

The complete mitochondrial genome and phylogenetic analysis of *Rattus tanezumi* (Niethammer, 1975), captured from North China

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ABSTRACT

Rattus tanezumi (Niethammer, 1975) is one of the commensal rodent species in South China. With the development of transportation and climate change, *R. tanezumi* has gradually migrated north and become the dominant rat species for the past few years. In this study, we assembled a complete mitochondrial genome of *R. tanezumi*, captured from North China. The mitogenome contains 16,307 nucleotide pairs, including 13 protein-coding genes, 2 ribosomal RNA genes, and 22 transfer RNA genes, as well as one non-coding control region. Based on whole mitogenome phylogenetic analysis showed that *R. tanezumi* captured from North China had a close phylogenetic relationship with that from Japan and South Korea. These findings are valuable for further studies on the evolution, genetic diversity, and taxonomy of Asian commensal rodent.

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Introduction


The commensal rodent, *R. tanezumi* (Figure 1) traditionally called Oriental house rat (Niethammer, 1975), native to South East Asia (Adhikari et al. 2018a). It has been introduced to East Asia and mainly distributed in the south of the Yellow River (Adhikari et al. 2018b; Guo et al. 2019). With the development of transportation and climate change, it has been found that *R. tanezumi* has gradually migrated north China and become the dominant rat species for the past few years (Liu et al. 2019). As one of the serious pests in farmland, *R. tanezumi* can cause damage to crops during harvesting and reducing crop yields (Brown et al. 2006; Htwe et al. 2019). Due to its seasonal migration and wide range of habitats, the *R. tanezumi* can carry various pathogens, which cause zoonotic diseases, representing a serious threat for human health (He et al. 2021; Zhang, You et al. 2021; Zhang, Li et al. 2021).

At present, there have been some studies on the gene evolution and phylogeny of the *R. tanezumi*, but this is little information on the whole mitochondrial genome of *R. tanezumi* (Teng et al. 2016; Li et al. 2023). Our study characterized the complete mitochondrial genome of *R. tanezumi* captured from North China, which will provide more molecular information for future research on the taxonomy and genetic evolution of Asian commensal rodent.

Materials and methods

The *R. tanezumi* specimen was collected in autumn 2019 from Handan (36°34'60.0"N, 114°28'60.0"E), Hebei Province, China. The tissue (hearts, lungs, livers, spleens and kidneys) was acquired after morphological analysis (Figure 1) on site, and stored at –80 °C in Hebei Provincial Center for Disease Control and Prevention (Shiyou Liu, lsy7@outlook.com) under the voucher number HD190053. Total genomic DNA was isolated from the liver tissue using the Tissue Genomic DNA kit (Biogerm, Shanghai, China). The extractive genome was sent to Personalbio (Shanghai, China) for sequencing use shotgun technology. DNA library with an insert size of approximately 400 bp was constructed and sequenced on the Illumina Miseq platform, with 250 bp paired-end reads. After filtering out of raw sequencing reads containing adapters and reads of low quality, the resultant sequences were assembled using SPAdesv3.9.0 (Bankevich et al. 2012) and correcting the results with Pilon v1.18 (Walker et al. 2014). The functional annotations were performed using the MITOS (<http://mitos.bioinf.uni-leipzig.de/>) (Bernt et al. 2013), and mitochondrial whole genome circles were mapped using the cgview. Phylogenetic analysis was performed using the MEGA 11, the complete mitochondrial genomes of 23 species were obtained from Genbank, the best nucleotide substitution model GTR + G + I was selected and the No. of bootstrap replications was 1000.

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Figure 1. The morphological characteristics of *R. tanezumi* (HD190053). The photo of specimen was supplied by Hebei Provincial Institute for Plague Control and Prevention.

