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Genetic evidence for allopatric speciation of the Siberian ibex *Capra sibirica* in India

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ABSTRACT: Montane systems, formed by a series of climatic oscillations and temporal topographic metamorphoses, have broken up the contiguous distribution of widespread species and accelerated allopatric speciation. We used a partial fragment of the mitochondrial cytochrome *b* (*cytb*) gene to address speciation across the entire range of the Siberian ibex *Capra sibirica*. We demonstrated that the Siberian ibex is a polytypic species, plausibly formed by a combination of at least 2 species and/or 3 to 4 sub-species. Bayesian phylogeny showed that the Indian-Tajikistan (I-T) clade is adequately diverged from the other clades based on the mean intra-specific distance criterion, and warrants recognition as a distinct species. We provide pragmatic evidence for the endorsement of the I-T clade as a distinct species of Siberian ibex and urge prioritization of the conservation of this species at global and regional scales.

KEY WORDS: Siberian ibex · Alpine ibex · Phylogeography · Taxonomic revision · Genetic analysis · Conservation · IUCN Red List

1. INTRODUCTION

Climatic oscillations and temporal topographic transformation have been 2 prominent drivers causing vicariance speciation in montane systems (Baker & Bradley 2006, Drovetski et al. 2013, Yang et al. 2016). Elevated ridges in the montane system often alter habitat and force changes in floral composition, consequently imposing challenges on native species for survival under changing climatic conditions (Manish & Pandit 2018, Muellner-Riehl 2019). Wild ungulates, which prefer to live in groups with smaller home ranges, have relatively lower ecological resilience, so during historic climatic oscillations and resulting topographic transformation they may plausibly have remained longer in deep clefts in the

mountains, facilitating high intra-specific variation (Lorenzini & Garofalo 2015, Mukesh et al. 2015, Kumar et al. 2017) and driving allopatric speciation (Outlaw & Voelker 2008, Pyron & Burbrink 2010, Xing & Ree 2017). The central Asian landscape is well-known for its mixed composite of varying habitats, consisting of a chain of low and high elevation mountain ranges that harbor a diverse group of species (Körner 2004, Tamma & Ramakrishnan 2015). This landscape has been subjected to a series of climatic oscillations and temporal topographic metamorphoses in the past, whereby several species/subspecies have evolved either through colonization and/or *in situ* diversification and allopatric speciation (Abramowski et al. 2006, Hörandl & Emadzade 2012).

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The distribution of the Siberian ibex *Capra sibirica* comprises diverse habitats ranging from cold deserts, rocky outcrops, steep terrain, highland flats and mountain ridges to low mountains and foothills (Bhatnagar 1997, Clark et al. 2006, Khan et al. 2016). In Mongolia, its distribution is patchy, and it is mainly found in rugged terrain throughout the western half of the country, as well as in central and south-central Mongolia (Clark et al. 2006). Its distribution further extends towards the Altai, Hangai, Gobi–Altai and Hurkh Mountain Ranges (Schaller et al. 1994, Clark et al. 2006), and it also inhabits the Sayan Mountains near the Russian border, with scattered populations in the small mountains of the Trans-Altai Gobi (Fedosenko & Blank 2001, Clark et al. 2006, Reading & Shank 2008, Khan et al. 2016). In Asia, the ibex is found in montane habitats, ranging in elevation from 500–6700 m a.s.l. in, for example, India, Kazakhstan, Kyrgyzstan, Tajikistan, Mongolia, Pakistan, southern Siberia and China (Bhatnagar 1997, Clark et al. 2006, Khan et al. 2016). In India, the Siberian ibex is found mainly in the Trans-Himalayan ranges of Jammu and Kashmir, Union Territory and Himachal Pradesh up to the river Satluj (Fox 1987, Bagchi et al. 2004, Grubb 2005).

Interestingly, it has not yet been established whether the Siberian ibex is a distinct species or a subspecies of *C. ibex* (Shackleton 1997). However, Wilson & Reeder (1993) advocated that the Siberian ibex should be considered a distinct species from *C. ibex*. Further, Pidancier et al. (2006) addressed the evolutionary history of the genus *Capra* and highlighted speciation and westward migration from the central Asian landscape.

