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Delineating Ecological Boundaries of Hanuman Langur Species Complex in Peninsular India Using MaxEnt Modeling Approach

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Abstract

Hanuman langur is one of the widely distributed and extensively studied non-human diurnal primates in India. Until recently it was believed to be a single species - *Semnopithecus entellus*. Recent molecular and morphological studies suggest that the Hanuman langurs consists of at least three species *S. entellus*, *S. hypoleucos* and *S. priam*. Furthermore, morphological studies suggested that both *S. hypoleucos* and *S. priam* have at least three subspecies in each. We explored the use of ecological niche modeling (ENM) to confirm the validity of these seven taxa and an additional taxon *S. johnii* belonging to the same genus. MaxEnt modeling tool was used with 19 bioclimatic, 12 vegetation and 6 hydrological environmental layers. We reduced total environmental variables to 14 layers after testing for collinearity and an independent test for model prediction was done using ENMTools. A total of 196 non-overlapping data points from primary and secondary sources were used as inputs for ENM. Results showed eight distinct ecological boundaries, corroborating the eight taxa mentioned above thereby confirming validity of these eight taxa. The study, for the first time provided ecological variables that determined the ecological requirements and distribution of members of the Hanuman langur species complex in the Indian peninsula.

Introduction

Species is one of the fundamental units of biodiversity and of great interest to taxonomists, evolutionary biologists, ecologists, and conservationists [1,2]. In spite of the importance, till recently, there has been little consensus regarding its definition that resulted in numerous species concepts [3]. However, de Queiroz [4] and Pigliucci [5] considered "species" as metapopulation lineages, a concept that attempts to combine various species concepts. Thus, it is generally accepted that species comprise of lineages, although, there still exists inconsistencies regarding how lineages are diagnosed as species [6,7]. An integrative approach which uses multiple lines of evidence is usually recommended for recognizing evolutionary lineages [8]. Thus, one of the challenges now is to obtain ample evidence to establish a clear demarcation of species boundaries.

In this regard, delimiting species boundaries using ecological niche modeling (ENM) approach (alongside molecular studies) has generated wide interest [9,10,11,12,13,14,15,16,17]. Martinez et al, [18] observe that these ecological niche models, by identifying consistent differentiation patterns in characters related to the ecological niche, might provide alternative means of recognizing putatively independent lineages and thus act as an effective tool in delimiting species boundaries. These models which largely employ empirical data are useful to characterize species ecological requirements [19,20,21], understand distributions, biogeography and dispersal barriers [10,22], identify effects of climate change [14,23], forecast species invasions [24], realize the effects of habitat alterations [25], delimit species boundaries [9,11,12,13] and predict unknown populations and species [26,27].

Ecological niche models are being utilized for a number of aforementioned reasons, and its application in the field of Primatology also seems to be slowly gaining momentum. Primates play an important role in seed dispersal thus helping to maintain and balance biodiversity [28,29,30]. However, studies using primates as model systems largely assess the status and potential distributions for setting up conservation priorities [18,26,31,32,33,34].

Of recognized 634 primates in the world, at least 304 of them are threatened with extinction [35]. It implies that nearly half of the world's primates are at risk. The primates as a whole are facing the worst odds in all the years they have been assessed and there are many more to follow [36]. However the number of "species" at risk of extinction still remains uncertain due to the ambiguities regarding the definition of species concepts itself indicating, that our understanding of primate diversity and taxonomy is by no means complete [37]. Taxonomy solely based on specific morphological traits often results in inadequate or misleading guides for phylogenetic distinctions at subspecies and species level [38,39]. Hanuman langurs (*Semnopithecus entellus*) are one such example, whose taxonomic status is hugely debated.
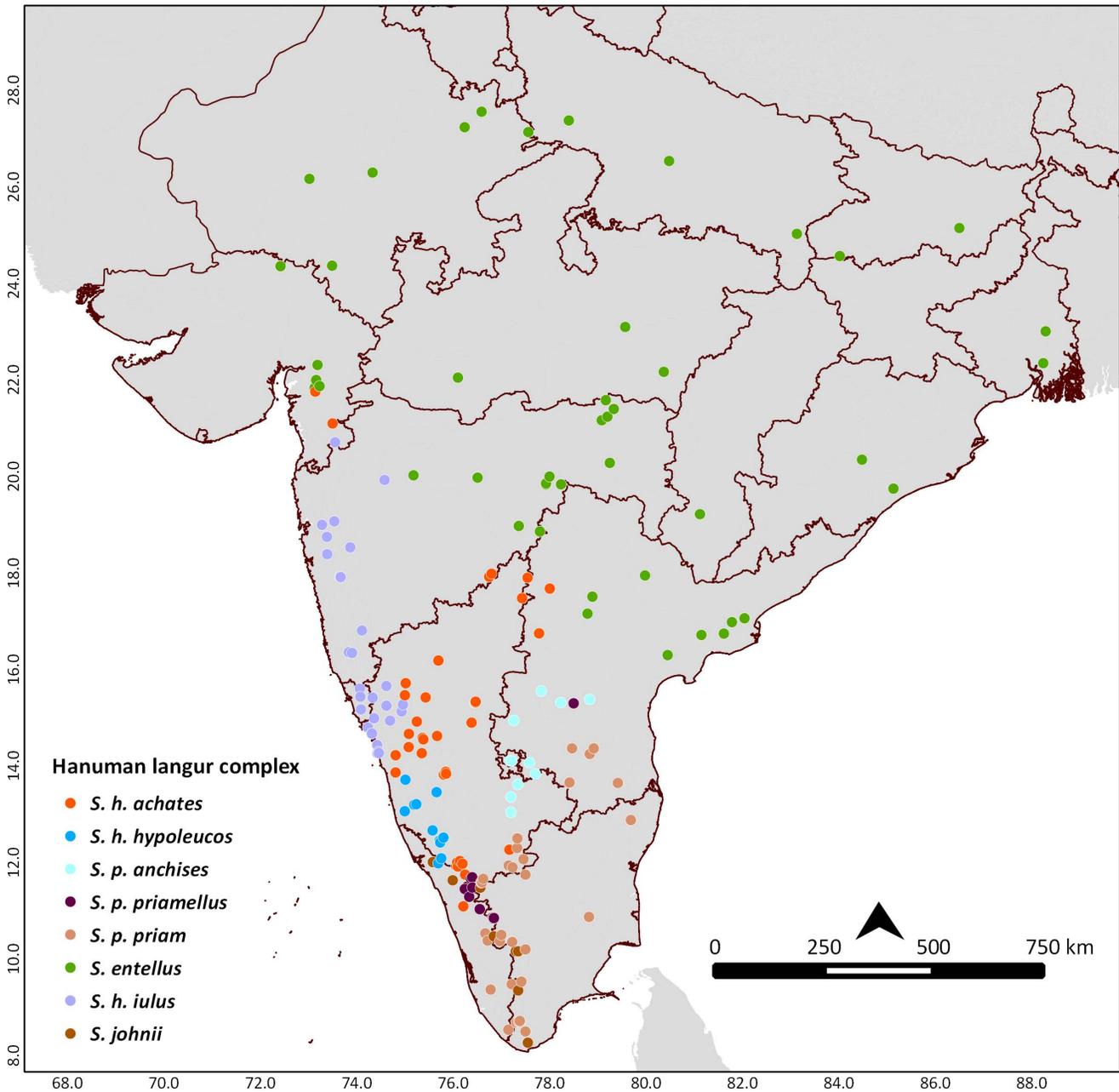
Figure 1. Occurrence points of eight taxa of langurs of peninsular India.
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Amongst the most widely distributed non-human primates in South Asia, Hanuman langurs or common langurs (Family: Cercopithecidae, Subfamily: Colobinae) are a common sight in Indian villages, towns and tourist areas, [40,41]. They are also revered by Hindus, and perhaps one of the most extensively studied non-human diurnal primates in India. Hanuman langurs are distributed throughout most of India and Sri Lanka [42,43,44] as well as in parts of Pakistan, Nepal [43,45,46], Bhutan and Bangladesh [47]. Hanuman langurs are acclimatized to a wide range of habitats [48,49,50,51,52,53,54,55,56,57] from arid regions on the edge of the desert in Rajasthan to the rainforests of Western Ghats. They have been recorded at altitudes from sea level to 4270m above msl in the Himalayas [58,59,60]. The annual rainfall in Hanuman langur habitats is known to range