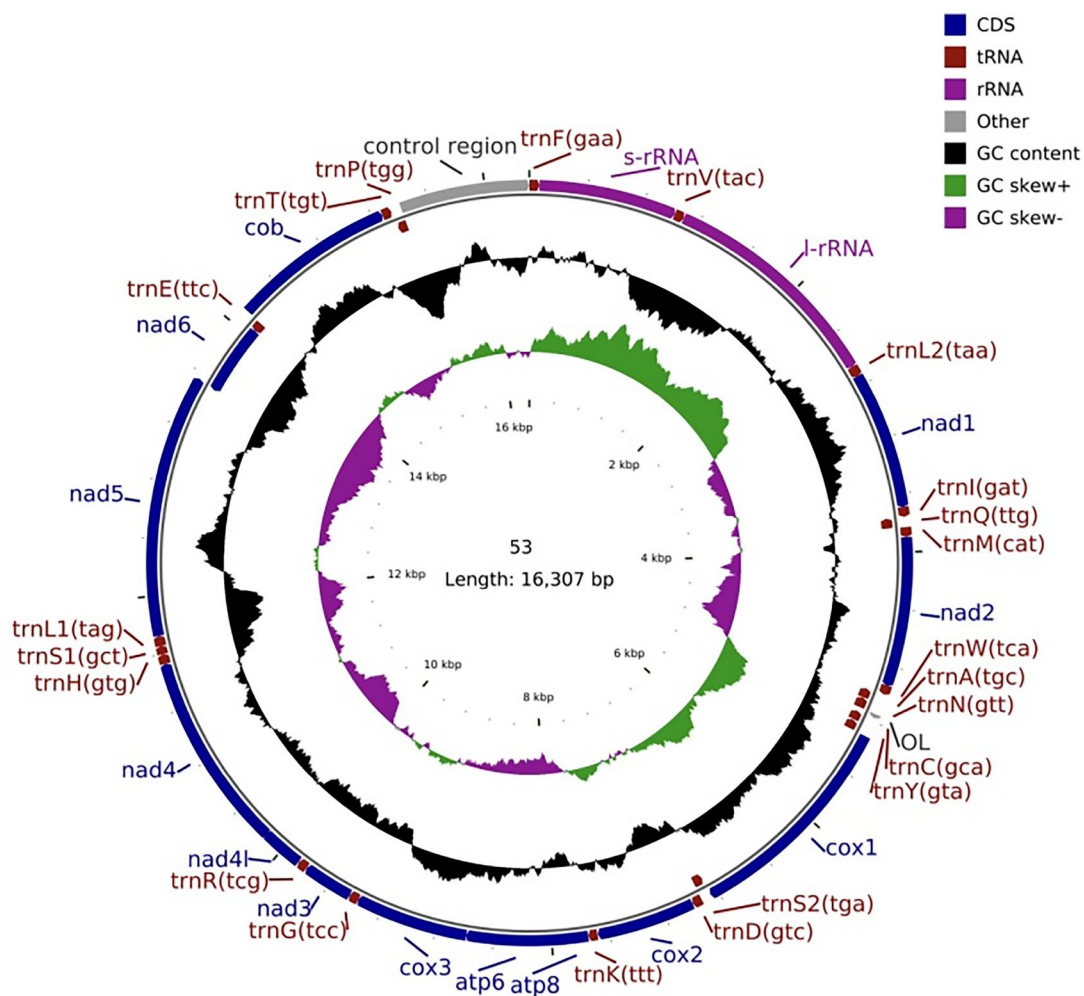


Figure 2. Complete mitochondrial genome map of *R. tanezumi*. The diagram displays the scale, GC skew, GC content, and arrangement of protein coding genes, tRNA genes, and rRNA genes on the genome. The first circle represents the scale, followed by the GC skew in the second circle, the GC content in the third circle, and the arrangement of genes in the fourth and fifth circles.

