife sanctuary as the preferred food by Sloth Bear among them 22 are natural, while the rest are cultivated plants. These plant species are consumed by Sloth Bear in the form of young & mature leaves, flowers & flowers bud, unripe & ripe fruits, and sometimes roots, shoots, bark, and seed (Chhangani 2002). While, animal matter was reported higher in Sloth Bear scats by Laurie & Seidensticker (1977), Josnsingh (1981), Gokula & Vardharajan (1995), Joshi et al. (1997), Ratnayake et al. (2007), Ramesh et al. (2009), Khanal & Thapa (2014), Palei et al. (2014, 2020), and Baskaran et al. (2015) possibly due to less availability of flashy fruits in the southern, eastern, and northeastern parts of the Indian sub-continent (Figure 2,3). Garshelis (1999) also noted higher animal matter than fruits in Sloth Bear scats in the Terai areas of the Indian sub-continent. Animal matter is composed of mainly termites, ants, honey bees, and bee wax.

In the majority of studies, plant-based food was recorded more abundantly than animal-based food, probably due to hard soil during the summer season make difficult to dig for ants and termites. It is also believed the greater importance of plant matter in the bear diet

during summer is due to seasonal flowering and fruiting. While almost equal dietary pattern was observed during the winter and monsoon seasons between plant and animal-based food (Figure 4). It is assumed that bears feed on ants and termites throughout the year while fruits are the most preferred food; when fruits are available, they shift their diet towards plant matter. With the availability of both fruits and insects, bears feed on fruits to fulfill nutrition requirements due to the bulk of availability and easy access of fruits than insects. Fleishy fruits are rich in sugar provide instant energy to Sloth Bears, and excess sugar can be converted and stored as tissue fat for further utilization (Palei et al. 2020). Although the insects are rich in protein than fruits but being a larger body size of Sloth Bears, an adequate amount of food required to quench the hunger in less time may influence the animal to shift on fruits (Baskaran et al. 2015).

Generally, Sloth Bears do not prey on carrion or other mammals, but McDougal recorded one instance in which a sloth bear was feeding on buffalo killed by a tiger during a tiger baiting program in western Chitawan (Laurie & Seidensticker 1977). A similar instance was recorded by Sanderson (1890) where Sloth Bears

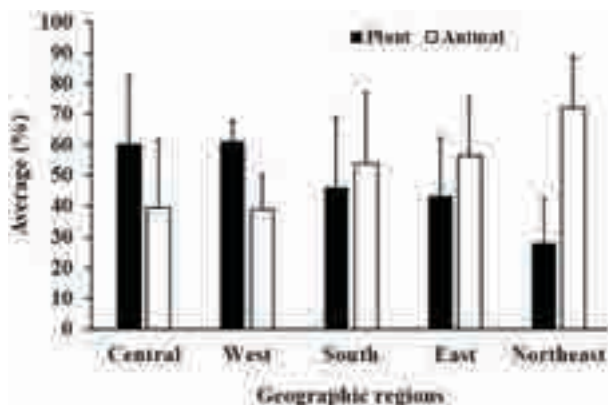


Figure 3. An average (%) diet composition of Sloth Bear across different geographic regions based on the available literature surveyed in this review (error bar represents standard deviation).

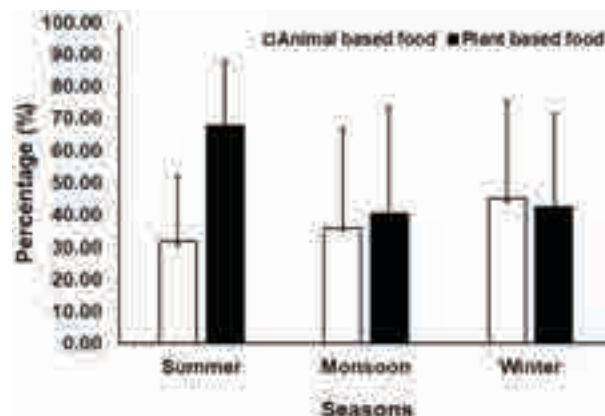


Figure 4. A mean percentage of seasonal diet of Sloth Bears, according to checked literature (error bar represents standard deviation).

scavenged on tiger kills and gnawed on cattle bones. A 37 cm long, digested snake was found in the scat of Sloth Bear by Hasted (1903). In Kumbhalgarh Wildlife Sanctuary, carcasses of dead wild & domestic animals are also recorded as a possible food content of Sloth Bear (Chhangani 2002). Remains of Sambar were reported in Mudumalai Tiger Reserve, Tamil Nadu (Ramesh et al. 2009). The incidence of observing animal carcasses in Sloth Bear scat has been dated to the late 19th century and no concrete proofs have been given in recent studies on Sloth Bear consuming carcasses. But recently, mammalian hairs were reported in Sloth Bear scats in Chitwan National Park, Nepal by Khanal & Thapa (2014). Similarly, mammalian hairs and bones were reported in Sloth Bear scats in Nawada Forest Division, Bihar by Kumar & Paul (2021) probably suggests carrion feeding behavior of Sloth Bear.

Feeding patterns of Sloth Bears have been also reported with some rare and extreme observations in western India. In the Jessore Sloth Bear Sanctuary (Gujarat), cicadas (*Platypleura spp.*) were found for the first time in the scats of Sloth Bears (Patel et al. 2017). Singh et al. (2017) reported two instances of Sloth Bear attractant towards house and temple in search of food. Similarly, two bears were feeding on sweets, coconuts, and licking the 'Sindoor' around a sacred fire at a pilgrimage site, Mount Abu, Rajasthan, India (Koli & Prajapati 2022).

Habitat selection

Habitat use by an animal largely depends upon the biological requirements of species based on the quality of habitat known by species-habitat relationships (Ramesh et al. 2012). According to Bargali et al. (2004), availability of dietary components greatly influences

Sloth Bear habitat use. Fruits and insects comprise the majority of Sloth Bear diet, but it varies seasonally and geographically across their range from Nepal through India, and Sri Lanka (Baskaran 1990; Dharaiya et al. 2016). Depending on the nutrition requirements, bears tend to feed on ants and termites (Noyce et al. 1997). Plant biomass directly or indirectly influences termite growth, thus favorable climate and soil texture increase productivity and biomass of plants, resulting in the high availability of termites in different habitats. While Launre & Seidensticker (1997) suggested that movement of bears is associated with fruiting species of the area, it can be concluded that habitat selection is driven by the availability and accessibility of food (Laurie & Seidensticker 1977; Dharaiya et al. 2016).

Factors affecting food selection

The food habit of sloth bears is determined by several factors that have been classified into four categories— food availability, seasonal variation, energy requirement, and geographic location. Many studies have reported seasonal food availability determines what food resource Sloth Bears use (Bargali et al. 2004; Sukhadiya & Dharaiya 2013; Khanal & Thapa 2014; Baskaran et al. 2015; Rather et al. 2020). Among all bear species, the Sloth Bear seems almost entirely depends on insects for protein requirements (Yoganand et al. 2005; Khanal & Thapa 2014). Moreover, to fulfill immediate energy requirements, Sloth Bears are reported to feed on fleshy fruits during the fruiting season (Palei et al. 2020). According to Palei et al. (2014), Sloth Bears feed on diverse food items in different seasons to avoid deficiency of protein, calcium, starch, and other necessary nutrients. Several authors have depicted diet pattern of Sloth Bears varies with geographical location

as per availability and accessibility of fruiting species and colonies of ants and termites (Schaller 1967; Joshi et al. 1997; Mewada & Dharaiya 2010).

Is the food responsible for Human-Bear interaction?

Sloth Bears are facing multiple threats, mainly due to the increasing trend of human population causes habitat fragmentation, degradation, decreased natural resources, and conflict with humans (Garcia et al. 2016). Mewada & Dharaiya (2010) suggested that bears use less human-dominated areas when forest is available. It is reported that Sloth Bear competes with humans for the same resource utilization like fruits and honey (Bargali et al. 2004). During the summer season, most fruits are ripe and eaten by Sloth Bears (Baskaran et al. 1997; Joshi et al. 1997; Akhtar et al. 2004) and also collected by local people for their own or to sell in the market. In monsoon, the human-bear encounter was reported higher at agriculture fields where humans and bears spend their time for own purpose (Debata et al. 2017). Also, during monsoon and winter, local villagers go to forest areas for grazing their livestock might be the reason of encounters due to less detection of Sloth Bears in increased vegetation. People continuing harvest of timber and firewood cause an extensive loss of habitat (Garcia et al. 2016). Similarly, Chhangani (2002) suggests that due to dispersion of ground cover by overgrazing and agriculture practices near the bear habitat, chances of human-bear interaction increase, which leads to conflicts in some situations. Potential mitigation ways to reduce Sloth Bear intake of human grown food, is to grow crops not preferred by Sloth Bears (Bargali et al. 2004) and proper burial or disposal of carrion. Beyond this, movement in larger groups in the forest during the collection of natural products may reduce human-bear conflict.

