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Research Articles

Natural History Note

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TYPE Review Article

Diversity of Caryophyllaceae Juss. in Uttarakhand (India)

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Abstract

In this paper, we reviewed the records of Caryophyllaceae family of flowering plants reported from Uttarakhand state of India. Family Caryophyllaceae Juss. in Uttarakhand is represented by 24 genera 75 species, 2 subspecies and 6 varieties. Based on the herbarium history, literature survey and additional observations by the authors, 11 taxa were excluded from the flora of Caryophyllaceae of Uttarakhand. *Cherleria sedoides* was reported for the first time from India and Uttarakhand. Hitherto, the misidentification of the *Cherleria sedoides* and *Cherleria biflora* in the Himalayas, and identification ambiguity between *Cerastium glomeratum* and *C. fontanum* subsp. *vulgare* were resolved. We found that *Stellaria depressa* is a new record to the flora of Uttarakhand. New combinations of *Stellaria patens* subsp. *patens*, *Stellaria patens* subsp. *semivestita* and *Sabulina kashmirica* var. *foliosa* have been proposed. Based on the taxonomic history, and observations of the live and herbarium specimens, the synonymy of *Sagina purii* for *S. apetala* was concluded. This study summarises and presents the updated flora of Caryophyllaceae family in Uttarakhand based on these new findings.

Keywords: Alpine meadows, *Arenaria*, *Cherleria*, new combination, new record, *Silene, Stellaria*

Introduction

Caryophyllaceae Juss. is a major family of Angiosperms and ranked as the 24th largest family with 93 genera and 2395 species (Thorne & Reveal 2007). Though, the number of species and genera of the family varies from different sources as Catalogue of Life (2024) reported 104 genera with 3397 species and Stevens (2024) reported 101 genera with 2625 species. The family Caryophyllaceae also known as the pink family or carnation family, occurs almost worldwide particularly in the Northern Hemisphere except for most of the wet tropics and is mainly centered in the Mediterranean area of the Old World. Members of the family Caryophyllaceae are distinctive in having nodes often swollen, with simple opposite leaves, an inflorescence of solitary flowers or dichasial cymes, and biseriate, actinomorphic, usually pentamerous flowers with distinct, clawed petals, stamen ten or lesser, obdiplostemonous, a superior ovary with distally free-central or basal placentation, and a capsular fruit opening by teeth or valve (Bittrich 1993 a; b).

With the advancement of molecular and phylogenetic techniques, a few of the very diversified genera of the family such as *Arenaria* L., *Minuartia* L., *Gypsophila* L. *Pseudostellaria* Pax, *Silene* L. and *Stellaria* L. have been re-circumscribed (Dillenberger & Kadereit 2014; Pusalkar & Singh 2015; Sadeghian *et al.* 2015; Zhang *et al.* 2017; Madhani *et al.* 2018, Sharples & Tripp 2019; Jafari *et al.* 2020). On the basis of these studies, some new genera such as *Minuartiella* Dillenb. & Kadereit, *Mcneillia* Dillenb. & Kadereit, *Himgiria* Pusalkar & D. K. Singh, *Shivparvatia* Pusalkar & D. K. Singh, *Balkana* Madhani & Zarre, *Hartmaniella* M. L. Zhang & Rabeler, *Nubelaria* M. T. Sharples & E. Tripp, *Graecobolanthus* Madhani & Rabeler, *Rabelera* (L.) M. T. Sharples & E. Tripp, *Petroana* Madhani & Zarre and *Engellaria* Iamonico have been described (Dillenberger & Kadereit 2014; Pusalkar & Singh 2015; Zhang *et al.* 2017; Madhani *et al.* 2018; Sharples & Tripp 2019, Iamonico 2021 a) and some genera such as *Cherleria* L., *Eremogone* Fenzl, *Dolophragma* Fenzl, *Facchinia* Rchb., *Odontostemma* Benth. ex G. Don, *Bolanthus* (Ser.) Rchb., Deut. Bot. Herb.-Buch, *Cyathophylla* Bocquet & Strid, *Heterochroa* Bunge, *Habrosia* Fenzl. *Sabulina* Rchb , *Schizotechium* Fenzl and *Dichodon* (Bartl. ex Rchb.) Boiss have been reinstated (Dillenberger & Kadereit 2014; Hernández-Ledesma *et al.* 2015; Pusalkar & Singh 2015; Sadeghian *et al.* 2015; Pusalkar & Srivastava 2016; Madhani *et al.* 2018; Iamonico 2021 b). Lidén (2018) and

Lidén & Oxelman (2023) correctly identified many erroneous identifications of *Silene* species in the Himalayan region.

The Himalayan mountainous area can be broadly divided into two biogeographic zones: the Trans-Himalayas and the Himalayas (Rodgers & Panwar 1988). The Himalayan region also harbours an enormous diversity of the family. There are nearly 29 genera and 150 species of the family are present in the Himalayan areas. Out of 150 species, 37 species (24.5%) are endemic to the Himalaya and 59 species (39.3%) are endemic to the Himalaya and adjacent Tibet (Xizang) (Chandra 2016). This study systematically evaluated the documented records of the Caryophyllaceae family of angiosperms within the state of Uttarakhand. Uttarakhand, by large, lies in the Himalayan region with the northernmost parts falling in the Trans-Himalayas (Rodgers & Panwar 1988). By conducting a meticulous review of published records augmenting with the authors' own observations, we aimed to provide a comprehensive overview of the species richness and distribution of the Caryphyllaceae plant family in this ecologically significant region.

Material and Methods

The study was carried out in the Himalayan state of Uttarakhand, India. It comprises of diverse topographies and habitats from plains and *Terai* regions in the south and east to dense forests and snow-covered peaks of the Himalayas in the north and west, facilitating plants of the family Caryophyllaceae flourish well in the state. For the present study, a detailed literature survey was carried out of various floras and checklists, *viz.*, Edgeworth & Hooker (1874), Williams (1898), Smith & Cave (1911), Shishkin (1936), Walters (1964), Majumdar & Giri (1983) Chowdhery & Wadhwa (1984), Grierson (1984), Ghazanfar & Nasir (1986), Majumdar (1993), Hajra *et al.* (1996), Srivastava (1998), Press *et al.* (2000), Majumdar (2002), Uniyal *et al.* (2007), Gogoi *et al.*, (2021). Regional floras such as Babu (1977), Duthie (1906), Gaur (1999), Gupta (1968), Naithani (1984), Pusalkar & Singh (2012), Pusalkar & Srivastava (2018), Raizada & Saxena (1978), Rana *et al.* (2003) were consulted. Various research papers, *viz.*, McNeill (1962), Pusalkar & Singh (2015), Pusalkar & Srivastava (2016), Lidén (2018) and Lidén & Oxelman (2023) *etc.* were also consulted. The threatened status of the species was assessed after Nayar & Shastry (1987-90) and Rao *et al.* (2003). A total of 73 research articles/reviews/books were consulted for the present work.

For the correct identity of the species protologues of the species were observed, and POWO (2024) was followed for accepted names. Herbarium specimens of Western Himalayan species housed in the herbaria of Botanical survey of India, Northern Circle, Dehradun (BSD), Hemwati Nandan Bahuguna Garhwal University, Srinagar, Garhwal (GUH), Forest Research Institute, Dehradun (DD), Wildlife Institute of India (WII), Kumaon University Nainital, Central National Herbarium (CAL) and Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India (GBPUH) were consulted. However, for type specimens and original collections of the species virtual herbarium as Kew Herbarium (http://apps.kew.org/herbcat/gotoHomePage.do) and Tropicos herbarium (https://www.tropicos.org/) were consulted.

Results and Discussion

Our review concluded that family Caryophyllaceae in the Uttarakhand is represented by 24 genera 75 species, 2 subspecies and 6 varieties. *Silene* L. is the largest genus of the family represented by 21 species followed by *Stellaria* L. (12 species) and *Eremogone* Fenzl (06 species). A list of genera and their respective species is provided in table 1. A detailed account of species along with their distribution, occurrence and other information is provided in appendix 1. Based on the herbarium history, literature survey and additional observations by the authors, 11 taxa have been excluded from the flora of Caryophyllaceae of Uttarakhand (appendix 2). Major findings of the present research are being presented here in details.

3.1. New Record to the Flora of India

During the study of the Caryophyllaceae of Uttarakhand, various new records have been reported by authors for the Indian and the Western Himalayan flora (Chandra & Rawat 2016; Chandra & Rawat 2017; Chandra & Rawat 2018; Chandra *et al.* 2020; Chandra & Rawat 2021). In the present study, we report *Cherleria sedoides* L. as a new record to the flora of India and Uttarakhand.

3.1.1. Collection of the *Cherleria sedoides* L. from Uttarakhand;

During a visit to the alpine Himalaya of Uttarakhand, the author (DSR) collected specimens of *Cherleria sedoides* L. from the Vasukital area of district Rudraprayag and both the authors collected specimens from the Ralam area of district Pithoragarh. The identity of the specimens was confirmed after Ghazanfar & Nasir (1986), Majumdar (1993), Halliday (1993) Dequan & McNeill (2001) Rabeler & Hartman (2005), Moore & Dillenberger (2017). The specimens were also matched photographically with the image of *Cherleria sedoides* L. provided by Moore & Dillenberger (2017).

Description of the species: *Cherleria sedoides* L., Sp. Pl. 1: 425. 1753; *Minuartia sedoides* (L.) Hiern, *Jour. Bot. (London)* 37: 321. 1899.

Herbs, perennial, caespitose, forming loose yellowish-green cushion up to 10 cm across. Taproot deep, woody stock giving rise to many prostrate stems. Vegetative branches on stem longer, 3–5 cm, spreading; flowering branches in the axil of leaves with broad sheathing base, erect, very short up to 1cm long. Leaves on vegetative shoots distant in proximal part, fascicled at distal end, opposite, connate at the base, basal tube pilose and up to 1mm long, lamina sessile, linear, entire, acute or rounded at apex, 5–7 × 0.75–1 mm, margin minutely pilose with few celled uniseriate trichomes in lower half, papillose in upper half, one nerve at base, obscurely 3 nerved in upper half; leaves on flowering shoot imbricate, concealing stem, linear–subulate, entire, acute or obtuse, central nerve conspicuous other obscure, 4–5 × 0.75–1 mm, upper lamina thickened, finely pubescent on the dorsal surface and leaf margin with uniseriate trichomes. Cymes terminal, 1- few-flowered. Bracts paired, lanceolate, pilose- like leaves, 3-nerved in the upper half. Flowers pedicellate, solitary or paired, axillary or terminal; pedicels finely pubescent with eglandular hairs, 2–3 mm, elongating in fruits. Flowers all bisexual, 2–3 mm across, greenish. Sepals 5, subequal, ovate-–lanceolate, obtuse or acute, 2.5–3.5 × 1–1.5 mm, scarious margined, 3 nerved, green, ligneous in fruit, finely pubescent on the abaxial side and lower half of margins, completely spreading at seed dispersal stage. Petals absent, when rarely present only 1 or 2 in a flower (seen in only 2 flowers of 55 examined), lanceolate, smaller to sepals, 1 nerved, 2 × 0.5mm, obtuse. Stamens 10 in two whorls, equal, all fertile, shorter than sepal, 2–3 mm long, antisepalous stamens with broader filament and a minute bilobed gland at base. Ovary ovoid, 1.5 mm; style 3, up to 1 mm long. Capsule ovate-elliptic, 2.5–3.5 mm long, glabrous, enclosed by persistent hardened calyx, equal to sepals, opening by 3 valves separate to base. Seed 7–12 per capsule, comma-shaped, light to dark brown, 0.5–0.75 mm, compressed, angular, smooth on sides, wrinkled on dorsal side, exstrophiolate, with U shaped outer darker area and hyaline center, embryo curved (Figure 1).

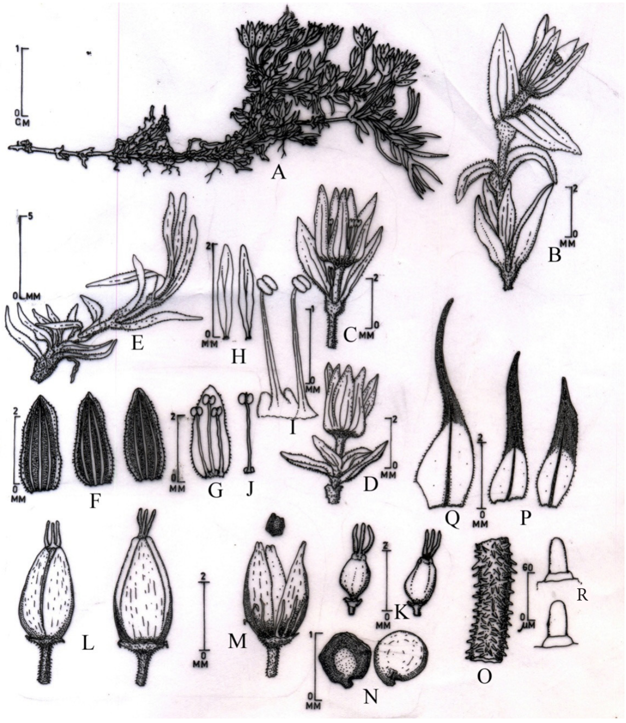

Figure 1. *Cherleria sedoides* L. A- Plant habit, B- Flowering shoot, C, D- Flower, E- Vegetative shoot, F- Sepal abaxial surface, G- Sepal with stamen, H- Petal, I- Stamen with prominent gland at base, J- Single stamen, K- Gynoecioum, L- Mature gynoecium, M- Fruit, N-Seed, O- Trichomes arrangement on stem, P- Upper leaf, Q- Lower leaf of flowering shoot, R- Eglandular trichomes.

Flowering: July–August

Fruiting: August–October

Distribution: The native range of the species is the European Mountains (POWO, 2024). Species present in moist alpine slopes, near the boulders, above 4000 m elevation in the Himalayas along with the moss species. Due to small size and cohabitating with the moss species this species was ignored by the botanical explorers.

Specimen Examined: India, Uttarakhand, Garhwal: Rudraprayag District, Kedarnath area, Vasukital area, 30°43’48.4”N 79°01’0.3”E, 4400m, 24.8.2005, *D.S. Rawat 609* B (GBPUH!). Kumaon: Pithoragarh District, Ralam valley, near Barjikang Pass, 30.310355, 80.272524, 4300m, 05.08. 2017, *D.S. Rawat & Satish Chandra 1205* (GBPUH!)

Note: The species was erroneously reported new to Indian flora by Majumdar & Giri (1983) as *Minuartia sedoides* (L.) Hiern (erstwhile name of *Cherleria sedoides* L.) on the basis of the specimens housed in CAL herbarium (specimens details provided below). During the present study, the authors studied the specimens cited by Majumdar & Giri (1983) for their finding. It was observed that those specimens actually belong to *Cherleria biflora* (L.) A.J.Moore & Dillenb. (*Minuartia biflora* (L.) Schinz & Thell.) and were erroneously identified as *Minuartia sedoides* due to the reasons mentioned further.

Table 1. List of genera of Caryophyllaceae and their respective species in Uttarakhand

S. no	Genus	Species
1	*Acanthophyllum* C.A.Mey.	01
2	*Arenaria* L. s.s.	03
3	*Cerastium* L.	03
4	*Cherleria* L.	01
5	*Dianthus* L.	03
6	*Dichodon* (Bartl. ex Rchb.) Rchb.	01
7	*Dryamria* Willd. ex Schult.	02
8	*Eremogone* Fenzl	06
9	*Gypsophila* L.	01
10	*Herniaria* L.	02
11	*Lepyrodiclis* Fenzl	01
12	*Odontostemma* Benth. ex G.Don	03
13	*Polycarpaea* Lam.	01
14	*Polycarpon* Loefl.	01
15	*Pseudostellaria* Pax	01
16	*Sabulina* Rchb.	01 (2 varieties)
17	*Sagina* L.	02
18	*Schizotechium* (Fenzl) Rchb.	03
19	*Shivparvatia* Pusalkar & D.K. Singh	03 (02 varieties)
20	*Silene* L.	21
21	*Spergula* L.	01
22	*Spergularia* (Pers.) J. Presl & C. Presl	01
23	*Stellaria* L.	12 (2 subspecies, 2 varieties)
24	*Thylacospermum* Fenzl.	01

Majumdar & Giri (1983) reported flowers of the *Minuartia sedoides* as unisexual. During our observation of the specimens, we found all the specimens having bisexual flowers. Due to small petals of the species, Majumdar & Giri (1983) might have omitted their presence. They considered capsule valves as petals. Consequently, capsule valves were termed as petals in male flowers and reported no gynoecium. They further described abortive ovaries in male flowers; because, to that time gynoecium became a capsule. Similarly in female flowers Majumdar & Giri (1983) reported no petals because what they thought of as petals were here considered as ovary and capsule. Bisexual flowers and the abortive nature of the ovary in *M. sedoides* were not reported in any of the flora (Shishkin 1936; McNeill 1962; McNeill 1984; Dequan & McNeill 2001). Halliday (1993) mentioned the abortive stamen and ovary of *M. sedoides* but the rest of the morphological characters described by him to *M. sedoides* did not match with the description by Majumdar & Giri (1983) and the specimens cited by them. The absence of petal is one of the key characteristics of *M. sedoides* (Halliday 1993). Based on the absence of petals (at least in the female flowers of specimens), Majumdar & Giri (1983) erroneously considered these specimens as *M. sedoides*. Majumdar & Giri (1983) further separated both *M. sedoides* and *M. biflora* on the basis of seed ornamentation pattern but that character is not promising and prominent (Halliday 1993). The rest of the morphological characters described by Majumdar & Giri (1983) to differentiate *M. sedoides* and *M. biflora* are not sufficient to distinguish both species (Halliday 1993).

During our observation of the specimens, we found that all flowers are bisexual and petals are present in all. The description of the specimens matches with the description of *M. biflora* (Ghazanfar & Nasir 1986; Majumdar 1993; Hal liday 1993; Dequan & McNeill 2001) and qualified them to be part of *M. biflora* populations. Hence, it can be concluded that *M. sedoides* reported earlier by Majumdar & Giri (1983) was, in fact, erroneous identification of *M. biflora* specimens.

