

Comparative analyses of past population dynamics between two subterranean zokor species and the response to climate changes

Lizhou TANG¹, Long YU¹, Weidong LU¹, Junjie WANG¹, Mei MA¹, Xiaodong SHI¹,
Jiangang CHEN^{1*}, Tongzuo ZHANG²

¹College of Biologic Resource and Environmental Science, Qujing Normal University, Qujing, Yunnan 655011, China

²Key Laboratory of the Qinghai-Tibetan Plateau Ecosystem and Biological Evolution and Adaptation, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining, Qinghai 810001, China

Received: 16.04.2012 • Accepted: 21.09.2012 • Published Online: 25.02.2013 • Printed: 25.03.2013

Abstract: The mitochondrial cytochrome *b* sequences of 34 haplotypes from 114 individuals of *Eospalax baileyi* and GenBank data of 40 haplotypes from 121 individuals of *Eospalax cansus* were used to investigate if the Quaternary glaciations on the Qinghai-Tibetan Plateau (QTP) influenced the distribution and colonisation of these 2 species, and if both species presented congruous past population dynamics. The phylogenetic tree indicated a valid status for the genera *Eospalax* and *Myospalax*, and an independent species status for *E. baileyi* and *E. cansus*. The demographical population history of *E. baileyi* showed that the effective population size remained stable between 1.00 and 0.50 million years ago (Mya), increased quickly to a peak and fluctuated dramatically between 0.50 and 0.20 Mya, after which a stable level was maintained from 0.20 Mya to the present. The past population dynamics of 3 subgroups of *E. cansus* exhibited a congruent fluctuation status with *E. baileyi* from 0.40 to 0.16 Mya, which coincided with the time of the Penultimate Glaciation. This uniform population dynamics changed during the same glacial dates, suggesting that the population history for these 2 species had been influenced by the Penultimate Glaciation of the QTP.

Key words: *Eospalax baileyi*, *Eospalax cansus*, subterranean rodent, past population dynamics, Qinghai-Tibetan Plateau, Penultimate Glaciation

1. Introduction

The purpose of comparative phylogeography is to compare population patterns of sympatric species and to subsequently identify factors that influenced common evolutionary history, and to evaluate whether the phylogeographical patterns for all of the species show a congruous response to geological or historical events as a whole (Avice, 2000; Arbogast and Kenagy, 2001). In general, plants or animals distributed in the same important regions are highly responsive to climatic shifts and either share a similar colonisation model or demonstrate a more complex model of diversification (Qu and Lei, 2009; Tang et al., 2010). However, these species may maintain their demographical population history due to seeking out favourable habitats or foods during periods of repeated climatic oscillations, especially the Quaternary glaciations (Qu and Lei, 2009).

Eospalax baileyi and *Eospalax cansus*, sympatric zokor species of genus *Eospalax*, are 2 types of subterranean rodents that are highly adapted to an underground lifestyle

and have an overlapping distribution in a region on the north-eastern edge of the Qinghai-Tibetan Plateau (QTP) (Fan and Shi, 1982). Owing to its unique topographical physiognomy, extreme environmental conditions, and distinct geological evolution history, the QTP has been proven to be an important area for inducing the distribution and diversification of endemic plants and animals (Tang and Shen, 1996; Meng et al., 2007; Tang et al., 2010). Geological studies of the QTP showed that there were 3 distinct glacial ages (Maximum Glaciation, Penultimate Glaciation, and Last Glaciation) which happened 0.80–0.60 million years ago (Mya), 0.32–0.15 Mya, and 0.08–0.02 Mya, respectively (Shi, 1998; Jiao and Shen, 2003). These periods of glaciation have been considered an important factor in influencing the population dynamics of local species since the Pleistocene (Du et al., 2010). As they are subterranean species, *E. baileyi* and *E. cansus* must move more slowly and expend more energy than aboveground species when trying to escape climatic changes such as those experienced during periods of glaciation on the QTP.

* Correspondence: cjg113@126.com

The restricted dispersal ability and similar underground behaviour of these 2 zokor species prompted us to examine the following hypothesis: if the Quaternary glaciations on the QTP could affect distribution and colonisation of 2 species then both species would present with congruent past population dynamics.