Application for Management

However, only 10% of the good quality of habitat for Sloth Bears is left in India (Yoganand et al. 2005). The Sloth Bears are inhabiting fragmented habitats, continuously facing habitat disturbance, retaliatory killing, and poaching. These days, resource sharing is emerging as a major threat between humans and Sloth Bears (Rajpurohit & Krausman 2000; Dharaiya & Ratnayeke 2009). Lack of natural food resources, habitat fragmentation, and increased anthropogenic activities would clearly support that most attacks happen outside protected areas. The availability of adequate food may reduce the movement of bears out of the protected area, which will result in fewer encounters with humans. This

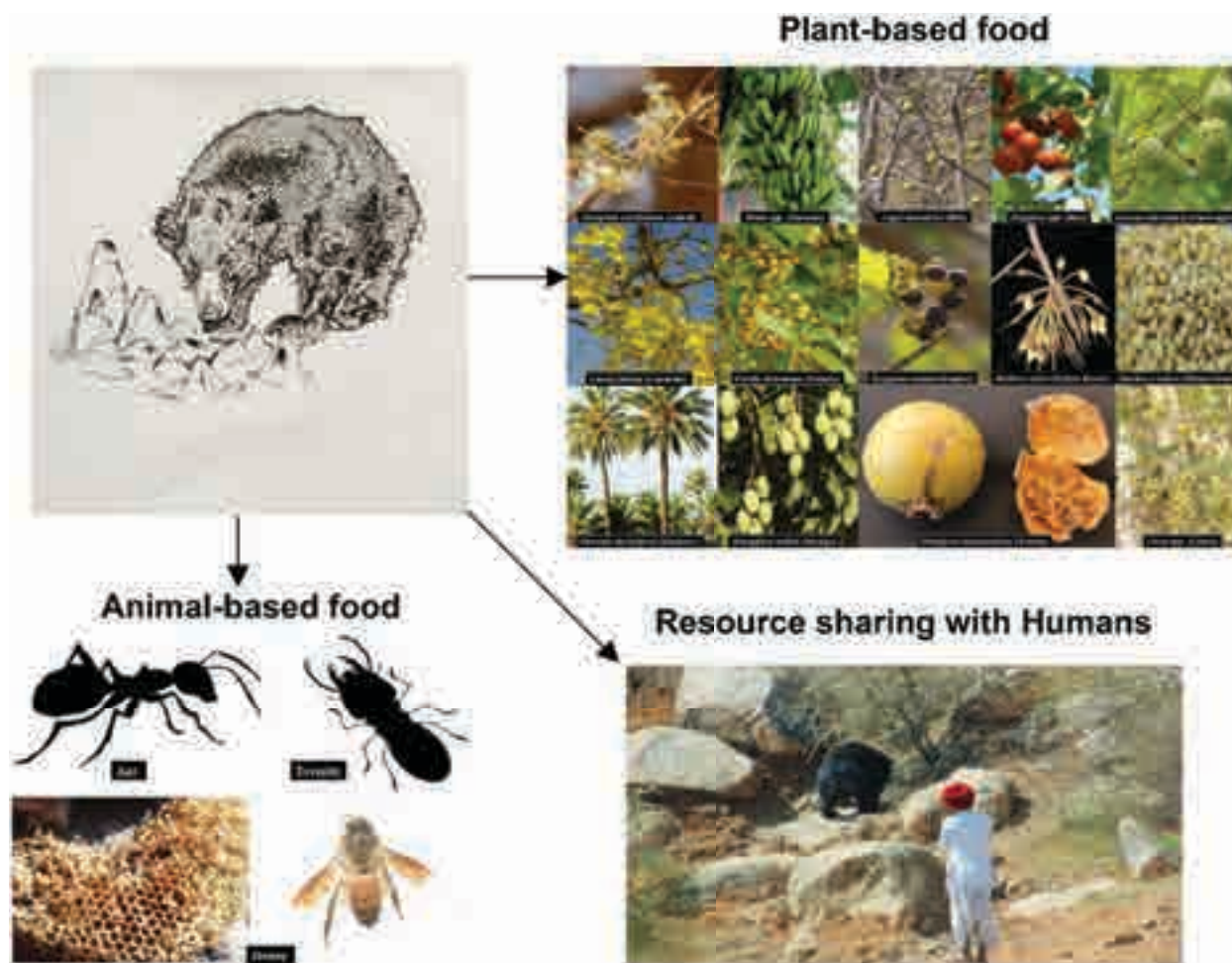
review reveals that important fruiting species play a vital role on the Sloth Bear movement and the plantation of such trees within the forest will increase food availability for Sloth Bears that can be the backbone of further management practices.

CONCLUSION

Studies on the feeding habits of Sloth Bears in the different regions reveal that Sloth Bears feed on both plants and animal matter based on food availability. It is clearly stated that their feeding habit change with the season, geographic region, as well as the availability of food resources. By knowing these different results, we can conclude that bears are playing a vital role as an indicator of climate because they are vulnerable to changes in the landscape influenced by deforestation, logging, habitat destruction, and changing plant phenology. They are an umbrella species in the protected areas, but their actual role in the forest ecosystem has been quite unclarified. The need of high nutritious food converts their feeding pattern towards the intake of fruit, making them more effective as a seed disperser.

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SHORT COMMUNICATION

Mercury in tuna from the western equatorial Atlantic Ocean and health risk assessment

Ana Paula Madeira Di Benedetto^{1*}, **Inácio Abreu Pestana^{2*}**, **Igor David da Costa³**, **Marcelo Gomes de Almeida⁴**, **Braulio Cherene Vaz de Oliveira⁵** & **Carlos Eduardo de Rezende⁶**

^{1,2,4,5,6} Universidade Estadual do Norte Fluminense Darcy Ribeiro, CBB, Laboratório de Ciências Ambientais, Av. Alberto Lamego 2000, Campos dos Goytacazes, RJ, 28013-602, Brazil.

³ Universidade Federal Fluminense, Instituto do Noroeste Fluminense de Educação Superior, Av. João Jasbick, s/nº, Santo Antônio de Pádua. RJ. 28470-000. Brazil.

* These authors contributed equally to this work.

¹anadibeneditto@gmail.com (corresponding author), ²inacio@uenf.br, ³igorbiologia@yahoo.com.br, ⁴marcelogaa@yahoo.com.br, ⁵brauliocherene@gmail.com, ⁶crezende@uenf.br

Abstract: This study analyses the mercury (Hg) concentration in the meat of *Thunnus albacares* and *Thunnus obesus* caught from the western equatorial Atlantic Ocean. The objective was to estimate the Hg intake via tuna ingestion and presents the possible health risk assessment. For *T. albacares* and *T. obesus*, the median concentration was 212 ng·g⁻¹ and 475.1 ng·g⁻¹ wet weight, respectively. The Hg concentrations were below the maximum tolerable limit established by international and Brazilian regulations for fishery products. The consumption would pose a risk for human populations that ingest more than 80 g·day⁻¹. Regular monitoring of both human consumption rates and Hg levels in fish are recommended.

Keywords: Hg, human health, *Thunnus albacares*, *Thunnus obesus*.

Portuguese: Este estudo analisa a concentração de mercúrio (Hg) na carne de *Thunnus albacares* e *T. obesus* capturados em pescarias no Oceano Atlântico equatorial ocidental, estima a ingestão de Hg via ingestão de atum e apresenta a avaliação de risco à saúde. Para *T. albacares* e *T. obesus*, a mediana das concentrações foi 212 ng·g⁻¹ e 475,1 ng·g⁻¹ peso úmido, respectivamente. As concentrações de Hg ficaram abaixo do limite máximo tolerável estabelecido pelas regulamentações internacionais e brasileiras. O consumo representaria um risco para as populações humanas que ingerem mais de 80 g·dia⁻¹.

In Brazil, the tuna fishery targeted mainly the yellowfin tuna *T. albacares* Bonnaterre, 1788) and the bigeye tuna *T. obesus* (Lowe, 1839), which are among the main tuna species caught worldwide (Guillotreau et al. 2017; Rodrigues et al. 2020). Tuna species are large and long-lived predatory fishes with wide distribution, becoming good bio monitors of contaminants, such as mercury (Hg) (Ferriss & Essington 2011; Jinadasa et al. 2019; Tseng et al. 2021). In the marine environment, >95% of the Hg in the meat of predatory fish is methylmercury, a potent neurotoxin (Lescord et al. 2018). This study analyzes the Hg concentration in tuna meat from western equatorial Atlantic Ocean, and estimates the Hg intake via tuna ingestion.