Specimen observed
INDIA, Kashmir, Frozen lake, 3500 m, 12.09.1961, *Wadhwa and Vohra 62* (CAL!); Khilan 13.07.1899, *Inayat* s.n. (CAL!); Deosai plains, shores of shersar lake, 13000-14000 ft, 15.09.1893, J.F. *Duthie* 14052 (CAL!); Baltistan, Chataninala, west of Dras, 29.08.1893, J.F. Duthie 13888 (CAL!) (Figure 2).

Key to identify species from allied species in the Himalayas:
Cherleria biflora (L.) A.J. Moore & Dillenb. is an allied species of *C. sedoides* in the Western Himalayas. Both species can be distinguished on the basis of the following characters:

1a. Flower usually more than 1 to 3, in cymes; petals present, equaling or exceeding sepals.......................................*Cherleria biflora*

1b. Flowers solitary; petals absent or if present shorter than sepals..*Cherleria sedoides*

3.2. New Record to the Flora of Uttarakhand:

Stellaria depressa Em. Schmid was previously described from the Ladakh region (India) and subsequently reported from Tibet (China). The current specimens of the species were collected from the Chhojan area, Byans valley of Pithoragarh district Uttarakhand by G.S. Rawat, G. Singh and B.S. Kalakoti. These specimens are housed in the herbaria of the Wildlife Institute of India, Dehradun, and Kumaun University, Nainital, respectively. After matching the characters of the specimens with the description of *Stellaria depressa* (Shilong & Rabeler 2001, Doležal *et al.* 2018, Schweingruber *et al.* 2020) its identity was confirmed. An image of the type specimen of the species, provided by Wang *et al.* (2020), was also consulted for further identification.

Description of the species: *Stellaria depressa* Em. Schmid in Repert. Spec. Nov. Regni Veg. 31(816–825): 41. 1932; C. Shilong & R. K. Rabeler, in Wu *et al.*, Fl. China 6: 22. 2001.

Herb, perennial. Stem diffused, loosely tufted, erect–decumbent, 8–15 cm long, glabrous. Leaves elliptic-oblong, 3–7×2–4 mm, glabrous, mid vein prominent. Flower 2–7 in dichasial cyme. Bracts 2, ovate-lanceolate, 3–4 mm long, fully scarious, glabrous, apex acuminate. Pedicel 2–4 mm long, glabrous. Sepals 5, ovate-–lanceolate, 3.5–4.5×1–1.5 mm, veins 3, margin scarious, apex acute–obtuse. Petals 5, white, 2–2.5 mm long, shorter than sepals, bipartite up to base, lobes lanceolate, apex obtuse. Stamens 10, antisepalous with a prominent gland at the base, 0.75–1.5 mm long, antipetalous stamens without gland at the base, 0.5 mm long. Ovary ovoid, 1–1.5×1 mm, style 3, 1 mm long. Capsule ovoid–orbicular.

Flowering: July–August

Fruiting: August–September

Distribution: This species was earlier recorded from Tibet (China) and Ladakh regions. In the flora of India, Majumdar (1993) excluded this species because he did not see specimens. In the present work, we include this species on the basis of specimens housed in the herbarium of the Wildlife Institute of India, Dehradun, and Kumaun University, Nainital, collected by G.S. Rawat, G. Singh and B.S. Kalakoti, respectively, from Chhojan area of Byans valley, Pithoragarh district. This was a new record for the flora of Uttarakhand.

Specimens examined: Kumaon: Pithoragarh district, Byans valley, Chhojan, 4800 m, 18 July 2004, *G.S. Rawat & G.S. 14547* (WII!); 18 July 1983, *B.S. Kalakoti 1788* (Kumaon University Herbarium).

Key to identify species:
Species of the *Stellaria* as *S. decumbens* Edgew., *S. congestiflora* H. Hara, and *S. umbellata* Turcz. are allied species of the *S. depressa* in the higher alpine zones of the Himalayas. Here keys are provided to differentiate them, considering *S. media* (L.) Vill. as a common representative of the *Stellaria* genus.

1a. Inflorescence terminal umbellate or sub umbellate cyme..*S. umbellata*

1b. Inflorescence axillary or terminal dichasial cyme………………2

2a. Plant cushion forming or tufted……………………….....………..3

2b. Plant diffused, prostrate, ascending or erect...…….......*S. media*

3a. Leaf apex obtuse; bract not foliaceous, fully scarious………...*S. depressa*

3b. Leaf apex acuminate, slightly pungent; bract foliaceous……....4

4a. Inflorescence dense cyme, forming corymb….*S. congestiflora*

4b. Flower solitary or 2–6 per cyme……..……..………*S. decumbens*

3.3. Taxonomic revisions/corrections including new combinations and synonymy

3.3.1. New Combination in *Stellaria patens*

The whole distribution range of the *Stellaria patens* D. Don and *S. semivestita* Edgew. were sampled in Uttarakhand and specimens were collected. After examining the protologue and type specimens of both species, it is proposed that *S. semivestita* should be treated as a subspecies of *S. patens.* Solitary flowering in the *S. semivestita* was the prominent character for distinguishing both species (Edgeworth & Hooker 1874). During the present study, it was found that S. *patens* has both solitary flowering at lower elevations and lax many-flowered cyme at higher elevations. Thus, the separation of both species on this ground is not justifiable.

After a critical examination of a large number of specimens from its entire distributional range (1500-4000 m elevation) *Stellaria semivestita* Edgew. is now being merged with *S. patens* D. Don and is proposed as a subspecies.

Stellaria patens D. Don subsp. *semivestita* (Edgew.) S. Chandra & D.S. Rawat *comb et stat. nov.*
≡*Stellaria semivestita* Edgew. in Hook. f., Fl. Brit. India 1: 230. 1874.

Type: (Syntype) INDIA, Uttrakhand: Kumaon, Lohaghat, 1500 m, May 1845, *T. Thomson s.n. K000723661* (K Image !).

Stellaria semivestita is morphologically very similar to *S. patens* D. Don and these two species were separated on the basis of inflorescence. *Stellaria patens* is mentioned as having axillary or terminal dichasial cyme and *S. semivestita* as having a solitary flower in protologue (Edgewoth & Hooker 1874). But after examining both species in nature we found that *S. patens* may have solitary or 2–3 flowers in cyme when present at lower elevations. Both share some characteristics as stem quadrangular, 10 –50 cm, decumbent, branched, yellow-green, and eglandular hairy in villous vestiture. Leaves linear-lanceolate, hairy at both surface and margin, mid vein conspicuous, apex acute. The flower is axillary or terminal, solitary in both when *S. patens* is present at lower elevations and inflorescence bracts are shorter than sepals, and scarious. Similarly, the sepal is glaborus, mid vein prominent and the other

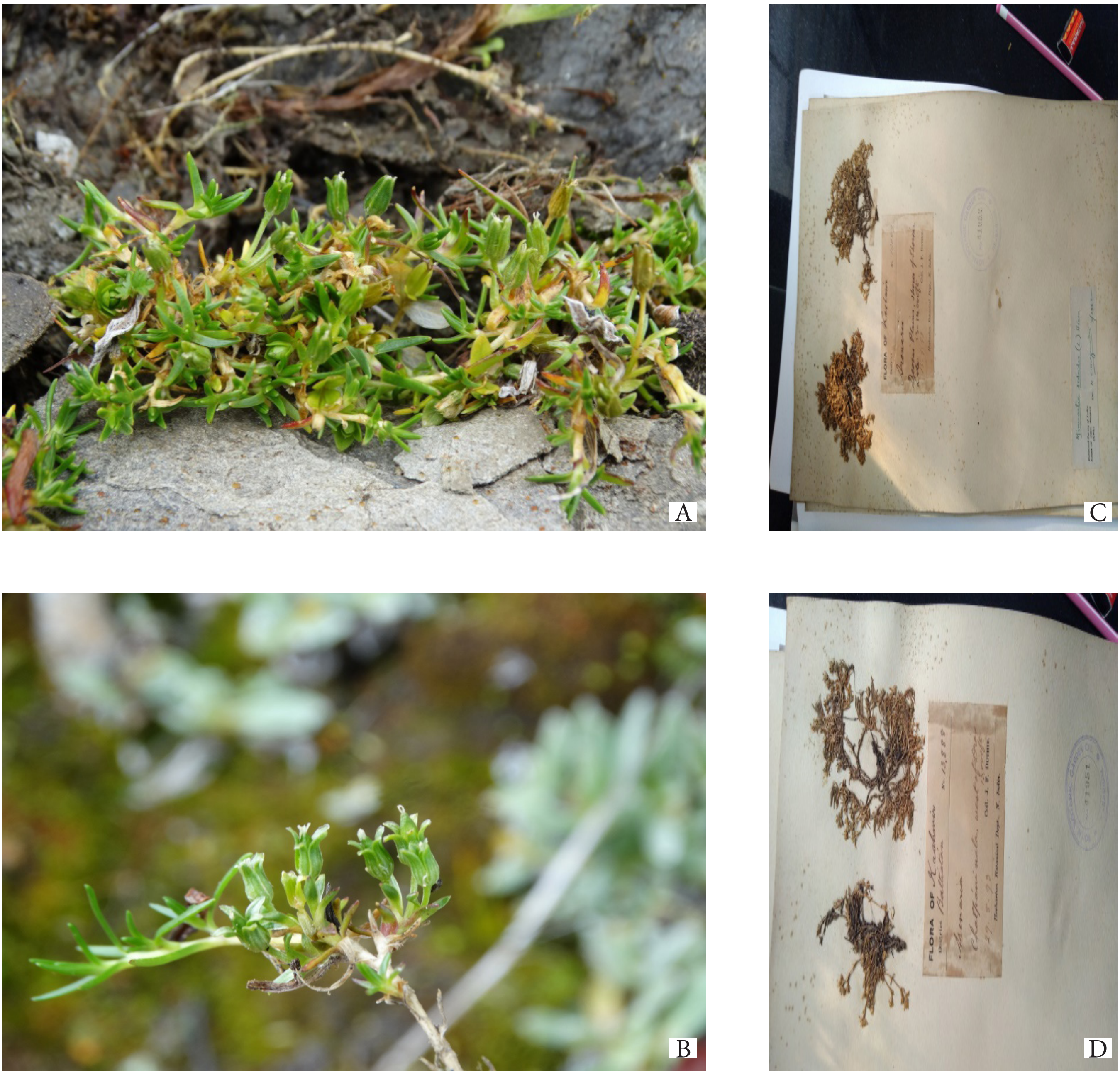

Figure 2. *Cherleria sedoides* L. A. habit, B. flowering shoot; *Cherleria biflora* (L.) A.J. Moore & Dillenb. C, D. herbarium specimen housed in CAL.

two veins are obscure in both species.

Keys to subspecies:

1a. Stem hairy with antrorse uniseriate eglandular hairs; hairs persistent; inflorescence usually dichasial cyme, rarely solitary axillary, inflorescence bract fully glabrous...*Stellaria patens* subsp. *patens*

1b. Stem hairy with appressed uniseriate eglandular hairs; hairs deciduous; flowers solitary axillary; inflorescence bract hairy at margins... *Stellaria patens* subsp. *semivestita*

3.3.2. New Combination of *Sabulina kashmirica*

Sabulina kashmirica (Edgew. & Hook. f.) Dillenb. & Kadereit var. *foliosa* S. Chandra & D.S. Rawat. comb. nov.

≡ Minuartia *kashmirica* var. *foliosa* (Royle ex Edgew. & Hook.f.) Pusalkar, Fl. Uttarakhand 1: 671 2018.

≡ *Minuartia foliosa* (Royle ex Edgew. & Hook.f.) Majumdar in J. Indian Bot. Soc. 44: 142 1965.

≡ *Arenaria foliosa* Royle ex Edgew. & Hook.f. in Hook.f. Fl. Brit. India 1: 236 1874.

Type: (Syntype) INDIA, North West Himalaya: 1832, *Royle, J.F. s.n. acc. no. K000742096* (K Image!)

The conventional genus *Minuartia* L. *s.l.* has been split into various new genera on the basis of phylogenetic relationship (Dillenberger & Kadereit 2014). The species *Minuartia kashmirica* (Edgew.) Mattf. is now transferred to the genus *Sabulina* as *Sabulina kashmirica* (Edgew.) Dillenb. & Kadereit. Consequently, the variety of the *Minuartia kashmirica* must be transferred to the *Sabulina kashmirica*. Thus, the new combination of the variety is proposed here.

3.3.3. Synonym of *Sagina purii* for *Sagina apetala*

The species *Sagina purii* R.D. Gaur was described by Gaur (1992) after distinguishing it from *Sagina saginoides* (L.) H. Karst. All the characteristics of this species match well with the description of *S. apetala* of the Western Himalayas except the presence of petals and the warty surface of seeds. Though, *S. apetala* do possess petals (Cullen 1984), these are absent in the Western Himalayan populations. In the protologue of *S. purii* ovary shape and capsule shapes are not described and petals are considered as broader than sepals. After examining type collection of *S. purii* (12807) housed in GUH herbarium, we found that petals are altogether absent and seeds are not warty as described in the protologue but wrinkled. Structures which are described as petals in the protologue are capsule valves, which are alternisepalous in position and erroneously considered as petals by Gaur (1992). Capsule open by 4 valves in *S. apetala* and each valve is equal to or slightly broader than sepals. In the publication by Gaur (1992), herbarium sheet number 12807 GUH was mentioned as holotype but his sheet contains two plants pasted over it thus making it syntype. After a critical examination of the material of *S. purii* and various collections of *S. apetala* from the Western Himalayas, we conclude that *S. purii* does not qualify as a separate species, but it should be merged with *S. apetala*.

3.3.4. Case of *Cerastium glomeratum* in the Himalayas

In Himalaya, *Cerastium fontanum* Baumg. is sometimes erroneously identified as *C. glomeratum* Thuill. Both these species are distinguished by their inflorescence. *Cerastium fontanum* contains lax cyme inflorescence while *C. glomeratum* contains dense cyme; flowers clustered together forming glomerule (Baumgarten 1816; Dequan & Morton 2001; Morton 2005; Thuiller 1799; Wyse Jackson 1995; 2000). Both of the species are native to Europe and introduced in other parts of the globe. In Uttarakhand, both species are reported (Uniyal *et al.* 2007; Pusalkar & Singh 2012). In the Flora of British India, Edgeworth & Hooker (1874) described both species under C. vulgatum L. as two varieties- *C. vulgatum* var. *glomerata* (Thuill) Edgew. & Hook. f. and *C. vulgatum* var. *trivialis* Edgew. & Hook. f. Edgeworth & Hooker (1874) distinguished both these varieties on the basis of fruiting pedicel. *Cerastium vulgatum* var. *glomerata* was mentioned as having fruiting pedicel shorter than sepals while *C. vulgatum* var. *trivialis* was mentioned having fruiting pedicel longer than sepals.

During the present study, we found that pedicel length varies with age of the plant in *Cerastium*. Young plants contain short pedicels and peduncles, hence inflorescence appears as glomerule of *C. glomeratum*. Pedicel and peduncle length increases as plant ages and inflorescence appears as lax cyme of *C. fontanum.* In the same population inflorescence may look like glomerule in the early season; while lax cyme in the late season. In the present study, we observed plants from different localities of Uttarakhand in different seasons and found the same pattern in pedicel and peduncle length and consequent inflorescence pattern. Specimens of both species, collected from Uttarakhand and housed in DD, BSD, GUH, Kumaon University herbarium and WII Dehradun, also show the same pattern in pedicel and peduncle length.

After studying the protologue of *C. glomeratum* (Thuiller 1799) it is clear that flowers in this species are strictly clustered together and form glomerule. But in the plants present in Uttarakhand this phase is not permanent rather it is transitory and ultimately forming lax cyme, hence cannot be considered as *C. glomeratum*. In lax cyme, the peduncle ultimately branches into three pedicels which is a characteristic feature of *C. fontanum* (Baumgarten 1816). *Cerastium fontanum* is also divided further into many subspecies and varieties. The most common - *C. fontanum* subsp. *vulgare* Greuter & Burdet can be distinguished from *C. fontanum* subsp. *fontanum* by the presence of both glandular and eglandular hairs; petals equaling sepals and capsules 9–13 mm long. While, *C. fontanum* subsp. *fontanum* is characterised by the presence of only eglandular hairs; petals 1.3-1.5 times as long as sepals and capsules 11–17 mm long (Morton 2005; Wyse Jackson 1995; 2000). The plants present in Uttarakhand possess both glandular hairs and eglandular hairs; petals as long as or slightly exceeding sepals and fruit length reaches up to 10 mm, hence belong to *C. fontanum* subsp. *vulgare* not the *C. glomeratum*.

3.4. Key to genera of Caryophyllaceae of Uttarakhand

1a. Leaves stipulate; sepals often with a dorsal subapical appendage; styles often fused at the base or for most of their length……………………………………………2.

