Genetic resolution of composition and phylogenetic placement of the Isabelline Bear

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Abstract: We sequenced part of the mitochondrial control region of 2 Himalayan *Ursus arctos isabellinus* individuals and compared it with that of other *U. arctos*. Results indicate that the valid allopatric subspecies *U. a. isabellinus* represents an ancient clade and includes the Gobi bear of Mongolia as a relict population.

Key words: Gobi bear, Ursidae, *Ursus arctos isabellinus*

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The Isabelline bear (*Ursus arctos isabellinus* Horsfield) of central Asia is allopatric with respect to conspecifics. Much of its historical range (Fig. 1) lies in the mountains north and west of the vast Taklimakan Desert, but it also extends southeast into the western Himalayas (Ognev 1932; Pocock 1932, 1941; Chestin 1996; Zhang et al. 1997; Schaller 1998; Nawaz 2007). To its west, north, and east lies the range of *U. a. arctos*; to the southeast, on the Tibetan Plateau, is the distinctive (Chestin 1996, Schaller 1998) *U. a. pruinosus*.

The Isabelline bear is reasonably coherent in terms of cranial (Pocock 1932, 1941; Chestin 1996) and pelage (Pocock 1932, 1941; Schaller 1998) traits. Schaller (1998) noted similarity of the pelage of the rare Gobi bear to that of the nearby *U. a. isabellinus*, and suggested affinity between them. We investigated this issue using mitochondrial DNA (mtDNA) data, and also investigated the phylogenetic relationship of *U. a. isabellinus* to other *U. arctos*.

Masuda et al. (1998) examined part of the 5′ portion (beginning with position 7 as enumerated on the light chain) of the mtDNA control region of one Gobi bear and 2 *U. a. pruinosus* and noted the large genetic difference between the 2 types of bear. The Gobi bear's small geographic range is situated near one end of the main *isabellinus* range (Fig. 1). We generated homologous control region data using mtDNA of 2 wild-caught *U. a. isabellinus* from the Deosai Plains of the western Himalayas, nearer the opposite end of the *isabellinus* range (Fig. 1). DNA extraction from ear pinna sections was performed following standard protocols in the Qiagen (Valencia, California, USA) tissue kit, our PCR methodology used mtDNA control region primers (Ward et al. 1991), and cycle-sequencing followed standard protocols (Adams et al. 2003). A short segment of DNA beginning with control region position 87 exhibited a variable number of thymine and cytosine nucleotides and was excluded from phylogenetic analysis, leaving 263 bases.

Maximum parsimony (MP) and maximum likelihood (ML) phylogenetic analyses were performed using data from across the extensive range of the *U. arctos–U. maritimus* ('*arctos* group') clade. MP and ML analyses were performed with PAUP (v.4.0b10,

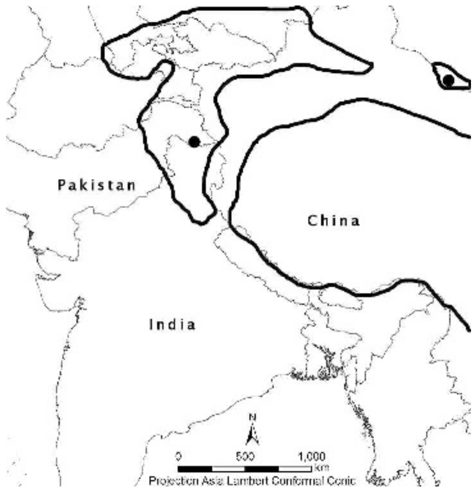

Fig. 1. Approximate historical geographic range of *Ursus arctos* in central Asia. The range of *U. a. isabellinus* is to the northwest, that of *U. a. pruinosus* to the southeast. Dots within the range of the former indicate mtDNA collection locations.