Tuna sampling was conducted in 2019 and 2020 at the fish market named *Mercado Municipal Central Leste*, located in São Paulo State, southeastern Brazil. The fish

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Table 1. Sample size, fork length, weight and Hg concentration (wet and dry basis) in the muscle of *Thunnus albacares* and *Thunnus obesus* from the western equatorial Atlantic Ocean. The values are shown as the median $\pm$ interquartile range (minimum and maximum). Lowercase letters compare the variables between sampling years for the same species. Uppercase letters compare the variables between species and consider the data from both samplings.

Species	<i>T. albacares</i>		<i>T. obesus</i>	
Year	2019 (n = 32)	2020 (n = 18)	2019 (n = 26)	2020(n = 6)
Fork length (cm)	123.5 $\pm$ 22.3 ^a (97–167)	139.5 $\pm$ 9.3 ^b (129–176)	104 $\pm$ 38 ^a (91–181)	104.5 $\pm$ 5.3 ^a (101–111)
Weight (kg)	25.5 $\pm$ 19.6 ^a (12.6–65.2)	39.5 $\pm$ 9.5 ^b (29.3–76.1)	18.2 $\pm$ 20.7 ^a (11.9–72.3)	18.5 $\pm$ 1.5 ^a (16.9–20.4)
Hg (ng·g ⁻¹ wet weight)	168.3 $\pm$ 80.2 ^a (82.1–455.1)	309.5 $\pm$ 98.8 ^b (103.2–570.3)	499 $\pm$ 195.9 ^a (204.1–1347.5)	457.7 $\pm$ 51.9 ^a (387.7–475.1)
Hg (ng·g ⁻¹ dry weight)	636.6 $\pm$ 303.4 ^a (310.6–1722)	1170.9 $\pm$ 373.9 ^b (390.5–2157.6)	1840.5 $\pm$ 722.5 ^a (752.8–4970.4)	1688.5 $\pm$ 191.4 ^a (1430–1752.5)
Log [Hg (ng·g ⁻¹ wet weight)]·kg ⁻¹	6.7 $\pm$ 2.5 ^a (3.4–11.9)	7.2 $\pm$ 2.7 ^b (3.5–19.1)	26.7 $\pm$ 8.4 ^a (4.9–49.7)	23.4 $\pm$ 2.8 ^a (20.2–28.1)
All samples	<i>T. albacares</i>		<i>T. obesus</i>	
Fork length (cm)	132.5 $\pm$ 23.5 ^A		104 $\pm$ 20 ^B	
Weight (kg)	32.4 $\pm$ 20 ^A		18.2 $\pm$ 9.1 ^B	
Hg (ng·g ⁻¹ wet weight)	212 $\pm$ 149.6 ^B		475.1 $\pm$ 107.8 ^A	
Hg (ng·g ⁻¹ dry weight)	802 $\pm$ 565.9 ^B		1752.5 $\pm$ 397.7 ^A	

sampled at the market were caught from commercial fishery done by the fishing fleet of *Areia Branca* Harbor. This fishing fleet operates in the western equatorial Atlantic Ocean (off northern Brazil), in the vicinity of one of the oceanic buoys of PIRATA Program (“Pilot Moored Array in the Tropical Atlantic”). The fishing area is located in waters that are 4,000 m deep, at 0°, 35 °W, and 600 km offshore (international waters). The sample size for *T. albacares* was 32 individuals in 2019, and 18 individuals in 2020; and for *T. obesus* it was 26 and six individuals in 2019 and 2020, respectively. Sample of meat from the fish belly (5 g of wet weight) was removed from each individual at the fish market. The samples were brought to the laboratory and kept frozen (-20 °C) in dry sterile vials, freeze-dried and homogenized with a mortar and pestle (Table 1). Mercury determinations followed Bastos et al. (1998) and were conducted with an ICP-OES (Varian, Liberty II Model 720 ES, Australia) with a cold vapor accessory (VGA-77). The recovery values for the certified reference material (DORM-4) ranged 85–95 %. The coefficients of variation of the triplicate analyses were <10%. The results were calculated as ng·g⁻¹ dry weight and converted to a wet weight basis. For *T. albacares*, the water loss after freeze-drying was 74 $\pm$ 1%, and for *T. obesus*, it was 73 $\pm$ 1%.

The maximum permissible limit for Hg in predatory fishes established by the World Health Organization—WHO (FAO/WHO 1991) and Brazilian Government (ANVISA 2021), is 1 mg·kg⁻¹ (or 1,000 ng·g⁻¹) wet weight. The estimated daily intake proposed in Caldas et al. (2016) was used for the intake analysis: EDI = C·IR/BW,

where EDI is the estimated dietary intake of Hg (μg·day⁻¹·kg⁻¹), C is the Hg concentration in the fish meat (μg·g⁻¹ wet weight), IR is the intake rate in Brazil (27 g·day⁻¹; Barone et al. 2017), and BW is the body weight (70 kg for a Brazilian adult). The provisional upper tolerable weekly intakes - PTWI limit for Hg is 1.6 μg·week⁻¹·kg⁻¹ or 0.57 μg·day⁻¹·kg⁻¹ (FAO/WHO 2003). We calculated the IR of tuna meat that would be necessary to reach the intake limit for Hg. We considered the median Hg concentration as a fixed variable and applied the EDI formula to estimate the IR needed to reach the intake limits established by the FAO/WHO (2003).

ANOVAs (aov function, base package; R Core Team 2022) were used to test the differences in Hg concentrations among the species and sampling years. Estimations of Hg intake due to tuna consumption by humans were conducted by Monte Carlo Method (Khitilishvili 2016) to incorporate the variability of each variable (Hg concentration in fish, human intake rate and body weight) in the final results.

Total Hg concentration varied between sampling years for *T. albacares*, and between the two tuna species (higher Hg concentrations in *T. obesus*) (Table 1). *T. albacares* sampled in 2020 was larger and heavier than in 2019, and the size difference was consistent with the Hg concentrations, reflecting Hg bioaccumulation during the fish growth (Lacerda et al. 2017). The enrichment of more bioavailable organic Hg complexes in deep waters, such as methylmercury, and the tuna foraging depths explain the interspecific differences in Hg concentrations (Choy et al. 2009; Ferriss & Essington

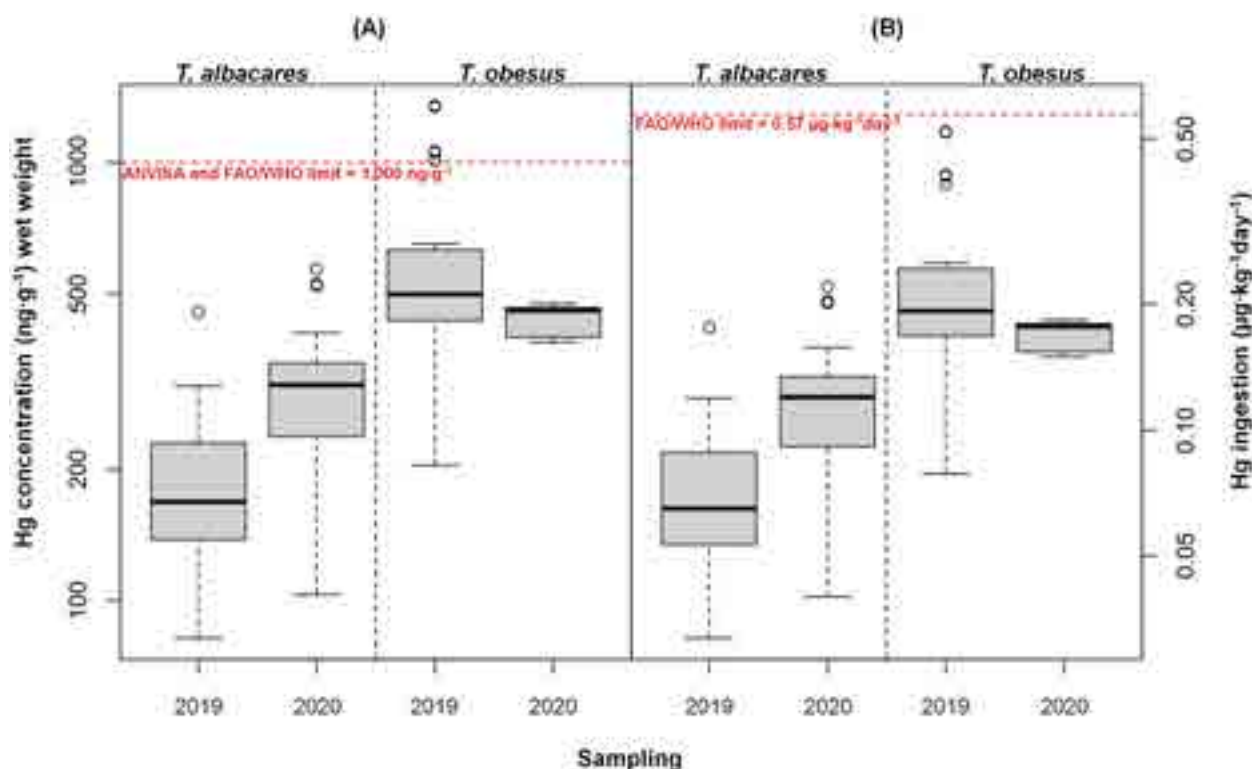


Figure 1. Medians, interquartile ranges, minimum and maximum values of Hg concentrations in *Thunnus albacares* and *Thunnus obesus* from the western equatorial Atlantic Ocean: A—Hg concentrations in muscle and the tolerable maximum limit established by ANVISA and FAO/WHO (dotted red line and value inside) | B—Hg intake estimates by adults and the tolerable intake limit (dotted red line and value inside). Open circles are outliers.