1b. Leaves exstipulate; sepals without a dorsal subapical appendage; styles free or very rarely fused……………………… 7

2a. Stipule inconspicuous; sepal apex obtuse; fruit utricle………… ……………………………………………*Herniaria*

2b. Stipule conspicuous; sepal apex acute or hooded; fruit capsule…………………………………………………3

3a. Style free throughout……………………………………4

3b. Style united at base or throughout………………………5

4a. Leaves usually pseudoverticillate; stipule not connate; style 5(3)……………………………………………*Spergula*

4b. Leaves decussate; stipules connate; style 3…………………… ……………………………………………*Spergularia*

5a. Sepals herbaceous; petals usually bipartite near to base, auriculate………………………………………*Drymaria*

5b. Sepals sacrious; petals usually entire to slightly emarginate, auricle absent……………………………………………6

6a. Leaves obovate–spathulate; sepal hooded; styles 3 distinct……………………………………*Polycarpon*

6b. Leaves linear–oblong; sepal not hooded; styles united………… ……………………………………………*Polycarpaea*

7a. Sepals free or only basally connate; petals not or only inconspicuously clawed; base of episepalous stamens often with a nectar gland……………………………………………8

7b. Sepals connate, often for most of their length; petals mostly clawed; base of episepalous stamens without nectar glan ds…………………………………………………23

8a. Root flashy, tuberous; flowers dimorphic ………*Pseudostellaria*

8b. Root not tuberous; flowers monomorphic…………………9

9a. Petal bipartite……………………………………………10

9b. Petal not bipartite………………………………………11

10a. Petals usually bipartite to base; capsule ovoid–globose, capsule open up to base or near to mid……………………12

10b. Petal bipartite to 1/3; capsule cylindrical, capsule open only at apex……………………………………………13

11a. Capsule open by valves as many as styles………………14

11b. Capsule open by valves twice as many as styles……………15

12a. Stem sub-scandent or stout; inflorescence many-flowered panicle of compound cyme; capsule with 1(–2) fertile enlarged seed ………………………………………………………………*Schizotechium*

12b. Stem decumbent or tufted; inflorescence many-flowered dichasial cyme; capsule with many seeds……………………*Stellaria*

13a. Styles 3; capsule open by 6 valves……………………… *Dichodon*

13b. Styles 5; capsule open by 10 valves…………………… *Cerastium*

14a. Petal apex lacerate; styles 2; capsule open by 2 valves……… ……………………………………………………………………*Lepyrodiclis*

14b. Petal apex obtuse or rounded; styles 3–5; capsule open by 3–5 valves………………………………………………………………………16

15a. Stem densely tufted, cushion forming; leaves 3–5 veined…………………………………………………………………*Cherleria*

15b. Stem erect or loosely tufted, not cushion forming; leaves strictly 3 veined……………………………………………………*Sabulina*

16a. Leaves linear; styles usually 4 or 5; capsule open by 4 or 5 valves………………………………………………………………………*Sagina*

16b. Leaves ovate–lanceolate; style 3; capsule open by 3 valves…………………………………………………………………………17

17a. Inflorescence umbellate cyme………………………………*Holosteum*

17b. Inflorescence various but not umbellate cyme………………18

18a. Plants cushion forming; leaves congested, linear–subulate, apex apiculate or spinose…………………………………………………19

18b. Plants not cushion forming; leaves not congested, variously shaped, apex not apiculate or spinos………………………………20

19a. Stamen longer than sepals; style 2 (3); capsule open by 4(6) valves…………………………………………………………*Thylacospermum*

19b. Stamen shorter than sepals; style 3; capsule open by 6 valves………………………………………………………………*Eramogone*

20a. Leaves distinctly petiolate, more than 3 cm long; styles 2; capsule open by 4 valves……………………………………*Brachystemma*

20b. Leaves sessile or subsessile; less than 3 cm long; styles 2–3; capsule open by 4–6 valves…………………………………………21

21a. Leaves and sepals saccate at base; styles 2–3………………… ……………………………………………………………………*Odontostemma*

21b. Leaves and sepal not saccate; styles 3……………………………22

22a. Leaf base broadly forming cupular cavity at node; flower solitary; petal twice as long as sepals……………………*Shivparvatia*

22b.Leaf base narrowly connate not forming cupular cavity at node; flowers numerous in cyme; petals shorter than or slightly exceeding sepals………………………………………………………*Arenaria*

23a. Style 3 or 5, never 2………………………………………………*Silene*

23b. Style 2, rarely 3……………………………………………………………24

24a. Calyx tubular, veins more than 20……………………… *Dianthus*

24b. Calyx campanulate to cylindrical, veins 5……………………25

25a. Leaves oblong–lanceolate; calyx conspicuously 5 winged…………………………………………………………………………*Gypsophila*

25b. Leaves ovate–spathulate; calyx campanulate, not winged... ……………………………………………………………………*Acanthophyllum*

Conclusion

The state Uttarakhand has varied topography including the Trans-Himalayan cold dessert, snow line, alpine meadows, temperate dense forests and open plains that allow for a high diversity of the Caryophyllaceae in the state. Our review concluded that family Caryophyllaceae in the Uttarakhand is represented by 24 genera 75 species, 2 subspecies and 6 varieties. Herbarium history, literature survey and observations by the authors revealed that 11 taxa of the family erstwhile reported from the Uttarakhand should be excluded from the flora of Uttarakhand. The taxonomic ambiguity of the *Cherleria sedoides* and *Cherleria biflora* in the Himalayas was resolved. The study reported *Cherleria sedoides* first time from India (Uttarakhand) and *Stellaria depressa* was recorded as new to the flora of Uttarakhand. In the present study, a new combination of *Stellaria patens* subsp. *patens, Stellaria patens* subsp. *semivestita* and *Sabulina kashmirica* (Edgew. & Hook. f.) Dillenb. & Kadereit var. *foliosa* are proposed. Synonymy of *Sagina purii* for *S. apetala* was concluded based on the taxonomic history and observation of the live and herbarium specimens. On the basis of morphological features, differences and occurrence of the *Cerastium glomeratum* and *C. fontanum* subsp. *vulgare* in Uttarakhand was also resolved.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

No data was used in this research.

ORIGINALITY STATEMENT

iThenticate software shows a high overlap (>40%) with the first author's PhD thesis; however, the overlapping document is only archived on internet and not published as a peer reviewed article.

AUTHORS' CONTRIBUTION

The first author (SC) conducted field and lab studies and wrote the manuscript. The second author (DSR) further improved the manuscript and added line diagram of *Cherleria sedoides*.

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Improved detection and classification of cryptic '*Phylloscopus*' warblers with integrated computer vision and deep learning method

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Abstract

The rapid advancement of image recognition technology, especially with the help of YOLO algorithms, has greatly improved the ability to identify and recognize different species. This research focuses on the *Phylloscopus burkii* cryptic species complex, distinguished by little phenotypic diversity making precise identification challenging. In order to make it easier to distinguish between members of this complex, we suggest an enhanced YOLOv5-based model that employs a part-based approach that keeps part detection and identification classification apart. Our goals included determining whether images taken in natural settings can produce statistically reliable identifications and examining the variation of image characteristics of the species within the burkii complex. A dataset of 289 annotated images was augmented to build 2,890 photographs for training purposes. After training on Amazon Elastic Cloud, the model achieved a mean average precision (mAP) of 94 and a recall of 97.3. The model demonstrated strong precision and recall metrics across species, and the results showed that it worked best at a confidence threshold of 0.50. *P. valentini* and *P. tephrocephalus*, in particular, showed considerable overlap, which sometimes resulted in misidentifications, although *P. poliogenys* and *P. whistleri* demonstrated excellent accuracy. We recommend training models based on species geographic distributions to improve identification accuracy. This strategy might successfully lower the rate of erroneous identifications, enhancing the caliber of data produced by citizen science projects. In the end, our results support current efforts to conserve biodiversity by offering a solid framework for automating species identification and guiding ecological study. Our goal is to improve species monitoring and data collection precision by utilizing innovative methods based on machine learning.

Keywords: Cryptic species, deep learning, image recognition, *Phylloscopus burkii* complex, YOLOv5.

Introduction

Studies on bird populations help us understand key indicators for environmental monitoring (Koskimies, 1989), and shifts in bird communities help track climate change (Lindström *et al.*, 2013). These surveys that monitor biodiversity often depend on numbers and counts (Larsen *et al.*, 2012). Through citizen science initiatives all around the world, the appeal of adopting a "Big Data" strategy over the past few years has made it possible to collect enormous amounts of data online. But all too frequently, this data lacks sufficient detail to clarify how the observations were made (Kelling *et al.*, 2018), and identification errors that have occurred (see de Freitas *et al.*, 2022). The difficulty of working with morphologically cryptic species is made apparent by this significant error rate in field identification, especially for smaller polytypic species like *Phylloscopus burkii*.

Traditional polytypic *P. burkii* is a classic example of cryptic speciation since distinct species have almost similar morphology and plumage (Päckert *et al.,* 2004). This group of sister species experienced a complex evolutionary history, resulting in both extant vicariance, vertical and possibly horizontal parapatry, and local sympatry throughout the Sino-Himalayan range (Alstrom & Olsson 1999, Martens *et al.,* 1999) with seven currently recognized species (Päckert *et al.,* 2004; Martens 2010). The distribution of four of these species (Figure 1) clearly indicates overlapping species ranges, although few of the species are almost completely separated altitudinally (Alström, 2020a, b, c, d). Martens *et al.* (2002, 2011) have earlier reported the presence of three of these

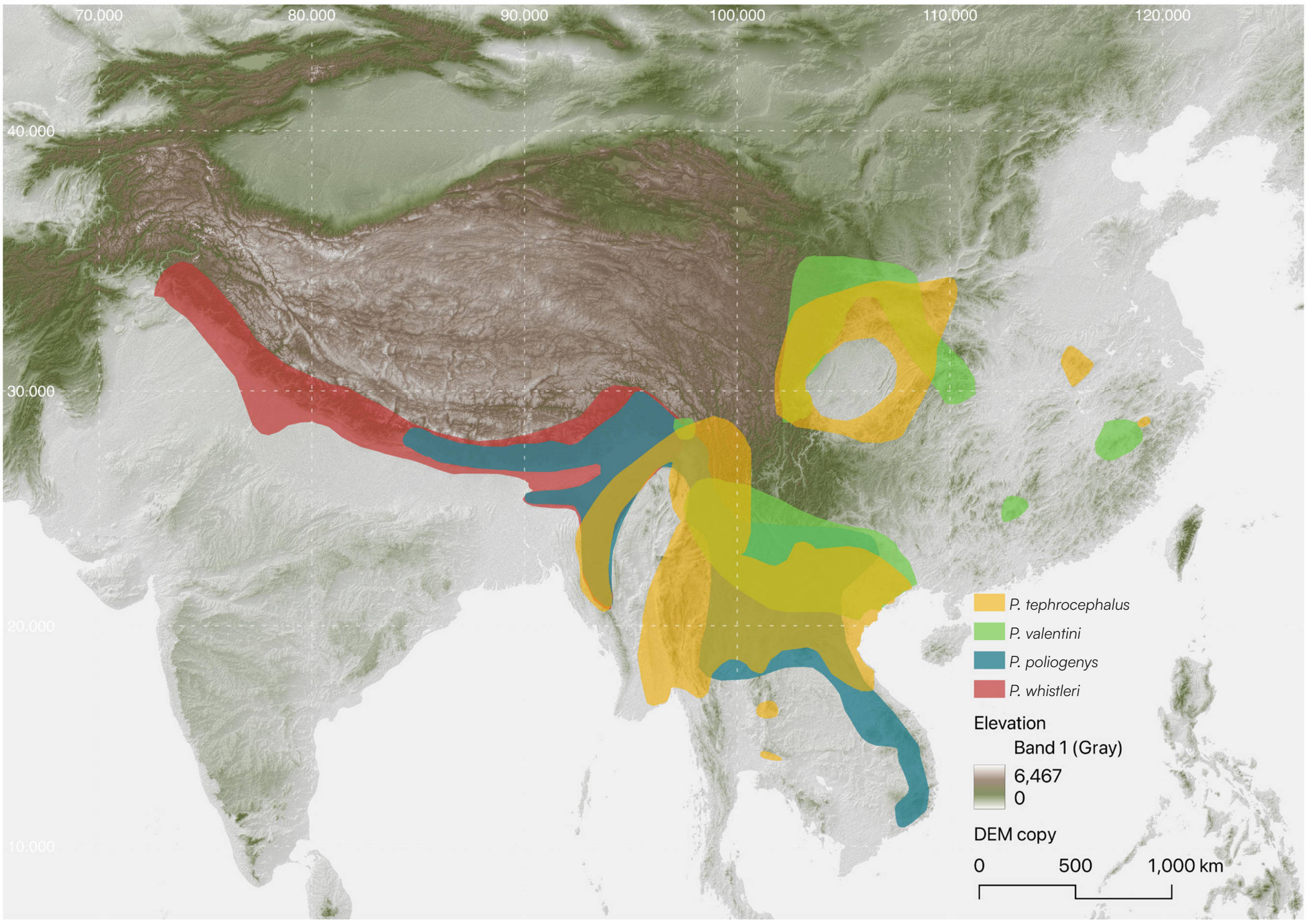


Figure 1. A distribution map of *P. tephrocephalus, P. valentini, P. poliogenys*, and *P. whistleri*, depicting their respective geographic ranges and areas of overlap. For understanding common zones where ranges converge, color-coded portions reflect each species' breeding grounds.

species along a single forested edge during the breeding season. Observation errors are most likely to occur in these regions where two or more of these species coexist.

In order to address these inaccuracies, image classification has evolved into an increasingly popular topic in deep learning and machine learning approaches (Alter & Wang, 2017). However, distinguishing between different bird species based on their distinctive shapes and appearances, considering background differences, controlling various lighting conditions in images, and adjusting to the dynamic postures of birds make it challenging to accurately identify bird species from images (Vo *et al.*, 2023). Our study used a part-based strategy to address this issue by splitting the identification process into two sections: part detection and identity classification. We provide an improved *You Only Look Once version 5* (further YOLOv5) based approach to part detection that can handle diverse ambient conditions and partial overlap between part objects.

The primary objective of this study was to determine whether the use of machine-learning and computer vision could make it easier to distinguish between members of the *P. burkii* complex based on morphological characteristics. Specific objectives of our investigation were to i) determine whether simple in-natural-setting photos could yield statistically accurate identification of visually similar co-occurring warblers of the *burkii* complex, and ii) record the degree to which any differences between groups show continuous or disjunct distributions in the image feature space; that might set up the foundation for developing a dependable and precise method for automating the identification of at least some *P. burkii* species using morphological data. The results will facilitate quick and precise recognition of cryptic species, thereby reducing identification errors while monitoring.

Materials and Methods

Data Collection and Pre-processing

In addition to our own image library, we enhanced our dataset by collecting images for four species (*tephrocephalus*, *whistleri*, *valentini* from *P. burkii* complex and *poliogenys*-outgroup) from various online databases (iNaturalist, eBird, Wikimedia Commons) and creating respective sets of images for each species. These images are licensed under Creative Commons, allowing for their use in research and analysis while complying with copyright regulations. We specifically considered two important filtering criteria: a) subtle indicators like the supercilium, wing bars, bill and leg color, flanks, and most importantly the eye ring are crucial for identification in species with limited distinctive features, like *Phylloscopus* warblers. It was necessary to focus on side-profile photos to make sure that all of these features were readily apparent due to the complexity of these markers (this filter nearly cut the dataset in half). The images that passed the filter check were uploaded and annotated manually with the polygon tool (instances and label borders; Supplementary Figure S1-A) in *Supervisely* v1.0.23 software. A total of 289 photographs (71 personal observations and 218 sourced) were annotated, which consisted of *tephrocephalus*-

69, *whistleri*-73, *valentini*-59, and *poliogenys*-88, and later augmented to create 2890 photographs. In order to improve the model's robustness in identifying the target species under varying environmental conditions and its ability to generalize across diverse scenarios, we improved the dataset tenfold by flipping, applying slight rotations, adding random noise, and adjusting contrast and saturation in the images. We further analyzed the label size and label position to understand the ratio between the abscissa of the label center and the image width ('x'), as well as the ratio between the ordinate of the label center and the image height ('y') (Supplementary Figure S1-B). The correlogram of tagged samples and the bounding boxes are provided in Supplementary Figure S2. Images that were not included in the training validation dataset were used to generate the test dataset, which was further used to evaluate the model performance.

Dataset Splitting and Annotation

The 2,890 augmented images were split into training, validation, and test sets to ensure robust model training and unbiased evaluation. From these images, 2,312 images (80%) were allocated for training the YOLOv5 model, while 578 images (20%) were used for validation to monitor performance and fine-tune hyperparameters. Additionally, a separate dataset of 370 images, collected alongside the original dataset, was reserved exclusively for testing. This independent test set was not included in the training or validation phases, ensuring an unbiased evaluation of the model's capability.

All images in the training and validation sets were annotated using the polygon tool in Supervisely software to provide precise bounding boxes for the target species. The test dataset underwent the same annotation process, enabling accurate comparisons of predictions with ground truth during performance evaluation. Our dataset splitting strategy followed standard deep learning practice to ensure robust model training while accounting for independent assessment of the model's ability to generalize to unseen data. By using this structured approach, the model's performance was evaluated in a manner that closely simulates real-world scenarios.

The CNN Model (YOLO Architecture)

Convolutional neural networks are ideally suited for image classifications since they are composed of numerous convolution layers that record information from input photographs and offer a typical pattern for detecting the species evaluated after training the datasets (Szegedy *et al.*, 2015). Within CNNs, YOLO is a family of detection models in which the input image is divided into grids, and each cell in the grid detects the object. It incorporates test time augmentation, model ensemble, and the evolution of hyper-parameters. We used YOLOv5 (Redmon *et al.*, 2016) with the YOLOv5x6 variant (where x stands for "extra-large" and 6 for P6 layers in the model architecture), which performs exceptionally well and allows high-resolution images using larger models. The algorithm combines the feature extraction network to extract the feature information of the input bird image and then uses the aggregation network for detection to obtain the required semantic information (Liao & Tian, 2021). This architecture is also excellent at handling bounding boxes, which makes it ideal for managing multiple predictions using Intersection over Union (IoU) thresholding and non-max suppression, as well as for recognizing bird species that have bounding boxes. We used a Nvidia V100 GPU on an AWS EC2 instance, on which this model obtained a mean average precision (mAP) of 94 with a recall of 97.3 and a model size of 270MB.

The Amazon Web Services (AWS) Elastic Cloud Compute (EC2) Backend

The model was trained on a custom backend connected as a compute cluster to Supervisely. We chose to proceed with this approach as the Supervisely platform already has the established service for Amazon Web Services (AWS)—Elastic Cloud Compute (EC2). Hence, all model training tasks were performed on the AWS backend. After the model was trained using annotated data to correctly identify the target species, it was validated with untrained annotated data to compare true and predicted results. Using cross-validation methods, hyperparameter tuning was carried out to improve model performance by optimizing learning rates, batch sizes, and network architecture. Real-time tracking of training progress and easy dataset management were made possible by the interface with Supervisely. Scalable resources were made available via AWS EC2, ensuring the effective processing of larger data sets. The model was further tuned for improved species identification accuracy after validation.

WORKFLOW PIPELINE

The workflow pipeline (Figure 2) outlines a methodical approach used in this machine-learning technique. First, the annotations are carefully examined and revised. This data was further used to train and evaluate the YOLOv5 machine-learning model. If the model converged, it was then tested on new footage. If the accuracy of the model was deemed adequate by the performance evaluation report, it was deployed; if not, the hyperparameters or dataset were adjusted, and the model was retrained to improve its detection ability. This iterative process guaranteed that the model was sturdy and dependable prior to its implementation in practical scenarios involving species identification.