between 10 cm to 740 cm [30,49,50,51,53,55,56,61]. Hanuman langurs are also known to show adaptation to strong seasonality from Himalayan habitats (-7°C) to extreme summer temperatures at Rajasthan ranging between 30°C to 46°C [57,59,62,63]. Being predominately folivorous, the Hanuman langur's diet includes mature leaves of deciduous and evergreen trees along with fruits, fruit buds and petioles [30,54,57,64,65].

There has been much disagreement in the literature on the subspecies or species status of various populations of Hanuman langurs [58]. Most authors consider Hanuman langurs to be a single species (*Semnopithecus entellus*), but classify it into 14, 15, and 16 subspecies while others split them into two, four and seven distinct species suggesting their taxonomy is in a flux [66]. A recent work by Nag et al, [58] observed at least six morphotypes of

Table 1. Non-overlapping occurrence data points of the various taxa used in the present study.

Sl. No.	Species/Subspecies	Field survey data	Secondary data	Total
1	*S. hypoleucos achates*	32	8	40
2	*S. hypoleucos hypoleucos*	7	5	12
3	*S. priam anchises*	10	2	12
4	*S. priam priamellus*	10	1	11
5	*S. priam priam*	22	13	35
6	*S. entellus*	21	24	45
7	*S. hypoleucos iulus*	26	4	30
8	*S. johnii*	6	5	11
Total occurrence points				**196**

Hanuman langurs in peninsular India by using a combination of five diagnostic morphological characters. Their study recommended Hill's [67] classification scheme for future studies to bring about some clarity in the taxonomy of these langurs. However, these results now need to be validated with other lines of evidence such as ecology and molecular data.

Hill [67] classified Hanuman langurs into four species namely *Semnopithecus schistaceus*, *S. entellus*, *S. hypoleucos* and *S. priam*. While the *S. schistaceus* and *S. entellus* have Northern type (NT) tail carriage distributed to the north of Narmada and Krishna rivers of peninsular India, *S. hypoleucos* and *S. priam* on the other hand have Southern type (ST) tail carriage and are predominantly distributed south of Narmada and Krishna rivers in South India and Sri Lanka. *Semnopithecus schistaceus* consists of five subspecies viz., *S. s. hector*, *S. s. schistaceus*, *S. s. achilles*, *S. s. ajax* and *S. s. lanius*, largely confined to the Himalayas. *S. entellus* is distributed in the plains of central and northern India (south of the Himalayan region) till the Narmada and Krishna rivers of peninsular India. The southern species *S. priam* consists of three subspecies namely *S. p. priam*, *S. p. thersites* and *S. p. anchises*; *S. hypoleucos* consists of six subspecies *S. h. hypoleucos*, *S. h. aeneas*, *S. h. elissa*, *S. h. iulus*, *S. h. dussumieri* and *S. h. achates*. However, Hill [67] was doubtful of the validity of *S. p. thersites* and *S. h. dussumieri* [58]. Thus one of the fundamental questions is that whether morphologically distinct species and subspecies of Hanuman langurs also exhibit distinct ecological niches?

The majority of Hanuman langur studies in India and Sri Lanka have directed their attention to behavioral studies (Nag, unpublished) and a few studies have looked at foraging ecology [30,51,54,57,68,69,70,71,72]. Furthermore, there has been a lack of a reliable distributional and ecological data on various species/subspecies of Hanuman langurs. Accordingly, there is an urgent need to delimit species and subspecies boundaries among Hanuman langurs and understand their requirements. This is particularly important given that the Hanuman langurs are used as model organisms for various biomedical, ecological, behavioral studies [66]. Thus in this paper, we test if the species and subspecies accepted by Hill [67] exhibit significant divergence in their ecological niches. Also, we have attempted to understand their ecological requirements and potential distributional ranges. In order to do so we concentrated on the southern species *S. priam* and *S. hypoleucos* and their subspecies. Furthermore we have also included *S. entellus* and *S. johnii* in the analysis.

Materials and Methods

Ethics statement

All the observations were made without any physical contact with the study animals. To carry out necessary field work we had necessary permissions from the Forest Departments of respective State Governments, which is a regulatory body (Andhra Pradesh State Rc. No. 29757/2009/WL.3 dated 24/8/2009; Kerala State WL.1 2-2937/2008 dated 26/7/2008; Karnataka State PCCF/C/CR-127/2007-2008 dated 20/5/2008 & D/WL/CR-/2007-2008 dated 23/1/2008 and Maharashtra state D-22(8)/Research/1340/2009-2010 dated 4th August/September 2009).

Target species and occurrence data

The study obtained 196 non-overlapping occurrence records of seven Hanuman langur morphotypes namely, Semnopithecus hypoleucos achates, S. h. iulus, S. h. hypoleucos, Semnopithecus priam priamellus, S. p. priam, S. p. anchises, S. entellus; and Nilgiri langur Semnopithecus johnii [58]. We followed Hill's [67] classification scheme to assign species/subspecies names for the morphotypes. We retained name priamellus as per Pocock, [73] for one population in Palakkad district. The occurrence records were collected from field surveys (details of field surveys are given in [58]) and literature records [74,75] representing the known distribution of the species (Table 1; Figure 1). The occurrence data are available with the corresponding author and will be sent on request.

Environmental Coverage Variables

The study considered 37 environmental variables for modeling ecological niches of Hanuman langurs in peninsular India. Of these 37 variables, 19 were bioclimatic [76], 12 were vegetation (Enhanced Vegetation Index – EVI) and 6 were hydrological layers. The layers were related to precipitation, temperature, topography, and ecological bioregions (Table S1 in File S1). All the layers were re-sampled to 1000 m resolution, on WGS84 Longitude-Latitude projection and clipped for Indian subcontinent (excluding Sri Lanka, Bangladesh, Pakistan, Nepal and Bhutan). Layers were tested for multicollinearity and layers that had r≤±0.85 (Pearson's correlation coefficient) following Elith et al, [77] were selected for further analysis. This resulted in 14 layers comprising of one vegetation layer, seven bioclimatic and six hydrological layers.

MaxEnt modeling algorithm

We used a maximum entropy algorithm available in MaxEnt [78,79]. Recent studies indicate [17,80,81] that MaxEnt performs well when compared with other ENM methods and has been widely used to delimit species boundaries and ecological niches [82,83,84,85]. MaxEnt is a machine learning program that estimates the probability distribution for a species occurrence based on environmental constraints [79]. MaxEnt is designed to make predictions from presence-only data using background environment of the study area. In other words MaxEnt is designed to characterize probability distribution from incomplete information. MaxEnt is also advantageous since it uses both continuous and categorical variables [86] and the output is a continuous prediction. MaxEnt has been in wide use because of its effectiveness even with small sample sizes. However, few drawbacks of MaxEnt approach such as model extrapolation or over fitting have been discussed in the literature [86].