2. Materials and methods

2.1. Population samples, Cyt b gene DNA extraction, PCR amplification, and sequencing of *E. baileyi*

A total of 114 individuals of *E. baileyi* were collected from 11 populations (Table) across the north-eastern region of the QTP. Muscle tissue samples were preserved in 95% ethanol. Total DNA was isolated from ethanol-fixed tissue by proteinase K digestion followed by standard phenol-chloroform extraction and 70% ethanol precipitation. To amplify the sequence of mitochondrial cytochrome *b* (Cyt *b*) gene: L14724 (5'-C GAA GCT TGA TAT GAA AAA CCA TCG TTG-3') and H15917 (5'-C GGA ATT CCA TTT TTG GTT TAC AAG-3') 2 primers were used (Zhou et al., 2004). The amplification and sequencing of samples were performed according to Tang et al. (2010).

2.2. Cyt b sequences downloaded for *E. cansus* and other species

The complete Cyt *b* sequences of 40 haplotypes from 121 individuals of *E. cansus* were downloaded from GenBank (accession numbers: GQ244363–GQ244402) (Lin, 2010) and named C01–40, respectively. The complete Cyt *b* sequences of the 6 *Eospalax* species, 2 *Myospalax* species, and *Rhizomys sinensis* (outgroup species) were also downloaded for phylogenetic analyses (Zhou and Zhou, 2008; accession numbers: *Eospalax baileyi*, AF326256; *Eospalax cansus*, AF326261; *Eospalax fontanierii*, AF326266; *Eospalax rothschildi*, AF326268; *Eospalax*

rufescens, AF326269; *Eospalax (Myospalax) smithi*, EF530740; *Myospalax aspalax*, AF326272; *Myospalax psilurus*, AF326271; *Rhizomys sinensis*, AF326274).

2.3. Data analysis

Sequences were aligned using CLUSTAL X 1.8 (Thompson et al., 1997) with default settings that were refined manually. The haplotypes were determined from 114 samples of *E. baileyi* using DnaSP 5.0 (Librado and Rozas, 2009). A maximum likelihood tree was constructed using the Tamura-Nei model and 10,000 bootstrap replications using the computer program MEGA 5.0 (Tamura et al., 2011). The best-fit model was estimated from nucleotide frequency, gamma value, and the transition/transversion ratio for Cyt *b* sequences of both zokor species using the program Modeltest 3.06 (Posada and Crandall, 1998). The past population dynamics of *E. baileyi* and *E. cansus* were obtained using the program MIGRATE 2.4.4 (Beerli, 2002) based on the above analyses of the phylogenetic relationship and parameter estimation (Drummond et al., 2005). The following parameters were used for the past population dynamics analyses: Bayesian inference strategy; 10 short chains of 1,000,000 steps followed by 3 long chains of 1,000,000 steps; chains were sampled every 100 steps following a burn-in of 30,000 steps and 1000 increments, and 3 replications to obtain convergent and consistent results. The θ -value was converted to N_e using the formula $\theta = x N_e \mu$ (where x means a multiplier: 1 for haploid markers transmitted through one sex only, such as mitochondrial DNA and Y chromosomes; N_e , effective population size; μ , substitution rate) with a substitution rate of $\mu = 4.12\%$ per million years (Tang et al., 2010). The age could be calculated based on generation periods of 1 year for these 2 species (Wei et al., 1998).

Table. The geographic location, number of samples, and haplotypes of the plateau zokor (*Eospalax baileyi*).

Population	Site/Village	Longitude (E)	Latitude (N)	Altitude (m)	Sample	Haplotypes
Pop1	Bayan	102.30°	36.19°	3230	16	B1,2,3,4,5,6,7,8,9,10
Pop2	Ashennu	102.20°	36.04°	2603	8	B3,8,
Pop3	Donggou	102.12°	36.90°	3040	11	B11,12,13,14,15,16,17
Pop4	Xizhang	101.68°	36.95°	3020	11	B18,19,20,21,22,23
Pop5	Bohang	101.11°	36.64°	3110	10	B23,24,25,26
Pop6	Hongxing	102.72°	34.10°	3230	8	B27
Pop7	Axi	102.89°	33.91°	3450	11	B27,28,29,30
Pop8	Tangke	102.53°	33.41°	3490	12	B31
Pop9	Azitan	103.25°	34.75°	3160	10	B32,33
Pop10	Wanmao	103.05°	34.37°	3270	11	B32,33,34
Pop11	Shenzang	103.55°	34.74°	3020	6	B32

3. Results

3.1. Phylogenetic relationship analysis

A total of 34 haplotypes (named B1-34) (accession numbers: FJ358648-FJ358653, FJ358659-358678, FJ358680-FJ358684, FJ358688-FJ358690) were identified from 114 individuals, which covered 11 populations of *E. baileyi* (Table). The maximum likelihood tree showed that 34 haplotypes of *E. baileyi* and 40 haplotypes of *E. cansus* formed a monophyletic group (Figure 1). The topology tree with high bootstrap values indicated a valid status for the genera *Eospalax* and *Myospalax* and an independent species status of *E. baileyi* and *E. cansus*, which was consistent with a previous study by Zhou and Zhou (2008).

