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Hongshan Yang

*Chinese Academy of Agricultural Sciences, China*

Genzhu Chang

*Chinese Academy of Agricultural Sciences, China*

Xiaoqin He

*Gansu Forestry Planning & Designing Academy, China*

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## Molecular authentication of the *Eospalax* of *Myospalax* by analyzing cytochrome b sequences

Hong-shan Yang<sup>1</sup>, Gen-zhu Chang<sup>1</sup>, Xiao-qin He<sup>2</sup>

1. Lanzhou Institute of Animal & Veterinary Pharmaceutics Sciences, CAAS, Lanzhou 730050

2. Gansu Forestry Planning & Designing Academy, Lanzhou 730020 E-mail: yanghsh123@126.com

**Key words** : subgenus *Eospalax*, cytochrome b gene, genetic diversity, conversion and transversion, phylogenetic

**Introduction** Nucleotide Complete sequences of mitochondrial DNA (mtDNA) cytochrome b gene (1140 bp) were used to determine the phylogenetic relationships among 21 samples of subgenus *Eospalax* of *Myospalax* genus from 11 different places of Gansu province, other sequences were acquired from GenBank. Four species from Gansu included Qin ling zokor (*Eospalax rufescens*), Gansu zokor (*M. cansu*), Bailey's zokor (*M. baileyi*), and Smith's zokor (*M. smithi*). The phylogenetic tree was constructed from the 43 near complete cyt b sequences. The sequences and UPGMA tree are integrity and credibility in our study, then the origin and evolution of subgenus *Eospalax* from molecular level were illustrated firstly in Gansu province.

**Materials and methods** 21 liver samples of five subgenus *Eospalax* were collected in our experiment from eleven places in Gansu province. (1) Total genomic DNA was extracted from the liver samples, treated using a procedure modified from *Hagelberg and Clegg* (1991). (2) PCR and Sequence Analysis: primer L7: 5'-ACCAATGACATGAA-AAATCATCGTT-3', H6: 5'-TCTCCATTTCTGGTTTACA AGAC-3' for complete mitochondrial cyt b gene.

**Results** (1) Sequence Analysis: Twenty one complete sequences of cytb for 5 species of zokors were obtained, the alignment of cytb sequences is 1143 nucleotides long, nucleotide insertion and deletion was not found. Nine groups of the 43 sequences were found more than 386 polymorphic sites. The content of A, G, C, T is this the highest concentration of T base pairs (31.2%), followed by A (30.8%), C third (25.8%), the lowest content of G (12.4%). There are different content of A, G, C, T in the third codon position, contents in the third position changes at the rate of T (19.2% to 30.7%), C (24.3% to 44.2%), A (41.4% to 44.5%) and G (0.8% ~ 3.6%), significantly higher than that position 1 and 2. 80% Conversion and transversion occurred in the third codon position. The levels of homoplasy measured for C-T, T-C substitutions are the highest in the third position, but the lowest in the first position. (2) Phylogenetic Reconstruction: A phylogenetic tree constructed from the 43 near-complete cytb sequences of 5 five species in the subgenus *Eospalax* is shown in Figure 1. Different zokors and areas, based to the three indices of geographical position, heredity and evolution, difference of species, form different short branches, and each of branches may include different species.

**Conclusions** The results indicated that (1) the Grassland zokor and Northeast zokor are the old ancient species in the *Myospalax* genus, the Chinese zokor is the secondly and the others are in one evolution branch. (2) There are two big evolution branch in the Subgenus *Eospalax* from Gansu, one is Bailey's zokor branch and the other is the big branch that composed with Smith's zokor, Gansu zokor and Qin ling zokor, in other words three species are the similar ancestors. (3) In the Bailey's zokor branch, the Bailey's zokor distributed in the deserted zone of Hexi Corridor first diverged from the others, and distributed in the southeastern of Gansu aggregated in one branch.

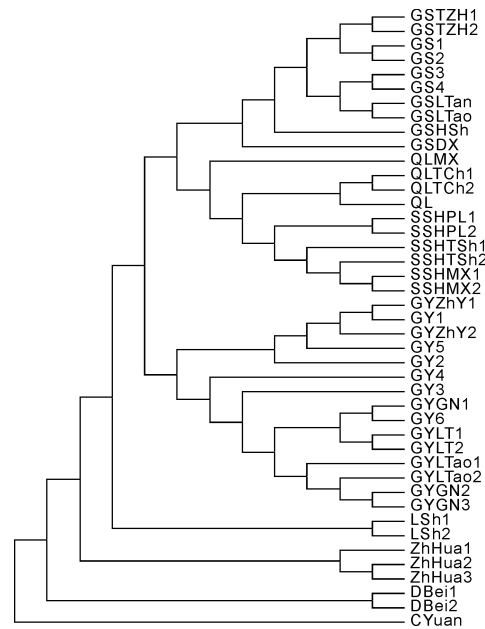


Figure 1 The most parsimonious tree of Complete cyt b gene sequence data of mtDNA.