MaxEnt was used with following changes in the model run. Random test percentage was set to 25%. Regularization multiplier was set to 1 and maximum number of background points for
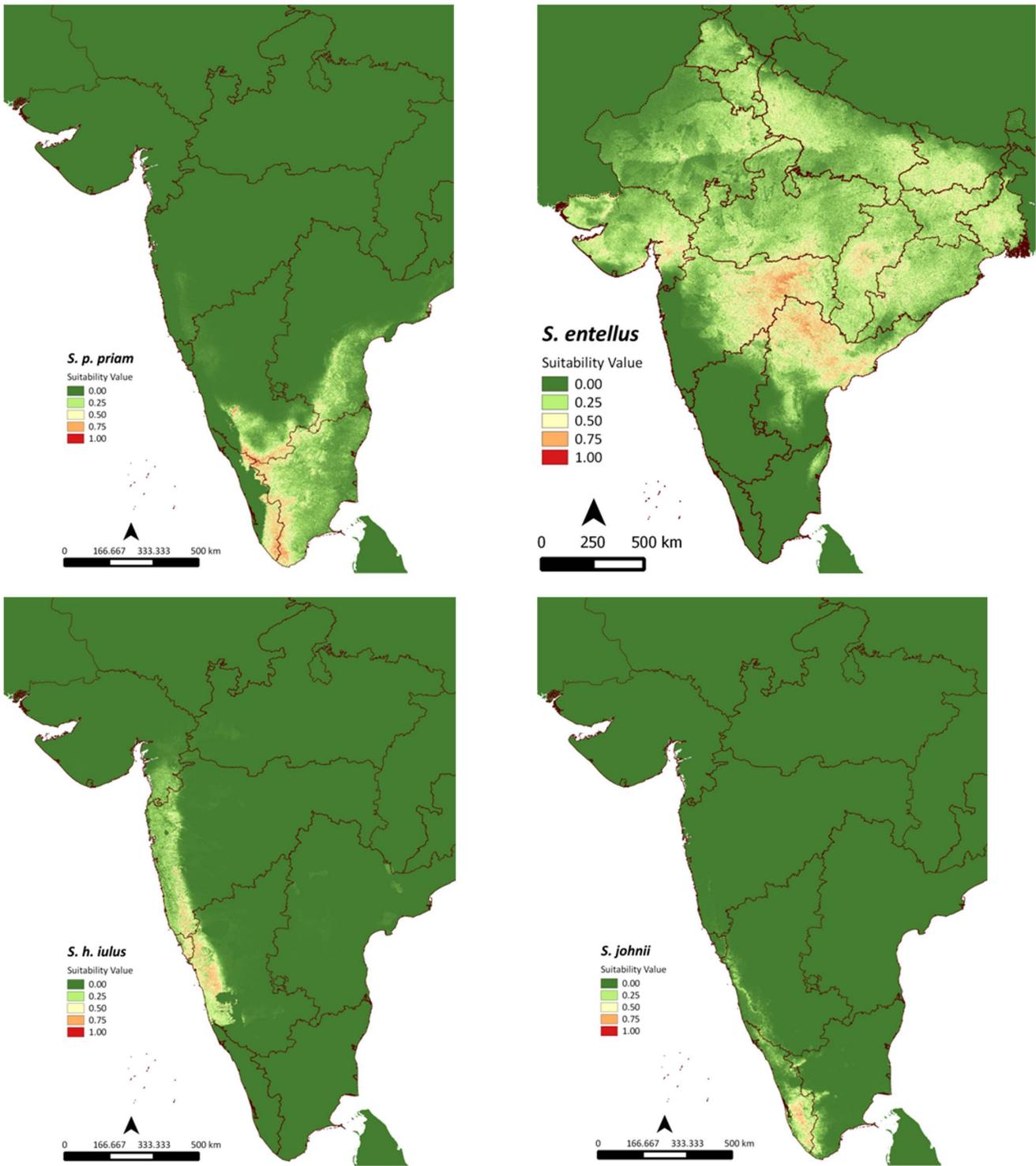
Figure 2. Maxent distribution modeling logistic output for *S. p. priam*, *S. entellus*, *S. h. iulus* **and** *S. johnii*.
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sampling was kept at 10,000. Extent of background selection points was restricted to the regions of mutually exclusive species occurrence points drawing minimum convex polygons using QGIS. We generated eight separate polygons. Within these polygons 10,000 random background points were selected. As species occurrence points used in the study was not collected

randomly, we provided bias files for each species separately during MaxEnt modeling. Each bias grid file is generated in QGIS applying Gaussian kernel function to 10,000 background points following Elith et al, [77]. We ran 15 replicates for each species and averaged the results. Maximum iterations were set to 5000, with $1*10^{-6}$ as convergence threshold. Auto feature of environ-
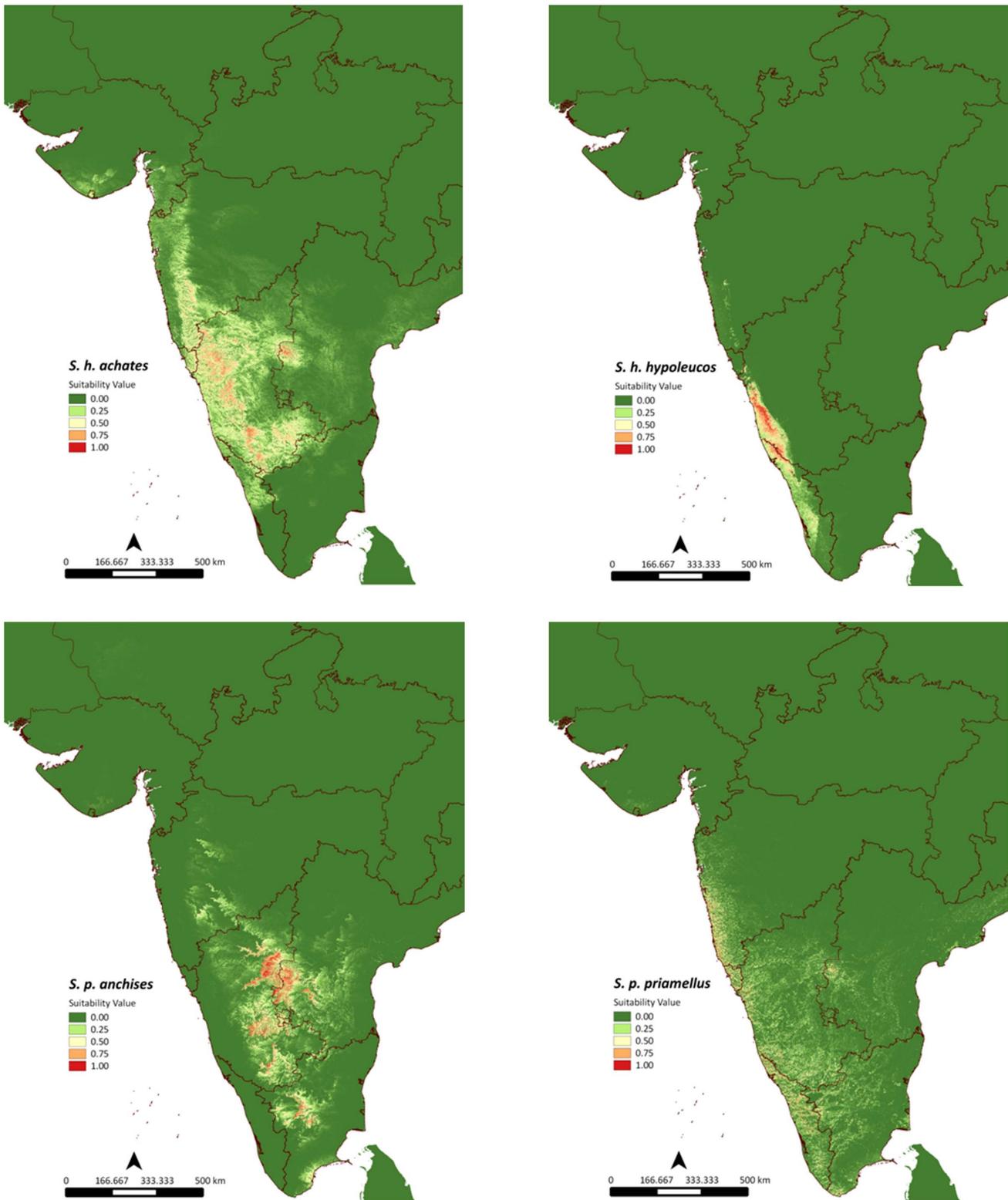
Figure 3. Maxent distribution modeling logistic output for *S. h. achates*, *S. h. hypoleucos*, *S. p. anchises* and *S. p. priamellus*.
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mental variables was selected. A 15 fold cross-validation was used to test model performance of each species. Jackknife procedure and percent variable contributions were used to estimate the environmental variable influence on each species. We performed