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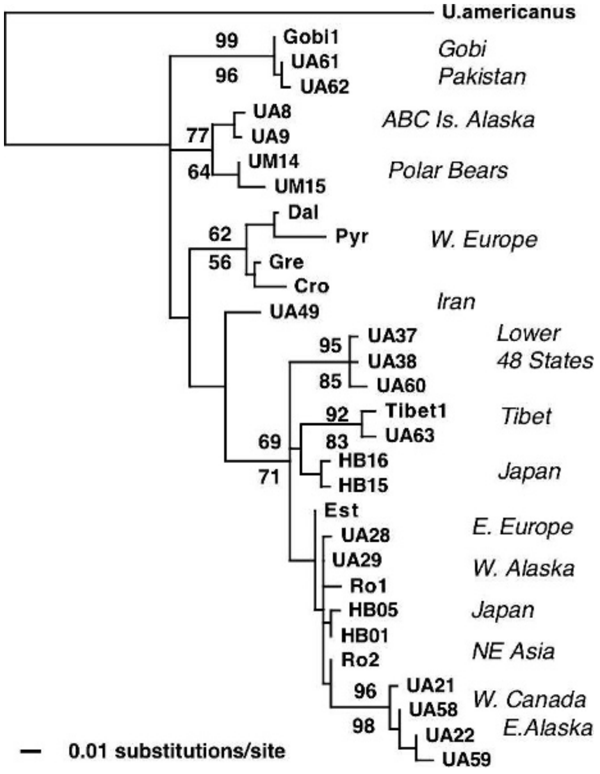

Fig. 2. Optimal maximum likelihood tree (HKY85 + I + G) for a dataset (L = −947) of 28 *Ursus arctos* and 2 *U. maritimus* haplotypes, using 263 base pairs of the mtDNA control region. *U. americanus* is used as an outgroup. Bootstrap values >50% are displayed, with maximum parsimony values (via 1000 replicates) above and maximum likelihood values (via 100 replicates) below each branch. Rough geographic provenance is indicated for certain clades. 'Tibet' includes the entire *U. a. pruinosus* range; samples are Tibet1 and UA63. Haplotypes HB01, HB05, HB15, and HB16 (Genbank AB013040, AB013050-013051, AB013062, AB013063-013064) are from Matsuhashi et al. (1999). Tibet1 and Gobi1 (AB010727-010728) are from Masuda et al. (1998). Cro, Dal, Est, Gre, Ro1, Ro2, and Pyr (X75867-75870, X75872-75873, X75878) are from Taberlet and Bouvet (1994). UA63 and UA49 (DQ914407-914408) were provided by P. Taberlet. UA8-9, UA21-22, UA28-29, UA37-38, and UA58-60 (EF033706, EF033710, EF033857-033858, EF033737, EF033765, EF033897, EF033916, EF033890, EF033896, EF034025), and UM14-15 (EF033728-033729) are from Waits et al. (1998). UA61-62 (DQ914409-914410) are our new Himalayan *U. a. isabellinus* sequences.

Swofford 2002) using heuristic searches with stepwise addition and tree–bisection–reconnection (TBR) branch swapping (Swofford et al. 1996). Confidence in the nodes was assessed by bootstrap replication (1,000 replicates MP, 100 replicates ML) with random addition of haplotypes (Felsenstein 1985). For ML analyses, the best-fit model of DNA substitution and parameter estimates for tree construction was chosen by performing hierarchical likelihood-ratio tests in Modeltest (v.3.06, Posada and Crandall 1998). The HKY + I + G model had the lowest likelihood score and showed significantly better fit than alternate models; the gamma value was 0.4799.

The *U. a. isabellinus* haplotypes from the Deosai Plains and the Gobi bear haplotype from south of the Altay Mountains are similar (UA61 and Gobi1 differ by one base pair) and form a clade with very strong ML (96%) and MP (99%) bootstrap support (Fig. 2). Mitochondrial data thus are compatible with the view that the taxon *U. a. isabellinus* includes the Gobi bear as a relict population. The *isabellinus* clade constitutes an early branch of the *arctos* group and is widely divergent genetically from haplotypes from such neighboring areas as Iran, Siberia, Japan, and the Tibetan region (Fig. 2).

The Isabelline bear represents an ancient lineage of *Ursus arctos*, presumably long isolated in the mountains of central Asia. The slight genetic difference between geographically distant populations of this subspecies suggests substantial range expansion during the Late Pleistocene–Holocene.

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