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# Integrative taxonomy confirms the species status of the Himalayan langurs, *Semnopithecus schistaceus* Hodgson, 1840

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## Abstract

Taxonomy is replete with groups where the species identity and classification remain unresolved. One such group is the widely distributed Hanuman langur (Colobinae: *Semnopithecus*). For most part of the last century, the Hanuman langur was considered to be a single species with multiple subspecies. Nevertheless, recent studies using an integrative taxonomy approach suggested that this taxon is a complex, with at least three species. However, these studies did not include the Himalayan population of the Hanuman langur whose taxonomic status remains unresolved. The Himalayan population of Hanuman langurs has been classified as a distinct species with multiple subspecies or been subsumed into other species. These classification schemes are wholly based on morphological characters which are sometimes insufficient to delimit different species. Here, we have integrated data from multiple sources viz. morphology, DNA, and ecology to resolve the taxonomy of the Himalayan langur and to understand its distribution limit. Our results with three lines of evidence corresponding to three different species concepts show that Himalayan langur is a species distinct from *Semnopithecus entellus* of the plains. Additionally, these results did not show any support for splitting of the Himalayan langur into multiple subspecies. Our study supports the classification proposed by Hill (Ceylon Journal of Science, XXI, 1939) and we recommend *Semnopithecus schistaceus* Hodgson, 1840 as species name for the Himalayan langur and subsume all the known subspecies into it.

**KEYWORDS**

gray langur, *Semnopithecus*, species concept, systematics, taxonomic ambiguity, temperate colobine

## 1 | INTRODUCTION

Species is the fundamental unit of study in many fields of biology such as systematics, ecology, evolution, behavior, and many more (de Queiroz, 2007). Thus, the first step in these studies is accurate and unambiguous identification of species for which it is imperative to have a well resolved taxonomy for the group of interest. Nevertheless, taxonomy is replete with "problematic groups" wherein species

identity and classification remain unresolved. One such group are the colobine monkeys broadly referred to as Hanuman langur or Gray langur or Sacred langur (Groves, 2001) or northern plains sacred langur (Roos et al., 2014) (Genus: *Semnopithecus* Desmarest, 1822, Subfamily: Colobinae). The Hanuman langur is a widely distributed primate in the Indian subcontinent (Newton, 1988) which exhibits extensive morphological variation across its range. A multitude of classification schemes has been proposed mostly during the early-mid 20th century to resolve the taxonomic status of Hanuman langurs (Brandon-jones, 2004; Ellerman & Morrison-Scott, 1966;

Groves, 2001; Hill, 1939; Napier & Napier, 1967; Pocock, 1928, 1939; Roonwal, 1984; Roonwal & Mohnot, 1977).

Hanuman langurs are broadly divided into two categories; the northern type (NT) which is characterized by a forward looping tail toward the head and the southern type (ST) with the tail looping backward away from the head (Roonwal, 1979, 1984). The Tapti-Godavari rivers in central India form the borderline with NT distributed to the north and ST distributed to the south of these rivers (Roonwal, 1979, 1984). Recent studies support the splitting of ST Hanuman langur into two species, namely *Semnopithecus priam* Blyth, 1844 and *Semnopithecus hypoleucos* Blyth, 1841, based on an integrative approach wherein multiple lines of evidence from molecular, morphological, and ecological data were used (Ashalakshmi et al., 2014; Nag et al., 2011, 2014). Similarly, genetic and morphological data suggest that the plains population of NT Hanuman langur is a separate species, *Semnopithecus entellus* Dufresne, 1797 (Karanth et al., 2010). However, the taxonomy of the Himalayan population (hereafter Himalayan langur) is still unresolved. To understand the exact number of species of Hanuman langurs in the Indian subcontinent, it is important to resolve the taxonomy of all the populations.

Himalayan langurs are the northernmost population of Hanuman langurs (Sugiyama, 1976) distributed in the Himalayan region of India, Nepal and parts of Pakistan and Bhutan (Blanford, 1888; Pocock, 1939). The altitudinal range extends from the Himalayan foothills up to 4,270 m asl (above sea level) (Bishop, 1977). These are one of the few colobine monkeys living in a temperate climate (Nijman, 2013) while the rest are distributed predominantly in tropical regions (Bishop, 1979). Himalayan langurs are classified in the NT category based on the tail loop character (Roonwal, 1979, 1984). Morphologically, the Himalayan langur can be distinguished from the plains population (*S. entellus*) by the tail carriage pattern (discussed in methods section; Figure 1) and by their pelage—the langurs from the Himalayas have a bushy white head which is very distinct from the darker gray-brown body (Figure 2) (Bishop, 1979). Many authors have also talked about differences in the behavioral characters between Himalayan langurs and their conspecifics in the plains, for example, reduction in vocalization profile such as whoop vocalization and canine grinding (Bishop, 1979; Dolhinow, 1978; Sugiyama, 1976); and reduction in use of certain aspects of sexual

behaviors like reduction in use of female headshake to initiate sex (Bishop, 1979). These behavioral differences could possibly indicate that Himalayan langurs and *S. entellus* are distinct lineages.

A variety of classification schemes have been proposed to resolve the taxonomy of the Himalayan langurs (Table 1). One of the earliest comprehensive classifications of Indian colobines was by Pocock (1928). He assigned the Himalayan langurs to five subspecies *Pithecus entellus schistaceus*, *Pithecus entellus ajax* Pocock, 1928, *Pithecus entellus achilles* Pocock, 1928, *Pithecus entellus lanius* Elliot, 1909, and *Pithecus entellus hector* Pocock, 1928 under the species *Pithecus entellus*. Later Pocock (1939) renamed *Pithecus* Geoffroy & Cuvier, 1795 as *Semnopithecus* with three subspecies under it; *Semnopithecus entellus schistaceus*, *Semnopithecus entellus ajax*, and *Semnopithecus schistaceus achilles*. The subspecies *P. e. lanius* and *Pi. e. hector* from Pocock's (1928) earlier classification were not included here. Hill (1939) considered the Himalayan langur to be a single species *Semnopithecus schistaceus* with five subspecies *Semnopithecus schistaceus hector*, *Semnopithecus schistaceus lanius*, *S. s. achilles*, *Semnopithecus schistaceus schistaceus*, and *S. s. ajax*.

Subsequent classification schemes (Ellerman & Morrison-Scott, 1966; Napier & Napier, 1967; Roonwal, 1984; Roonwal & Mohnot, 1977) synonymized *Semnopithecus* with *Presbytis* Eschscholtz, 1821 and subsumed all the Himalayan species into a single species *Presbytis entellus* along with the subspecies *Presbytis entellus entellus* from the northern plains. The subspecies *S. s. lanius* (Hill, 1939) was changed to *Presbytis entellus lania* and the subspecies *S. s. hector* (Hill, 1939) was removed.