3.2. Past population dynamics of *E. baileyi* and *E. cansus*

The phylogenetic tree also indicated that 3 subgroups diverged into the monophyletic group of *E. baileyi* and *E. cansus* from each other (Figure 1). The past population dynamics for the 3 subgroups of *E. baileyi* supported similarly consistent history curves (Figure 2). These 3 population dynamics curves of *E. baileyi* showed that the effective population size was maintained at a relatively stable state of 1,220,000 individuals between 1.00 and 0.50 Mya, and increased quickly to a peak and fluctuated dramatically between 0.20 and 0.50 Mya. A stable level of 600,000 individuals was then maintained from 0.20 Mya to the present (Figure 2). The demographic curves of the 3 subgroups of *E. cansus* showed similar fluctuations to *E. baileyi* in an effective population size from 0.60 Mya to the present (Figure 3). From the population dynamics curves, a stable period of an effective population size of 400,000 to 600,000 individuals occurred simultaneously for the 3 subgroups between 0.60 and 0.40 Mya. This was followed by a large increase to its highest peak, which then fluctuated and decreased briefly to its lowest values from 0.40 to 0.16 Mya (Figure 3). Lastly, the effective population size has been maintained in a nearly stable state from 0.16 Mya to present, with a slight fluctuation between 0.10 and 0.06 Mya (Figure 3).

4. Discussion

The analyses of the demographical history of *E. baileyi* and *E. cansus* showed that the effective population size fluctuated greatly between 0.16 and 0.40 Mya (Figures 2, 3). As subterranean rodents, with similar biological behaviours, these 2 species (parapatric sister species in the genus *Eospalax*) not only faced a weak dispersal ability because of the high energy costs of digging underground, but also because they had to adapt and respond to environmental variations (Fan and Shi, 1982; Zhang and Liu, 2003). The uniform population dynamics suggests that the population history for these 2 species could be affected equally by some factors, such as historical events or climate changes. Previous geological studies have suggested that