Evaluation Metrics

Average confidence: It was calculated by adding the confidence values for true positives, true negatives, false positives, and false negatives, then dividing the result by the total number of instances.

Accuracy: It measured the frequency with which a model correctly predicts positive and negative outcomes across all possible scenarios.

Accuracy = (True Positive+True Negative)/(True Positive+False Positive+True Negative+False Negative)

Precision (P) or True Positive Rate (TPR) or Sensitivity: An indicator of how frequently, a model makes a correct positive prediction out of all positive predictions.

Precision = (True Positive)/(True Positive+False Positive)

Recall (R) or True Negative Rate (TNR) or Specificity: It is a measure that, when applied to the actual positive samples, indicates how frequently a model forecasts the accurate positive prediction based on the data's ground truth.

Recall = (True Positive)/(True Positive+False Negative)

F1 Score: It computes the weighted average or harmonic mean of both Precision and Recall to merge them into a single value.

F1 Score = (2*Precision*Recall)/(Precision+Recall)

Intersection over Union (IoU): It metric that measures the overlap between the predicted bounding box and the ground truth bounding box to assess an object detector's accuracy for a given dataset.

IoU =(Overlap area of Ground Truth & Predicted bounding boxes) /(Total area of Ground Truth & Predicted bounding boxes)

Generalized Intersection over Union (GIoU): It measures the distance between boxes and an enhancement over IoU that accounts for situations where there is no overlap by calculating the overlap between predicted and ground truth-bounding boxes.

GIoU = IoU-(Area of minimum box enclosing both Ground truth & Predicted bounding boxes)/(Area of minimum box enclosing both Ground truth & Predicted bounding boxes)

Mean Average Precision (mAP): The trade-off between precision and recall is represented by the area under the precision-recall curve, which is the measure of average precision.

$$\text{mAP}=\frac{1}{n}\sum_{k=1}^{n} AP_k$$

In order to evaluate the model's performance in real-life scenarios, we generated a test set of frames using recently taken photos of the species under examination.

Results

Model training, validation, and testing

The model is most accurate at a confidence threshold of 0.75, suggesting that the performance of the model is good as the accuracy of the model holds up past the 0.5 confidence threshold mark (Figure 3A). Precision above 0.6 was estimated even at lower confidence thresholds of 0.2, suggesting that the model is mostly correct when it predicts a positive instance (Figure 3B). Through the precision and recall graph (Figure 3C), we can infer that the model has both good precision as well as a good recall. The model has a high recall value even at a high precision value suggesting that the model is correct when it predicts a positive instance and is also good at identifying all the positive instances in the data. The model also has a good recall at a confidence threshold of 0.75 (Figure 3D), suggesting that the model is good at identifying the positive instances in the data and holds up the recall past the 0.5 confidence mark. The model performance confusion matrix for performing predictions and model training performance trends over 100 epochs of training are provided. The confusion matrix suggests that the model has less confusion by its capability to perform predictions close to the ground truth, thereby having a prominence of scores across the diagonal (Figure 4A). The model training performance trends suggest improving training curves across 100 epochs of training (Figure 4B). The GIoU values for the same are presented in Supplementary Table T1. Based on the confidence scores per detection, model performance on a few detection samples is provided in Figure 5.

Inference of test results

The model accuracy for the test dataset (n_train = 880, n_ test=100) for poliogenys was 1, with a precision score of 1 and an average confidence of 0.86. Since *poliogenys* is the most distinct among the four species due to its characteristic fully grey head, this result can likely be attributed to this uniqueness. The model accuracy for the test dataset (n_train= 690, n_test=80) for *tephrocephalus* was 0.83, with a precision score of 0.6 and an average confidence of 0.62. The model was able to recognize 60 out of 80 test examples of *tephrocephalus* correctly, although there were a few cases where *valentini* was detected as *tephrocephalus* by this model. The model accuracy for the test dataset (n_train= 590, n_test=90) for valentini was 0.92, with a precision score of 1 and average confidence of 0.64. Due to a slight observable confusion that the model has between valentini and tephrocephalus due to the similarities between

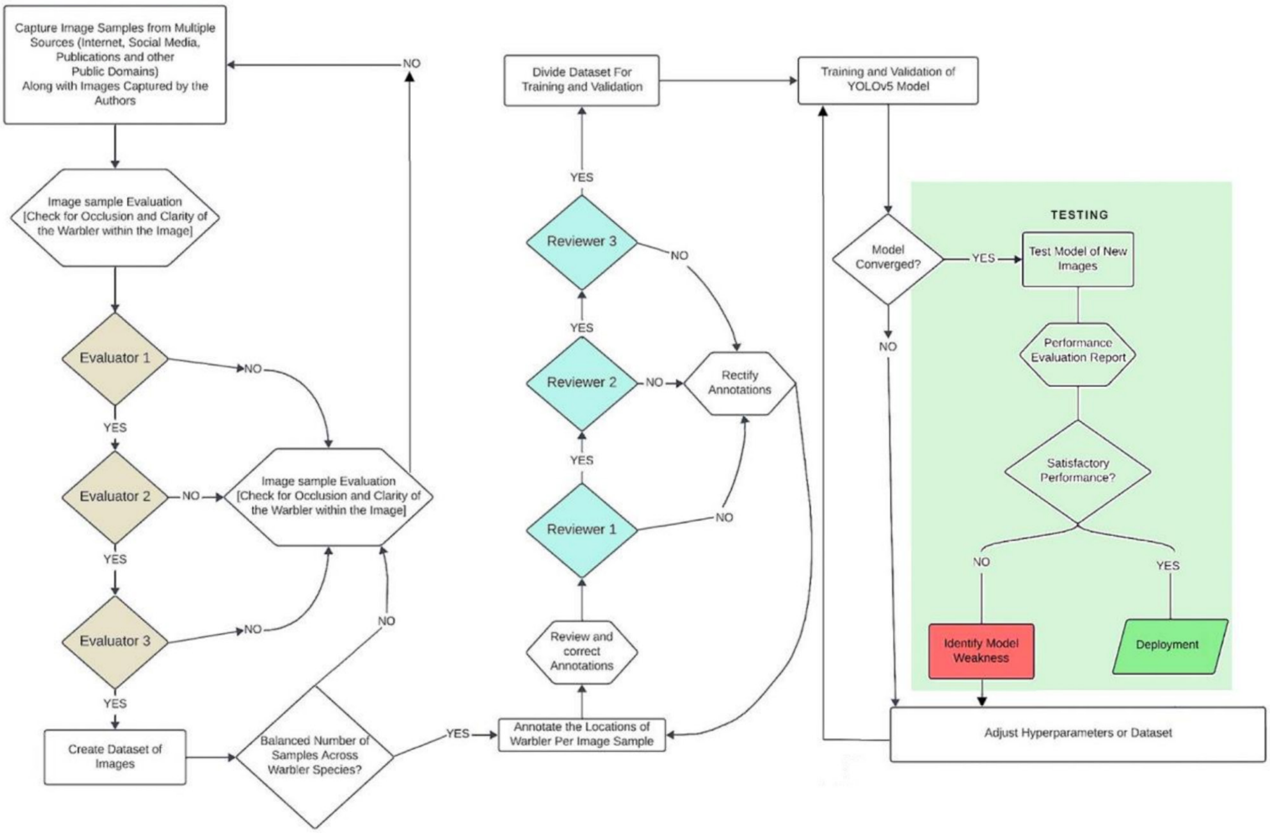


Figure 2. Workflow of image classification for annotations and subsequent model training and validation.

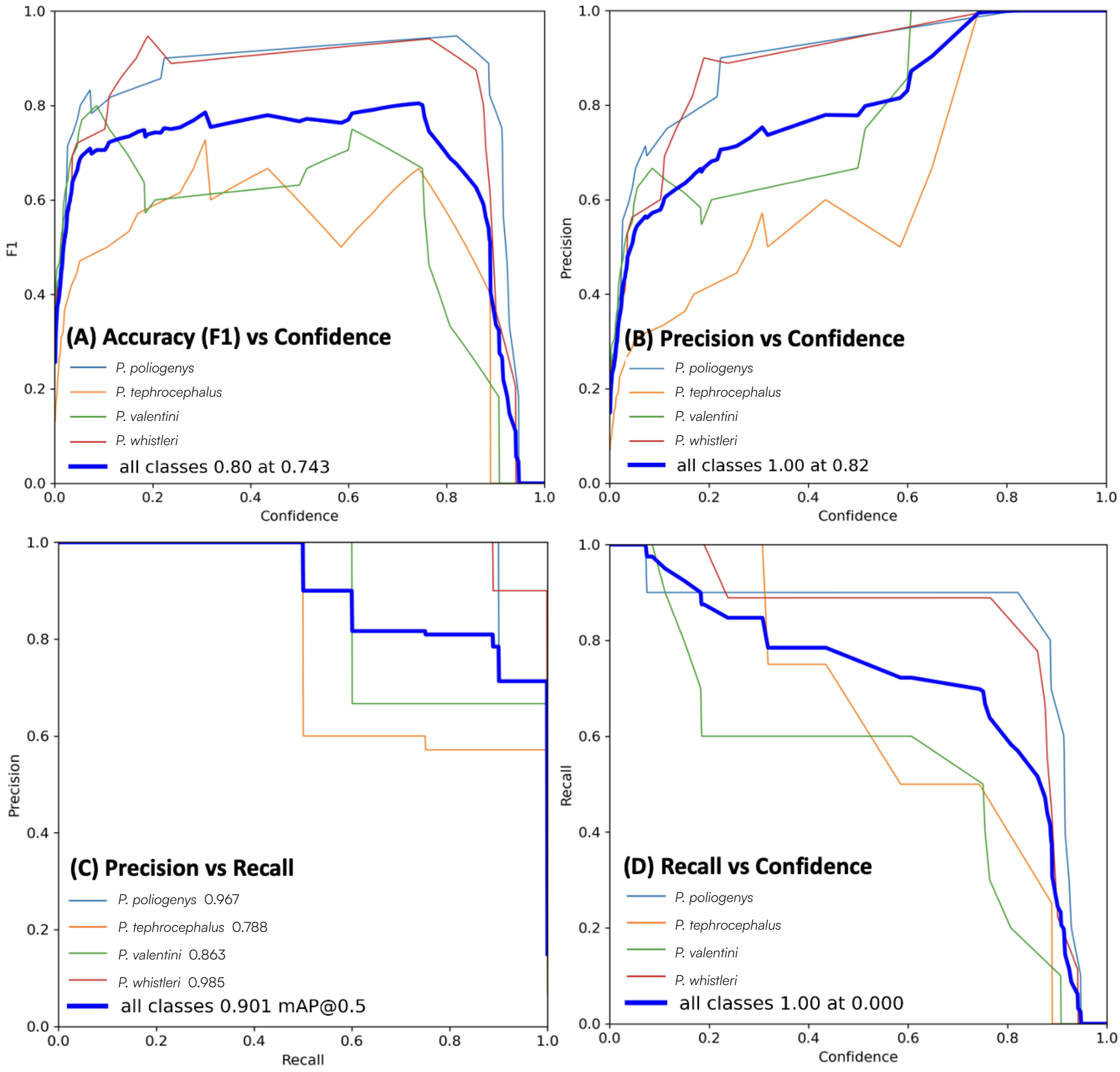


Figure 3. Based on the predictions that the model makes given an input image, for a set of test cases in the dataset, the following graphs are generated: (A) Graph showing the trade-off between the model's overall accuracy and confidence, (B) between the model's overall precision and confidence, (C) between the model's overall precision and recall, and (D) between the model's overall recall and confidence.

both species, the confidence scores for the valentini species have taken a major hit. Even though the model was able to detect valentini in 60 out of 90 test examples, the confidence scores were further reduced due to the model giving overlapping detections of tephrocephalus in 20 of these 60 correct detections, making only 40 purely correct detections for the valentini species. The model accuracy for the test dataset (n_train= 730, n_test=100) for whistleri was 0.97, with a precision score of 1 and an average confidence of 0.77. The model showed no observable confusion for this species, asserting that all other confusion within the model can be overcome by giving more training examples for each species. Results of average confidence, model accuracy, precision, and average confidence are provided in Table 1 and Figure 6, and respective true negative, false negative, true positive, and false positive values for each species and calculations performed (for precision, recall, and F1 scores) are provided in the Supplementary Table T2.

Discussion

The rapid development of image recognition technology, especially YOLO algorithms (see Jiang *et al.*, 2022) has tremendously promoted the research on species detection and identification. While there are several other models like Deep Learning Ensembles (Chen *et al.*, 2019), Transfer Learning Models (Wang *et al.*, 2020), Support Vector Machines (Shalika & Seneviratne, 2016), and Generative Adversarial Networks (Zhang *et al.*, 2023) available for species recognition, the application of YOLO v5 models for studies on mammals (Tan *et al.*, 2022, Xie *et al.*, 2023), birds (Ou *et al.*, 2020, Liao & Tian, 2021, Yi *et al.*, 2023), reptiles (Pandey *et al.*, 2023, Afonso *et al.*, 2024), and amphibians (Takaya *et al.*, 2023) has shown significant advancements in terms of accuracy, speed, and model efficiency.

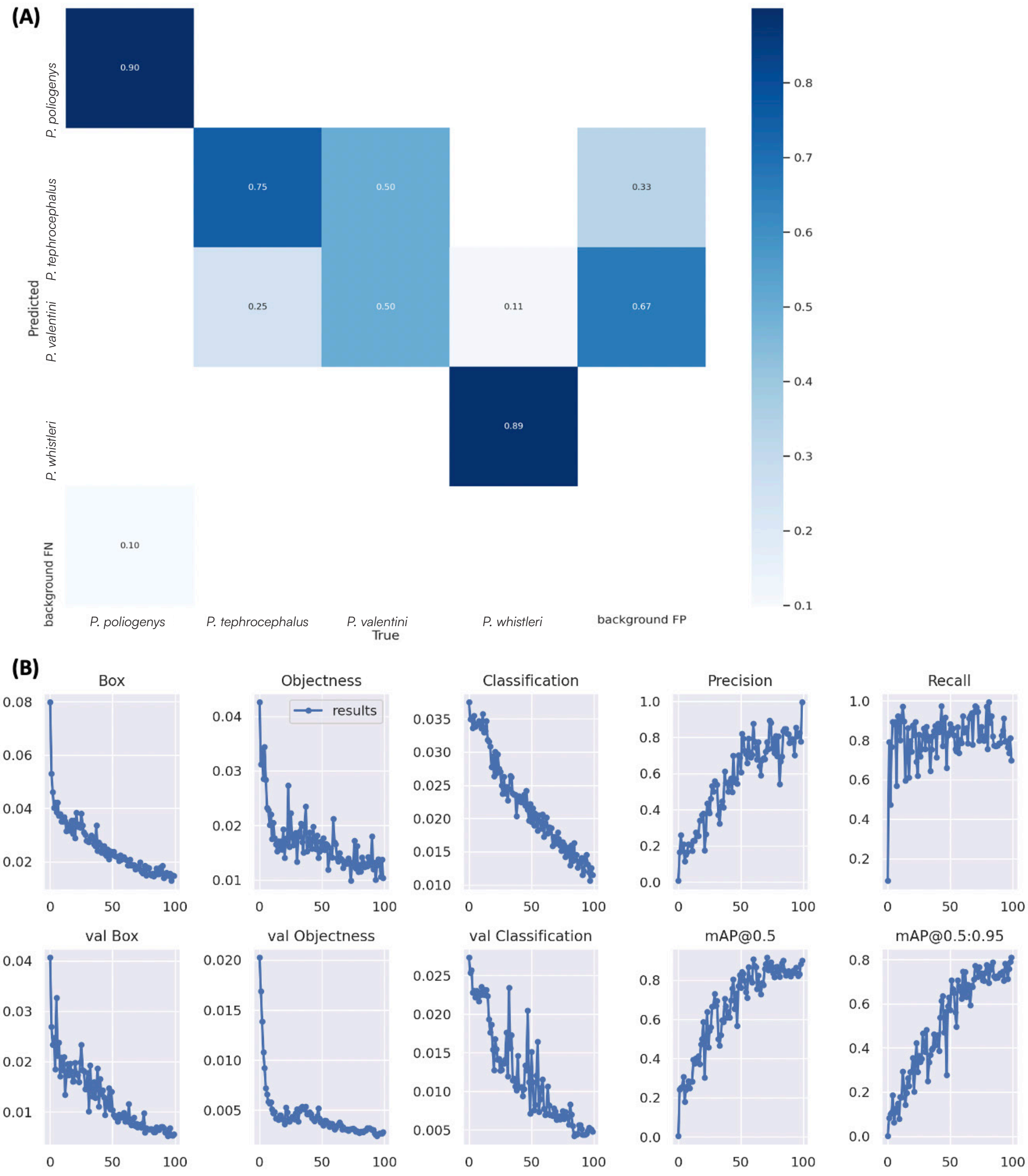


Figure 4. Comprehensive Evaluation of Object Detection Model Over 100 Training Epochs
(A) presents the confusion matrix, illustrating the classification accuracy of the detection model, where rows represent predicted species and columns show actual species. Correct predictions are displayed along the diagonal, while misclassifications are shown in off-diagonal elements.
(B) showcases various performance metrics, including loss metrics (Box, Objectness, Classification) and accuracy assessments (Precision, Recall, mAP@0.5, mAP@0.5:0.95) during training and validation phases.

However, cryptic species (*P. burkii* complex in our study) often possess minute variations in patterns that can be challenging for standard models. Thus, additional validation (see Ma & Yang, 2022) and specialized customization, such as enhanced data augmentation (Bati & Ser, 2023) and fine-tuning (Kim *et al.*, 2022) of model parameters, were necessary to improve detection accuracy and handle these subtle morphological differences in our study. Therefore, a comprehensive approach to preprocessing and model training was used to enhance species classification, which, in real-world scenarios, is both robust and adaptable. To maintain a clear and targeted research trajectory within this group of birds, we recommend that species belonging to different complexes be examined independently rather than merging them. This study addresses the challenges by proposing a deep learning method to improve the identification of bird species of the *burkii* complex, based on the YOLO-v5 algorithm.