2011; Lacerda et al. 2017).

The Hg concentrations were below the maximum tolerable limits established by ANVISA (2021) and FAO/WHO (1991) for fishery products, except for five *T. obesus* whose concentrations were higher than the limit (1,000 ng g⁻¹) (Figure 1A). The estimates for Hg intake due to tuna consumption were below the tolerable intake limit established by the FAO/WHO (Figure 1B). This result was expected since the Brazilian *per capita* intake of fishery products is half of the world intake (9.75 kg·year⁻¹ x 20.5 kg·year⁻¹) (Barone et al. 2017; FAO 2020).

Currently, Brazil exports whole large tuna to Indonesia, Vietnam and the United States (<https://www.volza.com/exports-brazil/brazil-export-data-of-whole+tuna>). Considering that whole large tuna are exported to other countries, it is important to conduct case-by-case health risk assessments. In this sense, tuna consumption from the western equatorial Atlantic Ocean would pose a risk for human populations that ingest more than 80 g·day⁻¹ tuna meat (IR based on our most contaminated fish: *T. obesus* individuals caught in 2019). Both tuna species are safe for intake as seafood, at least in the present, but we recommend regular monitoring of both consumption rates and Hg levels,

since the encouragement of seafood consumption has increased worldwide, as well the anthropic pollution that reaches the ocean basins.

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from Uttar Pradesh and Uttarakhand through to Nepal, northern West Bengal and Sikkim and then to western Assam and the forested valleys of Bhutan, which are below 1,100 m (Duckworth et al. 2015). The eastern limit of its range is through western Assam (Sankar & Acharya 2004) to the Sunderbans of West Bengal (India) and Bangladesh (Duckworth et al. 2015) and Sri Lanka is the southern limit (Schaller 1967). Chital occurs sporadically in the forested areas throughout the rest of the Indian peninsula (Sankar & Acharya 2004). Within Bangladesh, it currently exists only in the Sundarbans and some ecoparks situated around the Bay of Bengal, as it became extinct in the central and northeastern parts of the country (Duckworth et al. 2015). Introduced populations also occur within Andaman & Nicobar Islands. Chital is listed as 'Least Concern' on the IUCN RedList of Threatened Species because they possess a very wide range. The population is declining outside protected areas. Although they are widely distributed across India, there are no record of Chital from the Great Indian Bustard (GIB) Sanctuary, Maharashtra.

Study Area

The study area lies in the Deccan landscape which is a large plateau in western and southern India. The landscape is semi-arid region of India and receives very less rainfall which makes it suitable for GIB. The summer season, lasting from mid-February to mid-June (Habib 2007), is very dry and extremely hot, with temperatures regularly exceeding 48°C. The Great Indian Bustard Sanctuary, established in 1979, is a wildlife sanctuary for the Great Indian Bustard *Ardeotis nigriceps* at Solapur Maharashtra, India. The sanctuary is spread over seven talukas: Mohol, Mhada, northern Solapur, Karmala, Nevasa, Karjat, and Shrigonda. The original spread of the GIB Sanctuary was 8,469 km², which has been reduced to 1,222.61 km², including reserved forest, Gairan lands, and private lands (including grasslands) in 2011. This vast grassland is home for many resident wildlife species and a variety of migratory species, along with the GIB. The major floral species are *Azadirachta indica*, *Acacia nilotica*, *Ziziphus* spp., *Glericidia sepium*, *Hardwickia binata*, & *Albizia lebbek* and the prominent grasses are *Aristida funiculata*, *Aristida stocksii*, *Chrysopogon fulvus*, *Heteropogon contortus*, *Lodhopogon tridentatus*, & *Melanocentris jacquemontii* (Habib 2007). Also, the sanctuary has a good population of Blackbuck, Indian Wolf, Indian Fox, Golden Jackal, and Jungle Cat. There has been no previous record of the Spotted Deer from any part of the sanctuary.

MATERIALS AND METHODS

During the field work exercise for radio collaring of Indian Grey Wolves to monitor movement in the human-dominated landscape of Maharashtra, camera traps have been placed in the Gangewadi area of the GIB sanctuary. The trails and junctions of the area were targeted and Cuddeback Ambush/C1 camera traps (<http://cuddeback.com/cameras>) were placed. Cameras were tied up on tree trunks at the height of 25–35 cm from the ground at the animal trails. The camera delay was set at multi-shot mode with a delay of 5 seconds and were active for 24 hours.

RESULTS

Over the survey period, species photo-captured included the Indian Grey Wolf, Indian Fox, Jungle Cat, Black Buck, Wild Boar, porcupine, and Black-Naped Hare. A male Spotted Deer was captured by a single camera trap (17.83240°N, 76.00439°E) on 30 December 2020 at 0517 h (Image 1). This is the first record of Spotted Deer in the grassland ecosystem of Solapur region of Maharashtra (Image 1).

DISCUSSION

The Spotted Deer is endemic to southern Asia (Schaller 1967) and found in dry deciduous, moist deciduous, thorn forest, and mangroves. As per the IUCN RedList, the distribution data show that Spotted Deer are present in the entire state of Maharashtra. They are found almost exclusively in dry and mixed deciduous forest habitat intermixed with grasslands. They are most commonly associated with a mixture of forest and more open grass-shrub, but they occupy a wide range of habitats throughout their native range, often avoiding rugged terrain (Anderson 1999). It is one of the most common prey species for carnivores in the forest ecosystem. Carnivores that may prey upon Chital in the GIB Sanctuary include Indian Wolf *Canis lupus pallipes*. The sanctuary is dominated by a matrix of grasslands, barren lands and agricultural land, with small patches of *Azadirachta* sp. and *Gliricidia* sp. plantation. The sanctuary has long record of research activities on various flora and fauna (Kumar 1988; Rahmani 1988; Habib 2007; Habib & Kumar 2007; Kumar & Rahmani 2008; Vanak & Gompper 2010; Janakiraman & Jalal 2015; Varghese et al. 2016; Khan et al. 2019) but there is no earlier record of the Spotted Deer. The present work is the first record of Spotted Deer from this region. In the surrounding of the sanctuary various other wildlife sanctuaries are present. The closest sanctuary which has Spotted Deer population is Nayangaon Mayur Wildlife

