Research article

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PHYLOGEOGRAPHY OF *URSUS THIBETANUS* IN SOUTHEAST ASIA

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ABSTRACT

We investigated the phylogeography of the bear *Ursus thibetanus* in Southeast Asia, using mitochondrial DNA variation and Bayesian phylogenetic methodology. Included in our overall analysis were 24 new, locality-based samples from Southeast Asia and Pakistan. We present here a relatively detailed biogeography of haplotype clades within Southeast Asia. We suggest that the Chao Phraya basin acted as a barrier, substantially separating one clade from the other two.

Keywords: Asiatic black bear, Bayesian analysis, moon bear, mtDNA, phylogeography, Southeast Asia, *Ursus, Ursus thibetanus*

INTRODUCTION

The moon bear or Asiatic black bear (*Ursus thibetanus* Cuvier) today occupies a broad geographic range in southern and eastern Asia (Fig. 1). This species thrives in temperate deciduous forests with mast crops, but is also found in open forest (Peacock, 1933), shrubland (Pocock, 1932), and rainforest (Galbreath *et al.*, 2001). The boundaries of its global and local distributions must have ebbed and flowed during the climatic vicissitudes of the Pleistocene. Thus Kurtén (1968) reported Middle Pleistocene fossils from Europe, where the species does not presently occur. In contrast, Ferguson (1993) reported Pleistocene occurrence of alternation between deciduous forest and cold steppe (the latter not a viable moon bear habitat) in central China, where moon bears do occur today. At some point in the Pleistocene, moon bears spread into Southeast Asia.

We were particularly interested in identifying mitochondrial DNA (mtDNA) haplotype groups present in Southeast Asia, how they might nest within the larger mtDNA phylogeny of moon bears, and the extent to which the distribution of those haplotypes might reflect the large-scale topography of the region. Our study is not intended to represent an exhaustive phylogeography of the species, but it extends the geographic scope of the study by Wu *et al.* (2015), who found that moon bears of eastern mainland Asia are relatively closely related.

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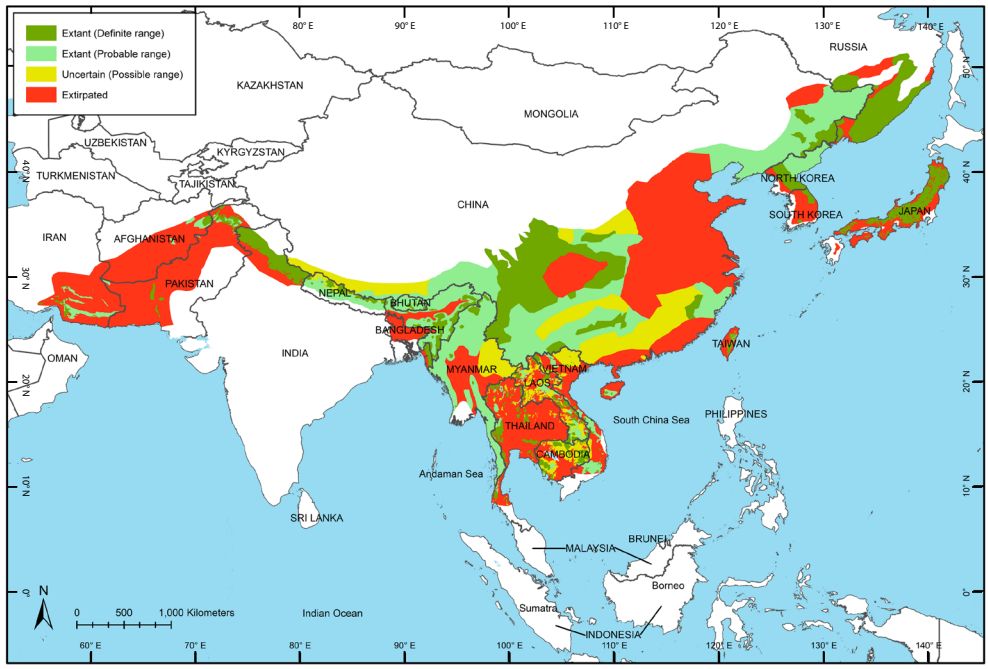

Figure 1. Geographic distribution of *Ursus thibetanus*, current and historical, courtesy of the IUCN SSC Bear Specialist Group.