Later, Groves (2001) reverted to using *Semnopithecus* for Hanuman langurs and recognized three species of Himalayan langurs *S. schistaceus*, *S. ajax*, and *S. hector*. He elevated the three subspecies from previous classification schemes to species level. Lastly, Brandon-Jones (2004) included all the Himalayan species as subspecies of *S. entellus*, except for *S. s. achilles* and *S. s. lanius* (Hill, 1939) which he did not include in the classification.

Thus, the Himalayan langur has a convoluted taxonomic history falling into three broad groups of taxonomic schemes (TS). TS1—Various populations of Himalayan langurs are placed in multiple subspecies under either *Pi. entellus*, *S. entellus*, or *P. entellus* (Brandon-Jones et al., 2004; Ellerman & Morrison-Scott, 1966;
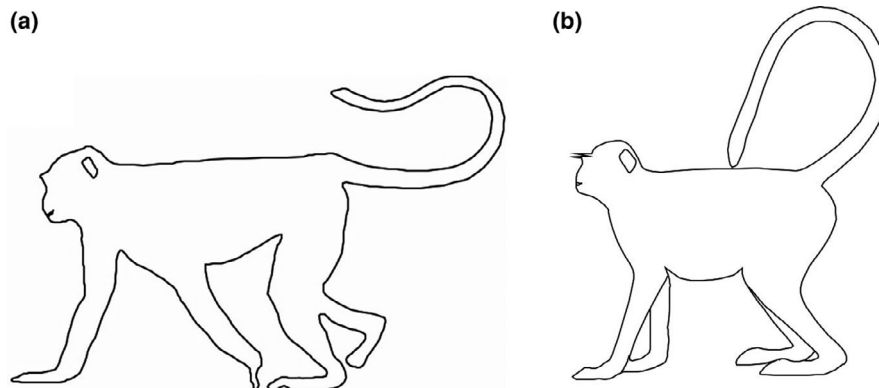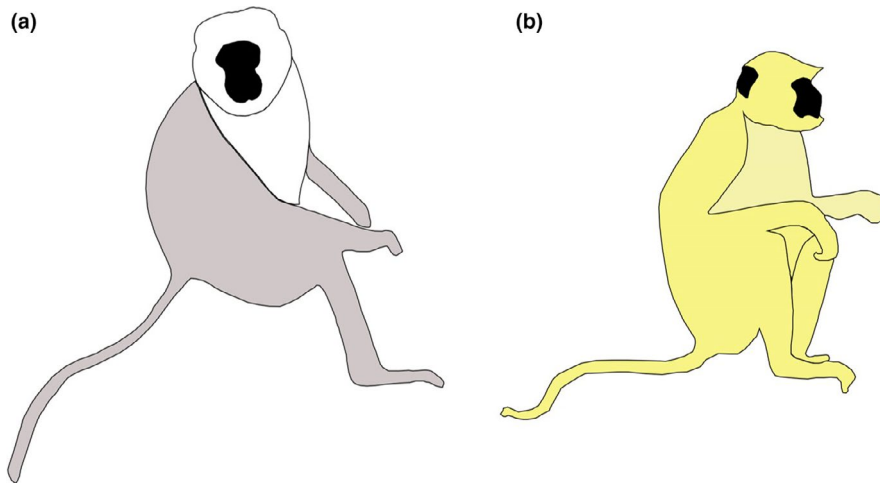


**FIGURE 1** Tail carriage pattern in the Himalayan langur (a) and *Semnopithecus entellus* (b)

**FIGURE 2** Contrast between the head and the dorsal region of the body in the Himalayan langur (a) and *Semnopithecus entellus* (b)

**TABLE 1** Different taxonomic schemes (TS) for Himalayan langur proposed by various authors

| Scientific names | TS 1 | | | | | TS 2 | TS 3 |
| | a | b | c | d[a] | e | f | g |
|---|---|---|---|---|---|---|---|
| Pi. e. schistaceus | X | | | | | | |
| Pi. e. ajax | X | | | | | | |
| Pi. e. achilles | X | | | | | | |
| Pi. e. lanius | X | | | | | | |
| Pi. e. hector | X | | | | | | |
| S. e. schistaceus | | X | | | X | | |
| S. e. ajax | | X | | | X | | |
| S. e. achilles | | X | | | X | | |
| S. e. hector | | | | | X | | |
| P. e. schistaceus | | | X | X | | | |
| P. e. ajax | | | X | X | | | |
| P. e. achilles | | | X | X | | | |
| P. e. lania | | | X | X | | | |
| S. schistaceus | | | | | | X | |
| S. s. ajax | | | | | | X | |
| S. s. achilles | | | | | | X | |
| S. s. lanius | | | | | | X | |
| S. s. hector | | | | | | X | |
| S. schistaceus | | | | | | | X |
| S. ajax | | | | | | | X |
| S. hector | | | | | | | X |

*Note: e, entellus; P, Presbytis; Pi, Pithecus; s, schistaceus; S, Semnopithecus.*

a = Pocock (1928); b = Pocock (1939); c = Roonwal and Mohnot (1977); d = Roonwal (1984); e = Brandon-Jones (2004); f = Hill (1939); g = Groves (2001).

X = name according to the respective classification, e.g., Pocock (1928) described Pi. e. schistaceus.

[a]Also includes Ellerman and Morrison-Scott (1966) and Napier and Napier (1967).

Napier & Napier, 1967; Pocock, 1928, 1939; Roonwal, 1984; Roonwal & Mohnot, 1977). TS2—Himalayan langurs are considered a distinct species itself with multiple subspecies (Hill, 1939). TS3—Himalayan langurs are split into multiple species (Groves, 2001).

With the advent of molecular techniques many recent studies have used genetic data to resolve taxonomic ambiguities in primates of the Indian subcontinent (Arekar et al., 2019; Ashalakshmi et al., 2014; Chakraborty et al., 2007; Karanth et al., 2008, 2010; Osterholz et al., 2008; Wangchuk et al., 2008). However, the use of molecular data does not guarantee a robust description and identification (Will et al., 2005). Molecular data are often considered as another singular data type, such as morphological data, which can be used as a line of evidence to characterize and describe species. To achieve a robust delineation of species, we need to integrate methods from different disciplines (Dayrat, 2005).

Given this background, we have used data from multiple sources viz. morphology, DNA, and ecology to address the following questions (a) Are Himalayan langurs a distinct species from *S. entellus*? (b) Do Himalayan langurs occupy a different niche than *S. entellus*? (c) Do Himalayan langurs comprise multiple species/subspecies? and (d) What is the distribution range of Himalayan langurs?