Correspondence analysis (CA), an ordination analysis on each species with their respective percentage contribution of the environmental variables. Correspondence analysis use Chi square

Table 2. Predicted area of distribution for all the taxa.

Species	Predicted area (km²)	Area ≥0.75 suitability (km²)	% of total area
S. h. achates	620559	4244	0.68
S. h. hypoleucos	91722	5695	6.2
S. p. anchises	367276	11660	3.18
S. p. priamellus	377501	3569	0.95
S. p. priam	380746	4736	1.24
S. entellus	2834717	18832	0.66
S. h. iulus	201720	1104	0.55
S. johnii	121693	1378	1.13

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distance (χ^2) to quantify the relationships among the dimensionally homogenous data set [87].

Logistic modeling output was chosen that displays suitability values from 0 (unsuitable) to 1 (optimal). For extracting the range values of environmental variables from logistic output, we considered threshold values ≥0.75 for all species following Liu et al, [88]. We derived threshold value from least valued ROC (Receiver Operator Characteristic) plot (Average ROC is 0.894±0.02 for *S. entellus*), wherein the threshold point (north westernmost point) lies at the intersection of the ROC curve and the line perpendicular to the diagonal of no discrimination following Lobo et al, [89].

The logistic model output is converted to binomial output with '0' to values from 0–0.75 and '1' to values ≥0.75. Area under curve (AUC) value is calculated for model validation. AUC reflects the model's ability to distinguish between presence records and random background points. AUC values ranged from 0.5 (not different from a randomly selected predictive distribution) to 1 (with perfect predictive ability). Models having AUC values >0.9 were considered to have very good, >0.8 good and >0.7 useful discrimination abilities [90]. We calculated partial receiver operator characteristics (pROC) as an additional measure to check model performances following Peterson et al, [91]. We used pROC calculator developed by Barve [92]. Z test was performed for statistical significance of the pROC values. Spatial overlaps between two species are calculated using QGIS and Idrisi® Taiga.

Using ENMTools software, niche overlap was measured among species distribution. Schoener's D as a measure of niche overlap [93] was estimated by taking the difference between species in

suitability score at each grid cell, after suitabilities were standardized [94,95,96]. This metric ranged from 0 (species without any niche overlap) to 1 (species with complete niche overlap/identical niches). D values >0.8 was considered as significant niche overlap [94].

Results

Species distribution

Based on maximum entropy modeling algorithm and using 14 environmental variables, we obtained eight distinct distribution maps corresponding to *S. h. achates*, *S. h. iulus*, *S. h. hypoleucos*, *S. p. priamellus*, *S. p. priam*, *S. p. anchises*, *S. entellus* and *S. johnii*. Figure 2 and 3 shows the distribution maps with warmer colours indicating more suitable habitat and cooler colours indicating unsuitable habitats.

Model prediction and assessment

Area of each species in predicted distribution is given in Table 2. Total area predicted is 49,95,934 km². *Semnopethicus entellus* has the highest area under the prediction (28,34,717 km²) while it was least in *S. h. hypoleucos* (91,722 km²). There was no significance correlation between the number of data points used for modeling and the area predicted (r = 0.65, p = 0.079). Percent suitability area predicted was highest in *S. h. hypoleucos* with 6.2% of total area predicted, followed by *S. p. anchises* with 3.18% and was least in *S. h. iulus* with 0.55%.

All the model performances exhibited high mean AUC values for 15 replicates in each species (Range: 0.894–0.989) (Table 3). The partial ROC values for each species was well over 1.0 (Range: 1.015–1.981) and were statistically significant (Z test, p<0.0001).

Variable range and their importance

Variables and permutation importance for each species are given in Table S2 in File S1. In addition the range of environmental variables in the predicted regions is given in Table S3 in File S1. Considering the importance values of ecological variables of each species, we performed correspondence analysis (Figure 4). Axis I and II explains 30% and 22% of variations in the data sets (Table S4 in File S1). Figures S1–S8 in File S1 provide response curve plots that show the type of correlation and influence of predicted suitability on the environmental variables (top five variables with high percentage contribution).

Temperature seasonality (Bioclim4) was primary variable influencing the niche of *S. p. anchises* and *S. p. priamellus* (25.4% and 30.3% respectively) niches. Similarly, it was precipitation during coldest quarter (Bioclim19) in *S. h. hypoleucos* and *S. p. priam*

Table 3. Area Under Curve (AUC) and Partial ROC values.

Species	AUC±SD	Partial ROC±SD
S. h. achates	0.942±0.051	1.814±0.129*
S. h. hypoleucos	0.948±0.105	1.520±0.372*
S. p. anchises	0.907±0.125	1.513±0.346*
S. p. priamellus	0.960±0.081	1.466±0.369*
S. p. priam	0.989±0.003	1.933±0.006*
S. entellus	0.894±0.02	1.485±0.063*
S. h. iulus	0.982±0.022	1.389±0.282*
S. johnii	0.967±0.077	1.986±0.002*

Note: * indicates Z test significance at P<0.0001.
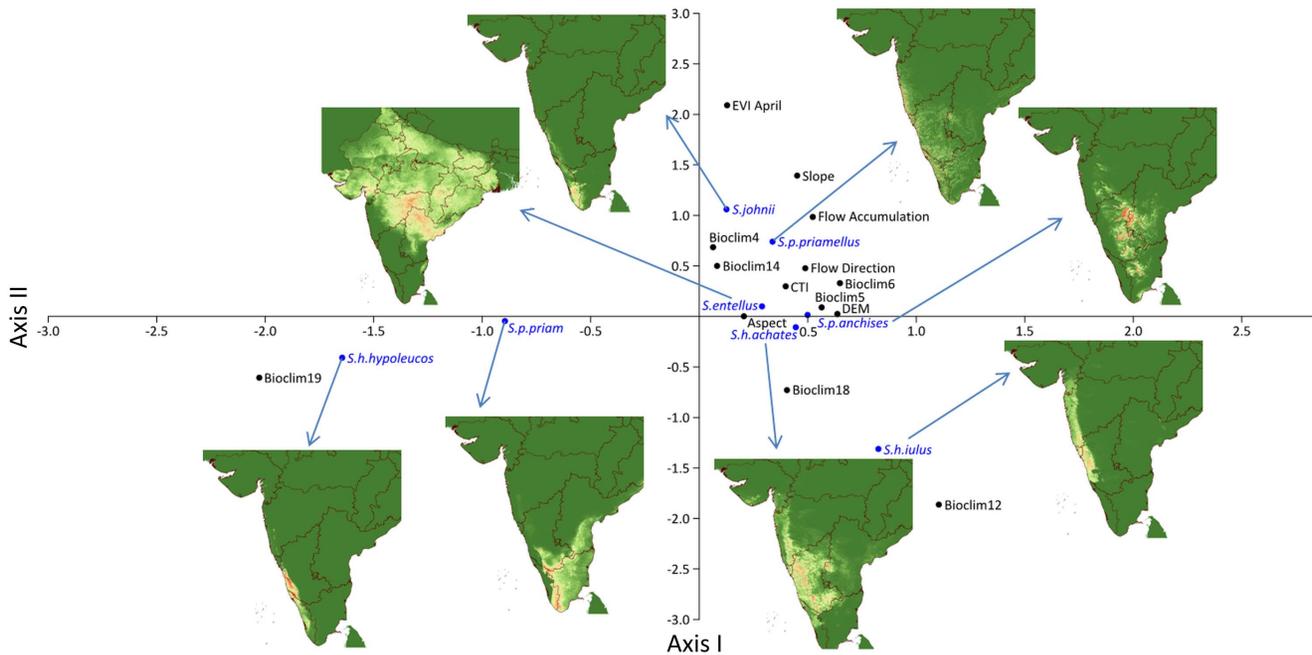doi:10.1371/journal.pone.0087804.t003