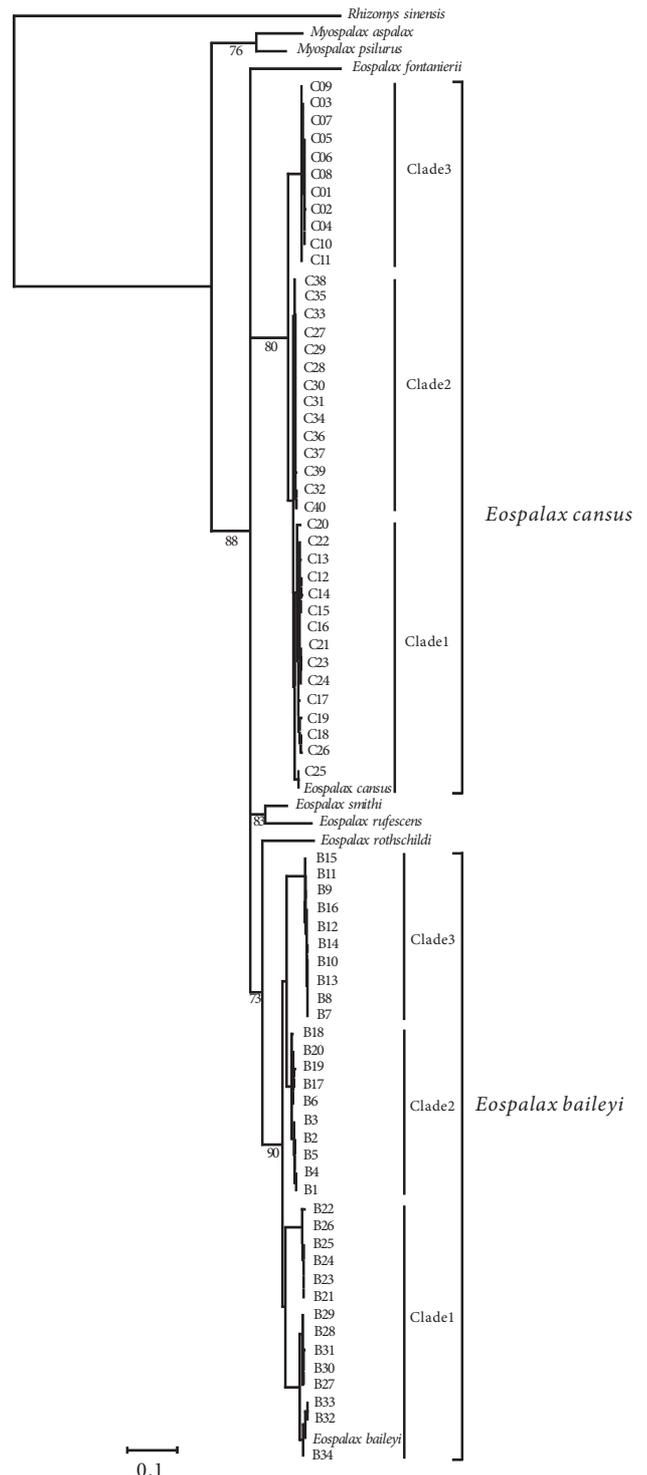


Figure 1. The maximum-likelihood (ML) tree of *Cyt b* sequences of zokor species. *Rhizomys sinensis* was used as the outgroup. Bootstrap values are shown above the branches of the ML tree.

climatic changes during Quaternary glaciations of the QTP may have greatly influenced the distribution area and colonisation of endemic species (Shi et al., 1998). The

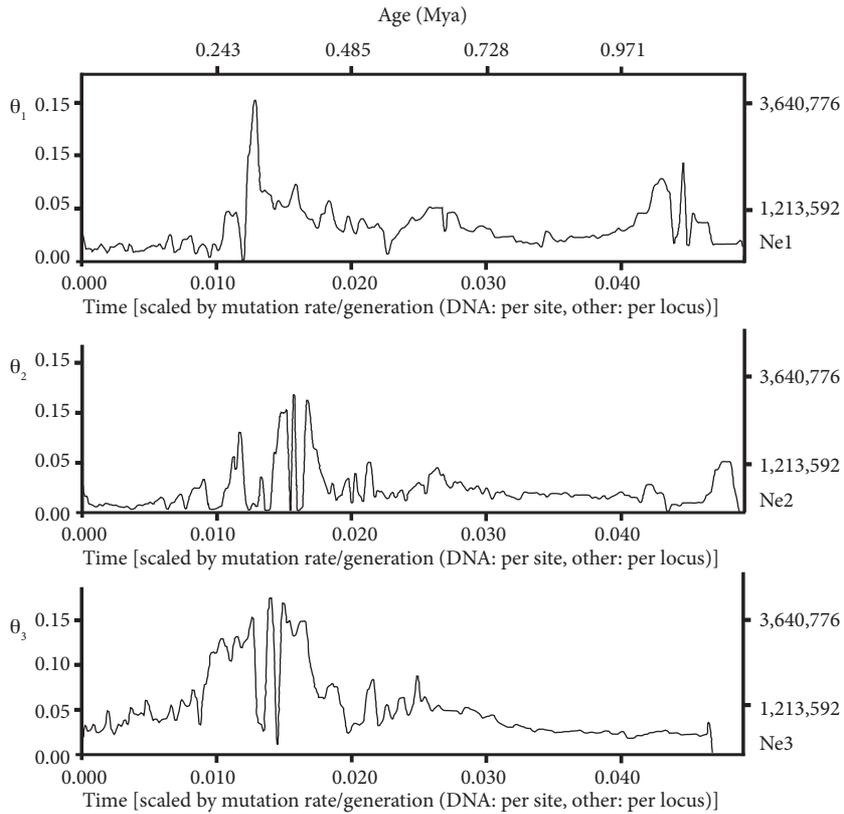


Figure 2. The effective population size through recent time for 3 clades of plateau zokor (*Eospalax baileyi*).