## 2 | MATERIALS AND METHODS

### 2.1 | Molecular data

#### 2.1.1 | Data collection

We conducted fieldwork in four Himalayan states of India—Jammu and Kashmir (J&K), Himachal Pradesh (HP), Uttarakhand, and Sikkim. These states were chosen for fieldwork based on distribution records from past studies (Bishop, 1979; Choudhury, 2001; Hill, 1939; Pocock, 1939; Sugiyama, 1976) as well as from IUCN website (www.

**(a)**



**(b)**



**(c)**
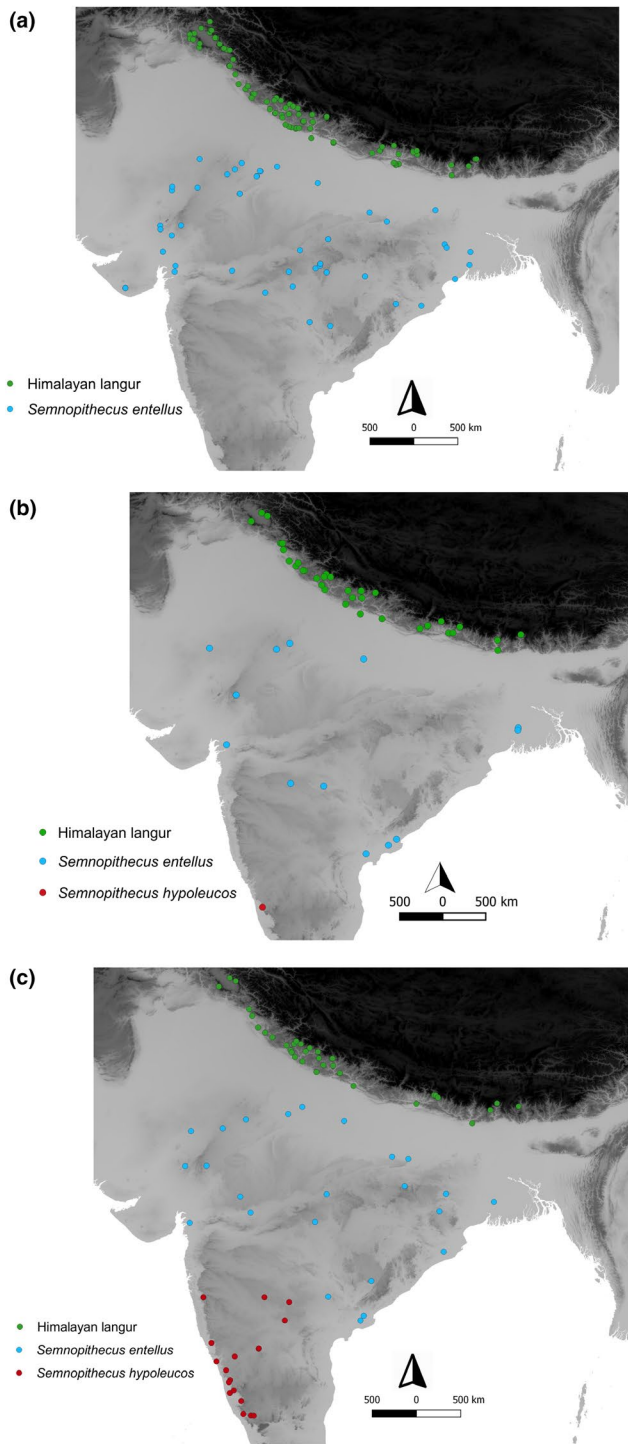


**FIGURE 3** Locations of samples used in ecological (a), molecular (b), and morphological (c) analyses

iucnredlist.org). We included our field data as well as published sequences from previous studies (Ashalakshmi et al., 2014; Karanth et al., 2010; Khanal et al., 2018).

We collected 176 fecal samples from 46 locations (Figure 3b, Table S1) across the distribution range of Himalayan langurs in India with multiple samples collected from each location. Additionally, five fecal samples of *S. entellus* were collected from one location in the northern plains (22.88220N, 88.39970E). Out

of these, we used 26 samples for the molecular phylogenetic analysis (Described in detail in Text S1 and Table S2). Fresh fecal samples were collected by following the troops in the morning and the evening hours. Samples were collected by two different methods—First, as described in Kawamoto et al. (2013), a sterile cotton swab was rolled multiple times over the surface of the feces and thoroughly rinsed in the lysis buffer (White & Densmore, 1992). The second method involved collecting the whole feces which was then stored in absolute alcohol. These samples were stored at –20°C in the laboratory until DNA extraction. Samples stored in lysis buffer were first treated with starch to remove potential PCR inhibitors like bilirubin and bile salts (Kawamoto et al., 2013; Zhang et al., 2006), and then DNA was extracted by using Wizard® SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA and stored in pure nuclease-free water at –20°C until further use. DNA from whole fecal samples was extracted using the commercially available QIAamp DNA stool mini kit (QIAGEN Inc.), following the manufacturer's protocol with slight modifications as mentioned in Mondol et al. (2009), however, we did not add the carrier RNA (Poly A) (Kishore et al., 2006). Each extraction had a negative control to monitor contamination. The quantity of extracted DNA was measured using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific Inc.).

### 2.1.2 | PCR amplification and sequencing

A 775 bp (amplicon length) region of mitochondrial *cytochrome b* (*Cyt-b*) gene was PCR amplified using the primer pair Cytb_278F (5′ – GCCTATTTCTACACGTAGGCCG – 3′) and Cytb_1052R (5′– CCAATTGCAATGAAGGGTTGGT – 3′). A 25 µl reaction was set with standard 1X reaction buffer premixed with 1.5 mM MgCl₂ (New England BioLabs® Inc.), 0.25 mM of dNTPs (Bangalore Genei), 0.3 µM of each primer (Sigma-Aldrich Inc.), 1.5 U Taq polymerase (New England BioLabs® Inc.), and 2 µl of template DNA (DNA concentration between samples varied from 20 to 80 ng/µl). We also added 2 µl of BSA (Bovine Serum Albumin, Fisher Scientific) to augment the PCR reaction. Further, the template DNA was diluted to 1:5 (DNA extract: water) ratio and used for the reaction to reduce the amount of PCR inhibitors. The PCR cycling conditions were carried out with initial denaturation at 94°C for 5 min followed by 50 cycles with denaturation at 94°C for 40 s, annealing at 57.5°C for 30 s and elongation at 72°C for 30 s and a final extension at 72°C for 10 min. The PCR products were outsourced for purification and sequencing to Medauxin, Bangalore.