Figure 4. Correspondence analysis of variables of importance based from MaxEnt modeling output.
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niches (82.7% and 48.5% respectively). Annual precipitation (Bioclim12) in *S. h. iulus* niche (73.6%). In *S. h. achates* and *S. entellus* niches it was maximum temperature in warmest month (Bioclim5) which had highest contribution (24.9% and 45.6% respectively). Precipitation during the driest month (Bioclim14) influence *S. johnii*'s niche (38.2%). Vegetation index of April was second important variable in *S. johnii*'s niche (33.1%). Among the hydrological parameters, digital elevation model, slope, aspect, flow direction and flow accumulation had significant contribution in determining ecological niches (Table S2 in File S1).

Niche overlap

Table 4, provides percentage niche overlap between species pairs. Percentage overlap ranged from 0 to 2.82%, with most species pairs showing zero overlap. Table 5, illustrates the pair wise niche overlap by each taxa. Here again none of the taxa pairs exhibited values beyond 8%, which clearly indicates distinct niches for each taxa. For an independent measure of modeled output, we measured D statistic values. None of the niche overlap estimate using D statistics showed any significant overlap (Table 6).

Discussion

Ecological niche modeling based on maximum entropy (MaxEnt) algorithm was used to determine the distinct ecological niches of various taxa of langurs of peninsular According to Hill [67], there are three species of Hanuman langurs in South India viz., *S. entellus; S. hypoleucos* and *S. priam* and a sister taxa Nilgiri langur (*S. johnii*). According to Nag et al, [58], based on morphology there are three subspecies in *S. hypoleucos* and three species in *S. priam*, making a total of eight taxa of langurs in peninsular India. In the present study, ENM clearly demarcated the ecological niches of these taxa mentioned above, with significant AUC and pROC in each of the distribution model. Selection of a threshold value to convert predicted model layer to binary layer has a significant influence on model accuracy, especially for presence-only data set [78,88]. With increase in threshold values there will be a decrease in predicted suitable area of the species. This has further implications on biodiversity assessment, protected/reserve area selection, climate change impact studies and government policies on conservation of a species [89,97]. For a sound reserve design and conservation

Table 4. Percentage Niche overlaps (%) between taxa pairs to their area of prediction.

Species	S.h. hypoleucos	S.p. anchises	S.p. priamellus	S.p. priam	S. entellus	S.h. iulus	S. johnii
S.h. achates	0.01	2.18	0.82	0.02	0.00	0.00	0.00
S.h.hypoleucos		0.00	2.82	0.00	0.00	0.74	0.01
S.p.anchises			1.14	0.01	0.00	0.00	0.00
S.p.priamellus				0.30	0.00	0.47	0.10
S.p.priam					0.00	0.00	0.47
S. entellus						0.00	0.00
S.h.iulus							0.00

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Table 5. Proportion of the predicted area of taxa A occupied by the predicted area of taxa B.

Species	S. h. achates	S. h. hypoleucos	S. p. anchises	S. p. priamellus	S. p. priam	S. entellus	S. h. iulus	S. johnii
S.h.achates	-	0.02	8.18	1.51	0.05	0.00	0.00	0.00
S.h.hypoleucas	0.02	-	0.00	4.58	0.00	0.00	0.88	0.02
S.p.anchises	2.98	0.00	-	1.48	0.01	0.00	0.00	0.00
S.p.priamellus	1.79	7.31	4.85	-	0.70	0.00	0.62	0.14
S.p.priam	0.04	0.00	0.02	0.53	-	0.00	0.13	0.61
S. entellus	0.00	0.00	0.00	0.00	0.00	-	0.00	0.00
S.h. iulus	0.00	4.53	0.00	1.99	0.54	0.00	-	0.00
S. johnii	0.00	0.07	0.00	0.36	2.10	0.00	0.00	-

For above the diagonal values, species in row-heads are taxa A and column-heads are taxa B. For below the diagonal values, taxa in row-heads are taxa B and column-heads are taxa A.
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programme on Hanuman langur complex one needs to carefully look at the threshold values used in binary model prediction layer.

Bioclim variables had a major contribution in determining the niche of a particular species followed by hydrological layers and EVI (Figure 4). It is well known that *S. johnii* inhabits evergreen forests of high elevation in south-west India between 8°–10°N latitude and the region receives rainfall throughout the year [58]. However, it is the precipitation in driest month and vegetation index of April predominantly influenced ecological niche of *S. johnii*.

Similarly, the niche of *S. h. hypoleucos* is determined predominantly by precipitation during the coldest quarter (82.7%) in the mid Western Ghats regions of south-west India between 12°–14°N. Ecological niche of *S. h. iulus* was between 14°–18°N on the Western part of south-west India predominantly determined by annual precipitation (73.6%). This particular region receives moderate rainfall [72].

In the present study, the distribution of *S. p. priam* was between 8°–13°N and strongly influenced by precipitation during coldest quarter. *S. p. priam* is confined to deciduous forests in southern Western Ghats of India [58]. For the most widely distributed taxa in India, *S. entellus*, it is the temperature during warmest month and temperature seasonality that determined the niche, followed by aspect. Earlier studies have indicated an influence of temperature on vegetation type [63,97].

Niche of *S. h. achates* is between 12°–17°N and 76°–79°E influenced by maximum temperature in warmest month followed by annual precipitation. This corroborated with the fact that *S. h. achates* occured in the regions with very low rainfall and dry vegetation [48,58]. Niche of *S. p. anchises* is influenced by temperature seasonality in the south-central India between 11°–17°N and 77°–79°E. It is also the taxa belonging to low rainfall and dry vegetation region [58]. Ecological niche of *S. p. priamellus* was also determined by temperature seasonality, warranting more studies.

Through ENM, the study looked at niche overlaps between taxa pairs to determine if there was divergence in their ecological axis. There were no significant niche overlaps between any taxa pairs, thereby suggesting that each taxa occupied a distinct ecological niche. This clearly supported the morphological distinction of Hanuman langurs as explained in Nag et al, [58]. *Semnopithecus johnii* has long been considered as a distinct species and can be easily distinguished from the members of the Hanuman langur species complex by their distinct pelage color and vocalizations [75,98,99]. They have a very restricted distribution and are confined to the wet evergreen forest of Central and Southern Western Ghats.Nevertheless, in some areas mixed species associations between *S. johnii* and *S. priam* have been reported [52,56,98,99] and sometimes they are also known to hybridize. Such polyspecific association and hybridization events have been reported for other Asian primates as well [51,68,69,100,101,102,103]. Nonetheless, ecological niche modeling provided a distinct, non-overlapping niche for *S. johnii* and supported it as a distinct species. Another interesting output of the model was the support for Pocock's [73] *priamellus* which has a very restricted distribution in Western Ghats. According to Nag et al, [58], *S. p. priamellus* form is morphologically distinct from other morphotypes of *Semnopithecus* and is confined to Nilambur, Silent

Table 6. Niche overlap estimate (D statistic) between species pairs.