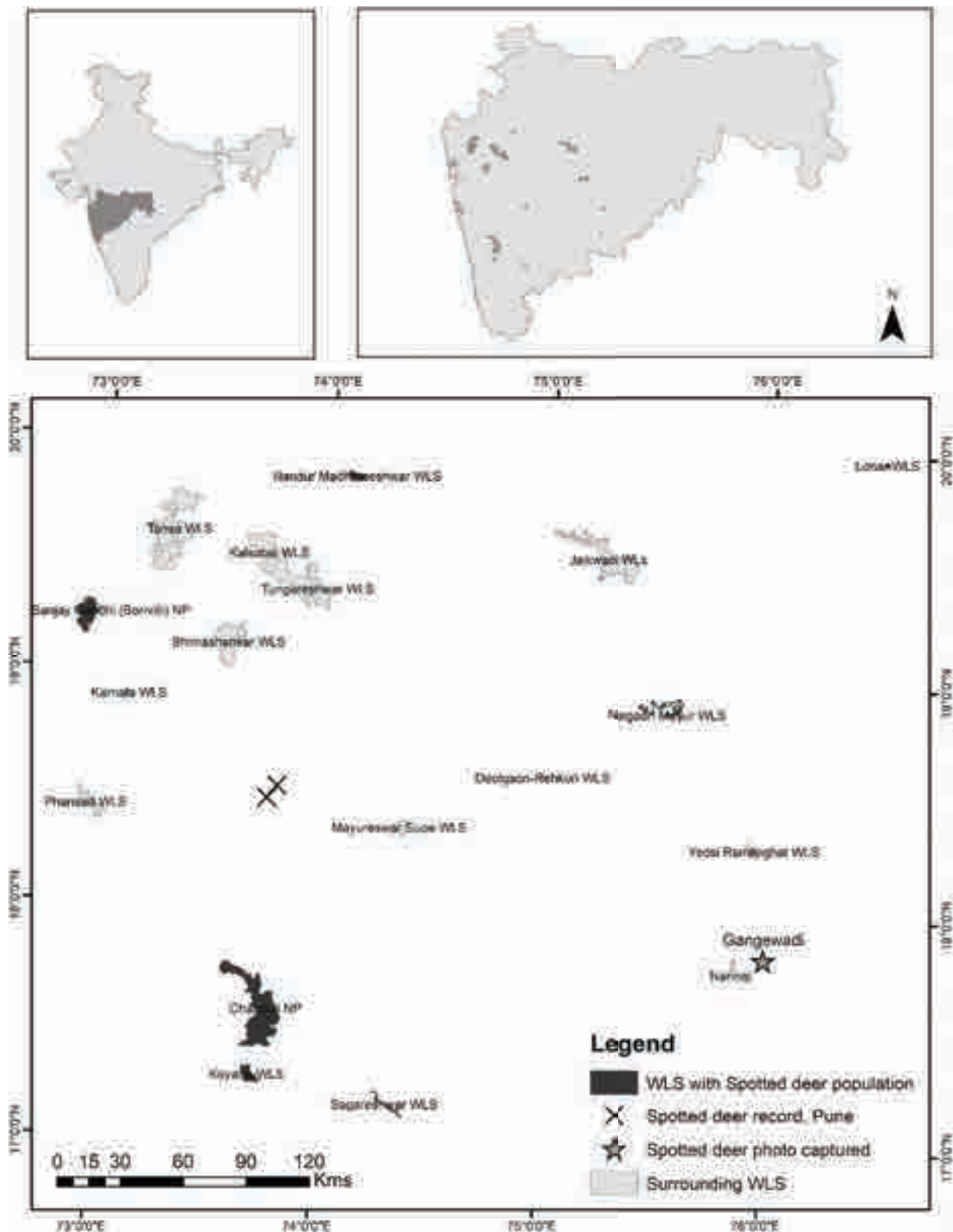


Figure 1. The location of the first photographic evidence of Spotted Deer *Axis axis* from Great Indian Bustard Sanctuary (marked with star symbol) along with the record of dead Spotted Deer from Pune (marked with cross in 2016 and 2017). The solid black colour polygons are the PAs where Spotted Deer population is present and the light grey polygons show surrounding PAs. Topleft: map of India showing the state of Maharashtra (topright), showing the PAs of Maharashtra around Great Indian Bustard Sanctuary. Bottom figure shows protected areas and Spotted Deer presence around the sanctuary along with the photographed location of Spotted Deer in Gangewadi area of the sanctuary.



Image 1. First photographic record of Spotted Deer *Axis axis* from Great Indian Bustard Sanctuary, Maharashtra.

Sanctuary (WS) (Show as symbol and name in legend in bottom map of Figure 1) which is about 124 km away from the photo-captured point. The other close by sanctuaries are Sagareshwar WS (190 km), Lonar WS (240 km), Nandur WS (305 km), and SGNP (356 km), where Spotted Deer population is present (Figure 1). There have been a few earlier records of Spotted Deer from Pune district (250 km away from Nannaj Bustard Sanctuary). In 2016, a dead male Spotted Deer was found at dumping site in Warje, Pune (The Golden Sparrow 2016) and in 2017 a male was killed by dogs in Khadakwasla area of Pune (Phadnis 2017). These two areas are close to each other and surrounded by forested area. Each year Pune division of the state forest department conducts waterhole census in four wildlife sanctuaries: Nannaj Bustard Sanctuary (10 km; part of GIB Sanctuary as Gangewadi area), Bhimashankar (292 km), Rehekhuri (145 km) and Mayureshwar (178 km). In the census during year 2021 no Spotted Deer was recorded from the above given wildlife sanctuaries, and the species was never recorded from Solapur district. This is the first wild record of Spotted Deer here. The other ungulates recorded from the Solapur region, including the GIB Sanctuary are Black Buck *Antelope cervicapra*, Chinkara *Gazella bennettii*, and Wild Boar *Sus scrofa*.

Systematic studies are necessary to assess whether populations of *A. axis* have started colonising the area or are using the area as a corridor. This data may support actions for conservation of regional biodiversity.

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Comparative study of morphology and keratin levels in hair from deer and goat

Sangeeta Patle¹, Divya Bagchi² & K.P. Singh³

¹Department of Forensic Science, Mangalayatan University Jabalpur, Madhya Pradesh 482001, India.

²Department of Biological Science Ranidurgavati University Jabalpur, Madhya Pradesh 482001, India.

³School of Wildlife Forensic and Health at Nanaji Deshmukh Veterinary Science University Jabalpur, Madhya Pradesh 482001, India.

¹sangeetapatle22@gmail.com (corresponding author), ²dbagchi_2000@yahoo.com, ³kpsinghbaghel@yahoo.com

Abstract: Hair is a defining character of mammals. In the present study, the hair samples of Chital *Axis axis*, Sambar Deer *Rusa unicolor*, and goat *Capra hircus* were collected from the back, neck, abdomen and tail regions of carcasses brought to the forensic laboratory for necropsy examinations. Cross-sections of hair, cuticle scale, and medullary patterns were analyzed to establish indices for species identification. Keratin levels were also analyzed by protein electrophoresis (SDS-PAGE). We determined that both microscopic and SDS-PAGE analysis of guard hair is useful for identifying species, particularly in forensic applications.

Keywords: *Axis axis*, *Capra hircus*, domestic animals, guard hair, protein electrophoresis, *Rusa unicolor*, SDS-PAGE, wild herbivores.

Abbreviations: kDa–kilo Dalton | MALDI-TOF–matrix-assisted laser desorption/ionization–time of flight | PMF–peptide mass fingerprinting | SD–standard deviation | SDS-PAGE–sodium dodecyl sulphate

No other animal possesses hair except mammals, and these hairs have the capability to resist putrefaction and may keep unpreserved for a long time. Hair being the most common biological material found at the scene of a crime, plays a crucial role in criminal

investigations related to wildlife, taxonomy, investigative dermatology, pathology, and other fields of forensic science (Sahajpal et al. 2009; Bahuguna et al. 2010). Guard hairs are usually procured for wildlife forensics, particularly species identification of wild animals (Tridico 2005; Knecht 2012). The hair has three internal parts: cuticle, cortex, and medulla, covered with a thin coating of derived proteins and tilted scales. Hair coloring is based on the presence of keratin protein in the hair cortex, scales of keratin overlapped by the cuticle layer (Deedrick & Koch 2004). The high content of cysteine and dead keratinocytes helps to protect the hairs from putrefaction and keep its chemical composition intact (Knecht 2012). Studies on human hair keratin show that it constitutes approximately 80% of the total mass of the hair and consists primarily of keratins having 40–65 kDa (molecular weight) and 6–30 kDa keratin-associated with proteins (KAPs) and may be isolated using SDS-electrophoresis (Gillespie 1990; Langbein et al. 2001; Nakamura et al. 2002). There are two subfamilies of

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keratin protein, type I (acidic; 40–50 kDa) and type II (neutral/basic; 55–65 kDa). These keratin proteins are also classified into high-sulfur proteins, ultra-high-sulfur proteins and high-glycine / tyrosine proteins based on their amino acid content (Fuji et al. 2013). Meager information is only available about the comparative morphology of guard hairs of domestic goats and deer families. Thus in many instances, poachers get the advantage of insufficient evidence of poaching for uncertainty regarding the seized hair, whether it belongs to goat or deer family. However, since illustrative research on morphological aspects of ungulates and carnivores has been done at the Wildlife Institute of India, the present study focused on the analysis of the hair of wild herbivores to generate hair index to identify and differentiate between the hair of domestic and wild animals for forensic uses. This will be helpful in the prosecution and conviction of poachers to overcome the wildlife crime.

MATERIALS AND METHOD

Hair samples are regularly brought to the School of wildlife forensic and health, NDVSU, Jabalpur, to identify whether the seized hair belongs to a wild animal or not. In the present study, hair samples of Chital *Axis axis*, Sambar Deer *Rusa unicolor*, and goat *Capra hircus* were collected and processed for identification and differentiation for forensic uses. Histological study of the hair cuticle, scale pattern, type of medulla, medullary index, and cross-section morphology was performed in the present study (Table 1) following the standard protocols of Trimori et al. (2018). The hairs of each animal's dorsal and ventral regions were collected in a sterilized container, washed separately using 95% ethanol, and dried before further analysis. Hair samples were examined under a light microscope after whole-mount and scale cast preparation.

