Pakistan J. Zool., vol. 50(4), pp 1545-1548, 2018. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2018.50.4.sc4

Short Communication

A Report on the Multivariable Sites of the Mitochondrial Genome of a Bamboo Rat, *Rhizomys pruinosus* from Wenzhou, China

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Article Information
Received 30 November 2017
Revised 01 March 2018
Accepted 06 March 2018
Available online 11 May 2018

Authors' Contributions
HL, KL and XJ planned the study. YL and KL designed the study. KL, YL, HZ, KM, MS and XJ performed the trial, KL and MAY analyzed the data. KL wrote the manuscript.

Key words
***Rhizomys pruinosus*, Mitochondrial genome, Sequence, Multivariable sites, Phylogenetic analysis.**

ABSTRACT

The sequencing of the complete mitochondrial (mt) genome of the pet bamboo rat *Rhizomys pruinosus,* which had a problem in standing and walking in a Bamboo farm in Wenzhou, China was carried out, using its tibia. The mt genome (16,579 bp) is composed of 13 protein coding genes, 22 tRNA genes and 2 rRNA genes. The ratio of the bases of the *mt* genome of *R. pruinosus* from Wenzhou are A (32.31%), T (31.04%), C (24.77%), G (11.88%), A+T (63.35%), respectively. The multivariable sites in rRNA were 1.98% and 5.53% in 12S rRNA and 16S rRNA, respectively. The multivariable sites in protein coding gene were ranged from 0 to 4.83%, while the multivariable sites in amino acids were ranged from 0 to 5.22%. The current results contributes to the perform prevention of leg problems in the bamboo rat industry.

Bamboo rats (*Rhizomys sinensis*, *Rhizomys pruinosus*, *Rhizomys sumatrensis* and *Cannomys badius*) are extremely popular in China for their medicinal value, delicious and nutritious meat, thick and soft fur. Because of successful breeding in captivity, bamboo rats industry was born in the 1990s (Liu *et al*., 2011). It was estimated that there were over 30 million farmed bamboo rats in 2011 (Liu *et al*., 2011; Tang *et al*., 2017).

The mitochondrial (mt) DNA have been widely utilized as molecular markers in the study of taxonomy, population genetics, phylogenetic and evolutionary analyses due to its maternal inheritance (Li *et al*., 2008, 2016a, b, 2017a, b; Yan *et al*., 2018). The *mt* DNA was reported to have a higher mutation rate than that of nuclear DNA (Zhao *et al*., 2014), and it was reported to be highly related to various diseases due to the alteration of *mt* DNA content (Zong *et al*., 2016; Chen *et al*., 2016). However, scare information is available about the *mt* characteristics of *Rhizomys pruinosus* (*R. pruinosus*) from Wenzhou, China, especially ailing bamboo rat. Therefore we carried out this research to sequnce the *mt* of *Rhizomys pruinosus* with a leg problem in this area to reveal whether it has relationship with the mitochondrial genome.

Materials and methods

Samples were collected after permission from the relevant institutions. All procedures were performed under the instructions and approval of Laboratory Animals Research Centre of Zhejiang province in P.R. China.

A 3 months old male, pet *R. pruinosus* with heavy leg problem (can not stand up) was obtained from the hospital of College of Animal Science, Wenzhou Vocational College of Science and Technology. This rat was fed on

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0030-9923/2018/0004-1545 $ 9.00/0

fresh bamboos. Under euthanasia tibia of this pet was removed and stored at -70 °C.

For sequencing *mt* DNA was isolated from an improved extraction method previously reported by Sorensen *et al*. (2006). After purification of *mt* DNA, short-insert libraries (insert size 350 bp) were constructed according to the manufacturer's instructions (Illumina). The short library sequences were sequenced by using a commercial Illumina Hiseq 4000 sequencing system at total genomics solution (TGS) Institute in Shenzhen, China.

For variation analysis the current *mt* genome was multiple aligned with previously reported 12S rRNA of *mt* sequences by MEGA (6.0). The reference sequences were *Rhizomys pruinosus* (AJ250358.1), *Rhizomys pruinosus* (KC789518.1) and *Rhizomys sinensis* (NC_026124.1). The phylogenetic analysis was performed to determine *Rhizomy* species using MEGA version 6 by pilotting methods of the neighbor-joining algorithm, and the distances were computed using the Tajima-Nei method. The stability of branches was assessed after bootstrapping with 1000 replicates (Li *et al*., 2016b). The reference *mt* genes are as follows: *R. pruinosus mt* (KC789518.1), *Rhizomys sinensis mt* (KM434232.1), *Eospalax rothschildi mt* (JN544420.1), *Eospalax baileyi mt* (JN540033.1), *Myospalax psilurus mt* (JX014234.1), *Eospalax cansus mt* (KC514112.1), *Rattus exulans mt* (KY814718.1), and *Myodes rufocanus mt* (KT725595.1).

Results and discussion

The sequence of the present *mt* genome of *R. pruinosus* was submitted to NCBI database with the Genebank accession number of MG193909. The *mt* genome (16,579 bp) is composed of 13 protein coding genes, 22 tRNA genes and 2 rRNA genes, which is in line with previous of KC789518 (Zhao *et al*., 2014) (Table I, Fig. 1). The ratio of the bases of the *mt* genome of *R. pruinosus* from Wenzhou are A (32.31%), T (31.04%), C (24.77%), G (11.88%), A+T (63.35%), which present a little difference with previous reported in southern China (KC789518) (Fig. 2). Interestingly, a lot of multivariable sites of the mitochondrial genome of *R. pruinosus* from Wenzhou, China were found when compared with KC789518. The multivariable sites in rRNA were 1.98% and 5.53% in 12S rRNA and 16S rRNA, respectively (Table I). The multivariable sites in protein coding gene ranged from 0 to 4.83%, while the multivariable sites in amino acids ranged from 0 to 5.22% (Table I).