2. MATERIALS AND METHODS

2.1. Sampling and PCR sequencing

We collected 30 fresh Siberian ibex faecal pellets (9 from Kibber Wildlife Sanctuary, Spiti Valley; 21 from Lahaul Valley) from Himachal Pradesh in northern India (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/n042p001_supp.pdf). Genomic DNA was extracted in a dedicated room using the QIAamp DNA Stool Mini Kit (Qiagen) following the manufacturer's instructions. Polymerase chain reaction (PCR) was used to amplify a partial fragment of the mitochondrial cytochrome *b* (*cytb*) gene, using the universal primers developed by Verma & Singh (2003). The PCR recipe and cycling conditions are detailed in Joshi et al. (2020). The

cycle sequencing of PCR products was cleaned up using the Big Dye Terminator kit (Applied Biosystems); purified products were subjected to DNA sequencing on an ABI 3730 genetic analyzer (Applied Biosystems). We retrieved Siberian ibex sequences from GenBank/NCBI (see Table S1 in the Supplement), encompassing the species' entire range in the countries/areas of the Altai Mountains, Kazakhstan, Kyrgyzstan, Russia, Mongolia and the Pamir region of Tajikistan.

2.2 Data analysis

The generated raw sequences were proofread with their electropherograms using the software Sequencher v.4.7 (Gene Codes Corporation), and species were validated using the similarity search tool in GenBank (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>). All sequences were identified as Siberian ibex with 91–99% identity. Genetic polymorphism, i.e. number of polymorphic sites and haplotypes and sequence divergences, were estimated using the Kimura 2 parameters (K2P) in MEGA v.10 (Kumar et al. 2018). The HKY+G model was found to be most suitable for the nucleotide substitution using the programme Model Generator (Keane et al. 2006). Bayesian inferences were conducted in BEAST v.2.1.3 (Bouckaert et al. 2014). For molecular dating, we used a normal prior with a mean of 2% per million years (myr), following Johns & Avise (1998), which is the universal evolutionary rate for the vertebrate *cytb* gene. We used a standard deviation equal to 10% of the mean to account for variation and uncertainty in the rate. Dating analysis was performed for 20 million generations while sampling every 1000th tree, with the first 10% of trees sampled treated as burn-in; FigTree v.1.3.1 (Rambaut 2009) was used to display and summarize annotated phylogenetic trees yielded by BEAST.

3. RESULTS AND DISCUSSION

To unravel the complexity in species recognition of the Siberian ibex, we collected 30 ibex faecal samples from the Lahaul and Spiti District of Himachal Pradesh, India, that yielded 2 haplotypes with an intra-specific sequence divergence (SD) of 0.00–0.004 (Tables S1 & S2). However, inclusion of published sequences of Siberian ibex from various countries/areas viz. the Altai Mountains, Kazakhstan, Kyrgyzstan, Russia, Mongolia and the Pamir region of

Tajikistan showed an intra-specific SD of 0.028–0.118 (Tables S1 & S3). All Siberian ibex sequences yielded 20 haplotypes, and Bayesian-based phylogeny clustered them into 3 major clades with high posterior probability (0.9; Fig. 1): the I-T clade, representing India and Tajikistan; the KZ clade, representing Kazakhstan; and the AMR clade, representing sequences from the Altai mountains, Mongolia and Russia. Surprisingly, the I-T clade (presently known as the Siberian ibex) was estimated to be more closely related to the the Alpine ibex (divergence during the early Pleistocene epoch, ~2.4 myr ago; 95 % CI: 1.4–3.8, SD 0.041) than to the Siberian ibex (divergence during the Miocene–Pliocene boundary, ~6.6 myr ago; 95 % CI: 4.8–9.4, SD 0.083) (Fig. 1). The I-T clade was found to be adequately diverged from the KZ (SD 0.083) and AMR clades (SD 0.110; Fig. 1) based on the set 10× threshold of mean intra-specific distance criterion (Kerr et al. 2009, Tobe et al. 2010). The present study indicates that the I-T clade, found in India and Tajikistan, has deeper divergence and is certainly not the same species as the Siberian ibex in the KZ and AMR clades. Interestingly, species divergence between the I-T clade and the Nubian ibex *Capra nubiana* occurred during the Pliocene epoch (~3.9 myr ago; CI: 2.4–5.7; SD 0.061). The estimated species divergence time (3–7 myr) reasonably corroborates with other closely related species, e.g. Cervidae (Pitra et al. 2004) and Bovidae (Bibi 2013). Interestingly, the results indicate that diversification events may have occurred during the Pleistocene era, when many areas served as refugia for mammals (Zink et al. 1995, Rodríguez 2004, Byrne et al. 2008, Pavelková Řičánková et al. 2014), as well in the Last Glacial Maximum (Pavelková Řičánková et al. 2015) in central Asia.