STUDY AREA

The geographic focus of our work was Southeast Asia, where we collected and sequenced 19 specimens, from Thailand, the Thailand/Myanmar border area, Laos, and Cambodia. We also collected and sequenced 5 specimens from Pakistan, yielding the first such sequences from that nation.

METHODS

Two criteria for use of specimens in our study were (1) credible locality data and (2) complete sequence coverage (i.e., no "missing" sequence data). We successfully sequenced for the 24 individuals a continuous 1,359 base pair (bp) sequence comprising the cytochrome b gene, the threonine tRNA gene, the complement of the proline tRNA gene, and the adjacent 84 bp of the control region. The bears were in captive circumstances, and only capture localities that were deemed certain or highly credible, after investigation, were accepted (Table 1). MtDNA was extracted from roots of plucked hairs using a Qiagen kit. Primers were those of GALBREATH *ET AL.* (2006), GALBREATH *ET AL.* (2008), and/or GALBREATH *ET AL.* (2012).

Accurate rooting of our Southeast Asian haplotypes required consideration of a broader phylogenetic context. Five Genbank *U. thibetanus* sequences from eastern Asia (AB863014 from Japan, PP820857 from Russia, EF667005 from Korea, EF076773 from Taiwan, and EF587265 from Vietnam) were used, along with our own 24 sequences, to produce a phylogram. Genbank sequences representing *U. americanus* (AF303109), *U. arctos* (AF303110), *U. malayanus* (FM177765), and *U. ursinus* (FM177763) were utilized as basal outgroups. Bayesian phylogenetic analysis was used to calculate posterior probability values (PPVs) using the MrBayes 3.2.7a program (Ronquist *et al.*, 2012), with settings nst=6, rates=invgamma, ngen=100,000, and samplefreq=10. A PPV value of 0.80 or higher was considered significant.

Table 1. Southeast Asian and Pakistan specimens used in the present study.

SPECIMEN	GENBANK ACCESSION #	LOCALITY INFORMATION
THAILAND1	PP932523	Vicinity of Ban Pang Kae, Nan Province, Thailand, on either side of Thailand/Laos border
THAILAND2	PP932524	Vicinity of Nan city, on either side of Thailand/Laos border
THAILAND3	PP932530	North of Chang Dao, Thailand, near the border with Myanmar
THAILAND4	PP932515	Vicinity of Mae Hong Son, Thailand, on either side of Thailand/Myanmar border
THAILAND5	PP932516	Vicinity of Namtok Surin National Forest, Thailand, on either side of Thailand/Myanmar border
THAILAND6	PP932517	Vicinity of Sop Moei District, Thailand, on either side of Thailand/Myanmar border
THAILAND7	PP932518	Vicinity of Tak Province, Thailand, on either side of Thailand/Myanmar border
THAILAND8	PP932519	Vicinity of Pala-U District, Prachuap Khiri Khan Province, Thailand, on either side of Thailand/Myanmar border
MYANMAR1	PP932520	Within 200 km of Dawei, Myanmar, on either side of Myanmar/Thailand border
LAOS1	PP932528	Sabot Mountains, Xiangkhouang Province, Laos
LAOS2	PP928972	Vicinity of Nam Et - Phou Louey National Park, Houaphanh Province, Laos
LAOS3	PP928970	Vicinity of La District, Oudomxay Province, Laos
LAOS4	PP928971	Mon Subdistrict, Sing District, Luang Namtha Province, Laos
LAOS5	PP932521	Vicinity of Pa Oudom District, Bokeo Province, Laos
LAOS6	PP932529	Vicinity of Sainyabuli Province, Laos, on either side of Laos/Thailand border

Table 1 (continued).

SPECIMEN	GENBANK ACCESSION #	LOCALITY INFORMATION
LAOS7	PP932522	Vicinity of Nam Phouy National Protected Area, Sainyabuli Province, Laos, on either side of Laos/Thailand border
CAMBODIA1	PP932525	Cardamom Mountains or to their immediate north, on either side of Cambodia/Thailand border
CAMBODIA2	PP932526	Kirirom National Park, Cambodia
CAMBODIA3	PP932527	Prey Nup District, Preah Sihanouk Province, Cambodia
VIETNAM1	EF587265*	Vicinity of Cat Tien, Vietnam
PAKISTAN1	PP932512	Khuzdar District, Pakistan
PAKISTAN2	PP868282	Khuzdar District, Pakistan
PAKISTAN3	PP928973	Vicinity of Darail, Pakistan
PAKISTAN4	PP932513	Vicinity of Makra Mountain, Hazara Region, Pakistan
PAKISTAN5	PP932514	Swat District, Pakistan

