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Mitochondrial genome analysis, phylogeny and divergence time evaluation of the *Strix aluco* (Aves, Strigiformes, Strigidae)

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Abstract

Background

Divergence time analysis can provide a reference for the evolution process of species and is also the basis for other further studies. In this study, a complete mitochondrial genome of the *Strix aluco* was reported for the first time, with a total length of 18,632 bp. There were 37 genes, including 22 tRNAs, 2 rRNAs, 13 protein-coding genes (PCGs), and 2 non-coding control regions (D-loop). This study provides a reference for the evolution history of the *S. aluco*.

New information

The second-generation sequencing of the complete mitochondrial genome of the *S. aluco* was conducted using the Illumina platform, and then Tytoninae was used as the out-group, PhyloSuite software was applied to build the ML-tree and BI-tree of the Strigiformes, and finally, the divergence time tree was constructed using Beast2.6.7 software, the age of *Miosurnia diurna* fossil-bearing sediments (6.0~9.5 Ma) was set as the internal correction point. The common ancestor of the *Strix* was confirmed to have diverged during the Pleistocene (2.58~0.01Ma). The dramatic uplift of the Qinling Mountains in the Middle Pleistocene and the climate oscillation of the Pleistocene together caused *Strix* divergence between the northern and southern parts of mainland China. The isolation of glacial-interglacial rotation and glacier refuge was the main reason for the divergence of the common ancestor of the *Strix uralensis* and the *S. aluco* during this period.

Keywords

Strix aluco, phylogeny, divergence time, Pleistocene, climate oscillation, mountains uplift

Introduction

Strix aluco belongs to Strigidae (Strigiformes), and is a medium-sized owl (Grytshyshina et al. 2016). It is a non-migratory and territorial nocturnal bird (Doña et al. 2016, Sunde et al. 2003). It is widely distributed in the mountain broadleaf forest and mixed forest in Eurasia, and Israel is the southernmost country in the Northern Hemisphere (Obuch 2011, Comay et al. 2022). Mammals, fish, amphibians, and even small birds such as sparrows can be its food (Obuch 2011), and voles are its most preferred food (Solonen 2022, Karell et al. 2009). This species was listed as "Least Concern" (LC) on the IUCN, and the current population trend is stable, the number of said individuals ranges from 1000000 to 2999999. The IUCN (2016): (<https://www.iucnredlist.org/>). In China, it has been listed as a national class II protected animal.

Mitochondria are characterized by maternal inheritance, high conservation, multiple copies in cells, low sequence recombination rate, and high evolutionary rate, widely used in phylogenetic studies (Yan et al. 2017, Sun et al. 2020), and be able to accurately infer phylogenetic relationships in birds (Tuinen et al. 2000), while complete mitochondrial genomes generally have higher accuracy than partial mitochondrial genes (Haring et al. 2001, Harrison et al. 2004). Previous studies have defined the phylogenetic position of *S. aluco* using a single gene or a combination of multiple mitochondrial genes (Heidrich and Wink 1994, Yu et al. 2021, Li et al. 2022, Fuchs et al. 2008, Wood et al. 2017). Earlier studies identified the monophyly of Strigiformes phylogeny through the cytochrome B (Cyt B) gene (Wink and Heidrich 2000), through skeletal comparison, Striginae were divided into three subfamilies: Striginae (13 genera), Surniinae (8 genera) and Asioninae (2 genera) (Salter et al. 2020). Phylogenetic relationships through the cytochrome B (Cyt B) gene also show that the Strigiformes can be divided into four parts, Tytoninae consists of Tytoninae (with *Tyto*) and Phodilinae (with *Phodilus*), Striginae can be divided into Striginae, Surniinae and Ninoxinae. Among them, Striginae consists of six branches (Bubonini+Strigini+Pulsatrigini+Megascopini+Asionini+Otini), and Surniinae consists of three parts: Surnini+Athenini +Aegolini. Ninoxinae is mainly composed of *Ninox*, possibly including *Sceloglaux* (Wink et al. 2009); Zhang et al. (2016) completed the whole mitochondrial genome sequencing of *Asio flammeus* and determined the parallel phylogenetic relationship among the three genera of *Otus*, *Ptilopsis* and *Asio*; Kang et al. (2018) completed the whole mitochondrial genome sequencing of *Strix uralensis*, and determined the inter-genus relationship of *Otus*+ (*Asio*+ (*Strix*+*Bubo*)) through the study of the mitochondrial genome of Strigidae. Uva et al. (2018) clarified the global distribution of Tytonidae and the time of divergence, and their analysis showed that Tytonidae and *S. aluco* split from a common ancestor dating back to about 45 million years ago. Koparde et al. (2018) identified The juxtaposition phylogenetic relationship between Striginae and Surniinae in the South Asian Subcontinent population with Tytonidae as the out-group. Their study showed that Strigidae and Tytonidae diverged at about 42.5~47.7Ma (mega-annum, million years). The timing of the divergence of the *Strix* is unclear.