### 2.1.3 | Phylogenetic analysis

The sequence files obtained were viewed and edited manually in ChromasLite v2.01 (Technelysium Pty. Ltd.). Sequences of Himalayan langur, as well as *S. entellus*, were also downloaded from previous molecular studies (Table S2) (Ashalakshmi

**TABLE 2** Coding system used for the six morphological characters used in this study

| | Characters | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | Crest | Streak | EOB | Tail loop (TL) | Tail carriage (TC) | HBC |
| Himalayan langur | 0 | 0 | 0 | 1 | 3 | 1 |
| S. entellus[a] | 0 | 0 | 3 | 1 | 0 | 0 |
| S. hypoleucos | 0 | 1 | 3/4[b] | 2 | 1 | 0 |
| S. priam[c] | 1 | 0 | 1 | 2 | 2 | 0 |

Note: The coding system is designed to be consistent with Nag et al. (2011), unless mentioned otherwise.

S, Semnopithecus.

Abbreviations: EOB, extent of blackness; HBC, head-body contrast.

Crest, Streak and HBC, 0 = absent, 1 = present; TL – 1 = Northern type, 2 = Southern type; EOB – not visible = 0, till finger tips = 1, till knuckles = 2, till wrist = 3, till elbow = 4; TC = tail carriage (Figure 1), TC3 = 0, TC4 = 3, TC1 (Figure 3c in Nag et al., 2011) = 1.

[a]Semnopithecus entellus = Nt from Nag et al. (2011).

[b]Semnopithecus hypoleucos contains two morphotypes (St1/St2; Nag et al., 2011).

[c]We did not use this species in the analysis.

et al., 2014; Khanal et al., 2018). The sequences were aligned using MUSCLE algorithm (Edgar, 2004) incorporated in MEGA v7 (Kumar et al., 2016).

We used jModelTest 2.1.3 (Darriba et al., 2012) to pick the best model of sequence evolution. Phylogenetic reconstruction was performed using Maximum Likelihood (ML) and Bayesian methods. ML analysis was performed in RAxML7.4.2 incorporated in raxmlGUI v1.3 (Stamatakis, 2006). We used the GTR + G model in RAxML as there is no provision in the GUI version to use other models. One thousand replicates were performed to assess support for different nodes. We used MrBayes 3.2.2 (Ronquist et al., 2012) to perform the Bayesian analysis with HKY + G nucleotide substitution model. Two parallel runs with four chains each were run for 10 million generations with sampling frequency every 1,000 generations. Convergence between the two runs was determined based on the standard deviation of split frequencies. The program Tracer v1.6 (Rambaut et al., 2013) was used to determine stationarity, an effective sample size (ESS) value of >200 for each parameter was used as a cut-off for run length. The first 25% of trees were discarded as burn-in.

### 2.1.4 | Hypothesis testing

We compared the Bayesian tree (Figure 1), with an a priori hypothesis where the phylogeny was constrained to be consistent with Groves' (2001) three species of Himalayan langurs; S. ajax, S. hector, and S. schistaceus. RAxML7.4.2 incorporated in raxmlGUI v1.3 (Stamatakis, 2006) was used to generate a constraint tree. The likelihood of the constraint tree was then compared with the best tree using the SH test (Shimodaira & Hasegawa, 1999) and AU test (Shimodaira, 2002) in PAUP* version 4.0a (build 164) (Swofford, 2001). For both the SH and AU test, we performed 10,000 bootstrap replicates and the non-parametric bootstrap with reestimated log likelihoods (RELL) approximation (Kishino et al., 1990) was used for resampling the loglikelihoods.

## 2.2 | Morphological data

### 2.2.1 | Data collection

Morphological data were collected by direct observations during the field survey as well as from photographs. We used a total of 85 samples for the morphological analysis—35 samples for Himalayan langur, 28 samples for S. entellus, and 22 samples of S. hypoleucos. For the Himalayan langur, out of the 35 samples, seven were from Nepal (photographs provided by Prof. Naomi Bishop, Prof. John Bishop, Prof. Andreas Koenig, Prof. Carola Borries, Mr. Ganga Ram Regmi, Mr. Sagar Dahal, Ms. Mehreen Khaleel), one was from Bhutan (Photograph provided by Ms. Himani Nautiyal) and the remaining 27 were from the Indian Himalayan region (collected for this study). Samples for S. entellus and S. hypoleucos were obtained from a previous study (Nag et al., 2011). Based on past studies (Nag et al., 2011) and our field observations, six color-independent morphological characters were used to differentiate between S. entellus and the Himalayan langur. These included the four characters described by Nag et al. (2011) and two characters unique to Himalayan langurs. Characters specific to Himalayan langur included tail carriage (Roonwal, 1979, 1984) and demarcation between the head and the body Head-Body contrast [HBC]) (Bishop, 1979; Groves, 2001; Hill, 1939; Pocock, 1939). Within the NT langurs, two forms of tail carriage are observed, in S. entellus from the northern plains the tail loops over the back and the tip of the tail hangs perpendicular to the ground, here onwards TC3 (Figure 1b), whereas, in the Himalayan populations the tail loops well behind the back and the tip ends above the base of the tail, here onwards TC4 (Figure 1a). We coded them TC3 and TC4 to be consistent with Nag et al.'s (2011) coding system. The tail carriage pattern was recorded when the individual was walking on a flat surface and not while climbing up or down the hill and nor while it was running or standing (as per Roonwal, 1984). The Himalayan langur has a distinct demarcation between the head and the body; the head is bushy and white-colored distinct from the gray-brown body (Figure 2a) (Bishop, 1979; Groves, 2001; Hill, 1939; Pocock, 1939). Langurs from the plains (S. entellus) have a uniform color without much
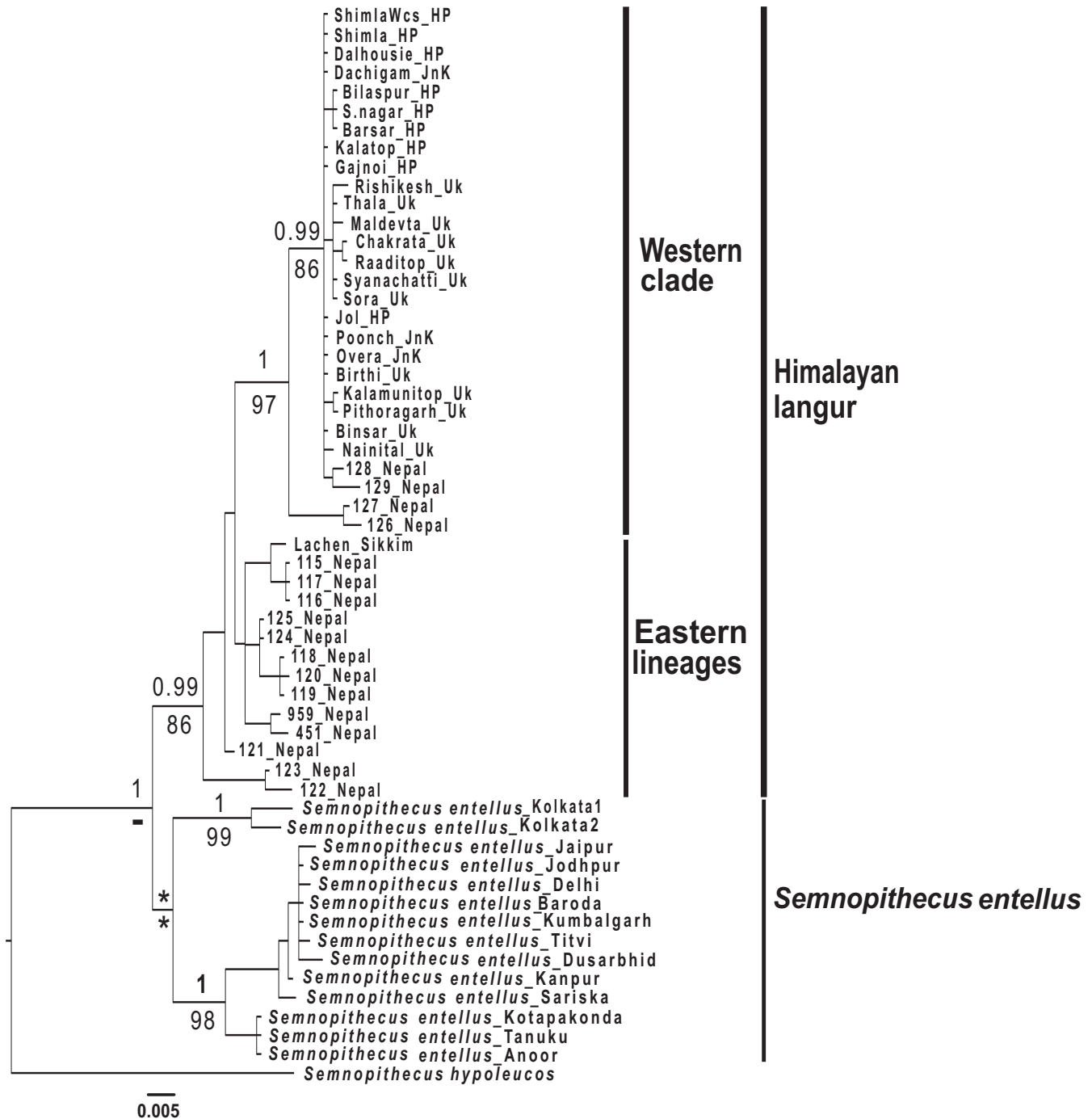
**FIGURE 4** Bayesian tree of Himalayan langurs based on the mitochondrial *cytochromeb* (*Cyt-b*) gene. The numbers at the nodes above the branches indicate Bayesian posterior probability (BPP) values whereas numbers below the branches indicate Maximum likelihood bootstrap support (MLBS) support. Support values are shown only for main nodes. *indicates BPP <0.85 and MLBS <85; - indicates MLBS values not available for that node. HP, Himachal Pradesh; JnK, Jammu & Kashmir; Uk, Uttarakhand