Microscopic examination of hair

The cuticle scale pattern was examined using the nail polish method described by Brunner & Coman (1974). The nail polish method is very convincing and quick. For cuticle scale examination, nail polish was spread on a clear glass slide and hair was placed on it and kept until dried. Then the hair was removed, and the impression was examined under a compound microscope at 40x magnification. The cuticle scale pattern was also examined using the gelatin casting method described by Cornally & Lawton (2016). For this, 20% gelatin was mixed in boiling water, and a thin gelatin film was spread on a clean glass slide. The hair shafts were superficially

placed in the gelatin film and left at room temperature overnight. The hairs were subsequently removed, leaving the scale imprint on the gelatin cast, which was examined under the microscope. Further, the same cleaned and washed hairs were kept in xylene for 72 h before examination of the medullary pattern under the compound microscope. The camera lucida drawings were made to compare the cuticular and medullary patterns of deer and goats.

Extraction of keratin

Guard hair of Chital, Sambar, and goat were washed with ethanol and a mixture of chloroform-methanol (2:1, v/v) for 24 h to remove lipid molecules on the surface of the hair. The washed hair (20 mg), dispensed in a solution (5 ml) containing 25 mM tris-HCl, pH 8.5, 2.6M thiourea, 5M urea and 5% 2-mercaptoethanol (2-ME), was kept at 50°C for 48 h in a hot air oven. The mixture was filtered using a muslin cloth and centrifuged at 15000 rpm for 20 min at room temperature. The light to dark brownish supernatant was further processed following the protocols of Nakamura et al. (2002). The Protein amounts were estimated using Bradford colorimetric method, and further SDS-PAGE electrophoresis process was done at a refrigerated temperature of 40°C to protect the electrophoresis chamber from excess heat. To differentiate the hair matrix protein (HMP) area by the position and intensity of the polypeptide band, the isolated proteins gel was stained with 0.1% Coomassie brilliant blue R-250 (dissolved in 10% acetic acid and 40% ethanol) for 24 h, then de-stained by adding acetic acid, methanol, and distilled water (1:3:6 ratio) following the method of Folin et al. (1996).

RESULTS

The cuticle pattern of the wild herbivores Chital and Sambar are smooth and irregular, whereas, in the goat, it is rough with a marginal gap within the cuticle and medulla. The margin and distance between the cuticular pattern and medullary pattern of the hair from the various regions, including proximal and distal regions, were also examined (Table 1), and it was seen that the cuticle scale pattern varied from species to species. While the medullary pattern of both the domestic and wild herbivores looks similar, the goat's hair medulla was a more compact mass than that of chital and sambar (Image 1, Table 2). The keratin extracted through SDS-PAGE revealed no remarkable differences between protein bands (40–65 kDa) of wild and domestic herbivores (Image 2).

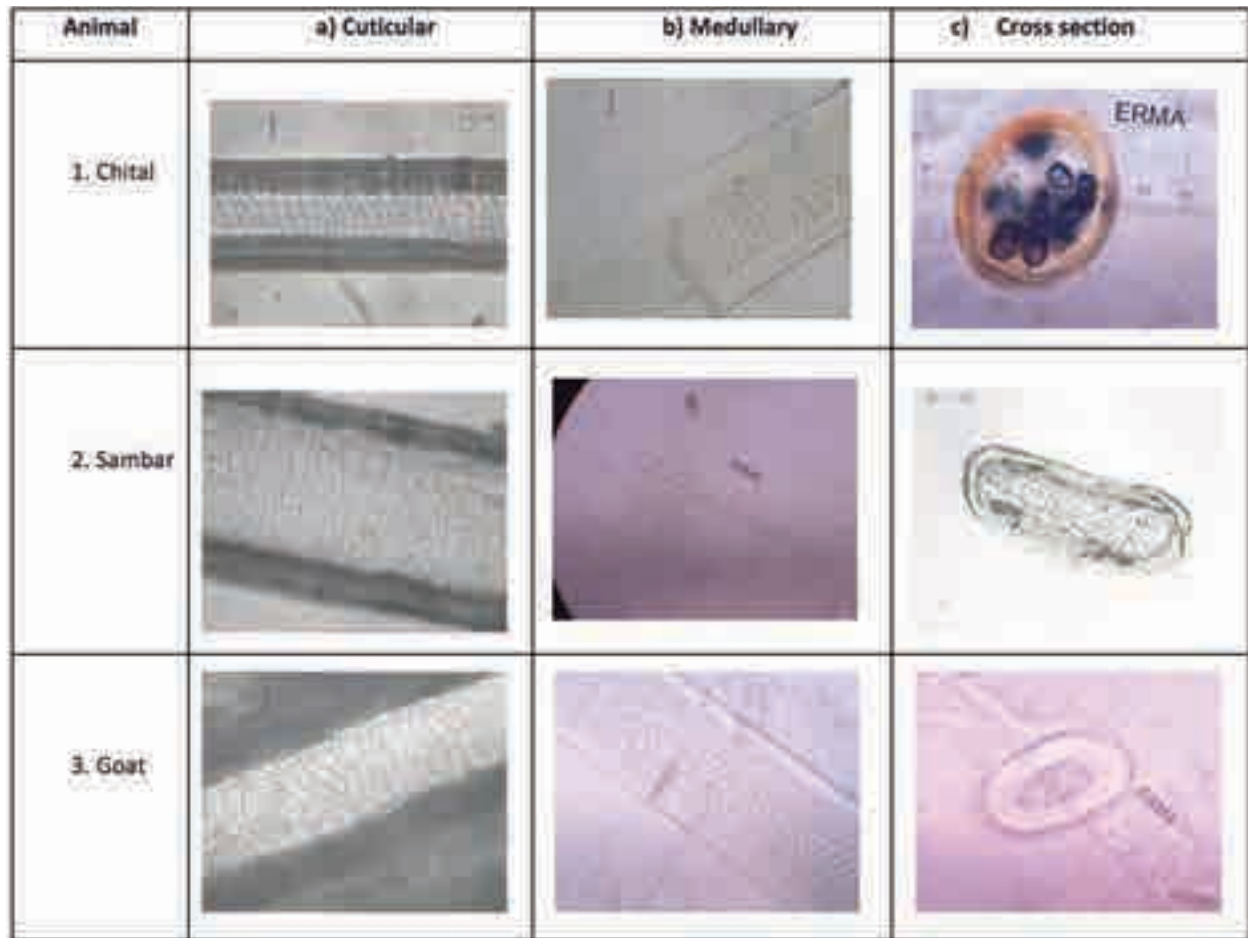


Image 1. Hair of wild and domestic herbivores.

Microscopic images of different hair: 1–Chital | 2–Sambar | 3–Domestic Goat showing (a) cuticular pattern, (b) medullary pattern, and (c) transverse section of hair using Leica DM 3000 compound microscope(40 X).

Table 1. Micrometry of wild and domestic animal's hair.

	Species	Hair length (mm)		Diameter of hair T.S. (mm)		Cuticle scale pattern			Medullary pattern
		Max	Min	Max	Min	Margin	Distance	Pattern	
1	Chital	30	15	0.087	0.025	Regular wave	Distant	Smooth	Multicellular in rows Cloisonné
2	Sambar	96	23	0.077	0.012	Rippled	Near	Irregular	Multicellular in rows Cloisonné
3	Goat	40	15	0.10	0.005	Irregular	Close	Rippled	Packed with cell

DISCUSSION

The results of the present study showed that the irregular pattern of hair cuticle has distinctive characteristics for certain animals sufficient to determine its origin. The distribution of the medulla is also an important characteristic feature; the medulla along the hair shaft differs in its continuous or discontinuous texture, showing species to species variations. In the present study, the hair index value of the goat was found

greater than that of the Chital and Sambar, the values varying between 92.5 ± 0.100 – 44.6 ± 0.200 , (44.4 ± 0.100) mean $\pm$ SD.