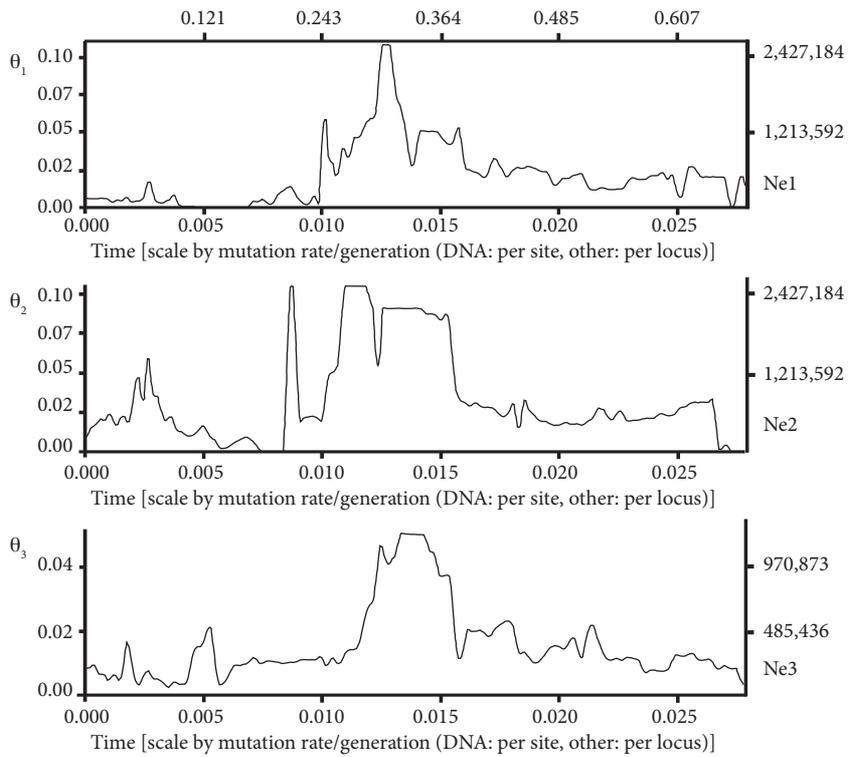


Figure 3. The effective population size through recent time for 3 clades of Gansu zokor (*Eospalax cansus*).

Penultimate Glaciation occurred at approximately 0.15–0.32 Mya, which coincides with the time of population fluctuation of both zokor species (Shi, 1998). Therefore, this result fully supports the theory that the population history of the 2 species may have been significantly affected by the Penultimate Glaciation of the QTP.

The sampled populations of *E. baileyi* and downloaded haplotypes of *E. cansus* were mainly distributed in regions on the north-eastern edge of the QTP (Table 1). During the Penultimate Glaciation, the density of the ice sheets, as well as the degree of area covered by them, was the second largest in scale when compared with the Maximum Glaciation (Jiao and Shen, 2003). The distribution regions for the 2 zokor species were at high and low altitudes which were largely covered by a big ice sheet in 0.40–0.16 Mya (Shi et al., 1998). Development of the ice sheet was induced by a decrease in temperature, which probably influenced the growth of plant species and the degradation or succession of suitable food plants. In addition, the growth and perishing rate of the plant species may have been restricted by the feeding behaviour of the zokors, because these 2 zokor species mainly feed on the roots, stems, and shoots of annual and perennial grasses, forbs, or shrubs (Wang et al., 2000). The presence of ice sheets

and the feeding habits of the zokors did not impact upon their survival but these factors may have reduced the rate of replacement of suitable plant species. So why did the effective population size of the 2 zokor species fluctuate acutely during the Penultimate Glaciation? Although there is a positive correlation between the population density of the zokors and the percentage of dicotyledons (food plants) in the total biomass (Zhang, 2000), these dicotyledonous plants may not have been able to sustain enough zokor individuals, particularly during climatic oscillations. Lastly, the effective population size fluctuated many times during the Penultimate Glaciation and the maintenance of stable population dynamics before or after this period probably resulted from a balance between food resources and population size.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31071915 to J-P Su; 31260087 to L-Z Tang), the Applied Fundamental Research Project of Yunnan Provincial Science and Technology Department (2010ZC149 to L-Z Tang), and the Key Project of Qujing Normal University Scientific Research Fund (2009ZD004 to L-Z Tang).