Results

Mitogenome organization

The whole mitogenome of *R. tanezumi* is 16,307 bp in length and has been deposited in GenBank (Accession number: OK054583.1). The nucleotide composition of the *R. tanezumi*

mitochondrial genome was as follows: A = 34.08%, T = 28%, G = 12.6%, and C = 25.33%. It consists of 13 protein-coding genes (PCGs), 2 ribosomal RNA genes (rRNAs), 22 transfer RNA genes (tRNAs), and a control region. Among these, 12 PCGs, 14 tRNA genes, and 2 rRNA genes were encoded in the heavy strand, whereas 1 PCG (NADH dehydrogenase subunit 6) and 8

tRNA genes were encoded in the light strand. Among the protein-coding genes, except for *nad3* and *nad5*, which had ATA as the start codon, the other 11 genes had ATG as the start codon. In addition, two protein-coding genes (*cox3*, *nad4*) ended with incomplete stop codons, while the remaining 11 protein-coding genes ended with complete stop codons TAA or TAG (Figure 2, Table S2). The genome coverage across the reference figure is detailed in the supplementary material (Figure S1).

Phylogenetic analysis

To investigate the phylogenetic relationship between *R. tanezumi* from China and other regions, we constructed the

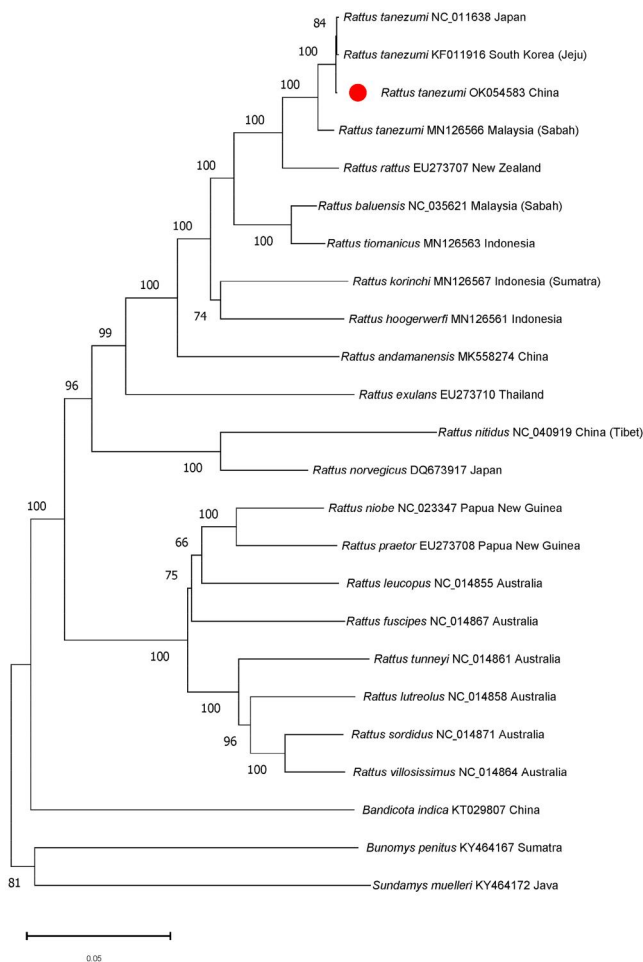


Figure 3. Phylogenetic tree of *R. tanezumi* and other related species based on mitochondrial genome sequences. Phylogenetic analysis was performed using MEGA 11 software. GenBank accession numbers of each mitochondrial genome sequences are given after the species name, and the bootstrap value based on 1,000 replicates is represented on each node. *Bunomys penitus* and *Sundamys muelleri* were used as outgroups to root the tree. The name of reported species was noted with a red dot. The following sequences were used: KF011916 (Adhikari et al. 2018), MN126566 (Camacho-Sanchez and Leonard 2020), NC_011638 (Robins et al. 2008), DQ673917 (Robins et al. 2010), NC_040919 (Zhu et al. 2016), MK558274 (Robins et al. 2008), EU273710 (Robins et al. 2010), EU273707 (Robins et al. 2010), EU273708 (Robins et al. 2010), NC_023347 (Adhikari et al. 2018b), NC_035621 (Camacho-Sanchez and Leonard 2020), MN126561 (Cai et al. 2021), MN126563 (Camacho-Sanchez and Leonard 2020), MN126567 (Camacho-Sanchez and Leonard 2020), NC_014855 (Robins et al. 2010), NC_014858 (Robins et al. 2010), NC_014861 (Robins et al. 2010), NC_014864 (Robins et al. 2010), NC_014867 (Robins et al. 2010), NC_014871 (Robins et al. 2010), NC_005089.1 (Zhang et al. 2021), KT029807 (Wang et al. 2016), KY464167 (Camacho-Sanchez et al. 2017), KY464172 (Camacho-Sanchez et al. 2017).