In contrast to other CNN architectures like ResNet, YOLOv5 was selected for classification in this study as it can effectively handle the unique difficulties of our task while providing notable benefits in terms of flexibility, performance, and deployment. It integrates categorization and object detection into a single, effective pipeline. YOLOv5 does not require a separate detection step, which is especially helpful for applications requiring localized species identification, in contrast to ResNet, DenseNet, or other CNNs that are solely focused on classification. The distinctiveness of the species' morphology in the dataset may determine how well visual cues alone may distinguish species differences. The accuracy of image-based categorization may be constrained when species have notable overlap in appearance or modest visual differences. Utilizing complementary information, and combining visual and auditory signals may increase categorization accuracy. However, the primary reason for excluding acoustic classification was that it was not be feasible

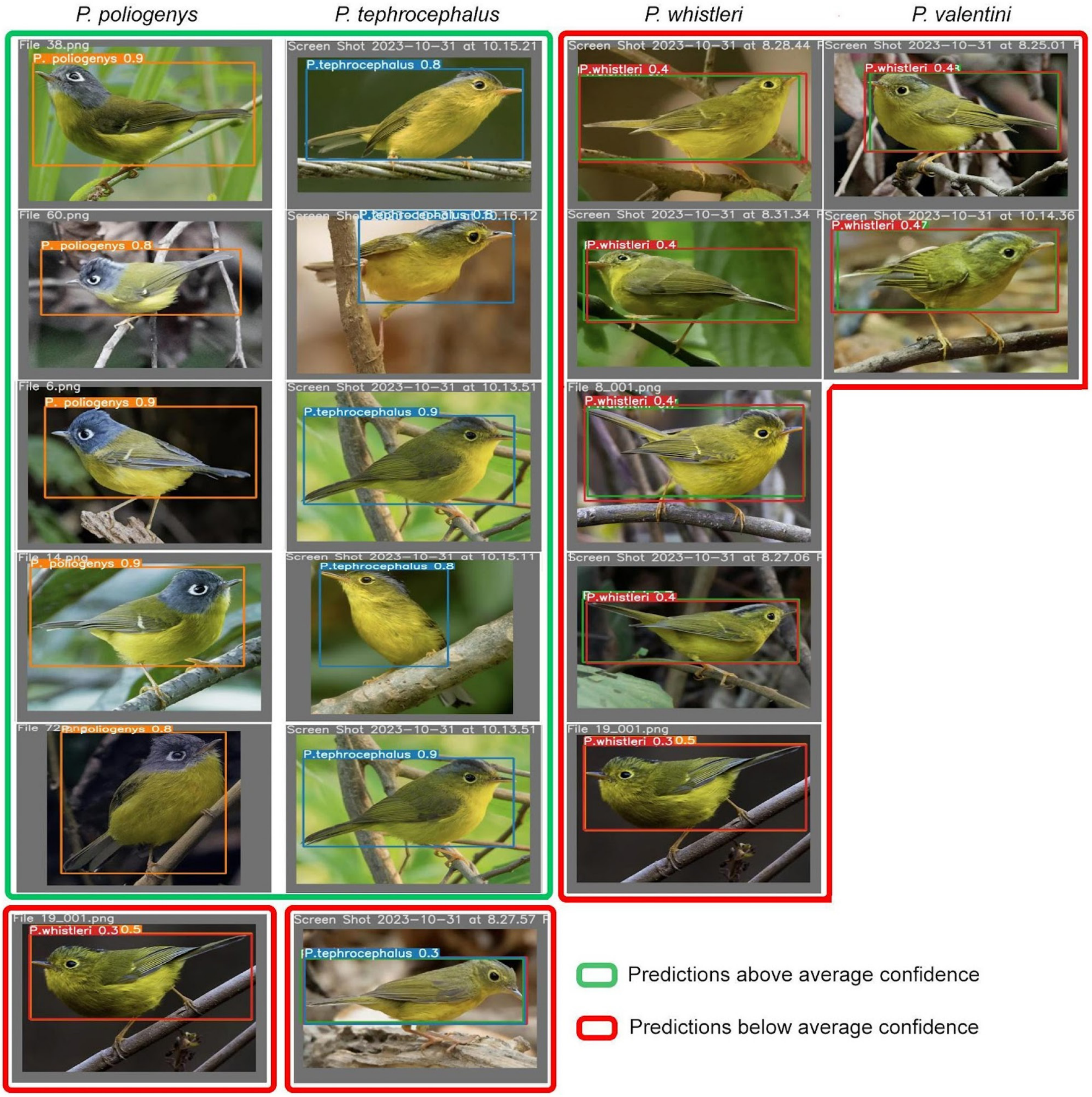


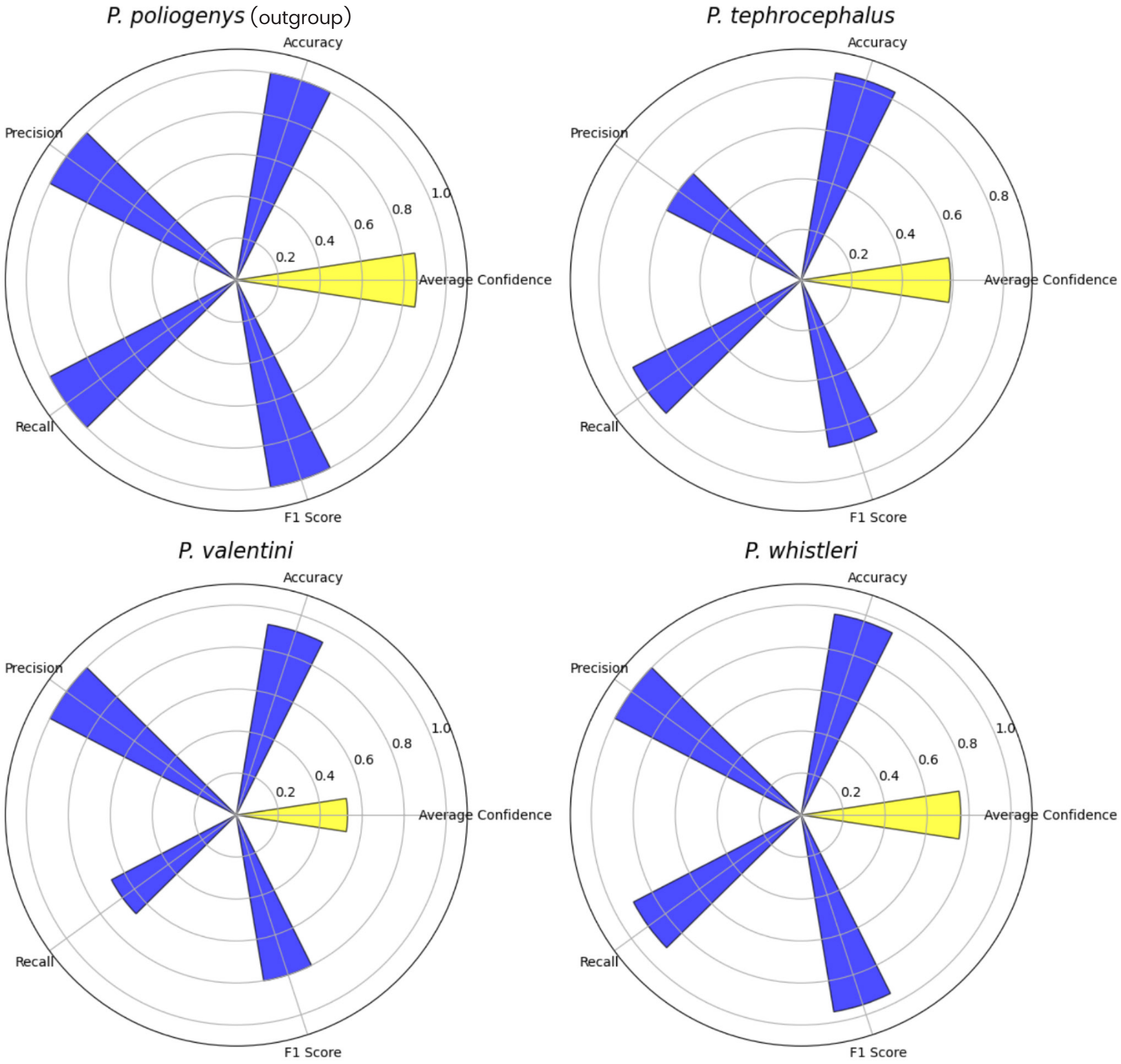
Figure 5. Sample set of model detections over the test dataset, categorized based on confidence scores for each detection. Links to the photographs featured in this figure, along with the names of the respective photographers, are provided in the supplementary section.

Table 1. The average performance metrics, obtained from the confusion matrix findings over multiple runs, for test datasets, including accuracy, precision, recall, and F1 score.

Species	Avg. Confidence	Accuracy	Precision	Recall	F1 Score
P. poliogenys (ougroup)	0.86	1	1	1	1
P. tephrocephalus	0.59	0.83	0.6	0.75	0.67
P. valentini	0.53	0.92	1	0.67	0.8
P. whistleri	0.76	0.97	1	0.9	0.95

to gather high-quality acoustic data for these species within the limitations of our analysis. It is more difficult to get reliable and consistent audio recordings of this species since the calls often consist of short pulses, and species identification primarily relies on songs.

The results of this study indicate that statistically valid morphology-based identifications of cryptic species can be obtained from basic images taken in natural settings. The YOLOv5 model's strong recall, accuracy, and precision rates reveal how machine learning algorithms use the unique morphological features available in these images even if they have subtle variations. The model demonstrated strong performance with high accuracy, precision, and recall at the 0.50 confidence threshold and maintained precision even at lower thresholds, which makes it ideal for such tasks. But in order to make it adaptive, we are currently focusing on increasing the training dataset as the model slightly confuses *valentini* (average confidence = 0.64). The model operates effectively



P. poliogenys (outgroup)

P. tephrocephalus

P. valentini

P. whistleri

Figure 6. Accuracy, Precision, Recall, F1 Score, and Average Confidence distributions for different species are shown in a heat plot, emphasizing the variation in performance across metrics.

with *tephrocephalus* and has excellent detection and differentiation ability between *poliogenys* and *whistleri* (average confidence = 0.0.86 and 0.77, respectively). The test dataset presents difficulties for tackling *valentini*, though, often leading to the simultaneous detection of tephrocephalus and *valentini*. Resolving these issues will possibly improve the model and guarantee accurate species identification in ecological research (Shah *et al.*, 2023).

Even though the image only included *P. valentini*, there have been instances where multiple bounding box detections occurred in the same image, generating bounding boxes for both *P. valentini* and *P. tephrocephalus*. This might have been influenced by the low imagecount of both *P. valentini* and *P. tephrocephalus* in the current dataset, as well as similarity in several features between the two species. To address this, we recommend (a) training the dataset by doubling epochs and (b) extending the dataset with more diverse and representative examples of both *P. valentini* and *P. tephrocephalus*. One way to reduce the model's confusion in differentiating between species like *valentini* and *tephrocephalus* is to train it independently depending on the geographic distributions of each species. Identification can be made much simpler by concentrating on the unique elevational gradients and ecological requirements (see Yang *et al.*, 2023, Parab *et al.*, 2023) preferred by each species (Alstrom, 2020). By using a training technique tailored to geographic distribution, the model can acquire the distinct traits linked to the preferred settings of individual species (see Opaev & Kolesnikova, 2019), resulting in increased recognition accuracy. Enhancing species identification in regions where overlap exists requires incorporating geographic distribution data into the training process (Alström, 2020a, b, c, d).

Although other applications like Merlin offer users a list of potential species, mostly aimed at skilled birders who use their knowledge to make the ultimate decision, our method starts the process of developing a more reliable model for the few focal species that are being considered. Our goal was to create a model that could make a single, solid prediction, allowing even inexperienced birders to progressively come to trust the model's accuracy and gain more knowledge by using it. We foresee adding a feedback mechanism that displays confidence scores next to predictions as a possible future improvement. This would provide an instructional component currently lacking in other applications and allow users to assess the model's forecasting precision, thereby fostering confidence.

In conclusion, the percentage of incorrect identifications on citizen science platforms can be considerably decreased by using specific models created for species identification. We may enhance the quality of the data gathered by citizen scientists by improving the precision of these models. This development is critical to successful conservation efforts because accurate species identification directly affects the quality of data (Sharma *et al.*, 2019) and our understanding of the ecosystem. The application of such models will ultimately result in improved ecological research outputs and more informed decision-making regarding biodiversity conservation.

Limitations and future work

The test dataset for this study did not specifically include non-target species, such as co-occurring *Phylloscopus* warbler species and other co-occurring non-warbler species. In order to assess YOLOv5's performance under controlled circumstances, the main goal was to concentrate on target species identification. To preserve clarity and consistency in performance evaluation, we opted to focus only on target species for the present study; however, the inclusion of non-target species would have provided more insights into the framework's generality. We acknowledge that adding non-target species would be useful for evaluating the resilience of the model in practical applications, and suggest that this be the primary target for future research.

TO DOWNLOAD SUPPLEMENTARY MATERIAL CLICK **HERE**.

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DECLARATION OF USE OF GENERATIVE AI

The authors declare that they have used generative artificial intelligence (Claude AI v3) for rephrasing and in the writing of descriptions of figures and tables. The authors thoroughly reviewed and edited the content generated by AI tool and take full responsibility for the content of the publication.

CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY

The code used for the analyses in this study is available at the following GitHub repository: https://github.com/Wild-Inst-Ind/yolov5_Warbler.

AUTHORS' CONTRIBUTION

T.P: conceptualization, investigation, methodology, writing original draft, annotations & formal data analysis, software, visualization, formatting, writing – review & editing.
G.B.H: conceptualization, investigation, methodology, annotations and formal data analysis, software, writing – review and editing, validation.
M.B and S.N: Annotations, Data Augmentation, Image Sorting and preprocessing.
A.C: Annotations.
S.R: conceptualization, investigation, methodology, annotations and formal data analysis, software, visualization, formatting, writing – review and editing, validation.

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Assessing the impact of Livestock Grazing on Fecal Glucocorticoid Metabolites Levels and Gastrointestinal Parasites in Himalayan Ibex in Spiti Valley, Western Himalayas.

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Abstract

Livestock grazing in protected areas is considered a threat and detrimental to many wildlife species and their habitats. In the current study, we examined the impact of livestock grazing on the physiological stress response and parasite prevalence in the Himalayan ibex population of Spiti valley, Himalayas. We standardized cortisol enzyme immunoassay to measure the fecal glucocorticoid metabolites (fGCM) concentrations. A total of 192 fecal samples were collected from eight different locations of livestock grazing and ungrazed areas of Spiti valley during 2015 and 2016. We also collected fecal samples from livestock grazing areas to examine the prevalence of parasites in Himalayan ibex. We found no significant difference in cortisol levels between livestock in grazed and ungrazed areas. However, parasite prevalence was higher in ibex fecal samples collected from livestock grazing locations. Our study provides preliminary insights into the stress physiology and parasitic prevalence of the Himalayan ibex. Further, long-term research with a larger sample size and more quantitative methods is needed to explore a comprehensive understanding of its physiology and pathology.

Keywords: Fecal glucocorticoid metabolites, Himalayan Ibex (*Capra sibirica hemalayanus*), Livestock grazing, Parasite prevalence, Physiological stress

Introduction

The Himalayan ibex (*Capra sibirica hemalayanus*) are mountain goats and are regarded as a subspecies of the Siberian ibex (*Capra sibirica*). However, a recent study suggested that Himalayan ibex is genetically distinct from Siberian ibex (Jabin *et al.*, 2023). The Himalayan ibex is found in the Trans-Himalayan regions of the northern Indian subcontinent and their ranges include the western Himalayas, particularly in areas like Himachal Pradesh and Ladakh (Reading *et al.*, 2020). This species thrives in high-altitude environments, is adapted to steep, rugged terrains, and is typically found at elevations ranging from 2,500 to 4,500 meters above sea level (m asl) (Schaller *et al.*, 1977). Their habitats are alpine meadows, rocky slopes, steep, mountain cliffs, and outcrops. They are known to graze on a variety of plant species, including grasses and lichens, which are abundant in their high-altitude environment. They often form herds that vary in size, with males and females typically segregating into different groups outside the breeding season (Fedosenko *et al.*, 2001).

The Himalayan ibex faces several threats, including hunting, habitat loss due to human encroachment, resource competition for grazing with livestock, and impacts of climate change, which have led to concerns about its conservation status. The Himalayan ibex has been categorized as a "Near Threatened" species by the International Union for Conservation of Nature (IUCN) Red List (Reading *et al.*, 2020). However, in India it is a protected species under Schedule I criteria of the Wildlife (Protection) Act, 1972. Interspecific competition among species at the same trophic level for limited resources negatively impacts species fitness. These changes cause conflict between wildlife and livestock and are responsible for the decline of wildlife populations and lead to the extinction of various species (Krausman *et al.*, 2009). The conflict between wild herbivores and livestock is upsurge in many landscapes (Ren *et al.*, 2021). Himalayan rangeland in Spiti supports a large domestic herbivore population, reaching up to ten times the biomass of wild ungulates (Bagchi *et al.*, 2004). The foraging competition between domestic and wild herbivores for grazing caused local extinctions of four wild herbivore species including wild yak, kiang, Tibetan argali, and chiru from spiti (Mishra *et al.*, 2002).

The short-term release of hormones enhances energy and improves health, but long-term release causes a negative impact on reproduction, development, social behavior, *etc.* An invasive technique for physiological stress analysis requires animal handling, which causes animal stress and it is not feasible to handle animals in the wild (Kumar *et al.*, 2019). Here, the non-invasive method plays an important role in assessing the glucocorticoid metabolites in feces enabling a cumulative evaluation of adrenal activity over time (Möstl *et al.*, 2002). Prolonged chronic stress can lead to immunosuppression, making an organism more vulnerable to parasitic infections (Sapolsky *et al.*, 2000). The occurrence of parasitic diseases in wild herbivores is rapidly increasing due to the interaction and co-existence of livestock and wild animals. This results in mortality, a significant decline in population, and contributes to local extinction events (Gortázar *et al.*, 2007). Studies have revealed that wild animals in human-dominated landscapes experience a high prevalence and diversity of directly transmitted parasites. This suggests that changes in habitat caused by human activities are increasing the risk of infectious diseases for wildlife (Hussain *et al.*, 2013).