of a distinction/contrast between the head and the body (Figure 2b). We call this character HBC character. Apart from these two characters we also used the four characters described in Nag et al. (2011), that is, presence or absence of crest, presence or absence of streak between the eye and the ear, Northern or Southern type tail loop (TL) and the extent of blackness (EOB) on the hand; these characters are not seen in Himalayan langurs and therefore were coded "0"(absent). We coded TL as 1 to indicate that Himalayan langurs belong to NT langur group with

a forward looping tail along with *S. entellus*. We recorded these characters by direct observations using 10 × 50 binoculars (Olympus) and through photographs taken from a digital camera (Canon PowerShot SX20 IS). These characters were scored for multiple adult individuals per location. Furthermore, additional data points were obtained from literature records (Roonwal, 1981, 1984). The point coordinates for these data points were extracted from google maps using the names of the locations provided by Roonwal (1981, 1984) (Figure 3c, Table S3).

**TABLE 3** Topology test results

| Tree | −ln *L* | Diff −ln *L* | SH | AU | *p*-value |
|------|---------|--------------|-----|-----|-----------|
| Best tree | 2,107.65046 | (best) | | | |
| Constrained tree[a] | 2,371.94530 | 264.29484 | 0.0000* | ~0* | *p < .05 |

[a]Constraint tree – The phylogeny was constrained to be consistent with Groves' (2001) three species of Himalayan langurs; *Semnopithecus ajax*, *Semnopithecus hector*, and *Semnopithecus schistaceus*.

## 2.2.2 | Analysis

We typed 85 adult individuals, as mentioned above, from 82 locations (Table S3). We also included one of the southern species from this complex, *S. hypoleucos* (St1 and St2 morphotypes in Nag et al., 2011) in the analysis. The characters were coded as described in Table 2. All the characters were coded in a way that they are consistent with Nag et al.'s (2011) coding system. The codes for *S. hypoleucos* were similar to ones used in Nag et al. (2011).

We prepared a character matrix with the terminal taxa in the rows and the six morphological characters in the columns. Using this matrix, first, the mean character difference was calculated between the individuals in PAUP* Version 4.0a (build 164) (Swofford, 2001). Then, a neighbor-joining (NJ) tree was built using these distances, with the default settings. Mid-point rooting was used to root the tree.

## 2.3 | Ecological data

### 2.3.1 | Data collection

For ecological data, we obtained 192 occurrence records of the Himalayan langurs (Figure 3a—occurrence data are available from authors on request). Out of these, 79 records were from the field surveys conducted for this study, 58 occurrence records were obtained from previous studies (Khanal et al., 2018; Minhas et al., 2012, 2018) and 55 occurrence records were downloaded from GBIF (Global Biodiversity Information Facility) database (www.gbif.org). For *S. entellus*, 69 occurrence records were used (Figure 3a), out of these, 21 records were obtained from Nag et al. (2014), and 48 records were downloaded from GBIF database (www.gbif.org). For the occurrence records downloaded from the GBIF database, we plotted these occurrence records on the map and included only those records which fell within the known distribution zones of the respective taxa. Further, we used 22 environmental layers, 19 were bioclimatic layers downloaded from www.worldclim.org, one altitude layer (USGS website), two more layers—slope and aspect were derived from the altitude layer in ArcGIS 10.2.1 (Table S4). All the layers were of 30 arcsec resolution, projected in WGS84 projection. These bioclimatic variables were clipped to the region from 68°E to 97.4°E and from 6.7°N to 37°N using ArcGIS 10.2.1. These clipped layers were then exported to ASCII format using QGIS 2.18.12. The 22 environmental layers were tested for multicollinearity by calculating Pearson's correlation coefficient (*r*). The layers with $r \leq |.75|$ were selected for further analysis (Table S5).

### 2.3.2 | Ecological Niche Modeling analysis

We first performed model selection using the maximum entropy algorithm available in Maxent v3.4.1 (Phillips et al., 2006). We tested 48 models, each for Himalayan langur and *S. entellus*, by employing different combinations of features and regularization multiplier (RM) (Table S6) in MaxEnt v3.4.1 (Phillips et al., 2006).