References

- Arbogast, B.S. and Kenagy, G.J. 2001. Comparative phylogeography as an integrative approach to historical biogeography. *J. Biogeogr.* 28(7): 819–825.
- Avice, J.C. 2000. *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, MA.
- Beerli, P. 2002. *Migrate: documentation and program*, part of LAMARC version 1.5. Available at: <http://evolution.genetics.washington.edu/lamarc/index.html> (last accessed 11 January 2013).
- Drummond, A., Rambaut, A., Shapiro, B. and Pybus, O. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol. Biol. Evol.* 22(5): 1185–1192.
- Du, Y.R., Guo, S.C., Wang, Z.F., Ci, H.X., Cai, Z.Y., Zhang, Q., Su, J.P. and Liu, J.Q. 2010. Demographic history of the Tibetan antelope *Pantholops hodgsoni* (chiru). *J. Syst. Evol.* 48(6): 490–496.
- Fan, N.C. and Shi, Y.Z. 1982. A revision of the zokors of subgenus *Eospalax*. *Acta Theriol. Sin.* 2(2): 183–199.
- Jiao, K.Q. and Shen, Y.P. 2003. The Quaternary glaciations and glacier properties in the Tanggula Range. *J. Glaciol. Geocryol.* 25(1): 3442.
- Librado, P. and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 25(11): 1451–1452.
- Lin, G.H. 2010. Morphological adaptations of digging apparatus and population genetics of the subterranean rodent *Myospalax cansus*, Doctoral thesis, Graduate University of the Chinese Academy of Sciences, Beijing, 78 pp.
- Meng, L.H., Yang, R., Abbott, R.J., Miehle, G., Hu, T.H. and Liu, J.Q. 2007. Mitochondrial and chloroplast phylogeography of *Picea crassifolia* Kom. (Pinaceae) in the Qinghai-Tibetan Plateau and adjacent highlands. *Mol. Ecol.* 16(19): 4128–4137.
- Posada, D. and Crandall, K.A. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics.* 14(9): 817–818.
- Qu, Y.H. and Lei, F.M. 2009. Comparative phylogeography of two endemic birds of the Tibetan plateau, the white-rumped snow finch (*Onychostruthus taczanowskii*) and the Hume's ground tit (*Pseudopodoces humilis*). *Mol. Phyl. Evol.* 51(2): 312–326.
- Shi, Y.F. 1998. Evolution of the cryosphere in the Tibetan Plateau, China, and its relationship with the global change in the Mid Quaternary. *J. Glaciol. Cryopedol.* 20(3): 197–208.
- Shi, Y.F., Li, J.J. and Li, B.Y. 1998. Uplift and environmental changes of Qinghai-Tibetan plateau in the Late Cenozoic. Guangdong Science and Technology Press, Guangzhou.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* 28(10): 2731–2739.

- Tang, L.Y. and Shen, C.M. 1996. Late Cenozoic vegetational history and climatic characteristics of Qinghai-Xizang Plateau. *Acta Micropalaeontol. Sin.* 13(4): 321–337.
- Tang, L.Z., Wang, L.Y., Cai, Z.Y., Zhang, T.Z., Ci, H.X., Lin, G.H., Su, J.P. and Liu, J.Q. 2010. Allopatric divergence and phylogeographic structure of the plateau zokor (*Eospalax baileyi*), a fossorial rodent endemic to the Qinghai-Tibetan Plateau. *J. Biogeogr.* 37(4): 657–668.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997. The Clustal_X windows interface: flexible strategies for multiple alignment aided by quality analysis tools. *Nucl. Acids Res.* 25(24): 4876–4882.
- Wang, Q.Y., Zhang, Y.M., Wei, W.H. and Bian, J.H. 2000. Food habit of the plateau zokor. *Acta. Theriol. Sin.* 20(3): 193–199.
- Wei, W.H., Wang, Q.Y., Zhou, W.Y. and Fan, N.C. 1998. Effects of predatory disturbance on population reproductivity of *Myospalax baileyi*. *Acta Ecol. Sin.* 18(2): 176–180.
- Zhang, Y.M. 2000. Effect of plateau zokor on characters and succession of plant communities in alpine meadow. *Zool. Res.* 21(6): 435–440.
- Zhang, Y.M. and Liu, J.K. 2003. Effects of plateau zokor (*Myospalax fontanierii*) on plant community and soil in an alpine meadow. *J. Mammal.* 84(2): 644–651.
- Zhou, C.Q. and Zhou, K.Y. 2008. The validity of different zokor species and the genus *Eospalax* inferred from mitochondrial gene sequences. *Integr. Zool.* 3(4): 290–298.
- Zhou, C.Q., Zhou, K.Y. and Zhang, S.L. 2004. Molecular authentication of the animal crude drug Sailonggu (bone of *Myospalax baileyi*). *Biol. Pharm. Bull.* 27(11): 1850–1858.