*Not our own sequence

RESULTS

There were 117 variable sites among the 29 *U. thibetanus* sequences. Our 24 new sequences yielded 15 new haplotypes. In the Bayesian phylogram (Fig. 2) our sequences from Southeast Asia and Pakistan were quite divergent, with the latter and the Japan sequence constituting useful sequential outgroups to the others. Consistent with the results of Wu *et al.* (2015), a Taiwan sequence was retrieved as the sister group of all those from mainland eastern Asia (now including our 19 Southeast Asian sequences), which possessed a joint PPV of 0.88. Each of four mainland mitochondrial clades possessed a PPV of 1. Those clades from eastern mainland Asia formed a statistical phylogenetic tetrachotomy. Thus, the three clades from Southeast Asia were closely related, but did not necessarily constitute a monophyletic group.

A locality map of Southeast Asia (Fig. 3) displays distributions of the clades present in the region (which we term here, for ease of discussion, clades A, B, and C). Clade A haplotypes are found in the mountainous western region along the Thailand/Myanmar border. To the east, clade B is widespread, being found as far south as western Cambodia and southern Vietnam. The distribution of its haplotypes in northern Thailand and Laos at least interdigitates with, and probably overlaps, that of the relatively northern clade C.

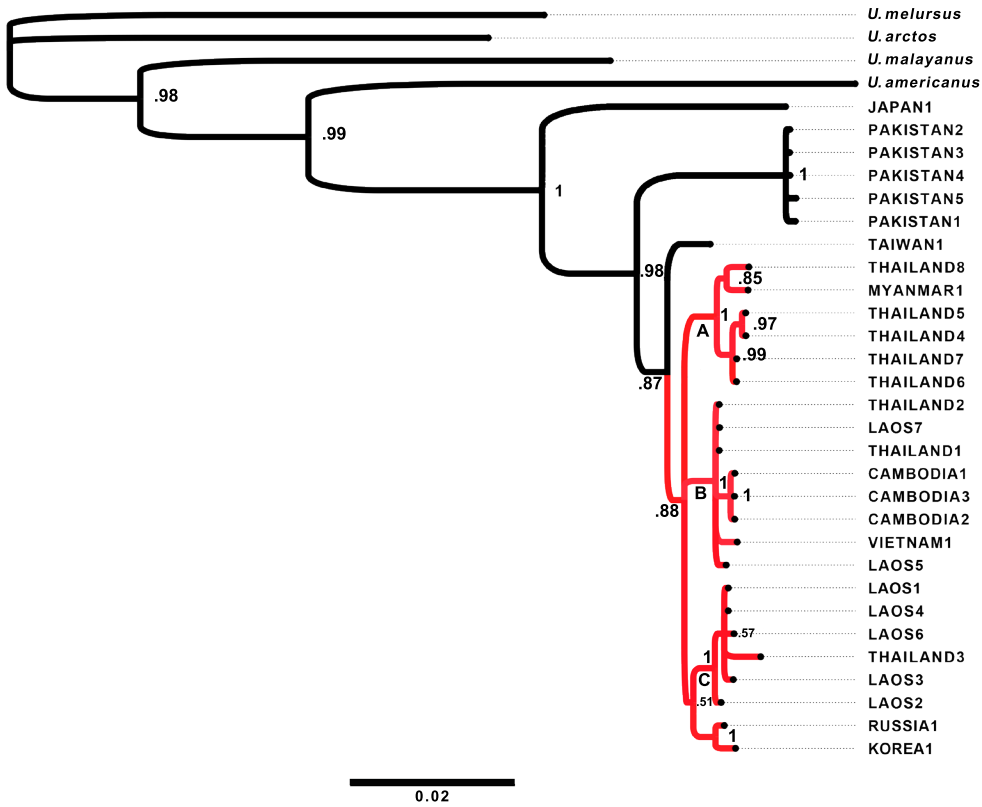

Figure 2. Bayesian mtDNA phylogram of 29 *Ursus thibetanus* sequences, each comprising 1,359 continuous bp representing the cytochrome b gene, the threonine and proline tRNA genes, and the adjacent 84 bp of the control region. Basal outgroups are Genbank sequences of *U. americanus* (AF303109), *U. arctos* (AF303110), *U. malayanus* (FM 177765), and *U. ursinus* (FM177763). Bayesian posterior probabilities are shown as nodal support values. The scale bar indicates nucleotide substitutions per site. The clade that represents specimens from Southeast Asia and northeastern mainland Asia is highlighted in red, and within it, clades A, B, and C are labelled.