phylogenetic tree using the complete mitochondrial genome sequence of *R. tanezumi* and the sequences from the other 23 species (supplementary material Table S1). *Bunomys penitus* and *Sundamys muelleri* were set as the outer groups and the sequences were obtained from GenBank. The phylogenetic tree indicates that *R. tanezumi* captured from North China was closely clustered with that from Japan and South Korea and completely separated from other species (Figure 3). This newly sequenced complete mitochondrial genome provides valuable information for exploring the genetic diversity and phylogenetic relationships of the Rodent family.

Discussion and conclusion

The sequence we assembled is 16307 bp in length and the other three are 16306 bp. We analyzed the mitochondrial sequences of four different species of *R. tanezumi*, and discovered that while the overall length of each sequence was slightly different, it was composed of 13 PCGs, 2 rRNAs, 22 tRNAs, and a control region. However, they distributed and been formed stable local communities in different countries (China, Japan, Malaysia, South Korea) and captured in different years. So these four *R. tanezumi* should have been evolved into different evolutionary branches.

In the 1950s, *R. tanezumi* in China was mainly distributed in the Yellow River basin, eastern and southern regions (Robins et al. 2008), however *R. tanezumi* has recently expanded its range to north of the Yellow River in the south of Hebei Province and partially replaced the native *R. norvegicus* subspecies (Li et al. 2023). Invasive species have an advantage in interspecific competition, and invasive species are often more aggressive than native species, and closely related species are more competitive than distantly related, encouraging invasive *R. tanezumi* to replace native species (Amarasekare 2002).

There was study suggest that *R. tanezumi* and *R. norvegicus* may chronically interact, and that such interactions may contribute to the invasive success and northward expansion of *R. tanezumi* and the decline of native *R. norvegicus* populations in natural habitats (Guo et al. 2017). Several factors, including global warming and higher resistance to common rodenticides compared with *R. norvegicus*, are believed to likely contribute to the invasive success of *R. tanezumi* (Guo et al. 2017). Rats as typical zoonotic hosts, harbor ectoparasites such as ticks, lice and mites, and a variety of internal parasites (Puckett et al. 2020). The change in population structure of native rat species will certainly have some impact on the original ecosystem and the prevalence of rat-borne diseases. The wider public health implications of the *R. tanezumi* invasion deserve our attention and further research.

The genus *Rattus* is highly speciose, the taxonomy is complex, and individuals are often difficult to identify to the species level (Robins et al. 2014). *R. rattus* and *R. tanezumi* were well known to be difficult to be morphologically differentiated from one another (Aplin et al. 2011; Huang et al. 2022). This suggests that the accuracy of traditional morphological identification is difficult to ensure and that molecular biology

methods can be used as a complementary tool (Camacho-Sanchez and Leonard 2020). The sequencing of mitochondrial genomes can better identify rat species at the molecular level and help us understand the composition of rat species in North China.

In conclusion, we identified the complete mitochondrial genome of *R. tanezumi* and elucidated the phylogenetic relationship with other *Rattus* by constructing a phylogenetic tree. These data provide important information for future taxonomic, systematic, and genetic studies on *R. tanezumi* and commensal rodent species in North China.

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Authors' contributions

SL, QL designed the study. SL and HT wrote the original manuscript, LL, XS, XH, YW, XH and YC performed sample collection, ZH, YZ, YX collected the data, SL, HT and WH supported the bioinformatics analyses. All the authors approved the manuscript.