While the studies have considered the impacts of livestock on vegetation and wild herbivore population performance (Huber *et al.*, 2003, Foley *et al.*, 2001), no study has yet considered the physiological and parasite load impacts of the co-existence of livestock and wild herbivores in Spiti valley, except the recent study by Iyer *et al.* (2022) on Indian Trans-Himalayas on physiological stress and parasite prevalence in blue sheep. Iyer *et al.* (2022) concluded that blue sheep had higher cortisol levels and parasite loads in grazed areas by domestic livestock. Furthermore, numerous studies show elevated glucocorticoid levels in free-ranging animals due to human-dominated landscapes and anthropogenic disturbance activities. For instance, tigers have been shown to have increased physiological stress due to high tourism activities in National parks (Tyagi *et al.*, 2019), while Asian elephants who participated in public processions and festivals showed significantly higher glucocorticoid metabolite levels (Kumar *et al.*, 2014, 2019, Vijayakrishnan *et al.*,2018). Moreover, African lion shows significantly higher fecal glucocorticoid metabolite levels in human-settled buffer zones than in conservation areas, and glucocorticoid concentrations decreased with the increasing distance from human settlements (Creel *et al.*, 2013). Van meter *et al.* (2009) showed that fecal glucocorticoids were elevated due to pastoralist activity in the form of anthropogenic disturbance and not because of tourism in wild spotted hyenas. However, some studies show no significant impact of cattle grazing on Algerian mice (Navarro *et al.*, 2017). We hypothesized that blue sheep and ibex use similar terrain and habitats as sympatric species and co-exist with the domestic livestock to share the available resources. A previous study (Iyer *et al.*, 2022) demonstrated the impact of livestock grazing on fecal glucocorticoid metabolite levels in blue sheep and the prevalence of human-associated parasites. Therefore, the current study aimed to examine whether ibex is similarly impacted by livestock grazing on their physiology and potential transmission of parasites from humans or livestock. The objectives of the current study were (1) to develop and standardize cortisol enzyme immunoassay (EIA) in Himalayan ibex, (2) to understand the impact of livestock grazing by measuring fecal glucocorticoid metabolites (fGCM) levels in Himalayan ibex, and (3) to examine the prevalence of gastrointestinal parasite infections.

Material and Methods

Study Area

The study was conducted in 12 locations *viz.*, Gechang, Chhomo, Thidim, Noor, Dhar Dum Bachen, Nimaloksa, Koksar, Ensa valley (Opposite and Right), Kilung, Kilung upper and Debsa in Spiti valley of Himachal Pradesh, India (Figure 1). Out of 12 locations, six were livestock grazed areas *i.e.*, Chhomo, Nima Loksa, Kilung, Debsa pass, Kilung upper, and Ensa valley Right, two were ungrazed areas *i.e.*, Gechang and Ensa valley Opposite (Table 1) and four locations (Noor, Thidim, Dhar Dum Bachen, and Koksar) were used for parasite screening sampling. We also collected samples from Gechang, Chhomo, and Ensa valley Right for parasite screening (Table 2). These locations serve as home to pastoralist and agro-pastoral communities, who depend substantially on these ecosystems for their livelihoods (Mishra *et al.*, 2003). These are also home to wildlife species of global conservation concern, such as the vulnerable snow leopard (*Panthera uncia*), blue sheep (*Pseudois nayaur*), wolves (*Canis lupus chanco*), and Himalayan ibex (*Capra sibirica hemalayanus*) (Pandit *et al.*, 2014). Livestock such as goat, cattle, and sheep share similar dietary habits with the Himalayan ibex (Bagchi *et al.*, 2004). In areas where goat, cattle, sheep, and ibex coexist, they may compete for herbs, shrubs, and small grass species during the grazing season. Moreover, in grazing season, the dietary overlap between livestock and ibex increases particularly for grasses and herbs available in alpine meadows. This increased grazing pressure leads to increased competition and stress during critical seasons (Bagchi *et al.*, 2004, Dias-Silva *et al.*, 2020).

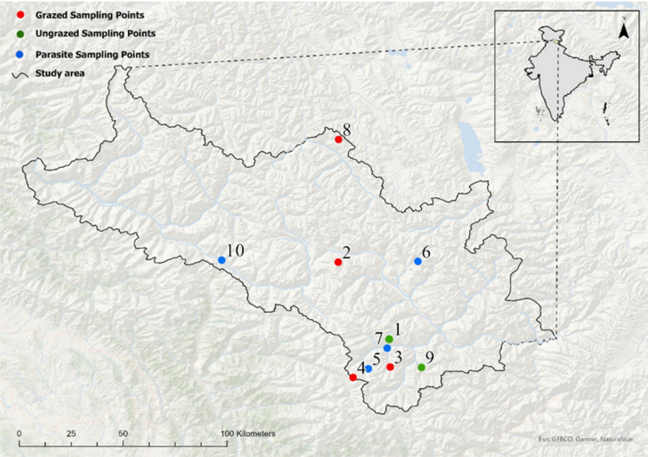

Figure 1. Map of Lahaul & Spiti valley, Himachal Pradesh, India with numbers denoting study points: (1) Gechang, (2) Nimaloksa, (3) Kilung and Kilung upper, (4) Debsa pass, (5) Noor, (6) Dhar Dum Bachen, (7) Thidim, (8) Chhomo, (9) Ensa valley (Right and Opposite), (10) Koksar

Table 1. Details of sample collection locations, human settlements and livestock grazing and mean fGCM concentrations in Himalayan ibex

Location	Livestock grazing/ non-grazing location	Number of Himalayan ibex fecal samples	Mean fGCM concentration ± standard error (ng/g)
Gechang	No	45	37.74 ± 2.30
Chhomo	Yes	12	19.76±1.94
Nima Loksa	Yes	14	44.06± 5.14
Ensa valley Opposite	No	14	17.85± 2.47
Kilung	Yes	08	29.37±7.58
Debsa	Yes	10	33.7± 7.58
Kilung upper	Yes	13	34.84± 3.92
Ensa valley Right	Yes	13	40.30± 6.68

Table 2. Details of sample collection for parasite analysis and parasite prevalence for the different populations

Location	Human & live-stock presence	Number of samples collected	Number of parasite species found	Percentage of samples infected	Mean number of parasite species per infected sample
Gechang	High	13	01	30.76	1
Chhomo	Low	15	01	20.00	1
Noor	High	2	01	50.00	1
Thidim	High	20	02	50.00	1.66
Dhar Dum Bachen	Low	06	01	45.00	1
Ensa valley Right	High	05	02	80.00	1.75
Koksar	Low	02	01	100.00	1

Sample Collection

A total of 192 fecal samples were collected from Himalayan ibex during 2015 and 2016. Among 192 fecal samples, 129 were used for hormone analysis and 63 were used for parasite analysis (Table 1). The fecal samples were collected from grazed and ungrazed areas. Fresh fecal pellets were collected that appeared isolated from others and based on pellet morphology such as pellet size, shape, and texture. For example, a larger pellet comes from larger individuals (*e.g.* adult males), and a smaller pellet indicates young or small individuals. We used 10x50 binoculars to locate herds of Himalayan ibex before carefully approaching them to gather fecal samples. Depending on the terrain, we kept a distance of 20 to 100 meters to prevent disturbing the animals. After identifying a herd, we used binoculars to see individual animals until they defecated. Each fecal deposit's exact position was noted using distinguishing local markers, including particular rocks or plants/shrubs. We collected the fecal samples after the herd had left the area. Two sections of each sample were separated: one for parasite screening and the other for hormone analysis. The fecal samples were dried in a conventional hot air oven at 70°C at the field station and pulverized into a fine powder for hormone analysis. For parasite analysis samples were stored in 10% formalin solution and transported to the LaCONES-CCMB, Hyderabad for further analysis.

Parasite Screening

We used sedimentation and flotation methods according to Chakraborty *et al.* (2019) for fecal gastrointestinal parasites. The percentage of samples containing a certain faunal taxonomic community was used to define the parasite prevalence (Iyer *et al.*, 2022).

Extraction of fecal glucocorticoid metabolites

Fecal glucocorticoid metabolites were extracted using the previously published procedure (Umapathy *et al.*, 2013, Mithileswari *et al.*, 2016). Approximately 0.2 g of fine fecal powder was weighed and boiled in 5 mL of 90 % ethanol for 20 min. The fecal extracts were centrifuged at 500 g for 10 min, the supernatants were transferred to fresh tubes and the pellet was resuspended in 5 mL of 90 % ethanol (Iyer *et al.*, 2022). The samples were then vortexed, centrifuged, and pooled with previous supernatants. The supernatants were dried at 40ºC and resuspended in 1 mL of absolute methanol. The fecal extracts were kept at -20ºC until used for enzyme immunoassay.

Cortisol Enzyme Immunoassay (EIA)

Fecal cortisol concentration was measured using polyclonal cortisol antibody (R4866, provided by Dr. Coralie Munro, University of California, Davis, CA, USA). The cortisol antibody showed cross-reactivity with cortisol 100%, prednisolone 9.9%, prednisone 6.3%, cortisone 5%, and <1% with corticosterone, deoxycorticosterone, 21-desoxycortisone, testosterone, androstenedione, androsterone and 11-desoxycortisol (Kumar *et al.*, 2014, 2019, Budithi *et al.*, 2016). The cortisol EIA was performed as described previously (Kumar *et al.*, 2014, 2019, Umapathy *et al.*, 2015, Iyer *et al.*, 2022). Parallel displacement curves were plotted to examine the parallelism between pooled serial dilutions of Himalayan ibex fecal extracts (endogenous antigen) and corresponding standards (exogenous antigen). The sensitivity of the cortisol assay was found to be 1.95 pg/well at 90% binding (Kumar *et al.*, 2014). The coefficient of variation (CV) for inter and intra-assay were 9.34% and 6.98%.

Statistical Analyses

fGCM concentrations are presented as mean ± SE. Mann–Whitney U test (M–W test) was performed to test the differences in fGCM concentrations in grazed and ungrazed habitats as data were not normally distributed. We carried out the Kruskal-Wallis test to determine the difference in fGCM concentrations among the locations. SPSS version 17 was used to perform all the statistical analyses.

Results

We found that ibex in Nima Loksa had significantly higher fGCM (mean = 44.06 ± 5.14 ng/g of dry fecal powder) than ibex in Ensa valley (mean = 17.85 ± 2.47; Mann-Whitney U test: W = 126, p = 0.001; Figure 2). The Nima Loksa location is a highly grazed area by livestock as this area has a population of sheep and goats compared to Ensa valley which is reported to have an ungrazed area. Similarly, Ensa valley Right had significantly higher fGCM (40.30 ± 6.68 ng/g; Mann-Whitney U test: W = 132, p = 0.002; Figure 2) than Ensa valley Opposite. However, we did not find a significant difference between Debsa, which is known to have a grazed area (mean = 33.70 ± 7.58 ng/g) and Gechang ungrazed area (mean = 37.74 ± 2.30 ng/g; Mann-Whitney U test: W = 230, p = 0.28; Figure 2). We found that fGCM concentrations were significantly varied among the (Kruskal-Wallis's test: X2 = 35.561, df = 7, p < 0.0001). However, when eight locations fGCM concentrations of eight locations were grouped into grazed and ungrazed categories, no significant difference was observed in fGCM concentrations between grazed habitats (Chhomo, Nima Loksa, Kilung, Debsa, Kilung upper and Ensa valley Right: mean = 34.04 ± 2.30) and ungrazed habitats (Gechang and Ensa valley Opposite; mean = 33.02 ± 2.14; Mann-Whitney U test: W = 4.790, p = 0.766, Figure 3).

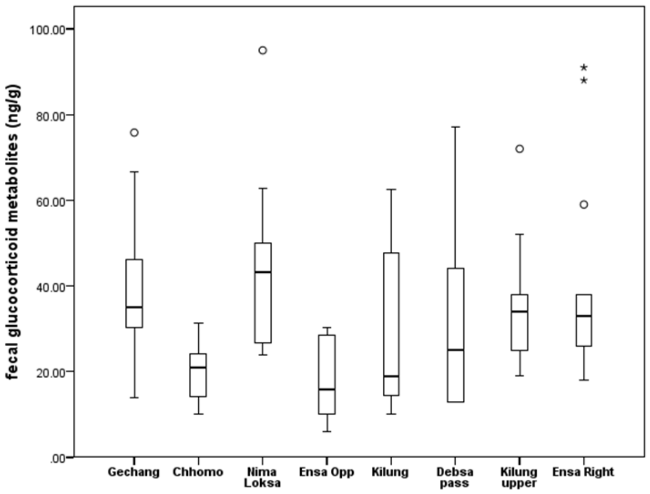

Figure 2. Box-plot showing fGCM concentrations in ibex populations of eight study areas

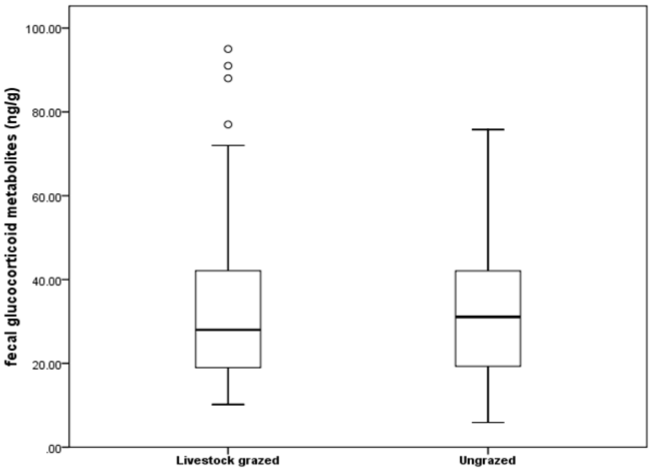

Figure 3. Box plot showing fGCM concentrations in livestock-grazed (Chhomo, Nima Loksa, Kilung, Debsa pass, Kilung Upper and Ensa valley Right) areas versus ungrazed areas (Gechang and Ensa valley Opposite).

Parasite prevalence

We found 3 parasite taxa consisting of two nematodes (Trichostrongyloides and Trichuris) and one protozoan (Coccidia). Thidim (Coccidia and Trichostrongyloides) and Ensa valley Right (Coccidia and Trichuris) had a higher number of parasite species followed by Gechang, Chhomo, Noor, Dhar Dum Bachen, and Koksar (Table 2). When infected samples were compared for the number of parasite taxa per sample, again Thidim (1.66) and Ensa valley Right (1.75) had the highest taxa per infected sample (Table 2). The percentage of samples infected by each of the three parasite species is given in (Table 3).

Table 3. Percentage of samples infected with the parasites (prevalence) in the different populations

Location	Coccidia	Trichostrongyloides	Trichuris
Gechang	30.76%	00.00%	00.00%
Chhomo	20.00%	00.00%	00.00%
Noor	50.00%	00.00%	00.00%
Thidim	50.00%	33.33%	00.00%
Dhar Dum Bachen	45.00%	00.00%	00.00%
Ensa valley	80.00%	00.00%	60.00%
Koksar	100.00%	00.00%	00.00%

Discussion and Conclusion

The interspecies competition between livestock and wild ungulates for available food resources is highly prevalent and the impact of this co-existence negatively affects the species fitness (Robinson *et al.*, 2014). In the current study, we investigated the effect of physiological stress and parasite prevalence in the Himalayan ibex in Trans Himalayas, India. We found no significant difference in fecal glucocorticoid levels between areas of domestic livestock grazing and non-grazing. However, the parasitic load was higher in ibex individuals from livestock grazing than in non-livestock grazing areas. Previous studies on other wildlife species show that competitive foraging and resource competition elevates fecal glucocorticoid metabolite concentrations. For instance, mountain ungulates such as musk deer and goral showed higher fecal glucocorticoid metabolite concentrations when their diet quality was the highest and they faced livestock grazing and resource competition (Srivastav *et al.*, 2021). Champoux *et al.* (1993) reported that cortisol levels and behavioural changes were increased during the period of high foraging demand in the adult female squirrel monkeys. However, a report suggests that high-ranking dominant female blue monkeys experienced lower glucocorticoid levels during times of high food competition which helps to reduce their energetic stress (Foerster *et al.*, 2011). On the contrary, Iyer *et al.* (2022) found significantly higher levels of fecal glucocorticoids concentrations in blue sheep in areas where domestic livestock grazed than in non-grazed areas. In addition, higher parasitic load was found in livestock grazing areas than non-grazing in blue sheep. These stressors make blue sheep more prone to parasitic infections (Iyer *et al.*, 2022). Although Himalayan ibex and blue sheep share the similar habitat variables such as in terms of slope angle, altitude, rock types, and rugged terrain to get away from the predators (Mallon *et al.*, 1991, Bhatnagar *et al.*, 1997). However, ibex usually avoids moderate slopes and areas devoid of snow, whereas blue sheep inhabit only the higher ranges and avoid altitudes that are too low. These differences in the habitat selection suggest that both species have evolved particular preferences in that specific mountainous region (Namgail *et al.*, 2007).

The co-existence between ibex and livestock is lower during summer as they do not utilize the same terrain and altitude type. However, the competition for available resources is higher during spring and summer. Moreover, ibex was observed to forage into the vicinity of the livestock and sometimes into the same livestock grazing group, which shows the likelihood of interference competition was minimal during the period of overlap (Bhatnagar *et al.*, 2000). Similarly, the current study did not find significant differences in fecal glucocorticoid metabolite levels in ibex while co-existing with livestock grazing and non-grazing periods. However, parasitic load was higher in grazing areas as compared to non-grazing areas.