Species	S. h. hypoleucos	S. p. anchises	S. p. priamellus	S. p. priam	S. entellus	S. h. iulus	S. johnii
S. h. achates	0.124	0.294	0.364	0.157	0.066	0.309	0.100
S. h. hypoleucos		0.205	0.336	0.061	0.002	0.171	0.435
S. p. anchises			0.381	0.150	0.029	0.028	0.244
S. p. priamellus				0.230	0.028	0.217	0.311
S. p. priam					0.020	0.023	0.244
S. entellus						0.032	0.001
S. h. iulus							0.064

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valley national park and Walayar regions of Northern Kerala. Interestingly, Hill's classification scheme discussed *priamellus* specimens as ''doubtful'' and he subsumed it under *S. prium*. However our analysis shows that its niche is distinct from all other taxa with very little overlap. Thus our results provide reasonably good support for retaining *priamellus* as a valid taxa as a subspecies of *S. prium*. However, a detailed survey of this population should be undertaken to better understand its distributional limits.

Although this exercise has provided greater clarity regarding niche separation between members of the Hanuman langur complex in south India, there are limitations and uncertainties in the modeling process, occurrence data and layers used in such studies as indicated in similar modeling studies elsewhere [104]. We used MaxEnt software for this study considering the use and performance [82,83,84,85], however there is no single best algorithm or software that addresses the uncertainties of modeling process [17]. A platform for ensemble forecasting called BIOMOD [23] is proposed to overcome the limitations of single model predictions. Also, small occurrence data (there were four species with <12 occurrence data) increase the uncertainty of predicting the ecological niches [105]. We cross validated individual model run to overcome this uncertainty.

Our observations in the present study should be cautiously weighed in the light of limitations of ENM, in that using only spatial variables may not give us complete niche separation of species. Thus, one has to look at other variables of species, *viz.*, canopy density, habitat preference, breeding behavior, feeding pattern, troop dynamics, and niche occupancy which can provide much deeper insight on realized niches of each species. Adding these variables might generate more precise species boundaries.

Supporting Information

File S1   Combined supporting information file containing Tables S1–S4, Figures S1–S8. Table S1. Derived bioclimatic, hydrological and vegetation layers used in the present study. Each layer is with 1000 m resolution and is clipped for Indian sub-continent. Table S2. Variables with percent contribution (in bold) and permutation importance in predicted distribution of species. Table S3. Range of Environmental variables in the predicted suitability regions. Table S4. Eigenvalue and percentage variation explained in correspondence analysis. Figure S1. Response curves of top five variables of importance in *S. h. achates*. Figure S2. Response curves of top five variables of importance in *S. h. hypoleucos*. Figure S3. Response curves of top five variables of importance in *S. p. anchises*. Figure S4. Response curves of top five variables of importance in *S. p. priamellus*. Figure S5. Response curves of top five variables of importance in *S. p. prium*. Figure S6. Response curves of top five variables of importance in *S. entellus*. Figure S7. Response curves of top five variables of importance *S. h. iulus*. Figure S8. Response curves of top five variables of importance in *S. johnii*.
(DOC)

Author Contributions

Conceived and designed the experiments: NC KKP GKV. Performed the experiments: NC GKV. Analyzed the data: GKV. Contributed reagents/materials/analysis tools: NC KKP GKV. Wrote the paper: NC KKP GKV.

References

1. Harrison RG (1998) Linking evolutionary pattern and process. The relevance of species concepts for the study of speciation. In: Howard DJ, Berlocher S, editors. *Endless Forms: Species and Speciation*. Oxford Univ. Press, New York. pp. 19–31.
2. Kunz W (2001) Taking more care in using different species concepts- an opinion. Parasitol Res 87:413–416.
3. Mayden RL (1997) A hierarchy of species concepts: the denouement in the saga of the species problem. In: Oaridge MF, Dawah HA, Wilson MR, editors. *Species: The Units of Biodiversity*. Chapman & Hall. pp. 381–424.
4. de Queiroz K (2007) Species concepts and species delimitation. Syst. Biol 56:879–886.
5. Pigliucci M (2003) Species as family resemblance concepts: the (dis-)solution of the species problem? BioEssays 25: 596–602.
6. de Queiroz K (1998) The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In: Howard DJ, Berlocher SH, editors. *Endless Forms: Species and Speciation*. Oxford Univ. Press, pp. 57–75.
7. Sites JW Jr, Marshall JC (2004) Operational criteria for delimiting species. Annu Rev Ecol Evol Syst 35:199–227.
8. Bond JE, Stockman AK (2008) An integrative method for delimiting cohesion species: finding the population-species interface in a group of Californian trapdoor spiders with extreme genetic divergence and geographic structuring. Syst Biol 57:628–646.
9. WiensJJ, Graham CH (2005) Niche conservatism: integrating evolution, ecology, and conservation biology. Annu Rev Ecol Evol Syst 36:519–539.
10. Peterson AT (2001) Predicting species geographic distributions based on ecological niche modeling. The Condor 103:599–605.
11. Raxworthy CJ, Ingram C, Rabibisoa N, Pearson R (2007) Applications of ecological niche modeling for species delimitation: a review and empirical evaluation using day Geckos (Phelsuma) from Madagascar. Systematic Biology 56:907–923
12. Graham CH, Ron SR, Santos JC, Schneider CJ, Moritz C (2004) Integrating phylogenetics and environmental niche models to explore speciation mechanisms in dendrobatid frogs. Evolution 58:1781–1793.
13. Rissler LJ, Apodaca JJ (2007) Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). Systematic Biology 56:924–942.
14. Guisan A, Zimmermann NE (2000) Predictive habitat distribution models in ecology. Ecological Modeling 135:147–186.
15. Zimmerman NE (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129–151.
16. Bett NN, Blair ME, Sterling EJ (2012) Ecological Niche Conservatism in Doucs (Genus Pygathrix). Int J Primatol 33: 972–988. DOI 10.1007/s10764-012-9622-3.
17. Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, et al. (2006) Novel methods improve prediction of species' distributions from occurrence data. Ecography 29:129–151.
18. Martinez TO, Gray VR, Meyer EM (2008) Predicted and verified distributions of *Ateles geoffroyi* and *Alouatta palliata* in Oaxaca, Mexico. Primates 49:186–194.
19. Austin MP, Meyers JA (1996) Current approaches to modelling the environmental niche of eucalypts: implications for management of forest biodiversity. Forest Ecology and Management 85:95–106.
20. Luoto M, Heikkinen RK, Saarinen K (2006) Determinants of biogeographical distribution of butterflies in boreal regions. Journal of Biogeography doi: 10.1111/j.1365-2699.2005.01395.x.
21. Diekötter T, Walther-Hellwig K, ConradiM, Suter M, Frankl R (2006) Effects of landscape elements on the distribution of the rare bumblebee species *Bombusmuscorum* in an agricultural landscape. Biodiversity and Conservation 15:57–68.
22. Bauer JT, Peterson AT (2005) Visualizing environmental correlates of species geographical range limits. Diversity and Distribution 11:275–278.
23. Thuiller W (2003) BIOMOD optimizing predictions of species distributions and projecting potential future shifts under global change. Global Change Biol 9: 1353–1362.
24. Thuiller W, RichardsonDM, Pyšek P, Midgley GF, Hughes GO, et al. (2005) Niche-based modelling as a tool for predicting the risk of alien plant invasions at a global scale. Global Change Biol 11:2234–2250.