Disclosure statement

The authors report there are no competing interests to declare.

Ethical approval

The sample used in this study was *Rattus tanezumi*, which is not involve endangered or protected animals, and the sampling did not violate any laws or regulations in China. The collection of field samples was approved by the Hebei Provincial Center for Disease Control and Prevention (HeBCDCIRB(S)2021-017), and the study strictly complied with the relevant regulations of China's animal welfare management.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (<https://www.ncbi.nlm.nih.gov/>) under the accession no. OK054583.1. The associated 'BioProject', 'SRA' and 'Bio-Sample' numbers are PRJNA1104746, SRR28818859, and SAMN41085771 respectively.

References

Adhikari P, Han S-H, Kim Y-K, Kim T-W, Thapa TB, Subedi N, Adhikari P, Oh H-S. 2018a. First molecular evidence of *Mus musculus bactrianus* in Nepal inferred from the mitochondrial DNA cytochrome B gene sequences. *Mitochondrial DNA A DNA Mapp Seq Anal.* 29(4):561–566. Epub 2017/05/20.

Adhikari P, Han S-H, Kim Y-K, Kim T-W, Thapa TB, Subedi N, Kunwar A, Banjade M, Oh H-S. 2018b. New record of the oriental house rat, *Rattus tanezumi*, in Nepal inferred from mitochondrial Cytochrome B gene sequences. *Mitochondrial DNA B Resour.* 3(1):386–390.

Amarasekare P. 2002. Interference competition and species coexistence. *Proc Biol Sci.* 269(1509):2541–2550.

Aplin KP, Suzuki H, Chinen AA, Chesser RT, Ten Have J, Donnellan SC, Austin J, Frost A, Gonzalez JP, Herbreteau V, et al. 2011. Multiple geographic origins of commensalism and complex dispersal history of Black Rats. *PLoS One.* 6(11):e26357.

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, et al. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol.* 19(5):455–477.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsche G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2):313–319.

Brown PR, Tuan NP, Singleton GR, Ha PTT, Hoa PT, Hue DT, Tan TQ, Van Tuat N, Jacob J, Müller WJ, et al. 2006. Ecologically based management of rodents in the real world: applied to a mixed agroecosystem in Vietnam. *Ecol Appl.* 16(5):2000–2010.

Cai H, Wang Q-Q, Zhao X-X, Yao Q-Q, Wu N, Zhang J-S, Liu Z. 2021. Sequencing and analysis of the complete mitochondrial genome of *Micromys erythrotis* from China and its phylogenetic analysis. *Mitochondrial DNA B Resour.* 6(5):1617–1620.

Camacho-Sanchez M, Leonard JA. 2020. Mitogenomes reveal multiple colonization of mountains by *Rattus* in Sundaland. *J Hered.* 111(4):392–404.

Camacho-Sanchez M, Leonard JA, Fitriana Y, Tilak M-K, Fabre P-H. 2017. The generic status of *Rattus annandalei* (Bonhote, 1903) (Rodentia, Murinae) and its evolutionary implications. *J Mammal.* 98(5):1340–1355.

Guo S, Li G, Liu J, Wang J, Lu L, Liu Q. 2019. Dispersal route of the Asian house rat (*Rattus tanezumi*) on mainland China: insights from micro-satellite and mitochondrial DNA. *BMC Genet.* 20(1):11.

Guo HL, Teng HJ, Zhang JH, Zhang JX, Zhang YH. 2017. Asian house rats may facilitate their invasive success through suppressing brown rats in chronic interaction. *Front Zool.* 14(1):20.

He W, Fu J, Wen Y, Cheng M, Mo Y, Chen Q. 2021. Detection and genetic characterization of seoul virus in liver tissue samples from *rattus norvegicus* and *rattus tanezumi* in urban areas of Southern China. *Front Vet Sci.* 8:748232.