Khanyari *et al.* (2024), show that domestic livestock rarely shares the pastures with ibex due to seasonal movement and therefore have low parasitic loads. We found high parasite load in Thidim and Ensa valley Right locations where human disturbance and livestock grazing were higher. Previous reports also showed higher parasite prevalence in human-dominated areas and species richness of gastrointestinal parasites in lion-tailed macaque groups which were directly associated with increased human activities, and high host density (Hussain *et al.*, 2013). The parasites recorded in the current study were strongyloides, trichostrongyloides, and coccidia, which are commonly available in goat, sheep, and humans (Sanyal *et al.*, 1996). These parasites are extremely harmful to the animals and could lead to severe infections such as anemia, reproductive disorders, intestine infection, and the risk of life of juveniles after weaning (Roberts *et al.*, 2009). The results from the current study suggest that parasites could be transmitted from domestic livestock to ibex since most of the water bodies are shared between domestic livestock, wild animals, and humans. Furthermore, pastures are also shared by domestic livestock and wild animals whereby open defecation is a common practice by humans. Several studies reported the transmission of parasites from domestic animals to the wild animals including ungulates (Martin *et al.*, 2011) and also recommended limiting the interaction between domestic and wild mammals (Pedersen *et al.*, 2007)

The current study suggests that livestock grazing did not have a significant effect on fecal glucocorticoid levels in Himalayan ibex. However, we found the presence of livestock and human parasites in Himalayan ibex. Future studies should focus on investigating host-parasite relationships in an ecological significance context to understand the parasite dynamics and their species-specific impacts. Moreover, parasite presence alone may not have much impact on the species, rather theits occurrence of extreme parasitic load may lead to pathological effects. Although our study aimed to provide baseline information on the stress physiology and parasitic load of the Himalayan ibex, additional long-term research data is required with a larger sample size and quantitative methods for a clear understanding of Himalayan ibex physiology and pathology.

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CONFLICT OF INTEREST

Govindhaswamy Umapathy is an academic editor at the Journal of Wildlife Science. However, he did not participate in the peer review process of this article except as an author. The authors declare no other conflict of interest.

DATA AVAILABILITY

Data are available from the corresponding author on request.

AUTHORS' CONTRIBUTION

Conceptualization and design of the study: Priya Iyer and Govindhaswamy Umapathy; Sample and data collection: Priya Iyer; Data Analysis: Vinod Kumar, Anuja Pandey; Original draft Writing: Anuja Pandey, Vinod Kumar; Review and editing: Anuja Pandey, Vinod Kumar, Govindhaswamy Umapathy

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Documentation of Vulnerable Bull Shark (*Carcharhinus leucas*) Occurrence in the Hooghly River, East Coast of India

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Abstract

The bull shark (*Carcharhinus leucas*) is a species of requiem shark distributed worldwide, which inhabits estuaries, nearshore areas, and the continental shelf waters and opportunistically in rivers within tropical and sub-tropical regions. In the present study, we report the first occurrence of bull shark from inland waters of the Hooghly River in the Ganga River Basin based on molecular and morphometric investigations. The identification of the specimen was confirmed through distinct morphological features, including a blunt snout, large dorsal fin, crescent-shaped mouth with sharp triangular teeth, and a muscular asymmetrical upturned tail. The percent identity of the specimen was 100% based on both the 16s rRNA and ND4 sequences. Phylogenetic analysis revealed that *Carcharhinus leucas* and *Glyphis glyphis* are closely related, forming a well-supported sister clade, while *Carcharhinus falciformis* was identified as a more distantly related sister taxon. This report documents the occurrence of the bull shark in the Ganga River system at the farthest inland distribution limit of the species in India. Our findings extend the known range of the bull shark within India's inland waters and contribute valuable baseline data on biodiversity, highlighting the ecological significance of this vulnerable species within the Ganga River system.

Keywords: Aquatic conservation, distribution, freshwater, molecular assessment, range extension

Introduction

River ecosystems face numerous challenges from water development projects, overfishing, habitat destruction, pollution, and climate-induced changes (Pittock *et al.*, 2008). These stressors collectively or acting independently have resulted in a decline in many freshwater and marine megafaunas (He *et al.*, 2024). Sharks serve as apex predators and play a significant role in local and regional trophic dynamics (Hammerschlag *et al.*, 2022). Despite, their crucial role in ecological processes they are exploited for meat, fins and liver oil for human consumption (Clarke *et al.*, 2005). Globally, the shark populations are under severe threat due to direct and indirect human activities and concerns are being voiced in response to reports of declining numbers due to destructive fishing, industrial activities, and habitat degradation (Airoldi *et al.*, 2008; Dulvy *et al.*, 2014). Despite their recognition of trophic dynamics, interventions focused on the conservation of sharks are impeded by a scarcity of distribution records of many species (Gausmann & Hasan, 2022; Haque *et al.*, 2021).

The bull shark (*Carcharhinus leucas* Müller & Henle, 1839) is a euryhaline migratory species belonging to the family Carcharhinidae. The global decline in the bull shark population, primarily due to overfishing, has led to its classification as Vulnerable on the International Union for Conservation of Nature (IUCN) Red List (Rigby *et al.*, 2021a; Postaire *et al.*, 2024). The species has a wide global distribution, inhabiting tropical to warm temperate waters (Compagno, 1990). Fossil records indicate the species has existed for 23 million years, with evidence spanning the former Tethys Sea, from present-day Peru to the Mekong River (Gausmann, 2021). The bull shark occupies a range of habitats, including rivers, estuaries, nearshore areas, and continental shelf waters in tropical and subtropical regions (Rigby *et al.*, 2021a). Their occurrences in inland rivers are not rare; historically, the species apart from the coast region has been reported inland worldwide (Gausmann, 2021). Recently, the species has been reported in new inland distributions in five different river basins in Indonesia (Gausmann & Hasan, 2022). Bull shark can survive for extended periods in freshwater systems (Thorson *et al.*, 1973; Chen *et al.*, 2015) and rivers and their mouths are known to be essential refuges for neonates and juveniles, offering safer environments and abundant food resources (Pillans *et al.*, 2020; Simpfendorfer *et al.*, 2005). In India, the bull shark has only been reported from the western coast and eastern coast (Purushottama *et al.*, 2013), with no inland records. Accurate identification of the bull shark remains a persistent challenge due to their close resemblance to other shark species, particularly the Ganges shark (*Glyphis gangeticus*) and other members of the genus Carcharhinus (Compagno, 2007; Haque & Das, 2019; Rigby *et al.*, 2021b). The morphological similarity could lead to the misidentification of the shark species, where multiple species are known to occur (Compagno, 2007). Therefore, in the present study, we utilized a combination of morphometric and molecular assessments to report the first inland record of a bull shark from India.

Methodology

Study Area

The Hooghly River, a significant distributary of the Ganga River in eastern India's West Bengal state, originates from the confluence of the Bhagirathi and Jalangi rivers at Nabadwip (Ranjan & Ramanathan, 2018). The Hooghly River estuary is located in the south-western flank of the Ganga–Brahmaputra delta, which flows through Nadia, Hooghly, North 24 Parganas, South 24 Parganas, Howrah and East Medinipur districts before it drains into the Bay of Bengal at Ganga Sagar (Chugh, 1961) (Figure 1). The dynamic interaction between freshwater and estuarine environments along the Hooghly River supports diverse habitats that sustain a wide range of aquatic resources, including numerous fisheries of high ecological and economic importance (Rakshit *et al.*, 2017). The water from the Ganga River is diverted *via* the Farakka Barrage through a feeder canal, which supplies the Hooghly River with sufficient water. The physicochemical parameters such as dissolved oxygen (DO), conductivity, total dissolved solids (TDS), salinity, pH, and NO3- recorded at the specimen collection site reflect the freshwater characteristics of the Hooghly River (Prakash *et al.*, 2023) (Supplementary Table S1).

Specimen collection

The specimen of a potential bull shark (*C. leucas*) was captured near Chandni Ghat, Hooghly (22° 54' N; 88° 23' E), situated 180 km upstream from the mouth of the Hooghly River, on April 26, 2021. The specimen was preserved in 70% ethanol for morphological and molecular assessment. The specimen was opportunistically captured in a fishing net during a moderate tidal phase from a meandering river channel with a width of 420 meters and a depth of 8.8 meters. At the time of collection, the DO concentration was measured at 7.66 mg/L, and salinity was recorded at 0.19 ppt. Additionally, 16 active fishing nets, 12 fishing boats, and 8 ferry boats were observed in the vicinity of the collection site (Supplementary Table S1).

Morphological assessment

We used the approach of Irschick & Hammerschlag (2014) to measure morphometric characteristics, and the specimen was identified following the field identification guide (McAuley *et al.*, 2002). We obtained the following morphometric and meristic traits using a standard metric flexible tape (accurate to 1 mm): total body length (TBL), fork length (FL), pre-caudal length (PCL), pre-orbital length (POB), pre-pectoral length

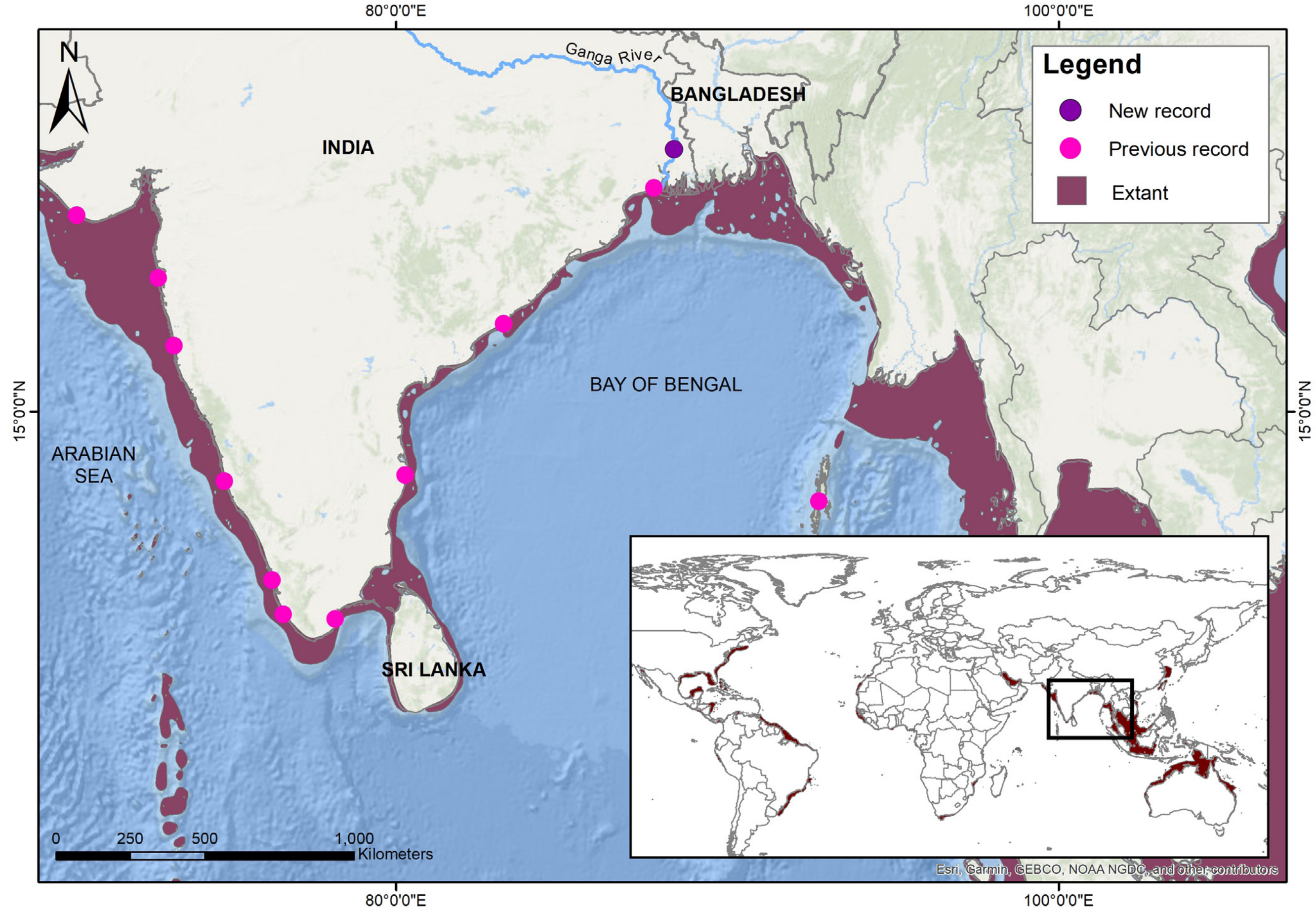

Figure 1. Occurrence records of bull shark (*Carcharhinus leucas*) from the eastern and western coast of India and the first inland record from Hooghly River, India.

(PPL1), pre-pelvic length (PPL2), pre-anal length (PAL), girth at first dorsal fin (GDF), snout-vent length (SVL), mouth length (ML), mouth width (MW), head length (HL), tail height (TH), sex, weight. These morphometric measurements were used to confirm species identification as well as age class.

Molecular assessment

DNA extraction, PCR amplification, and DNA sequencing

A small section of tail tissue was collected from the specimen for molecular assessment. Total genomic DNA was extracted using a DNeasy blood and tissue kit following the manufacturer's protocol (QIAGEN Inc. USA) and quantified using a Quantus™ Fluorometer (Promega Corporation, Woods Hollow Road, USA). We used two partial mitochondrial DNA (mtDNA) fragments 16s rRNA and NADH dehydrogenase subunit 4 (ND4) for species identification and inferring phylogenetic relationship (Table 1).

Table 1. Details of primers used for amplification of mitochondrial DNA 16s rRNA and ND4 for molecular assessment.

Gene/ fragment	Primer sequence (5’─3’)	Reference
16s rRNA	CGCCTGTTTATCAAAAACAT	Chapela *et al.* (2002); Palumbi (1991)
	CTCCGGTTTGAACTCAGATC	
ND4	TGACTACCAAAAGCTCATGTACAAGC	Engstrom *et al.* (2002)
	CCTATTTTAGAGCCACAGTCTAAT	

Polymerase chain reaction (PCR) was carried out in a 10 µL reaction volume containing 5 µL of QIAGEN multiplex PCR master mix, 0.25 µL (3 pmol) of each primer, and 1 µL of template DNA (20-40 ng/µL) and 3.5 µL of RNase-Free water. The PCR was conducted under the following conditions: initial denaturation at 95°C for 15 minutes followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at Ta=56°C for 40 seconds, and extension at 72°C for 90 seconds and final extension at 72°C for 30 min. The amplified PCR products were maintained at 4°C until further use. The amplified PCR products were visualized in 2% agarose gel electrophoresis. The amplified PCR products were purified using Exo-nuclease I and FastAP buffer and sequenced using BrilliantDye™ v3.1 Terminator Cycle Sequencing Kit (NimaGen Inc.) and sequenced in ABI Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) following manufacturers protocol.

The sequences generated were first inspected manually for any error and the quality (Phred Score) of generated DNA sequences was checked in FinchTV Version 1.4.0 (Geospiza Inc. Seattle, WA, USA). The species identification of the generated sequences was confirmed by nucleotide BLAST (Basic Local Alignment Search Tool; https://blast.ncbi.nlm.nih.gov/Blast.cgi). The generated sequences were then aligned with sequences of *C. leucas* other closely related species downloaded from GenBank (www.ncbi.nlm.nih.gov/genbank/).

Data analysis

Phylogenetic analysis and haplotype network

We constructed a Bayesian phylogenetic tree based on the concatenated dataset of 1292 bp (16s rRNA - 571 bp and ND4 - 721 bp) of *Carcharhinus leucas* along with other shark species (Table S2). *Chimaera monstrosa* was taken as an out group species to construct the phylogenetic tree. The appropriate nucleotide substitution model was selected based on the Akaike information criterion (AIC) values using the program jModelTest Version. 2.1.10 (Darriba *et al.*, 2012). The tree was constructed in MrBayes Version 3.2. (Ronquist *et al.*, 2012) using the GTR+I+Gamma model. Two independent MCMC chains of 100 million simulations sampling at every 10,000 generations and 25% of the initial runs as burn-in was performed. The phylogenetic tree was visualized using FigTree version.1.4.4 (Rambaut *et al.*, 2018) and the haplotype network was constructed to assess genealogical relationships using a median-joining network in PopArt (Leigh *et al.*, 2015). Phylogenetic analysis is the most reliable method for reconstructing evolutionary connections and distances between nucleotide sequences. The pairwise nucleotides differences and evolutionary link between the DNA sequences were estimated in MEGA v11.0 (Tamura *et al.*, 2021).

Results

The total length (TL) of the specimen was 83 cm and the weight was 4.5 kg (Table 2). The morphological and meristic features indicate that the captured specimen was a juvenile bull shark. The species identification was confirmed by its distinct morphological features such as, blunt snout, large dorsal fin, crescent-shaped mouth with sharp triangular teeth, and a muscular asymmetrical upturned tail (Figure 3). The coloration was grey on the upper side and pale underneath. As a cartilaginous fish, it possesses two dorsal fins without a skin ridge between them. The characteristic of having five-gill slits, with the last one to three located over the pectoral fin. The eyes were round, and equipped with internal nictitating eyelids (Figure 2).

Figure 2. Lateral view of bull shark (*Carcharhinus leucas*) specimen captured from the Hooghly River, India.

The detailed morphometric measurements of the bull shark specimen recovered from the Hooghly River are provided in Table 2. The gill slits are moderately long. An inter-dorsal ridge is absent. The first dorsal fin is large and broadly triangular, featuring a pointed or sharply rounded. The origin of the first dorsal fin is typically over or just behind pectoral fin insertions, occasionally closer to their free rear tips. The inner margin of the first dorsal is short, measuring less than a third of the dorsal base or slightly less. The second dorsal fin is large and tall and located near the anal origin. Pectoral fins are generally large and broad, featuring narrow, pointed apices.

Molecular analysis

The 571 bp ND4 and 721 bp 16s rRNA fragments were sequenced for accurate identification of the specimen. The BLAST query of both fragments showed 100% similarity with the bull shark sequence (Accession No. OP007121.1). The sequences generated were submitted to GeneBank (Accession No. PP748259 and PP780003). The phylogenetic tree constructed using a concatenated dataset of 16s rRNA and ND4

Figure 3. Specimen of bull shark (*Carcharhinus leucas*) captured from the Hooghly River, India. (A) dorsolateral view, (B) ventral view, (C) dorsolateral view of head showing gill slits. (1) notch in the anal fin, (2) subterminal notch in the caudal fin, (3) minute eyes.

positioned the sequence with a sequence from the Arabian Sea with strong Bayesian posterior probability (Figure 4). The pairwise genetic distance among the sequences analyzed ranged between 0.0 (United Arab Emirates, Japan, Australia and Papua New Guinea) and 0.02 (Seychelles, Thailand & Sri Lanka) (Table S2).