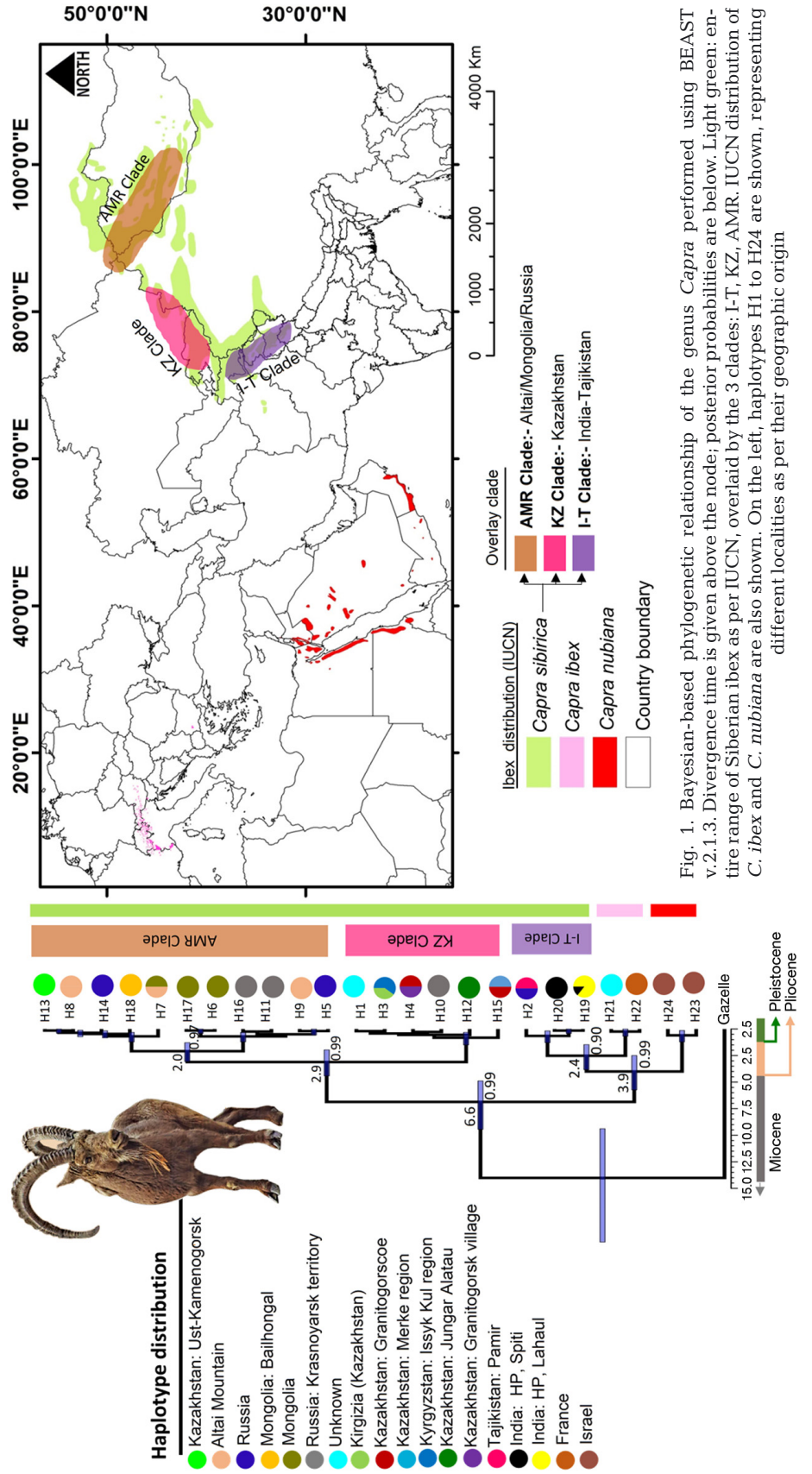


Fig. 1. Bayesian-based phylogenetic relationship of the genus *Capra* performed using BEAST v.2.1.3. Divergence time is given above the node; posterior probabilities are below. Light green: entire range of Siberian ibex as per IUCN, overlaid by the 3 clades: I-T, KZ, AMR. IUCN distribution of *C. ibex* and *C. nubiana* are also shown. On the left, haplotypes H1 to H24 are shown, representing different localities as per their geographic origin