25. Manel S, Buckton ST, Ormerod SJ (2000)Testing large-scale hypotheses using surveys: the effects of land use on the habitats, invertebrates and birds of Himalayan rivers. J Appl Ecol 37:756–770

26. Kumara HN, Ullah MI, Kumar S (2009) Mapping potential distribution of slender Loris subspecies in peninsular India. Endangered Species Research 7:29–38.

27. Raxworthy CJ, Martínez-Meyer E, Horning N, Nussbaum RA, Schneider GE, Ortega-Huerta MA, Peterson AT (2003) Predicting distributions of known and unknown reptile species in Madagascar. Nature 426:837–841.

28. Ganesh T, Davidar P (2001) Dispersal modes of tree species in the wet forests of southern Western Ghats. Current Science 80:394–399.

29. Prasad S, Chellam R, Krishnaswamy J, Goyal SP (2004) Frugivory of *Phyllanthus emblica* at Rajaji National Park, north-west India. Current Science 87:1188–1190.

30. Punekar SA (2002) Some food plants of Hanuman langur *Semnopithecus entellus* (Dufresne) in the Western Ghats of Maharashtra, India. Zoos' Print Journal 17:797–801.

31. Thorn JS, Nijman V, Smith D, Nekaris KAI (2009) Ecological niche modelling as a technique for assessing threats and setting conservation priorities for Asian slow lorises (Primates: Nycticebus). Diversity and Distributions 15:289–298.

32. Torres J, Brito JC, Vasconcelos MJ, Catarino L, Gonçalves J, et al. (2010) Ensemble models of habitat suitability relate chimpanzee (*Pan troglodytes*) conservation to forest and landscape dynamics in Western Africa. Biological Conservation 143:416–425.

33. Garcia FV, Silva JCS (2011) Potential distribution of Mexican primates: modeling the ecological niche with the maximum entropy algorithm. Primates. Published online: 16th March 2011. DOI 10.1007/s10329-011-0246-6.

34. Boubli JP, Lima MG (2009) Modeling the geographical distribution and fundamental niches of *Cacajao spp.* and *Chiropotes israelita* in Northwestern Amazonia via maximum entropy algorithm. International Journal of Primatology 30:217–228.

35. Anonymous (2010) World's most endangered primates revealed. Available: http://www.iucn.org/?4753/Worlds-most-endangered-primates-revealed. Accessed 2013 Nov 20.

36. Mittermeier RA, Schwitzer C, Rylands AB, Taylor LA, Chiozza F, et al. (2012) Primates in Peril: The World's 25 Most Endangered Primates 2012–2014. IUCN/SSC Primate Specialist Group (PSG), International Primatological Society (IPS), Conservation International (CI), and Bristol Conservation and Science Foundation, Bristol, UK. Pp. 40.

37. Alroy J (2002) How many named species are valid? PNAS USA 99:3706–3711.

38. Avise JC (1989) A role of molecular genetics in the recognition of conservation of endangered species. TREE 9:279–281.

39. Steinberg ER, Nieves M, Ascunce MS, Palermo AM, Mudry MD (2009) Morphological And Genetic Characterization of *Saimiribobiviensis* (Primates: Cebidae): Implications for Management and Conservation. Conservation Genetics 30:29–41.

40. Pirta RS, Gadgil M, Kharshikar AV (1997) Management of the rhesus monkey *Macacamulatta* and Hanuman langur *Presbytis entellus* in Himachal Pradesh, India. Biological Conservation 79:97–106.

41. Chaudhuri S, Murmu A, Talukder B, Alfred JRB (2004) A population survey of Hanuman langurs in the district of Purulia, West Bengal. Rec. Zool. Surv. India 103(3-4):47–54.

42. Groves CP (2001) Primate taxonomy. Smithsonian Institution Press, Washington D.C.

43. Oates JF, Davies AG, Delson E (1994) The diversity of living colobines. In: Davies AG, Oates JF, editors. *Colobine Monkeys: Their Ecology, Behaviour and Evolution*, Cambridge University Press, Cambridge. pp. 45–73.

44. Ellerman JR, Morrison-Scott TCS (1966) Checklist of Palaearctic and Indian mammals, 1758-1946. British Mus. (Nat. Hist.), London. 2nd ed., pp. 810.

45. Minhas RA, Ahmed KB, Awan MS, Dar NI (2010) Social Organization and Reproductive Biology of Himalayan Grey Langur (*Semnopithecus entellus ajax*) in Machiara National Park, Azad Kashmir (Pakistan). Pakistan journal of Zoology 42: 143–156.

46. Roonwal ML (1984) Tail form and carriage in Asian and other primates, and their behavioral and evolutionary significance. In: Roonwal ML, Mohnot SM, Rathore NS, editors. *Current primate research, Jodhpur, India*: Jodhpur University Press. pp. 93–151

47. Choudhury AU (2007) The eastern limit of distribution of the hanuman langur *Semnopithecus entellus* Dufresne. Journal of the Bombay Natural History Society 104:199–200.

48. Sugiyama Y (1965) On the social change of Hanuman langurs (*Presbytis entellus*) in their natural condition. Primates 6:381–418.

49. Kumara HN, Singh M (2004) Distribution and abundance of primates in rain forests of the Western Ghats, Karnataka, India and the conservation of *Macaca silenus*. International Journal of Primatology 25:1001–1018.

50. Jathanna D, Karanth KU, Johnsingh AJT (2003) Estimation of large herbivore densities in the tropical forests of southern India using Distance sampling. Journal of Zoology 261:285–290.

51. Singh M, Kumara HN, Kumar MA, Sharma AK, Defalco K (2000) Status and conservation of lion-tailed macaque and other arboreal mammals in tropical rainforests of Sringeri Forest Range, Western Ghats, Karnataka, India. Primate Report 58:5–16.

52. Chellam R (1995) Langurs of Mundanthurai. Blackbuck 1:20–26.

53. Ahsan MF, Khan MAR (2006) Eco-ethology of the common langur *Semnopithecus entellus* (*Dufresne*) in Bangladesh. U J Zool Rajshahi U 25:3–10.

54. Sharma SK (2001) Food habits of Hanuman langur (*Semnopithecus entellus*) during dry season at Mount Abu wildlife sanctuary. Zoos' Print Journal 16: 669.

55. Chhangani AK, Mohnot SM (2004) Crop raid by Hanuman langur *Semnopithecus entellus* in and around Aravalis (India) and its management. Primate Report 69: 35–47.

56. Ramachandran KK, Joseph G (2001) Distribution and demography of diurnal primates in Silent Valley National Park and adjacent areas, Kerala, India. Journal of Bombay Natural History Society 98:191–196.

57. Sayers K, Norconk MA (2008) Himalayan *Semnopithecus entellus* at Langtang National Park, Nepal: Diet, Activity Patterns, and Resources. International Journal of Primatology 29:509–530.

58. Nag KSC, Pramod P, Karanth KP (2011) Taxonomic implications of a field study of morphotypes of Hanuman Langurs (*Semnopithecus entellus*) in peninsular India. International Journal of Primatology 32:830–848.

59. Bishop NH (1979) Himalayan langurs: temperate colobines. J Hum Evol 8:251–81.

60. Hrdy SB (1977) The langurs of Abu-female and male strategies of reproduction: Harvard University Press, Cambridge.

61. Oppenheimer JR (1977) *Presbytis entellus*, the Hanuman langur. In: Bourne GH, editor. *Rainier III (Grimaldi) Prince of Monaco, Primate conservation*: Academic Press, New York. pp. 469–512.