We identified a total of 15 haplotypes in 364 bull shark sequences across 15 countries, including India. The haplotype CLH01 was the most dominant exhibited in 115 (31.7%) sequences, followed by CLH09 found in 61 (16.8%) sequences (Figure 5). The CLH01 has the widest distribution and it is found across ~60% of the countries including Australia, Indonesia, Japan, Taiwan Strait, Thailand, Sri Lanka, Papua New Guinea, United Arab Emirates and India. Our sequence also exhibited CLH01 haplotype, indicating a closer affinity and shared genetic lineage across regions.

Discussion

We report the first occurrence of bull shark in the Hooghly River, West Bengal, eastern India. Both morphological and molecular assessments strongly support the identification of the specimen, as a juvenile bull shark. Considering the ability of bull shark to thrive in freshwaters and recent records of the species in inland waters globally, its occurrences in the Hooghly River are no surprise (Compagno *et al.*, 2005; Hasan *et al.*, 2021) (Figure 1). It has also been observed that bull shark exhibits habitat preference based on size, with small individuals such as juveniles utilizing riverine habitats, while larger individuals prefer marine systems (Brunnschweiler & Barnett, 2013). The inland record of juvenile bull shark in the present study also supports the findings that young individuals utilize riverine habitats preferably guided by predator avoidance, and prey distribution (Glaus *et al.*, 2019). The bull shark species acts as a 'mobile link' species and plays an important role in the stability and functioning of the marine and freshwater ecosystems

Table 2. Morphometric measurements (cm) of the bull shark (*Carcharhinus leucas*) specimen collected from the Hooghly River, India.

Morphometric Measurements (cm)	CMFRI (2005)	Batcha & Reddy (2007)	Purushottama et al., (2013)	Sureandiran & Karuppasamy (2022)	Present study
Total length (TL)	356	330-350	82	180-248	83
Fork length (FL)	...	...	65	145-197	67
Pre-Caudal length (PCL)	...	...	...	...	60
Pre-Orbital length (POB)	...	...	...	...	3
Pre-Pectoral length (PPL1)	...	...	...	...	16
Pre-Pelvic length (PPL2)	...	...	...	...	39
Pre-Anal length (PAL)	...	...	...	...	51
Girth at first dorsal fin (GDF)	...	...	...	...	43
Snout-vent length (SVL)	...	...	43	122-143	43.2
Mouth length (ML)	...	...	7.9	21.5-25.7	7.2
Mouth width (MW)	...	...	9.2	25.5-29.6	8
Head length (HL)	...	...	18.3	55.3-57.9	17
Tail height (TH)	...	...	9.8	52-56	9.7
Sex	F	F	M	F	F
Weight (kg)	320	325-335	3.7	129-235	4.5

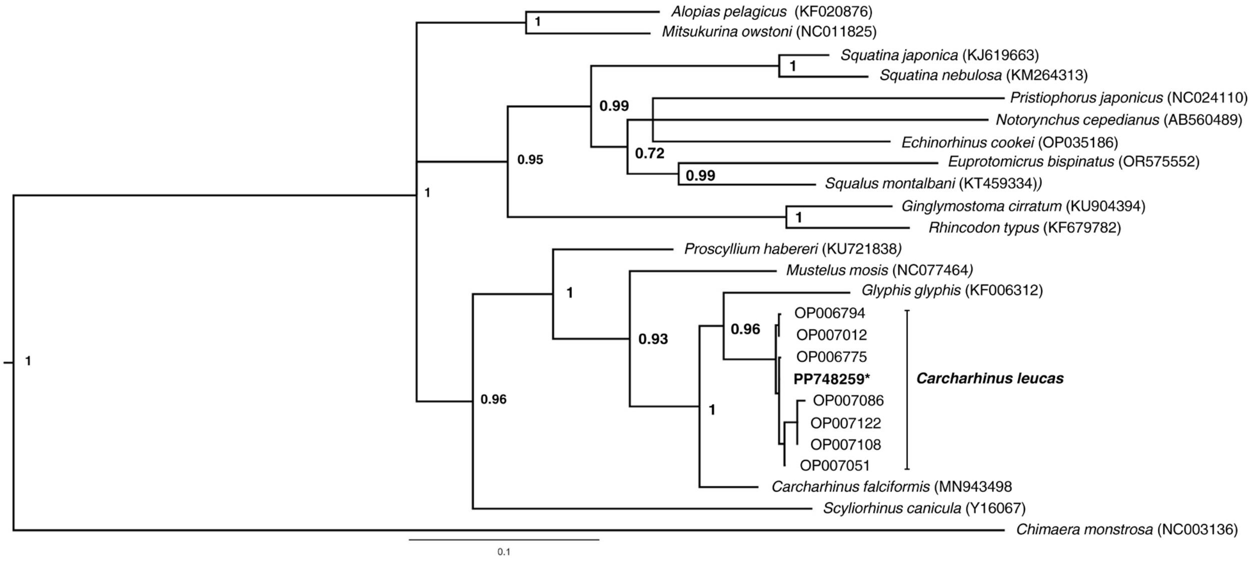

Figure 4. Phylogenetic tree of bull shark (*Carcharhinus leucas*) specimen using 1292 bp of concatenated mitochondrial 16s rRNA and ND4 sequence.

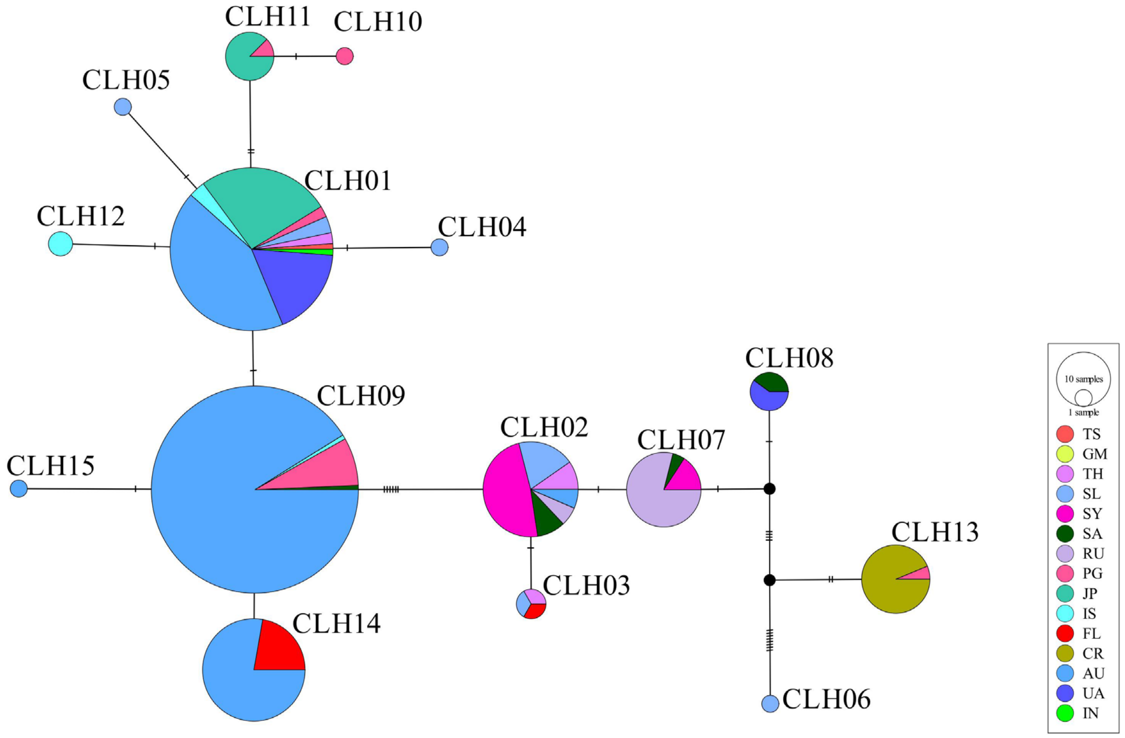

Figure 5. Haplotype network of bull shark (*Carcharhinus leucas*) specimen, constructed using 16s rRNA. Circle size reflects haplotype frequency. Different colors indicate haplotypes detected in different countries. TS-Taiwan Strait, GM- Gulf of Mexico, TH- Thailand, SL- Sri Lanka, SY-Seychelles, SA-South Africa, RU- Reunion, PG-Papua New Guinea, JP- Japan, IS-Indonesia, FL-Fiji, CR- Costa Rica, AU-Australia, UA- United Arab Emirates and IN- India.

(Lundberg & Moberg, 2003; McCann *et al.*, 2005). The increasing occurrences of bull shark in inland water systems worldwide have prompted the necessity for research on the factors that contribute to their presence in the inland waters (Werry *et al.*, 2012). Additionally, bull shark is known to exhibit a unique method of salinity regulation by actively moving between areas with differing salinity levels, rather than relying solely on physiological osmoregulation (Curtis *et al.*, 2013).

The identification of bull shark based on morphological traits and their phylogenetic placement remains somewhat ambiguous due to their notable resemblance with other species in the genus Carcharhinus and Ganges shark (da Cunha *et al.*, 2017; Haque & Das, 2019). Consequently, both the species - bull shark and Ganges shark - are often misidentified for each other (Martin, 2005; Compagno, 2007). Moreover, anecdotal records of these species, particularly Ganges sharks, might actually refer to bull shark and vice versa (Compagno, 1997), potentially explaining the scarcity of bull shark sightings in inland Indian waters. Therefore, identification through an integrated approach utilizing key morphological characteristics and molecular markers is crucial for the accurate identification of these species (Haque & Das, 2019). Key morphological features such as minute eyes, a notch in the anal fin, and a subterminal notch in the caudal fin are helpful in distinguishing the bull shark from the Ganges shark (Compagno, 1997) (Figure 3). Our findings based on the 16s rRNA and ND4 genes, confirmed the identification of the specimen as bull shark, and phylogenetic analysis revealed that C. leucas forms a distinct lineage, exhibiting a sister clade relationship with Glyphis glyphis and C. falciformis. The widespread distribution of haplotype CLH01 indicates substantial gene flow across regions, including India. In contrast, the dominance of CLH09 in other regions such as Australia, Papua New Guinea and Indonesia highlights potential regional structuring (Devloo-Delva *et al.*, 2023). However, the limited number of sequences from India may have introduced bias in these interpretations, emphasizing the need for additional sampling to achieve a more comprehensive understanding of population connectivity within bull shark populations. Further studies have highlighted that genetic divergence in bull shark is primarily driven by significant biogeographic barriers and their philopatric behavior, which restricts gene flow and population isolation (Karl *et al.*, 2011; Devloo-Delva *et al.*, 2023; Postaire *et al.*, 2024).

Understanding the ecological drivers behind the inland distribution of bull sharks remains critical. Bull sharks are known for their remarkable tolerance to both freshwater and marine environments, attributed to their unique osmoregulatory capabilities. This adaptability allows them to migrate hundreds of kilometers upstream in large river systems like the Ganga and its tributaries, including the Hooghly River. One of the primary drivers of their inland movement is the fluctuation of salinity levels in riverine systems. Seasonal variations in salinity, particularly during monsoon and post-monsoon periods, create favorable conditions for bull sharks to move further upstream (Pillans *et al.*, 2020). Juveniles are often found in low-salinity or freshwater zones, which are thought to serve as nursery grounds, providing a refuge from larger predators and supporting early growth stages (Blanco-Parra *et al.*, 2022).

Overall, the first record of bull shark in the inland water system constitutes a noteworthy addition to our understanding of the aquatic biodiversity of the Ganga river basin. Effective conservation efforts, especially for critical nursery habitats, are crucial for the survival of these populations. This highlights the importance of the ongoing efforts made under the 'Namami Gange' program in protecting and revitalizing these crucial ecosystems, by enhanced river monitoring and interventions aimed at preserving the river and its biodiversity (Hussain & Badola, 2020).

Furthermore, understanding behavior, habitat preferences, and migratory patterns of bull sharks can provide insights into the ecological process and interconnectedness of marine and freshwater ecosystems (Heupel *et al.*, 2010; Curtis *et al.*, 2013). Such understanding is essential for the effective management of populations and, for mitigating negative interactions between sharks and humans (Pinel *et al.*, 2023).

Conclusion

Marine apex predators, particularly elasmobranch species like bull shark, play a critical role in maintaining the stability and functioning of marine and estuarine ecosystems. However, they are increasingly threatened by anthropogenic pressures and global climate change. Effective management and conservation strategies for these species require a thorough understanding of their movement patterns and spatial distribution. Due to the limited knowledge of the long-term migration patterns of bull sharks in riverine and estuarine habitats, the report of bull shark in Hooghly River provides valuable insights crucial for conservation and management.

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CONFLICT OF INTEREST

S. A. Hussain is an academic editor at Journal of Wildlife Science. However, he did not participate in the peer review process of this article except as an author. The authors declare no other conflict of interest.

DATA AVAILABILITY

Data will be made available upon reasonable request.

AUTHORS' CONTRIBUTION

G.C.D collected the biological samples and did the morphometric measurements, designed the methodology and wrote the original manuscript. S.P.S and S.T. designed the methodology for molecular analysis, performed the experiment, data curation, data analysis and wrote the original manuscript. S.A.H. acquired resources, and permission to collect biological samples, develop the concept and design the framework and reviewed the manuscript. All the authors approved the final version of the manuscript.

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TYPE Natural History Note

A report of a hybrid offspring between capped langur and Phayre's langur in the state of Assam, India

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Abstract

A hybrid progeny of capped langur *Trachypithecus pileatus* and Phayre's langur *Trachypithecus phayrei* was observed in the Dargakona Tea Estate, Assam, India. The whisker, ventrum, and tail of the hybrid langur were a mixture of both langurs. At the same time, the other characters resembled either of the langurs, indicating the hybridization of the two langurs. A detailed study is suggested to understand their genetic, behavioral, and ecological aspects.

Keywords: Hybrid langur, Indo-Burma, mixed-group species, polyspecies, *Trachypithecus phayrei*

Introduction

The distribution of the endangered Phayre's langur (*Trachypithecus phayrei*) in India is limited in southern Assam, Mizoram, and Tripura (Chetry & Ahmed, 2021) and they share the habitats with capped langur (*Trachypithecus pileatus*). While surveying the primates in southern Assam, a hybrid individual was observed exhibiting the morphological characteristics of both Phayre's langur and capped langur in the Dargakona Tea Estate, bordering the Assam University, Silchar campus, India.

The mixed species group and their hybrid progeny were earlier reported from both old-world and new-world primates (Al-Razi *et al.*, 2023). Kumara *et al.* (2024) reported the mixed troop of Nilgiri langur (*Semnopithecus johnii*) and Hanuman langur (*Semnopithecus hypoleucos*) and Mahato *et al.* (2024) reported the mixed troop of Nilgiri langur (*Semnopithecus johnii*) and tufted grey langur (*Semnopithecus priam*) from the Western Ghats of India. Lu *et al.* (2021) observed the mixed species association of tufted gray langur (*Semnopithecus priam thersites*) with purplefaced langur (*Semnopithecus vetulus philbricki*) and their natural hybrid in Kaludiyapokuna Forest Reserve in Sri Lanka. Choudhury (2008) found a natural hybrid of capped langur (*Trachypithecus pileatus*) and golden langur (*Trachypithecus geei*) in the Zhemgang areas of Bhutan. Aguiar *et al.* (2008) observed the natural hybrid of Alouatta caraya and Alouatta clamitans in Mata do Bugio, southern Brazil.

Morphological characteristics of the hybrid langur

The Phayre troop consisted of two adult males, four adult females (including the hybrid), two juveniles, and three infants. The identified adult hybrid female in the forest patch of Dargakona Tea Estate had mixed morphological features resembling both the capped langur and Phayre's langur (Figure 1A-I). During the survey, an isolated adult male capped langur was observed at several instances near the Phayres' troop. The dorsum of the capped langur was gray or brownish gray, Phayres' langur was light black, while the back side of the hybrid was blackish to ash grey color, *i.e.*, a mixture of both parents. It was lighter than the adult female Phayre's langur of the troop. The upper ventrum of the male capped was yellowish buff or light yellowish orange in the wet season and golden-yellow or yellowish orange in the dry season. But the lower ventrum of the male capped langur was light yellow and the female capped langur was orange-yellow (Figure 1D-F). The ventrum of Phayre's langur was creamy white irrespective of gender and seasons (1B and 1C) while the abdomen of the female hybrid langur was observed more like the female capped langur (Figure 1F, 1I).

The crown of the head resembled the cap of capped langur, but it took the color of the cap of Phayre's langur. The fur color on the tail was dark grey, a mixture of both parents. It was darker than the 'father capped' langur and lighter than the mother Phayre's langur. Skin color at the lip regions of the hybrid langur was whitish like phayre's langur, but the color of the white patch was narrower than the color of Phayre's langur. Color of the pupil, iris and sclera of the hybrid langur was black, light orange and the light black while the color of the pupil, iris, and sclera of capped langur was black and orange and the color of the pupil, iris, and sclera of the Phayre's langur was black. The hybrid langur had a distinct white patch around the eyes similar to mother Phayre's langur but the patch was narrower than the mother's. The fur color near the eyes of the hybrid langur was identical to the father-capped langur. The whisker of the hybrid langur was orange at the base and light grey at the terminal part while the whisker color of capped langur is yellowish-orange and the Phayre's langur is grey to black (Figure 1B, 1D, 1F).

Discussion

Although Phayre's leaf monkey and capped langur are sympatric and habitats often overlap with one another in the region (Choudhury, 1990; Mukherjee, 1994; Feeroz *et al.*, 1995), yet the mixed troop of the two species was not reported in any other case except the study by Al-Razi *et al.* (2023) in northern Bangladesh. The birth of the hybrid individual in the present case can be attributed to the immigration of a male capped langur into the Phayre's troop. One of the major causes of mixed-species troop formation is habitat fragmentation (Detwiler, 2019; Al-Razi *et al.*, 2023). The hybridization as reported here indicates that it is crucial to initiate the conservation of the habitats of the langurs.

Figure 1. Morphological characterization of Phayre's langur (A-C), capped langur (D-F) and the hybrid langur (G-I)

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CONFLICT OF INTEREST

The authors have no conflicts of interest.

DATA AVAILABILITY

Data will be made available upon reasonable request.

AUTHORS' CONTRIBUTION

BS and NRT conceived and designed the study. BS, NRT carried out field study. BS, NRT and ASC analysed the data. BS & ASC wrote the first draft of the MS. PC & NRT reviewed and edited the final draft.

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