DISCUSSION

The three Southeast Asian mitochondrial clades displayed an interesting geographic pattern. During its spread and internal diversification, the westernmost one (clade A) may have been substantially isolated from the others by the marshes and swamps (and occasional partial marine inundation) of the great lowland drainage basin of the Chao

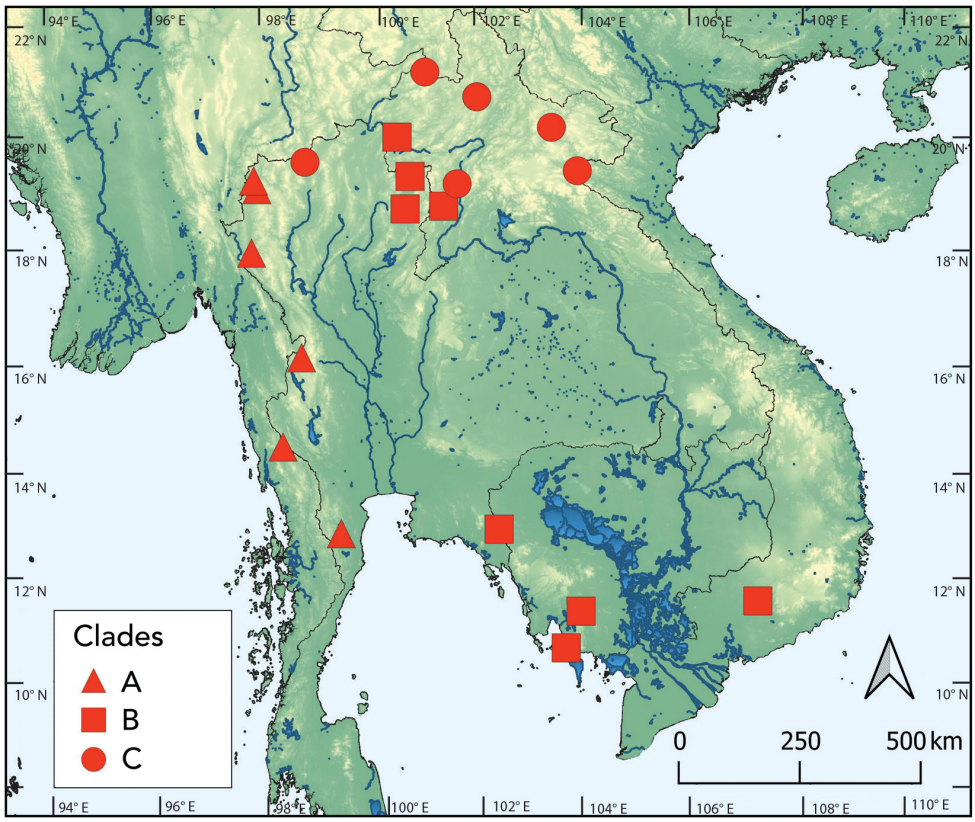

Figure 3. *Ursus thibetanus* localities of our Southeast Asian specimens. Mitochondrial clade identity is indicated by marker shape.

Phraya River and its Pleistocene extended correlate, the North Siam River. This seems more likely than that the river itself formed an effective barrier, given the ease with which bears swim.

The current distributions of mitochondrial clades B and C may be explained by either of two scenarios. Both clades may have originated in northern Southeast Asia (and/or southern China) and then intermingled somewhat, with clade B spreading farther southward than clade C. Alternatively, the two clades may reflect an early presence of two north/south disjunct populations east of the Chao Phraya basin, followed by a merger of the populations that has allowed some geographic intermingling of their haplotypes.

ACKNOWLEDGEMENTS

We declare no conflicts of interest. Authors' contributions: GJG planned this study, supervised the Southeast Asian collection of specimens (in which SMM participated), and

largely wrote the paper. F-i-A supervised the collection of specimens in Pakistan. Much of the DNA extraction and sequencing was conducted by JCM.

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