62. Curtin RA (1982) Range use of gray langurs in highland Nepal. Folia Primatol 38:1–18.

63. Chhangani AK (2002) Group composition and sex ratio in Hanuman langurs (*Semnopithecus entellus*) in the Aravali Hills of Rajasthan, India. Zoos' Print Journal 17:848–852.

64. Koenig A, Borries C, Chalise MK, Winkler P (1997) Ecology, nutrition, and timing of reproductive events in an Asian primate, the Hanuman langur (*Presbytis entellus*). Journal of Zoology 243:215–235.

65. Kirkpatrick RC (2007) The Asian colobines: diversity among leaf-eating monkeys. In: Campbell CJ, Fuentes A, MacKinnon KC, Panger M, Bearder SK, editors. *Primates in perspective*: Oxford University Press, New York. pp. 186–200.

66. Karanth KP (2010) Molecular systematics and conservation of the langurs and leaf monkeys of South Asia. J Genet 89:393–399.

67. Hill WC (1939) An annotated systematic list of the leaf-monkeys. Ceylon Journal of Science, Colombo 21:277–305.

68. Sushma HS, Singh M (2006) Resource partitioning and interspecific interaction among sympatric rain forest arboreal mammals of the Western Ghats, India. Behavioral Ecology 17:479–490.

69. Hladik CM (1977) A comparative study of the feeding strategies of two sympatric species of leaf monkeys: *Presbytis senex* and *Presbytis entellus*. In: Clutton-Brock TH, editor. *Primate ecology: studies of feeding and ranging behavior in lemurs, monkeys, and apes*: Academic Press, London, pp. 323–353.

70. Vasudev D, Kumar A, Sinha A (2008) Resource Distribution and Group Size in the Common Langur *Semnopithecus entellus* in Southern India. American Journal of Primatology 70:680–689.

71. Schuelke O (2001) Differential Energy Budget and Monopolization Potential of Harem Holders and Bachelors in Hanuman Langurs (*Semnopithecus entellus*): Preliminary Results. American Journal of Primatology 55:57–63.

72. Roy K, Singh M, Sushma HS, Singh M (2010) Stand structure of a primate rich rainforest region in the central Western Ghats of Southern India. Journal of Threatened Taxa 2:930–939.

73. Pocock RI (1939) Primates and carnivore: Taylor and Francis, London. pp. 97–163.

74. Kurup GU (1984) Census survey and population ecology of Hanuman langur, *Presbytis entellus* (Dufresne, 1797) in south India, Proceedings of Indian National Science Academy 50:245–26.

75. Brandon-Jones D (2004) A taxonomic revision of the langurs and leaf monkeys (Primates: Colobinae) of South Asia. Zoos' Print Journal 19:1552–1594.

76. Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

77. Elith J, Kearney M, Phillips S (2010) The art of modelling range-shifting species. Methods in Ecology and Evolution 1:330–342.

78. Phillips SJ, Dudik M, Schapire RE (2004) A maximum entropy approach to species distribution modeling. In Proceedings of the 21st international conference on machine learning, AMC Press, New York: 655–662.

79. Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecological Modelling 190:231–259.

80. Araújo MB, Guisan A (2006) Five (or so) challenges for species distribution modeling. Journal of Biogeography 33:1677–1688.

81. Merow C, Smith MJ, Silander JA Jr (2013) A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. Ecography 36:1–12.

82. Smith SA, Donoghue MJ (2010) Informing large-scale biogeography with niche models in Lonicera (Caprifoliaceae, Dipsacales) subgenus *Caprifolium*. Systematic Biology 590:322–341.

83. Dupin M, Reynaud P, Jarošík V, Baker R, Brunel S, et al. (2011) Effects of the Training Dataset Characteristics on the Performance of Nine Species Distribution Models: Application to *Diabrotica virgifera virgifera*. PLoS ONE 6: e20957. doi:10.1371/journal.pone.0020957

84. Ross KG, Gotzek D, Ascunce MS, Shoemaker DD (2010) Species delimitation: A case study in a problematic ant taxon. Systematic Biology 59:162–184.

85. Reeves PA, Richards CM (2011) Species delimitation under the general lineage concept: An empirical example using wild North American hops (Cannabaceae: *Humulus lupulus*). Systematic Biology 60:45–59.

86. Baldwin RA (2009) Use of Maximum Entropy Modeling in Wildlife Research. Entropy 11:854–866.

87. Legendre P, Legendre L (1998) Ordination in reduced space. In: *Numerical Ecology*: Elsevier Science B.V. Amsterdam, The Netherlands. pp. 451–476.

88. Liu C, White M, Newell G (2013) Selecting thresholds for the prediction of species occurrence with presence-only data. Journal of Biogeography 40:778–789.

89. Lobo JM, Jiménez-Valverde A, Real R (2008) AUC: A misleading measure of the performance of predictive distribution models. Global Ecology and Biogeography 17:145–151.

90. Khanum R, Mumtaz AS, Kumar S (2013) Predicting impacts of climate change on medicinal asclepiads of Pakistan using Maxent modeling. Acta Oecologica-International Journal of Ecology 49:23–31.

91. Peterson AT, Papeş M, Soberon J (2008) Rethinking receiver operating characteristic analysis applications in ecological niche modeling. Ecological Modelling 213:63–72.

92. Barve N (2008) Tool for Partial-ROC (Biodiversity Institute, Lawrence, KS), ver 1.0.

93. Schoener TW (1968) Anolis lizards of Bimini: resource partitioning in a complex fauna. Ecology 49:704–726.

94. Warren DL, Glor RE, Turelli M (2008) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. Evolution 62:2868–2883.

95. Warren DL, Seifert SN (2011) Ecological niche modeling in MaxEnt: the importance of model complexity and the performance of model selection criteria. Ecological Applications 21:335–342.

96. Rebelo H, Jones G (2010) Ground validation of presence-only modeling with rare species: A case study on Barbastelles *Barbestella barbastellus* (Chiroptera: Vespertilionidae). Journal of Applied Ecology 47:410–420.

97. Heany LR (1991) A synopsis of climatic and vegetational change in Southeast Asia. Climatic Change 19:53–61.

98. Hohmann G (1989) Group Fission in Nilgiri Langurs (*Presbytis johnii*). International Journal of Primatology 10:441–454.

99. Hohmann GM (1992) Comparative analyses of age- and sexspecific patterns of vocal behaviour in four species of old world monkeys. Folia Primatol 56:133–156.

100. Burton FD, Chan L (1996) Behavior of mixed species groups of macaques. In: Fa JE, Lindburg DG, editors. *Evolution and Ecology of Macaque Societies*: Cambridge University Press, Cambridge (UK), pp. 389–412.

101. Mackinnon JR, Mackinnon KS (1980) Niche differentiation in a primate community. In: Chivers D, editor. *Malayan Forest Primates: Ten Years Study in Tropical Rainforest*: Plenum Press, New York. pp. 167–190.

102. Bernstein IS (1967) Intertaxa interactions in a primate community. Folia Primatol 7:198–207

103. Grueter CC, Yong LD, Shun-Kai F, Bao-Ping R (2010) Niche partitioning between sympatric rhesus macaques and Yunnan snub-nosed monkeys at Baimaxueshan Nature Reserve, China. Zoological Research 31:516–522.

104. Wiens JA, Stralberg D, Jongsomjit D, Howell CA, Snyder MA (2009) Niches, models, and climate change: assessing the assumptions and uncertainties. Proceedings of the National Academy of Sciences 106:19729–19736.

105. Hernandez PA, Graham C, Master LL, Albert DL (2006) The effect of sample size and species characteristics on performance of different species distribution modelling methods. Ecography 29:773–785.