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Author(s)	Onuma, Manabu; Suzuki, Masatsugu; Ohtaishi, Noriyuki
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Possible conservation units of the sun bear (*Helarctos malayanus*) in Sarawak based on variation of mtDNA control region.

Manabu Onuma^{1,2)}, Masatsugu Suzuki¹⁾ and Noriyuki Ohtaishi^{1,3)}

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Abstract

The mitochondrial DNA control region of the sun bear (*Helarctos malayanus*) was sequenced using 21 DNA samples collected from confiscated sun bears to identify conservation units, such as evolutionarily significant units and management units, in Sarawak, Borneo Island. A total of 10 haplotypes were observed, indicating the presence of at least two lineages in the sun bear population in Sarawak. Presumably, these two lineages could represent evolutionarily significant units. However, the geographical distributions of the two lineages remained unknown due to the lack of information regarding the exact capture locations of the confiscated sun bears. It is essential to elucidate the geographical distributions of these lineages in order to create a proper conservation plan for the sun bears in Sarawak. Therefore, further studies examining the haplotype distributions using DNA samples from known localities are essential.

Key Words : mitochondrial control region, *Helarctos malayanus*, Sarawak

The sun bear (*Helarctos malayanus*) is distributed throughout Southeast Asia, including Borneo and Sumatra^{1,5,10)}. Their populations are thought to be in decline due to habitat loss and excessive human-caused

mortality¹¹⁾. The most recent comprehensive research on the sun bears on Borneo Island was conducted by Meijaard in 1999⁷⁾, and indicated that the loss of 30-60% of their total habitat on the island between 1960-1990 may

¹⁾Laboratory of Wildlife Biology, Graduate School of Veterinary Medicine, Hokkaido University, N18 W9 Kita-ku, Sapporo, Hokkaido, 060-0810, Japan

²⁾Biological Resource Laboratory, Laboratory for Intellectual Fundamentals for Environmental Studies, National Institute for Environmental Studies, 16-2, Onogawa, Tsukuba, Ibaraki, 305-8506, Japan
TEL : 029-850-2498 FAX : 029-850-2673

e-mail : onuma.manabu@nies.go.jp

³⁾Laboratory of Wildlife Management, Department of Biosphere and Environmental Sciences, Faculty of Environment Systems, Rakuno Gakuen University, Bunkyo-dai-Midorimachi, Ebetsu, Hokkaido, 069-8501, Japan

have led to a similar decrease in the sun bear population size. Furthermore, according to information supplied by local communities in Sarawak, Borneo Island, the sun bear is one of the animals most often reported to be in rapid decline²⁾. Therefore, the wild population of sun bears in Sarawak could be the one of the populations most in danger of extinction and a conservation plan must be established as soon as possible to avoid extinction.

Extinction risks for endangered species are evaluated based on demographic and environmental factors. Genetic data also provide important information for evaluating these risks, since the data can be applied to detect any loss of genetic diversity that may lead to inbreeding depression. Thus, genetic data represent one of the important sources of information for creating conservation plans for endangered species. In particular, data for the mitochondrial DNA (mtDNA) control region can provide information that not only allows evaluation of the genetic diversity but also defines the population structure. In addition, the sequence information is useful for defining such conservation units as evolutionarily significant units (ESUs) and management units (MUs)^{8,9)}. In fact, sequence information for the brown bear mtDNA control region has been used not only for phylogeographic studies but also to define conservation units^{3,6,14,18)}. To date, however, the amount of information available for the mtDNA control region in the sun bear remains limited. The aim of the present study was to obtain information regarding variation in the sun bear mtDNA control region to identify possible conservation units in Sarawak, Borneo Island.

Blood and hair samples were collected from 21 sun bears in quarantine at the Semenggoh Wildlife Rehabilitation Centre veterinary clinic from 1993 to 1999. All of these animals had been confiscated from the gen-

eral public in Sarawak's major towns, specifically Kuching (12 individuals : 10 females and 2 males), Sibul (3 individuals : 1 female and 2 males), Bintulu (3 individuals : 2 females and 1 male) and Miri (3 individuals : 2 females and 1 male) (Fig. 1). However, we were unable to obtain any information regarding the exact capture locations of the bears. A total of 21 DNA samples were analyzed in the present study. Specifically, 17 DNA samples were extracted from blood using QIAamp DNA Blood Mini Kits (QIAGEN), and 4 DNA samples were extracted from hair samples using ISOHAIR (NIPPON GENE).

The mtDNA was amplified using the primer set L15774 and H16498¹³⁾, which amplifies a partial sequence containing cytochrome b, tRNA-Trp and tRNA-Pro and a partial sequence of the mtDNA control region. All amplifications were performed using Ready-To-Go PCR Beads (Amersham Biosciences) in 25- μ l reaction mixtures containing 1.5 units of Taq DNA polymerase, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP, 0.5 μ M of each primer and 25-50 ng of extracted DNA. Amplification was conducted in a thermal cycler (PE2400 ; Perkin Elmer) under the following conditions : 95°C for 5 min ; 30 cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 30 sec ; and a final extension at 72°C for 5 min. The PCR products were purified using a MiniElute QIA quick PCR purification kit (QIAGEN), and sequenced using the same primers described above for the PCR. Sequencing was performed using BigDye Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems) and an ABI PRISM 310 sequencer with the POP 6 polymer (Applied Biosystems). The obtained sequences for the mtDNA control region were aligned with ClustalW¹⁷⁾ and checked by eye. The phylogenetic relationships between the observed haplotypes were examined by the neighbor-