Fig. 1. The complete *mt* genome map of *R. pruinosus* from Wenzhou.

Table I.- Comparison of the *mt* genome of *R. pruinosus* from Wenzhou (WZ) with previously reported from South China (SC) KC789518.

Gene	Length (gene / amino acid)		Gene variable sites (bp / %)	Amino acid variable (No. / %)
	SC	WZ		
12S-rRNA	958	938	19 (1.98%)	
16S-rRNA	1556	1477	86 (5.53%)	
NADH1	957/318	942/314	25 (2.61%)	4 (1.26%)
NADH2	1050/349	1035/345	38 (3.62%)	6 (1.72%)
COI	1545/514	1542/514	20 (1.29%)	1 (0.19%)
COII	684/227	681/227	13 (1.90%)	1 (0.44%)
ATP8	207/68	201/67	10 (4.83%)	2 (2.94%)
ATP6	681/226	678/226	14 (2.06%)	5 (2.21%)
COIII	784/261	783/261	13 (1.66%)	2 (0.77%)
NADH3	348/115	344/115	12 (3.45%)	6 (5.22%)
NADH4L	297/98	294/98	6 (2.20%)	0
NADH4	1377/459	1374/458	24 (1.74%)	4 (0.87%)
NADH5	1815/604	1779/593	59 (3.25%)	17 (2.81%)
NADH6	531/176	525/175	11 (2.07%)	1 (0.57%)
CYTB	1140/379	1134/378	22 (1.93%)	3 (0.79%)

Though, a low multivariable sites in protein coding gene were found, the NCBI blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) and phylogenetic analysis revealed that the *mt* genome of *R. pruinosus* from Wenzhou is 99% in homology with KC789518 by pilotting Neighbor Joining method with available *mt* sequences of *R. pruinosus mt* (KC789518.1), *Rhizomys sinensis mt* (KM434232.1), *Eospalax rothschildi mt* (JN544420.1), *Eospalax baileyi mt* (JN540033.1), *Myospalax psilurus mt* (JX014234.1), *Eospalax cansus mt* (KC514112.1), *Rattus exulans mt* (KY814718.1), and *Myodes rufocanus mt* (KT725595.1) (Fig. 3).

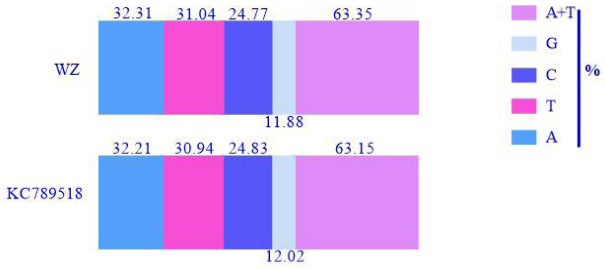

Fig. 2. The ratio of the bases of the *mt* genome of *R. pruinosus* from Wenzhou.

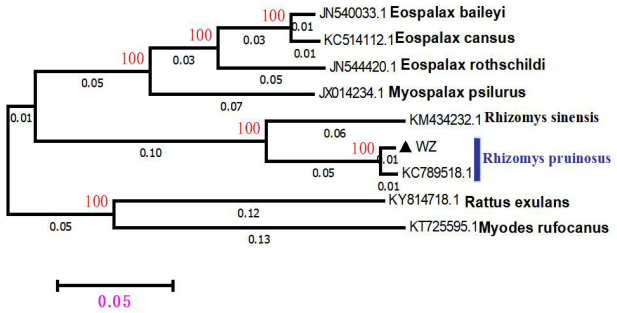

Fig. 3. Phylogenetic analysis of the *mt* genome of *R. pruinosus* from Wenzhou using Neighbor Joining method. The numbers at clades indicate bootstrap values.

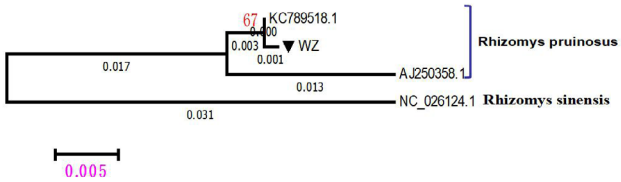

Fig. 4. Phylogenetic analysis of the 12S rRNA of *mt* genome of *R. pruinosus* from Wenzhou using Neighbor Joining method. The numbers at clades indicate bootstrap values.

We also performed the phylogenetic analysis of 12S rRNA by employing Neighbor Joining method with available sequences of *R. pruinosus* (AJ250358.1), *R. pruinosus* (KC789518.1) and *R. sinensis* (NC_026124.1), which also demonstrated the high homology 99.99% and 99.987% of *R. pruinosus* from Wenzhou with KC789518.1 and AJ250358.1, respectively (Fig. 4).

Conclusion

Though the *R. pruinosus* from Wenzhou shows a homology with previous reported one, low multivariable sites in protein coding gene were found with the leg problem rat, which may contribution to the perform prevention of leg problems in the bamboo rat industry.

Acknowledgements

This study was supported by the General Project of Education of the Zhejiang province in 2017 (Y201737824), and the Startup Project for Doctoral Scientific Research of Wenzhou Vocational College of Science and Technology in 2016 (No. 201604).

Statement of conflict of interest

The authors state that there are no competing interests.

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