There are many reasons for species divergence, among which geological and climatic influences on species diversification cannot be ignored (Claramunt and Cracraft 2015). The Cretacean-Tertiary extinction event was a mass extinction event in Earth's history that occurred 65 million years ago and wiped out most of the animals and plants of the time, including the dinosaurs. It also wiped out the direct ancestors of tree-dwelling, water birds on Earth today, The few that survived evolved rapidly thereafter (Field et al. 2018). Bird ancestry began to increase exponentially at the end of the Eocene, from 100 species to 10,000 today (Ksepka and Phillips 2015). Since the late Miocene, many birds in the Palaearctic have been migrating on a large scale and changing ranges have led to gene flows that have provided opportunities for the origin of various bird subfamilies (Drovetski 2003, Holm and Svenning 2014). Climatic oscillation during the Quaternary Period, especially throughout the Pleistocene (2.58~0.01Ma), promoted the evolution of species on a global scale (Hewitt 2000, Hewitt 2004, Lamb et al. 2019), Pleistocene glacial gyre played a positive role in speciation (Kozma et al. 2018, Zhao et al. 2013, Hung et al. 2014, Mays et al. 2018) and BRITO (2005) studied 14 populations of *S. aluco* in Western Europe and found that *S. aluco* in Europe could be divided into three branches originating from three glacial sanctuaries in the Iberian Peninsula, Italy and the Balkan Peninsula. This finding supports the "glacier refuge hypothesis" to describe the origin of *S. aluco* in Western Europe. The origin and divergence of *S. aluco* in mainland China are still a mystery.

Divergence time analysis can provide a reference for the evolution process of species and is also the basis for other further studies. In order to clarify the divergence time of species, it is necessary to obtain the gene sequence of species first, and then select an appropriate evolutionary model, and reliable calibration, such as the determining age of fossils (Ho and Duchêne 2014, Ho and Phillips 2009). To clarify the phylogenetic position, divergence time and reasons of *S. aluco* from China, this study determined the complete mitochondrial genome of *S. aluco*, and used the mitochondrial genome combined with the mitochondrial genome of other birds in Strigiformes, The phylogenetic tree of Strigiformes was reconstructed. Fossil data are usually used to evaluate the divergence time of birds, and the divergence time of the Surniinae fossil is used as the correction point to analyze the divergence time of *Strix*, and the possible reasons for its divergence are fully discussed.

Materials and methods

Sample origin and DNA extraction

Part of muscle tissue was extracted from the leg of a *S. aluco* that died of unknown cause in the Rescue Center of Leigong Mountain National Nature Reserve, Qiandongnan Prefecture, Guizhou Province (26° 49' 26.40" N, 104° 43' 33.60" E). Stored in refrigerated boxes with built-in thermometers, keeping the temperature near freezing. Transported back to the lab for DNA extraction. To extract DNA, we used the standardization CTAB method (Lutz et al. 2011). And the DNA Sample Prep Kit was used to construct genomic DNA libraries.