Separate Maxent analyses for the Himalayan langur and *S. entellus* were implemented in MaxEnt v3.4.1. Maxent was run with the following modifications. Random test percentage was set to 30%, maximum number of background points was set to 10,000 and the replicates were set to 10 with replicated run type changed to Subsample. Five thousand iterations were performed with the convergence threshold set to $1 \times 10^{-5}$. Jackknife test was used to estimate the contribution of each environmental variable. The feature type and RM value were based on the best model selected (Table S6). To overcome the sampling bias, a bias file was created in ArcGIS 10.2.1 by applying Gaussian kernel density function to 10,000 background points (Elith et al., 2010). The output format was chosen as Cloglog (Phillips et al., 2017). AUC values were examined to check for the predictive ability of the model.

Schoener's *D* (Schoener, 1968) was calculated as a measure of niche overlap between the two distribution models (Warren & Seifert, 2011) implemented in ENMTools (Warren et al., 2010).

## 3 | RESULTS

### 3.1 | Phylogenetic analysis

Our final alignment contained 746 bp of *Cyt-b* sequence from 57 samples (Table S2). It includes 26 samples sequenced in this study, 30 sequences downloaded from previous studies and 1 *S. hypoleucos* sequence used as outgroup (Table S2, Text S1). Sequences generated in this study have been deposited in GenBank, Accession numbers MT919045–MT919070 (Table S2). The final alignment for the *Cyt-b* dataset is provided in Alignment S1.

The Bayesian and ML analysis recovered two major clades, the *S. entellus* clade—containing the sequences from the northern plains; and the Himalayan clade—containing sequences from the Himalayan region. Within the Himalayan clade was a well-supported subclade consisting of haplotypes from the western Himalayas (west from 28.07N to 83.26E, which was nested within samples from eastern Himalayas (east from 28.19N to 83.65E). Both, the Bayesian and ML
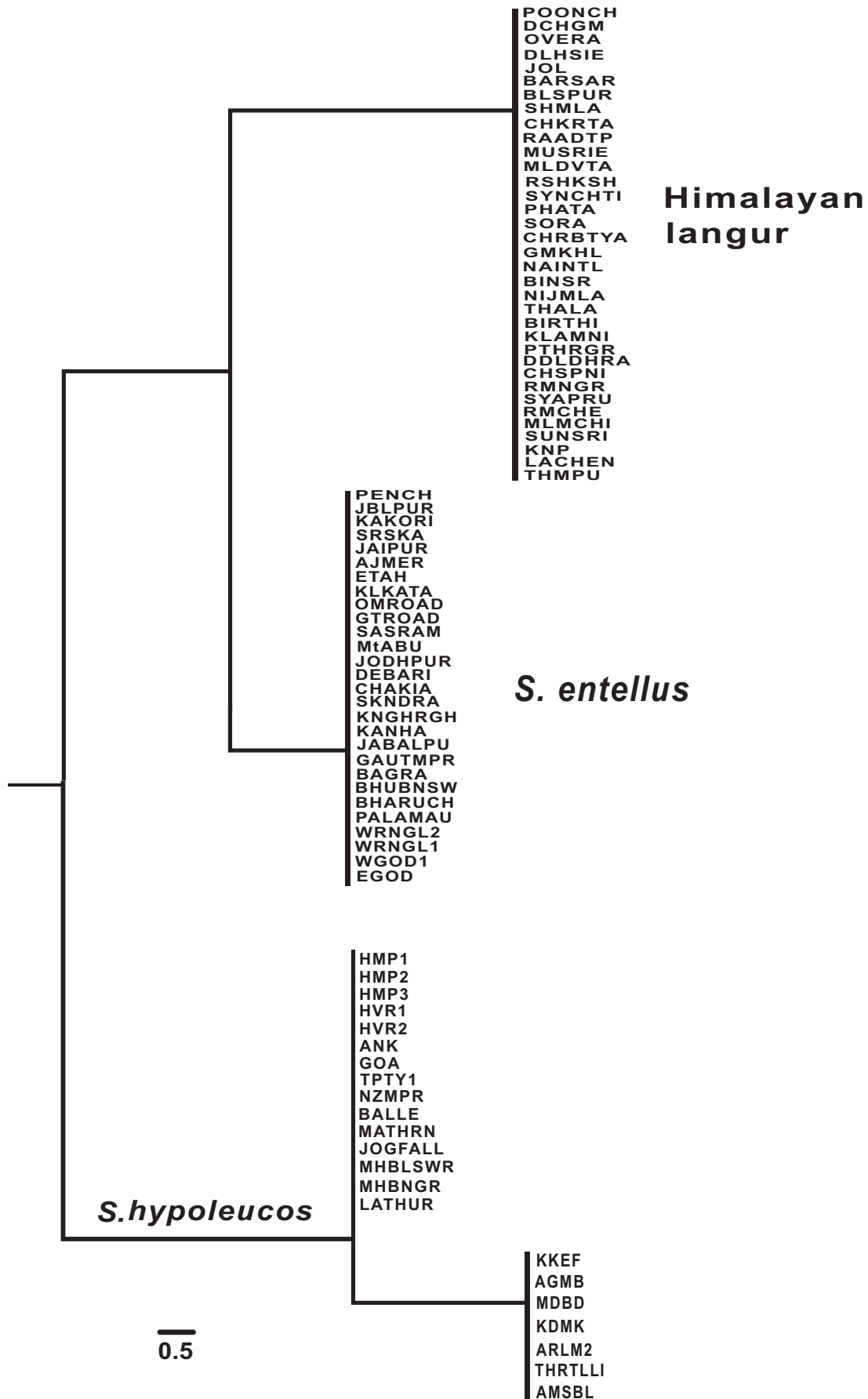
**FIGURE 5** Neighbor-joining tree based on six color-independent characters. *S. hypoleucos* is one of the two species of Hanuman langur from peninsular India