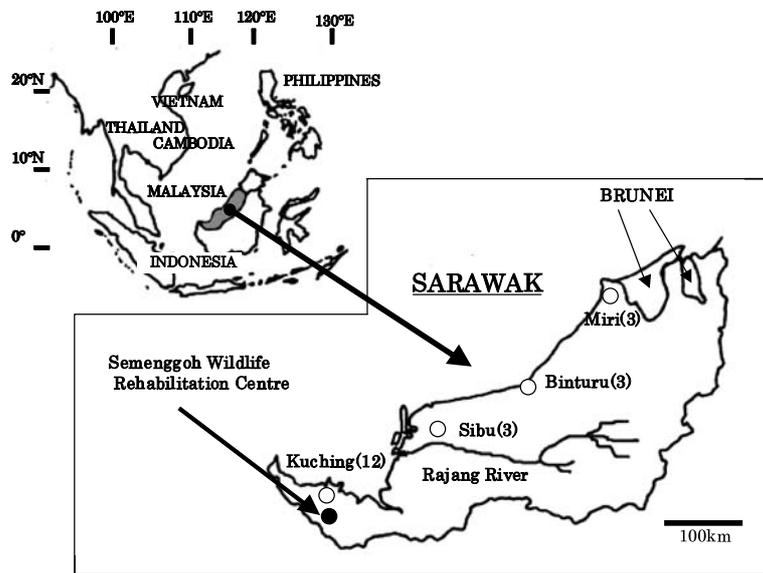


Fig. 1 . Location of the study area, the state of Sarawak, on Borneo Island. The DNA samples were collected at the Semenggoh Wildlife Rehabilitation Centre veterinary clinic. The numbers in brackets indicate the numbers of confiscated sun bears.

Table 1 . Haplotypes observed in the control region of 21 sun bears. Variable nucleotide positions refers to the sequence of Sarawak 1.

Haplotype	Occurrences	Nucleotide position														Accession number	
		9	17	30	52	56	87	89	174	211	213	220	235	256	282		290
Sarawak 1	6	C	T	G	T	C	T	C	T	A	C	C	C	A	C	A	AB098542
Sarawak 2	1	.	C	A	C	T	T	T	T	.	.	.	AB098543
Sarawak 3	3	T	AB098544
Sarawak 4	3	T	AB098545
Sarawak 5	1	T	T	AB098546
Sarawak 6	1	.	C	A	C	T	.	.	G	T	T	.	.	T	.	AB098547	
Sarawak 7	1	.	C	A	C	T	.	T	C	G	T	T	.	G	.	G	AB098548
Sarawak 8	3	T	T	AB098549
Sarawak 9	1	.	C	A	C	T	C	.	G	T	T	T	AB098550
Sarawak10	1	T	AB098551

joining method¹²⁾ using MEGA version 2.1⁴⁾. The giant panda (*Ailuropoda melanoleuca* ; accession number : AY390361) was used as an outgroup. The nodal support of the phylogenetic analysis was estimated using bootstrap values with 1000 replications.

The 300 bp mtDNA control region was

successfully sequenced for all 21 sun bears. All the sequences of the haplotypes have been deposited in EMBL/GenBank/DDBJ with accession numbers AB098542- AB098551. A total of 15 variable sites were observed and all the nucleotide substitutions were transition-type substitutions (Table 1). Overall, 10 haplo-

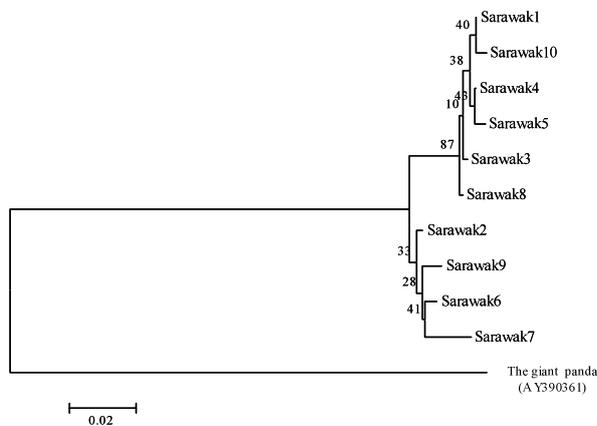


Fig. 2. Neighbor-joining tree of the mtDNA control region of the 10 haplotypes observed in the 21 sun bears. The numbers on internal branches are bootstrap values with 1000 replications. The scale indicates the numbers of nucleotide substitutions per site calculated using Kimura's two-parameter model.

types were identified and the most frequent haplotype was found in 6 individuals (Table 1). The mean pairwise differences among the haplotypes were 2.0% and the interspecific differences between the sun bear and giant panda were 28.0%. The phylogenetic analysis revealed that there were at least two lineages in the sun bear population in Sarawak (Fig. 2). Presumably, these lineages could be ESUs for Sarawak, since ESUs can be identified by significant phylogenetic structuring of the mtDNA and significant differences in nuclear allele frequencies⁸⁾. However, it was impossible to examine the geographical distributions of the 10 haplotypes, because we were unable to confirm the exact capture locations for any of the sun bears in this study. Therefore, we cannot provide any geographical information regarding the two ESUs. One possible assumption is that these two ESUs may be divided by the Rajang River, which is one of the major rivers in Borneo (Fig. 1). However, further studies are essential for determining the haplotype distributions using DNA samples from known localities. Collec-

tion of feces and hair in the field for DNA extraction has been successfully applied to studies of wild brown bear^{3, 15, 16, 19)}. The same technique should thus be applied to the wild sun bear population in order to obtain geographical information regarding the haplotypes in Sarawak for conservation purposes.

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