We observed strong phylogeographic structure with respect to the geographical origin of the samples, including different haplotypes in the Lahaul and Spiti Valley from India that support our hypothesis of long isolation histories, possibly due to the influence of climatic–topographic oscillations (Reading & Shank 2008). Further, the paraphyletic arrangement of clades in the Bayesian phylogeny supports that there are species complexes of Siberian ibex, plausibly comprising at least 2 species (i.e. one being the I-T clade and the other, the KZ/AMR clades), which are then divided into 3 to 4 subspecies (one each of the I-T and KZ clades and 2 of the AMR clade). Evolutionary history suggests that the genus *Capra* radiated from central Asia (Pilgrim 1947) from ‘ibex type’ and ‘bezoar-type’ ancestors, and among these, ibex subsequently bifurcated westward and isolated as 2 separate species i.e. *C. ibex* and *C. nubiana* (Pidancier et al. 2006). Subsequently, ibex dispersed to India, forming an edge population that was restricted to the Trans-Himalayan ranges of Himachal Pradesh in the western Himalayas.

Our results are based on only a partial fragment of the mitochondrial *cytb* gene, as this was the only data available for comparison from the entire range of the ibex. However, genes such as *cytb* and/or cytochrome *c* oxidase subunit I (COI) have been used in a number of studies, ranging from insects to mammals, for delineating species boundaries (Hebert et al. 2003, Verma & Singh 2003, Tobe et al. 2010). It is possible that increased sample sizes across the entire ibex distribution and/or using more genes or the whole genome may bring updated insights into the evolutionary history, demography and species diversification of the ibex. However, we believe this would not change the overall species assignment. We seek collaboration to sequence the genomes of ibex from the entire range.

In conclusion, the present study demonstrates that ibex found from India to Tajikistan are adequately diverged from the Siberian ibex found in the Altai Mountains, Russia and Mongolia and show relatively recent divergence from the alpine and Nubian ibex. Further, the Siberian ibex of the Altai Mountains, Russia and Mongolia also significantly diverged from the Kazakhstan population, proving a strong signature of genetic isolation throughout this large landscape. With this study, we advocate that ibex from India to Tajikistan be considered a distinct species, distinct from the Siberian ibex but relatively closer to *C. ibex*. If the I-T clade is recognized as a distinct species, it will provide the necessary attention to upgrade its conservation status under the IUCN Red

List of Threatened Species and facilitate a call to immediate attention for the conservation and management of the ibex at a global and regional scale.

Author contributions. B.D.J., A.Sh. (A. Sharief), V.K., M.K. and A.Si. (A. Singh) undertook field survey and collected samples. B.D.J., G.J. and S.K.S. participated in data generation, quality check and primary data analysis. T.M. prepared maps and provided all GIS-related support. B.D.J., A.Sh. and G.J. wrote the primary draft of the manuscript. B.D.J. and G.J. raised ideas and conceptualized under the supervision of M.T. and L.K.S. B.D.J., L.K.S. and M.T. finalized the manuscript. L.K.S. and M.T. coordinated the project funded under the National Mission Himalayan Studies (NMHS) of Ministry of Environment, Forest and Climate Change (MoEF&CC). K.C. supervised the overall activities and provided all the logistic support and administrative approval.

Acknowledgements. We thank the Principal Chief Conservator of Forest (PCCF) and Chief Wildlife Warden (CWLW), Department of Forest, Government of Himachal Pradesh for granting the necessary permission to undertake field surveys. The authors are thankful to Divisional Forest Officers, Lahaul and Spiti Forest Divisions for their consistent support during the field study. We acknowledge the National Mission for Himalayan Studies, Ministry of Environment, Forest and Climate Change (MoEF&CC) for funding (Grant No. NMHS/2017-18/LG09/02/476).

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