FIGURE 6 Species distribution maps of Himalayan langur (a) and *Semnopithecus entellus* (b)
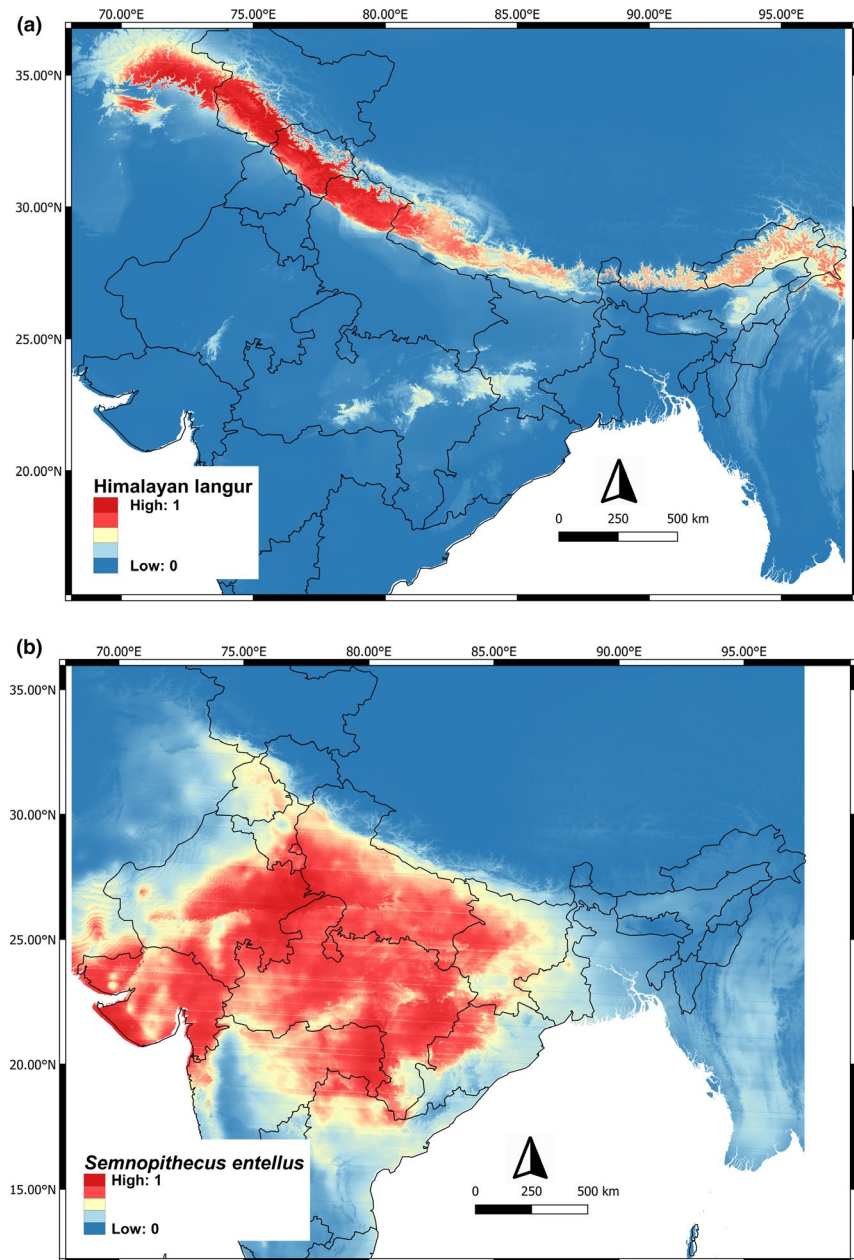


trees, showed similar topology wherein all the major clades were retrieved. Furthermore, in the Bayesian tree (Figure 4), two samples, that is, 128_Nepal and 129_Nepal were placed within the clade containing samples from the Indian Himalayan Region (IHR), whereas in the ML tree (Figure S1), these two samples were sister to the above-mentioned clade.

## 3.2 | Hypothesis testing

The likelihood score of the best tree was significantly higher than that of the constraint tree for both SH test and AU tests. Therefore, these trees based on the molecular data did not support the splitting of Himalayan langurs into three species (Table 3).

## 3.3 | Morphological data

Our final NJ tree (Figure 5) retrieved a distinct cluster consisting of all the Himalayan samples that were sister to a cluster with all the *S. entellus* samples. The UPGMA method also generated a similar topology (tree not shown). We retrieved two clusters of *S. hypoleucos*, this was similar to the result generated by Nag et al. (2011).

## 3.4 | Ecological niche modeling

### 3.4.1 | Model prediction

The best model selected for the Himalayan langurs had features "LQPTH" and an RM value of 2.5; whereas for *S. entellus*, the best

model had features "Auto" with an RM value of 3 (Table S6). Based on these models and the environmental variables selected, we obtained different distribution maps for *S. entellus* and the Himalayan langurs (Figure 6). In the distribution maps, the warmer color indicates suitable area whereas the cooler colors indicate unsuitable areas. The AUC values for the training and test data for the Himalayan langur dataset were 0.9663 and 0.9621, respectively. And the AUC values for the training and test data for *S. entellus* were 0.889 and 0.87, respectively. These AUC values indicate that the potential distribution of these species fits well with our data.

### 3.4.2 | Variable selection and their importance

For the Himalayan langur, precipitation of driest quarter (Bio17) was the highest ranked variable (Table 4). Jackknife test also illustrates the importance of Bio17 (Figure S3). The response curve (Figure S2) shows that the habitat suitability increases with the precipitation of the driest quarter (Bio17) but eventually attains stationarity. Annual mean temperature (Bio1) and mean diurnal range (Bio2) were the next two contributing variables (Table 4).

Precipitation seasonality (Bio15) was the most contributing variable toward the distribution of *S. entellus* (Table 4). The response curve for Bio15 (Figure S4) shows that the values for habitat suitability were high toward higher values of precipitation seasonality (Bio15), suggesting that *S. entellus* prefers areas with high variation in rainfall. Mean temperature of warmest quarter (Bio10) and annual mean temperature (Bio1) were the next two highly contributing variables (Table 4).

Niche overlap between *S. entellus* and Himalayan langur was 17% (Schoener's *D* value = 0.17). However, when we compare the areas with a high probability of distribution (>0.75), there was no overlap.

## 4 | DISCUSSION

The so-called Hanuman langur has been known to be a species complex for a long time. Nevertheless, recent studies have brought some clarity to their confused taxonomy. These studies suggest that the so-called Hanuman langur consists of at least three species: *S. entellus*, distributed in the plains of North India (Karanth et al., 2010), *S. hypoleucos* distributed in peninsular India and *S. priam* distributed in peninsular India and Sri Lanka (Ashalakshmi et al., 2014; Nag et al., 2011, 2014). However, the taxonomic status of the Himalayan population of this complex remained unresolved. Here, we address this issue by applying multiple lines of evidence to resolve the taxonomic status of the Himalayan langur.

Our molecular analysis based on mitochondrial *Cyt-b* gene retrieved a monophyletic Himalayan langur distinct from *S. entellus* of the plains. The Himalayan langur is also morphologically distinct and occupies a specific niche in the Himalayas. Thus, three lines of evidence, corresponding to different species concepts, suggest that the Himalayan langurs are a separately evolving metapopulation

lineage (de Queiroz, 1998). The phylogenetic species concept (PSC) II (Donoghue, 1985; Mishler, 1985; de Queiroz, 1998) identifies species as monophyletic groups based on shared derived characters. Our genetic data show that Himalayan langur and *S. entellus* are reciprocally monophyletic. Results from the morphological analysis suggest that the Himalayan langur is a separate species as per the Phenetic Species Concept (Sokal & Crovello, 1970). Further, our niche modeling analysis too shows that these two lineages occupy distinct ecological regions and thus show separation along the ecological axis (Van Valen, 1976).