Htwe NM, Singleton GR, Johnson DE. 2019. Interactions between rodents and weeds in a lowland rice agro-ecosystem: the need for an integrated approach to management. *Integr Zool.* 14(4):396–409.

Huang EYY, Law STS, Nong W, Yip HY, Uea-Anuwong T, Magouras I, Hui JHL. 2022. The screening for anticoagulant rodenticide gene VKORC1 polymorphism in the rat *Rattus norvegicus*, *Rattus tanezumi* and *Rattus losea* in Hong Kong. *Sci Rep.* 12(1):12545.

Li J, Huang E, Wu Y, Zhu C, Li W, Ai L, Xie Q, Tian Z, Zhong W, Sun G, et al. 2023. Population structure, dispersion patterns and genetic diversity of two major invasive and commensal zoonotic disease hosts (*Rattus norvegicus* and *Rattus tanezumi*) from the southeastern coast of China. *Front Genet.* 14:1174584.

Liu S, Wei Y, Han X, Cai Y, Han Z, Zhang Y, Xu Y, Qi S, Li Q. 2019. Long-term retrospective observation reveals stabilities and variations of hantavirus infection in Hebei, China. *BMC Infect Dis.* 19(1):765.

Puckett EE, Orton D, Munshi-South J. 2020. Commensal rats and humans: integrating rodent phylogeography and zooarchaeology to highlight connections between human societies. *Bioessays.* 42(5):e1900160.

Robins JH, McLenachan PA, Phillips MJ, Craig L, Ross HA, Matisoo-Smith E. 2008. Dating of divergences within the *Rattus* genus phylogeny using whole mitochondrial genomes. *Mol Phylogenet Evol.* 49(2):460–466.

Robins JH, McLenachan PA, Phillips MJ, McComish BJ, Matisoo-Smith E, Ross HA. 2010. Evolutionary relationships and divergence times among the native rats of Australia. *BMC Evol Biol.* 10(1):375.

Robins JH, Tintinger V, Aplin KP, Hingston M, Matisoo-Smith E, Penny D, Lavery SD. 2014. Phylogenetic species identification in *Rattus* highlights rapid radiation and morphological similarity of New Guinean species. *PLoS One.* 9(5):e98002.

Teng H, Zhang Y, Shi C, Mao F, Hou L, Guo H, Sun Z, Zhang J. 2016. Whole-genome sequencing reveals genetic variation in the Asian house rat. *G3 (Bethesda).* 6(7):1969–1977.

- Walker BJ, Abeel T, Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, et al. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One*. 9(11):e112963.
- Wang S, Cong H, Kong L, Motokawa M, Li Y. 2016. Complete mitochondrial genome of the greater bandicoot rat *Bandicota indica* (Rodentia: muridae). *Mitochondrial DNA A DNA Mapp Seq Anal*. 27(6):4349–4350.
- Zhang S, Deng Y, Huang X, Li N, Fan H, Xiang B, Wu Y, Cheng X, Chen X. 2021. The expression characteristics of cytochrome C oxidase subunit I in mitochondrial of MRL/lpr lupus mice. *Clin Exp Rheumatol*. 39(1): 44–51.
- Zhang M, Li Q, Wu F, Ou Z, Li Y, You F, Chen Q. 2021. Epidemiology, genetic characterization, and evolution of hunnivirus carried by *rattus norvegicus* and *rattus tanezumi*: the first epidemiological evidence from Southern China. *Pathogens*. 10(6):661.
- Zhang M, You F, Wu F, He H, Li Q, Chen Q. 2021. Epidemiology and genetic characteristics of murine kobuvirus from faecal samples of *Rattus losea*, *Rattus tanezumi* and *Rattus norvegicus* in southern China. *J Gen Virol*. 102(9):1882–1884.
- Zhu D, Huang J, Kang C, Song X, Yue B, Zhang X. 2016. The complete mitochondrial genome of the *Leopoldamys edwardsi* (Rodentia: muridae). *Mitochondrial DNA A DNA Mapp Seq Anal*. 27(3):1882–1884.