Sequencing and assembly

Enter subsection textThe Whole Genome Shotgun (WGS) strategy was used to construct the library (Roe 2004). The Next Generation Sequencing (NGS) technology was used for paired-end sequencing(PE), based on the Illumina NovaSeq sequencing platform.

The concentration and purity of DNA extracted from the samples were detected by Thermo Scientific NanoDrop 2000, and the integrity was detected by agarose electrophoresis and Agilent 2100 Bioanalyzer. Using the Covairs machine to break up DNA and fragment it. The gene library was constructed according to the shotgun method of Roe (2004). Agilent 2100 Bioanalyzer was used to detect the size of the library, and fluorescence quantitative detection was used to detect the total concentration of the library. The optimal amount of the library was selected and sequenced on Illumina. A single-stranded library was used as the template for bridge PCR amplification, and sequencing was performed while the synthesis.

After DNA extraction, purification, library construction and sequencing, the Raw image file obtained by sequencing is the first, and the Raw Data that can be read in FASTQ format is generated after multi-step transformation, that is, the offline data. The data transformation work is automatically completed by the sequencing platform. According to the statistics of Raw data, 7,947,240 Reads (each sequence read is called one read) were obtained, the total number of bases was 1192,086000bp, the percentage of fuzzy bases (uncertain bases) was 0.0016%, and the GC content was 44.58%. And base recognition accuracy of more than 99% accounted for 95.61% and base recognition accuracy of more than 99.9% accounted for 90.44%. The quality of the off-machine data should be tested through quality control, and the software used is FastQC(<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>).

Sequencing data contains some low-quality reads with connectors, which will cause great interference to subsequent information analysis. In order to ensure the quality of subsequent information analysis, Fastp software (version 0.20.0) is needed to remove the contamination of sequencing connectors at the 3' end. Low-quality sequences (sequences with an average Q value less than 20 and sequences with sequence length less than 50bp) were removed; The number of high-quality reads obtained was 7611,984, accounting for 95.78% of the raw data, and the number of bases of high-quality reads was 1123739765 bp, accounting for 94.27% of the raw data (Chen et al. 2018).

A5-miseq v20150522 (Coil et al. 2015) and SPAdesv3.9.0 (Bankevich et al. 2012) were used for the de novo assembly of high-quality next-generation sequencing data. Construct contig and scaffold sequences. The sequences were extracted according to the sequencing depth of de novo splicing sequences, and the sequences with high sequencing depth were compared with the nt library on NCBI by blastn (BLAST v2.2.31+), and the mitochondrial sequences of each splicing result were selected. Integration of splicing results: The mitochondrial splicing results obtained by different software above were combined with reference sequences, and collinearity analysis was performed using

mummer v3.1 (Kurtz et al. 2004) software to determine the position relationship between contigs and fill gaps between contigs. The results were corrected using pilon v1.18 (Walker et al. 2014) software to obtain the final mitochondrial sequence. The complete mitochondrial genome sequence obtained by splicing was uploaded to the MITOS web server (<http://mitos2.bioinf.uni-leipzig.de/index.py>) for functional annotation (Bernt et al. 2013). Among them, RefSeq 81 Metazoa is selected for Reference, The Genetic Code is set to a second set of vertebrate codons, and the rest are set according to the default parameters set by MITOS.

Through the above methods, the base composition of the whole mitochondrial genome, protein-coding genes, and rRNA genes was obtained. CGview visualization software was used to draw the mitochondrial complete genome circle map (Stothard and Wishart 2005).

Mitochondrial genome data collection in Strigiformes

Currently, there are 30 species with mitochondrial genomes greater than 10000bp in GenBank, including 27 species of Strigidae, and 3 species of Tytonidae, All taxonomic classifications of the species follow the current version of the IOC WORLD BIRD LIST (12.2) (<http://dx.doi.org/10.14344/IOC.ML.12.2>), the registration number as shown in Table 1.