Keratin proteins and their variations have also opened a means to recognize species through keratin protein molecular weight. SDS-PAGE technique helps to isolate the protein that can be validated using the western blot technique with specific antibodies raised in a particular species and by two-dimensional gel

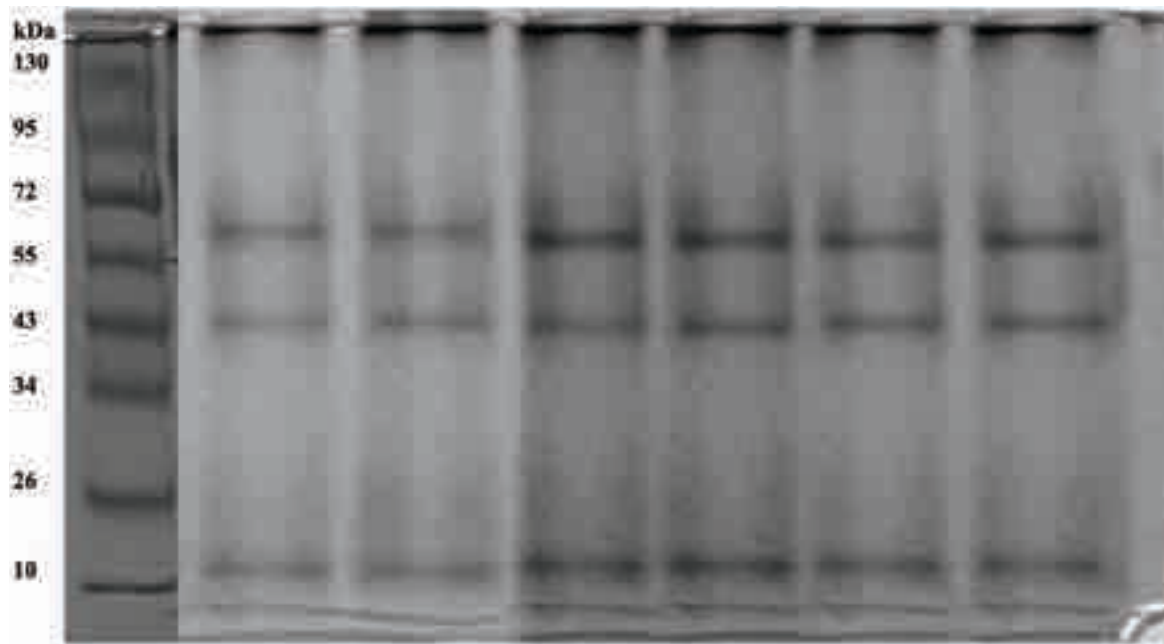


Image 2. Isolated keratin pattern of Chital *Axis axis*, Sambar *Cervus unicolor*, and goat *Capra hircus* by SDS-PAGE. SDS-PAGE data represent the isolation pattern of keratin protein based on their molecular weight of Chital, Sambar, and goat. Keratin separation was done in between 40–60 kDa.

Table 2. Hair index of wild and domestic animal's hair.

Species	Chital	Sambar	Goat
Scale count index	9.72–9.90 (9.81±0.100)	1.58–1.56 (1.57±0.010)	6.04–6.0 (6.02±0.020)
Medullary index	0.83–0.82 (0.825±0.005)	0.92–0.94 (0.93±0.010)	0.51–0.52(0.51±0.010)
Hair index	44.4–44.8 (44.6±0.200)	44.5–44.3(44.4±0.100)	92.6–92.5 (92.5±0.100)

electrophoresis. Nakamura et al. (2002) also reported similar results as in the present study. Another protein validation method is based on specific peptide markers by using peptide mass fingerprinting (PMF) with the MALDI-TOF technique to accurately identify amino acid sequences in a particular sample (Caroline et al. 2013; Carnally & Lawton 2016; Cortellini et al. 2019). The keratin extracted consisted of hard keratin with a molecular mass of 40–60 kDa, matrix proteins with 12–18 kDa, and minor components with 110–115 kDa & 125–135 kDa (Nakamura et al. 2002). Our study supports the fact that the keratin band separated (40–60 kDa) in the present study may be further categorized through serological tests for species identification by gel precipitation tests. The methods, morphological data, and molecular characterization may help to study the genetic variation and post-translational modification among the species in the matured keratinized tissues, hairs, and horns. The medullary index, cuticular pattern and cross-section thickness of the hair of different wild

animals and domestic animals also could be used as an identical feature for species differentiation.

CONCLUSION

The morphological study of Chital, Sambar and goat hair reveals the variations in cuticular scale pattern, medullary structure and shape of medulla visible in the cross-transverse sections. It is evident from the study that there are definite differences regarding the diameter, scale type, scale margin and medullary configuration of the dorsal guard hair of the three species. Further confirmatory species identification is also possible through species-specific antibodies that can be raised in a specific animal. The microscopic hair characteristics corroborated with keratin pattern studies are a competent basis for species identification and successful implementation of the Indian Wildlife (Protection) Act 1972 as scientific evidence for prosecution and conviction of wild animal poachers.

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Is trade the reason for the unusual colour morph of Cobra from Goa? Response to Sawant et al.

Raju Vyas¹ & Harshil Patel²

¹Shashwat Apartment, 23 Anandnagar Society, BPC Road, Alkapuri, Vadodara, Gujarat 390007, India.

²Thackeray Wildlife Foundation, Mumbai, Maharashtra 400051, India.

¹razoovyas@hotmail.com (corresponding author), ²harshilpatel121@gmail.com

Recently, Sawant et al. (2022) published a record of an unusual colour morph of the Indian Cobra *Naja naja* (Linnaeus, 1758), based on a specimen rescued from Modelo wado, Assonora (15.618°N; 73.897°E), Goa, India. Also, the authors provided three coloured figures, and some basic scalation data for this interesting case of the colour morph of a cobra. The colour description was provided as the dorsal body colour is brownish-black and brownish-grey on the ventral side, and with a scarcely visible spectacle mark on the hood. Sawant et al. (2022) state that the colour morph usually inhabits northwestern India (Whitaker & Captain 2004), and there is less chance that such animal would have come through transportation from the region where they are commonly found; hence, the authors interpreted this as the first report of such pigmentation in the species, which they reported for the first time from the region.

The record of this unusual colour morph and published images of Indian Spectacle Cobra by Sawant et al. (2022) indicates that the specimen is not from Goa but elsewhere, most probably from states of northwestern India. Such typical colour morph of cobra is found widely in parts of Gujarat State (Patel et al. 2019b), and published cobra images show it is not a typical normal specimen, especially the head portion behind the eyes. In a typical specimen of the species, this portion is slightly developed and appears like a bulge/swelling

because it is the site of the venom gland in the species (Image 1). This portion is not enlarged but depressed in the published images of the cobra (see Images 1–3 of Sawant et al. 2022). Such cobra is usually found in snake charmers' baskets because snake charmers remove fangs and the venom glands from that portion, which later results in a depression in the temporal region. Also, the scalation data provided by the authors (Sawant et al. 2022) is not complete and not at par with that of which was used by researchers for the genus *Naja* (see Wüster 1998). Sawant et al. (2022) provide the dorsal scalation as counted at neck, midbody, and tail; however, the authors do not provide the scales around the hood, which is an important count while studying the genus. Wüster (1998) has provided the dorsal scales at midbody of the northwestern population of *N. naja* as 19–21 scales and the rest of the Indian population have 23–25 scales. The specimen reported by Sawant et al. (2022) also had 21 dorsal scales at midbody, falling in the range of the northwestern population. Sawant et al. (2022) have said that cunate scale is present on both sides but did not provided the numbers of cunate scales; based on the images provided (Figure 2 of Sawant et al. 2022), the specimen had one cunate scale on the right side and two cunate scales on the left side. Sawant et al. (2022) have reported that the specimen had 25 undivided subcaudal scales. However, the subcaudal scales range reported in

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literature is 50–67 (Wüster 1998). The subcaudal count reported by Sawant et al. (2022) is very less but they fail to mention any plausible reason for the same; we believe that the specimen reported by them had incomplete tail. Wüster (1998) had provided a detailed account of the species along with the morphological variations of different populations; however, Sawant et al. (2022) did not refer to this publication and relied solely on a field guide for their observation. Field guides and popular books are useful for preliminary observations and may help in identification of a species but when it comes to scientific studies, authors should refer to scientific publications. Based on the limited data provided by Sawant et al. (2022) and studying the available literature, we believe that the specimen reported by them is a typical specimen found in the northwestern part of the country.

We believe that the specimen might have ended up in Goa via some sort of transport activity; it may have been brought illegally by some snake charmer or trader and it escaped from the snake charmer's basket or from an unknown captive facility. In recent years, several youngsters keep snakes illegally in the house as a hobby without the knowledge of the authorities. Such snakes come to the notice when they escape or the local forest officials take some legal action. In the last decade, we came across three such rescues of snakes by locals in the state of Gujarat; including two deadly venomous snakes (Banded Krait *Bungarus fasciatus* and Monocled Cobra *Naja kaouthia* [Image 2]) and a non-native species (Ball Python *Python regius* [Image 3]). Details of the rescue of such cases are provided in Table 1. All such instances of non-native species are a result of either escape from custody or pet trade or coming through some sort of transportation. The two venomous snakes belonging to the family Elapidae are found in the new urban areas, out of their natural distribution range, requires immediate attention due to their medical importance (Whitaker & Martin 2015)

The best example of illegal suspected activity in the state is confiscated partial albino specimen of Indian Red Sand Boa *Eryx johnii* along with a few normal morphs of the same species at Surat railway station from snake charmer of Rajasthan (Vyas et al. 2012; Parmar & Kaiser 2022). This incidence indicates the activity of illegal keeping and transporting snakes from one state to another.

