

SHORT COMMUNICATION

Limited Heredity Diversity of the Critically Endangered Guizhou Golden Monkeys

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ABSTRACT

Guizhou golden monkey (*Rhinopithecus brelichii*) was a unique, endangered and endemic primate species in Guizhou Province. It was an isolated population caused by habitat loss and fragmentation due to the human disturbance in the recently 30 years in China, only distributed in Fanjingshan National Natural Reserve, Guizhou Province. To know the background with demonic population structure, we sequenced 867 bp of the mitochondrial DNA D-loop from 312 fresh fecal samples, results showed there 11 haplotypes among these samples, h was 0.517, π was 0.00413. It indicated that this species had the lowest genetic diversity among four golden monkeys in China and need strengthen the conversation concern for this species immediately.

1. Background

Snub-nosed monkeys (*Rhinopithecus*, of *Colobinae*, *Cercopithecidae*) included five species, four species are endemic to China, these were *R. roxellana*, *R. bieti*, *R. brelichii*, *R. strykeri*; just the species of *R. avunculus* were distributed in northern area of Vietnam. Among the five snub-nose monkeys species, the most comprehensive studied objects were the Sichuan and Yunnan snub-nosed monkeys, while the remaining three species were only limited to general ecological studies, such as habitat selected and utilization, population size surveys, and diet selection, especially the *R. brelichii* and *R. strykeri*. The lack of in-depth research on *R. brelichii*, *R. strykeri* and *R. avunculus* has led to many problems in the evolutionary biology and behavioral adaptations of these rare monkeys. At present, with the development of conservation genetics

achievements, and Chinese government pay more attention to the great importance on the biodiversity conservation, the populations of *R. roxellana*, *R. bieti* and *R. strykeri* have recovered significantly, however, the population size of *R. brelichii* has decreased significantly according to Guo ^[1], at the same time, its endangerment level was raised from Endangered to Critically Endangered ^[2], so it is urgent to know the population genetic background of the altered species, which can help to scientifically. Therefore, after to know the true genetic background of this isolated population, which can help to protect it scientifically and effectively.

2. Materials and Methods

We used mitochondrial D-loop molecular markers to sequence 312 fresh fecal samples from the wild population of Guizhou golden monkeys in Fanjingshan

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National Nature Reserve. Forward and reverse sequencing was performed for each sample, and sequences were aligned using Clustal X and rechecked by eye. Haplotypes were identified using Mega 7.0. Genetic diversity within populations was estimated as haplotype diversities (h) index and nucleotide diversities (π) using DnaSP 3.0. The network tree was drawn with NETWORK 4.6.0.0 using the median-joining method. In Structure 2.3.4, model-based Bayesian model was used for population genetic STRUCTURE analysis.

3. Results

Out of 312 samples, 288 samples were successfully amplified with a full length of 867bp, 327bp in the highly variable region and 540bp in the conserved region. 32 variant loci were found, and the proportion of polymorphic loci was 3.7 %. The average content of T, C, A and G bases was 28.4%, 28.1%, 28.9% and 14.6%, respectively; A+T was 57.3%; G+C was 42.7%. The GC content was lower than the AT content, which was consistent with the primates base content pattern. The population of Guizhou snub-nosed monkeys had 11 haplotypes, with haplotype diversity index (h) was 0.517 and nucleotide diversity (pi) index was 0.00413. Population Structure diagram showed that *R. brelichi* were divided into red clade, green clade and blue clade, and ΔK and L(K) parameters indicated that $K = 3$ was the best (Figure2). The nonsignificant values of the neutrality tests, including both Tajima's D and Fu's FS indicate no departure from the null hypothesis of demographic stability within the 95% confidence interval. This result was further supported by the multimodal patterns of the mismatch distribution of *R. brelichi* to a stable and small isolated population (Figure 3).

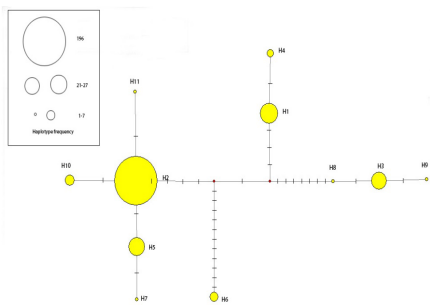


Figure 1. Median-joining network of mitochondrial D-loop haplotypes for *R. brelichi*

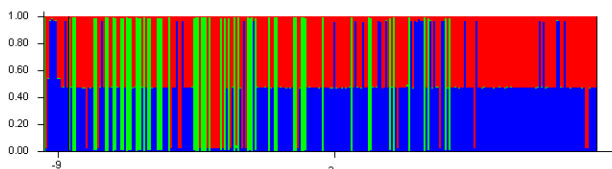


Figure 2. STRUCTURE out put of clusters obtaining of *R. brelichi* ($K=3$)

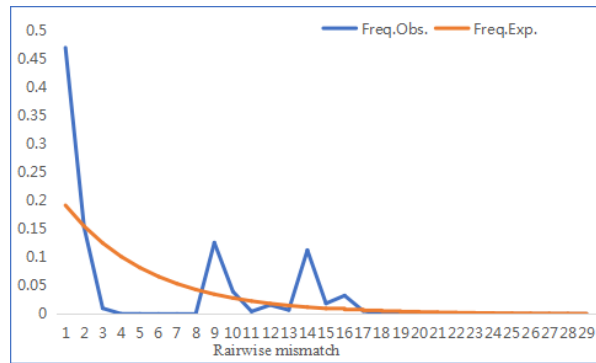


Figure 3. Mismatch distribution of *R. brelichi* mitochondrial D-loop sequences.

4. Discussion

Comparing the genetic diversity of mitochondrial control regions of *R. roxellana* and *R. bieti*, Guizhou golden monkeys had the lowest genetic diversity among the three. 157 samples of *R. bieti* monkeys resulted in 30 haplotypes, with haplotype diversity of 0.944 and nucleotide diversity of 0.036^[3]; 60 samples *R. roxellana* samples, with 12 haplotypes, haplotype diversity of 0.845 and nucleotide diversity of 0.034^[4]. Both *R. roxellana* and *R. bieti* have geographic populations, while Guizhou snub-nosed monkeys currently have only one population, which is one of the reasons for the low genetic diversity of Guizhou snub-nosed monkeys. The genetic diversity of *R. roxellana* in Shennongjia^[5], an isolated population lived in Shennongjia, Hubei, had the lowest genetic diversity compared to other Sichuan snub-nosed monkey geographic populations, with a haplotype diversity of 0.5011 and a nucleotide diversity of 0.0018, so it indicated that the Guizhou golden monkeys in Fanjingshan National Natural Reserve was also an isolated populations. In Yang's research published in 2012^[6], 146 fecal samples were collected from the same reserve, five haplotypes with a haplotype diversity of 0.457 and a nucleotide diversity of 0.014. Although the sampling volume was increased, neither haplotype diversity nor nucleotide diversity increased significantly when compared with our results. Therefore, according to the IUCN criteria for classifying species as endangered, we suggest that Guizhou golden monkeys be upgraded from Endangered to Critically Endangered by assessing the number of species, habitat area, presence of geographic populations, genetic background, and strength of human disturbance.

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