Among the plethora of classification schemes, only Hill (1939) placed the Himalayan langurs in a separate species, *S. schistaceus*, with multiple subspecies—*ajax, achilles, lanius,* and *hector* (Table 1). Our study supports species status for the Himalayan langur; however, we do not recommend further splitting of this taxon into multiple species/subspecies (see the result under hypotheses testing). Even though the western populations do form a well-supported subclade, this subclade is nested within the Himalayan langur clade. Importantly, the distribution of the western subclade does not correspond to the distributions of any of the species and subspecies mentioned in Groves (2001) and Hill (1939), respectively. Furthermore, the morphological data do not support the splitting of the Himalayan langurs into multiple species/subspecies. Here, we recommend *S. schistaceus* Hodgson, 1840 as a species name for all the Himalayan langurs and subsume all the earlier described species and subspecies within it until further detailed taxonomic studies address this issue.

Two of the characters used in this study—HBC and forms of tail carriage (TC3 and TC4), can be used as field identification characters for differentiating *S. schistaceus* and *S. entellus*. We do not recommend the use of any of the external morphological characters listed in the earlier classification schemes (Table 1) for distinguishing between different subspecies of Himalayan langurs. The morphological characters used in earlier classification schemes to describe these subspecies are highly plastic, variable, and subjective (Nag et al., 2011). For instance, in the identification key Pocock (1939) describes *schistaceus* as follows "General colour paler, salty or greyish-buff; coat shorter and less woolly" and for *achilles* he writes, "General colour dark earthy brown; coat thick and woolly." Hill (1939) describes *schistaceus* as "A slatey-grey race, with shorter, less woolly coat than those found at higher altitudes." Our personnel observations suggest that these characters tend to differ based on what month of the year the langur is being observed and what is the altitude at that location. Roonwal (1981) describes four types of tail carriage in the NT langurs; however, we recorded only two tail carriage types that are used in this study, the other two tail carriage types have not been observed.

We used ecological niche modeling (ENM) to determine the distribution range of *S. schistaceus*. It predicted the distribution range of the *S. schistaceus* (Figure 6a) with high accuracy (AUC = 0.96). The distribution was mainly governed by precipitation of the driest quarter (Bio17). Response curves (Figure S2) for the top three contributing variables (Table 4) suggest that *S. schistaceus* prefers areas with high precipitation but with moderate temperatures. Interestingly,

**TABLE 4** Maxent results showing the most important variables ranked on the basis of the amount of variation they explain in the predicted distribution of *Semnopithecus entellus* and the Himalayan langur along with the permutation importance for the variables

| Semnopithecus entellus | | | Himalayan langur | | |
|---|---|---|---|---|---|
| Variable | Percent contribution | Permutation importance | Variable | Percent contribution | Permutation importance |
| Bio 15 | 35.5 | 39.6 | Bio 17 | 42.1 | 5.9 |
| Bio 10 | 26.5 | 7.2 | Bio 1 | 20.6 | 48.2 |
| Bio 1 | 20.7 | 19.0 | Bio 2 | 15.5 | 16.5 |
| Bio 12 | 11.7 | 19.9 | Bio 4 | 13.7 | 23.7 |
| Bio 18 | 2.5 | 5.7 | Bio 12 | 8.1 | 5.7 |
| Bio 3 | 1.0 | 5.4 | Slope | 0.1 | 0.0 |
| Bio 8 | 0.8 | 0.0 | Aspect | 0.0 | 0.0 |
| Slope | 0.3 | 0.1 | | | |
| Bio 9 | 0.3 | 1.7 | | | |
| Aspect | 0.3 | 0.7 | | | |
| Bio 14 | 0.2 | 0.6 | | | |
| Bio 19 | 0.1 | 0.0 | | | |
| Bio 4 | 0.1 | 0.0 | | | |

slope and aspect did not contribute to the model prediction. A recent study (Khanal et al., 2018) also showed that precipitation plays a major role in the distribution of *S. schistaceus* in Nepal. Langurs in the Himalaya inhabit broadleaf subtropical forest at lower altitudes and temperate broadleaf forest at higher altitudes (Bishop, 1979; Curtin, 1982; Minhas et al., 2013; Sayers & Norconk, 2008; Sugiyama, 1976). These forests receive high rainfall during the monsoon as well as precipitation in the form of snowfall in winter (Bhattarai & Vetaas, 2003; Singh & Singh, 1987; Singh et al., 1995).

We also used ENM to determine if the ecological niches of *S. schistaceus* and *S. entellus* are separate. The ENM distinctly demarcated ecological niches of these two species. The AUC value for both the distribution models was significant, implying that the results greatly differ from the random predictions. Precipitation was the most important variable in demarcating these two species, with *S. entellus* requiring less precipitation and the Himalayan langurs need higher precipitation. Bishop (1979) also pointed out that the temperate climate is the factor that governs the distribution of *S. schistaceus* and *S. entellus*. We also checked for niche overlap between these two species to determine if they are divergent in their ecological axis. The niche overlap between these two taxa was not significant suggesting that their ecological niches are separate. However, it must be noted that the presence of langurs in hot and drier areas like Jodhpur (23.2389N, 73.0243E) could be due to provisioning of food by the local inhabitants (Mohnot, 1971). This type of food provisioning can positively affect different life-history traits and reproductive success in these populations (Borries et al., 2001).

This study provides comprehensive evidence for elevating the Himalayan langur to a species that is distinct from *S. entellus* of the northern plains. Based on our results, we recommend that this species be assigned to *S. schistaceus* as per Hill (1939).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**Figure S1.** Maximum likelihood tree of the Himalayan langur reconstructed using mitochondrial *cytochrome – b* (*Cyt – b*) gene.

**Figure S2.** Response curves for top three variables of importance in the Himalayan langur.

**Figure S3.** Jackknife test of regularised gain.

**Figure S4.** Response curves for top three variables of importance in *Semnopithecus entellus*.

**Table S1.** Locations of all the faecal samples collected for the study.

**Table S2.** List of samples with their accession numbers, Sample IDs and location coordinates.

**Table S3.** Details of the locations and characters used for the morphological tree.

**Table S4.** Different environmental layers used in this study. Each layer is of 30 arcsec resolution and is clipped to the region from 68°E to 97.4°E and from 6.7°N to 37°N.

**Table S5.** Correlation matrix between the 22 variables used in this study.

**Table S6.** Model selection for Maxent analysis: The table shows AUC values for different models.

**Text S1.** Dataset for molecular phylogenetic analysis.

**Alignment S1.** Alignment of *Cyt-b* dataset.

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