However, the records of three non Indian reptiles from Gujarat show such invasive species distributed in Gujarat are the result of anthropogenic activities, including the Robust Rock Gecko *Hemidactylus robustus*



Image 1. Hood of the dark morph of the Indian Spectacle Cobra *Naja naja*, the posterior eye portion is always well developed and bulges due to venom glands.



Image 2. An adult Monocled Cobra *Naja kaouthia* rescued from the urban area of Bharuch city, Gujarat, India. © Nitin Bhatt.



Image 3. A non-native species Ball Python *Python regius* rescued from the urban area of Vadodara city, Gujarat, India. © Harshil Patel.

Table 1. Details of significant snake species recorded from Gujarat, India, being a non-native form and species.

	Common and scientific name	Age group	Date	Name of location site of record	Native distribution range	Source
1	Banded Krait <i>Bungarus fasciatus</i>	Adult	25 January 2013	Sarkhej, Ahmadabad City,	The states of northern and eastern India	Sivbhadrasinji Jadeja
2	Monocled Cobra <i>Naja kaouthia</i>	Adult	09 February 2013	Bharuch City, Bharuch District	Northern and eastern India	Nitin Bhatt
3	Ball Python <i>Python regius</i>	Juvenile	17 May 2021	Manjalpur, Vadodara City, Vadodara District	Western and central Africa	Nitin Patel

(Bauer et al. 2012), Striped Bronzeback Tree Snake *Dendrelaphis caudolineatus* (Patel et al. 2019a), and Red-eared Sliders *Trachemys scripta* (Munjpura 2014; Patel & Vyas 2019; Vyas 2019).

The Robust Rock Gecko is present in the new seaport complex at Porbandar, Gujarat. This gecko species was earlier known as *Hemidactylus porbandarensis* (Sharma 1981), and its DNA sequences indicated that it was in fact an introduced population from Abu Dhabi (Bauer et al. 2012). The Striped Bronze-back Tree Snake *Dendrelaphis caudolineatus* is a native species of Thailand to Sundaland (Peninsular Malaysia, Sumatra, Billiton, and Borneo), but a single specimen was found in an urban industrial complex from Udhana, Surat, India (Patel et al. 2019b). The Red-eared Slider is a native species of the eastern United States, but now it is widely found in many freshwater habitats of India, thanks to popular pet demands in national and international markets (Vyas 2021). Two of them, the gecko and the turtle, are now well established and breeding in the natural habitats in India.

The state of Goa is situated on the west coast of India, a maritime state and a well-known tourist destination for many national and international tourists. There were reports of invasive turtles found in the freshwater habitat of Goa (Jadav et al. 2018), which supports our prediction that the cobra would be the result of escape from the captive condition. So the unusual colour morph cobra is not a case of higher melanism in that individual snake as quoted by Sawant et al (2022), but it is a result of some illegal anthropogenic activities in the area.

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Corrections to 'An unusual morph of *Naja naja* (Linnaeus, 1758) from Goa, India (Serpentes: Squamata)'

Nitin Sawant¹, Amrut Singh², Shubham Rane³, Sagar Naik⁴ & Mayur Gawas⁵

^{1,3,4,5}Department of Zoology, Goa University, Taleigao Plateau, Taleigao, Goa 403206, India.

²Animal Rescue Squad, Bhaili peth, Bicholim, Goa 403504, India.

¹nitin.sawant@unigoa.ac.in (corresponding author), ²amrutsnake@gmail.com, ³shubhamrane7036@gmail.com,

⁴sagarnaik1018@gmail.com, ⁵mithilgawas0987@gmail.com

An unusual morph of *Naja naja* from Goa (Sawant et al. 2022) was published recently to which Vyas & Patel (2022) have raised various concerns. In response, we wish to agree to the fact that there has been a clear oversight of an important reference and gaps in data collection which led to the misinterpretation. As pointed out by Vyas & Patel (2022) it could very well be a case of illegal wildlife trade for reasons such as photography or false beliefs. We did not refer to Wüster (1998) and hence failed to confirm the proper identity of this individual. With the current findings we conclude the individual to be a northwestern Indian form of Spectacled Cobra which might have come here through illegal wildlife trafficking and later released or might escaped from captivity.

There have been no reported cases of snake charmers involved in illegal trade of Spectacled Cobra in the state of Goa. Hence, we discarded the chances of this case could be of such illegal trade. Though we fail to

provide any conclusive remarks on the surprisingly low subcaudal count, we would like to confirm that the tail was complete as it tapered and ended into a terminal scute. The snake was in the custody of the Goa Forest Department, and was examined for a brief period of time. Apart from the dorsal, ventral, and the sub caudal scales, rest of the scale count was performed by taking closeup photographs of all the aspects of the body.

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Dr. Hemanta Kafley, Wildlife Sciences, Tarleton State University, Texas, USA

Other Disciplines

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Dr. Mandar S. Paingankar, University of Pune, Pune, Maharashtra, India (Molecular)
Dr. Jack Tordoff, Critical Ecosystem Partnership Fund, Arlington, USA (Communities)
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Communications

A preliminary survey of moss flora of Chail Wildlife Sanctuary, Himachal Pradesh, India

– Meenal Sharma, Anju Rao & S.S. Kumar, Pp. 22207–22214

New distribution record and DNA barcoding of *Sapria himalayana* Griff. (Rafflesiaceae), a rare and endangered holoparasitic plant from Mizoram, India

– Laldinfeli Ralte, Hmingremhlua Sailo, Sagolshem Priyokumar Singh, Laldinliana Khiangte & Y. Tuginba Singh, Pp. 22215–22220

Species distribution modeling of a cucurbit *Herpetospermum darjeelingense* in Darjeeling Himalaya, India

– Debasruti Boral & Saurav Moktan, Pp. 22221–22231

An updated catalogue of true flies (Insecta: Diptera) from northern Pakistan

– Noor Fatima & Ding Yang, Pp. 22232–22259

Desert Carabidae (Insecta: Coleoptera) of India

– S.V. Akhil, Sabu K. Thomas & Sanjeev Kumar, Pp. 22260–22269

Photographic evidence of fish assemblage in artificial reef site of Palk Bay - an implication for marine resource management

– Koushik Sadhukhan, T. Shanmugaraj, Ramesh Chatragadda & M.V. Ramana Murthy, Pp. 22270–22276

Systematics of the enigmatic and narrowly endemic toad genus *Bufo* Pillai & Yazdani, 1973: rediscovery of *Bufo kemp* (Boulenger, 1919) and expanded description of *Bufo meghalayanus* (Yazdani & Chanda, 1971) (Amphibia: Anura: Bufonidae) with notes on natural history and distribution

– R.S. Naveen, S.R. Chandramouli, Gautam Kadam, S. Babu, P.V. Karunakaran, H.N. Kumara & N. Parthasarathy, Pp. 22277–22292

Avifaunal diversity in Indian Institute of Technology Guwahati Campus, Assam, India

– Umang H. Rathod & Rupam Bhaduri, Pp. 22293–22308

Reviews

Threatened flora of Uttarakhand: an update

– D.S. Rawat, Satish Chandra & Preeti Chaturvedi, Pp. 22309–22328

A systematic review on the feeding ecology of Sloth Bear *Melursus ursinus* Shaw, 1791 in its distribution range in the Indian subcontinent

– Vasantkumar Rabari & Nishith Dharaiya, Pp. 22329–22336

Short Communications

Mercury in tuna from the western equatorial Atlantic Ocean and health risk assessment

– Ana Paula Madeira Di Benedetto, Inácio Abreu Pestana, Igor David da Costa, Marcelo Gomes de Almeida, Bráulio Chereze Vaz de Oliveira & Carlos Eduardo de Rezende, Pp. 22337–22340

First photographic record of Spotted Deer *Axis axis* (Erxleben, 1777) (Artiodactyla: Cervidae) in Great Indian Bustard Sanctuary, Maharashtra, India

– Shaheer Khan, S. Ramesh Kumar & Bilal Habib, Pp. 22341–22345

Comparative study of morphology and keratin levels in hair from deer and goat

– Sangeeta Patle, Divya Bagchi & K.P. Singh, Pp. 22346–22350

Response & Reply

Is trade the reason for the unusual colour morph of Cobra from Goa? Response to Sawant et al.

– Raju Vyas & Harshil Patel, Pp. 22351–22353

Corrections to 'An unusual morph of *Naja naja* (Linnaeus, 1758) from Goa, India (Serpentes: Squamata)'

– Nitin Sawant, Amrut Singh, Shubham Rane, Sagar Naik & Mayur Gawas, P. 22354

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