Construction of phylogenetic trees

Using PhyloSuite software (download from: <https://github.com/dongzhang0725/PhyloSuite/releases>) (Zhang et al. 2020), Drag the 30 GenBank format files downloaded from NCBI and the GenBank format files of *S. aluco* sequence obtained by this sequencing into the main interface.

First series of standardized operations, the choice for Mitogenome sequence types, and then export the annotation error tRNA file, upload the ARWEN website (<http://130.235.244.92/ARWEN/>) modify comments, will be the site of the modified comments copy paste to modify after box. Secondly, 13 PCGs and 24 RNAs need to be extracted. The second set of 2 Vertebrate mitochondrial codes is selected here, and the extracted 13 PCGs and 24 RNAs are imported into MAFFT for multiple sequence alignment. Select the 37 gene files exported by MAFFT and import them into 'concatenate sequence', use the '-auto' strategy and 'normal' alignment mode, and click start. Select the concatenated completion file and open the PartitionFinder 2.0 (Lanfear et al. 2017), performed a greedy search using the Bayesian, and calculated the optimal partitioning strategy and model selection. Using a separate GTR+G model for each data block.

Select the result file of PartitionFinder 2.0 and complete the ML method in IQ-tree mode (Minh et al. 2020). Set *Phodilus badius*, *Tyto alba* and *Tyto longimembris* as out-group. Under Edge-linked partition style for 10,000 replicates of ultrafast bootstrap (Hoang et al. 2018, Sterli et al. 2013), also select the result folder of PartitionFinder 2.0, open MrBayes, set the out-groups, define parameters as Partition Models, run algebra as 2 parallel runs, 4 chains, 2,000,000 generations (must ensure the average standard deviation of split

frequencies values were below 0.01), sampling freq is one sampling run for 1000 times, a burn-in number of initial 25% were burned.

Divergence time evaluation

Miosurnia diurna fossils provide an approximate date of the origin of Surniinae. the age of the fossil-bearing sediments of the *M. diurna* is 6.0-9.5 Ma (Li et al. 2022), Surniinae may be composed of the species of Surnia, *Athene*, *Ninox*, and *Glaucidium* (Wink et al. 2009), *M. diurna* fossil features are closer to the clade of Surnia +*Glaucidium*, Therefore, the origin times of *Glaucidium brasilianum*, *Glaucidium brodiei brodiei*, and *Glaucidium cuculoides* were set at 6Ma and 9.5Ma. The 'NEX' file obtained by concatenating 37 genes using the 'concatenate sequence' function in PhyloSuite was imported into BEAUti 2.6.7, (<http://www.beast2.org/>), Hasegawa–Kishino–Yano (HKY) model, with four gamma categories, Strict clock with 1.0 Clock rate, and with a Yule process (speciation) prior. Choose the "*Glaucidium brasilianum*, *Glaucidium brodiei brodiei*, *Glaucidium cuculoides*" (Sequence file name) to add Prior, Check the "monophyletic" option, set Mean to 6.0 /9.5 and Sigma to 0.1. A Markov chain Monte Carlo (MCMC) Bayesian analysis with a chain length of 10 000 000, and with states recorded every 1000 iterations, save to run using BEAST 2.6.7. Log files were assessed using TRACER 1.7.2 (<http://tree.bio.ed.ac.uk/software/tracer/>) to ensure posteriors were normally distributed and all statistics had attained effective sample sizes of >200, if ESS<200, try to optimize by adding 5000000 iterations (chain length) each time. A burn-in of 10% was discarded, and a maximum clade credibility tree was determined and Mean heights were chosen using TreeAnnotator 2.6.7. Finally, FigTree 1.4.4 was used to check the divergence time. Finally, use Adobe Illustrator 1.0.0.2 for visual editing.

Results

Genome annotation

The total length of the mitochondrial genome sequence was 18,632 bp (Gen Bank entry number: OP850567). The genome annotation results showed that the total number of genes was 39, including 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, 2 O_H genes, and 0 O_L genes. Among them, 8 tRNA genes (trn-Q, trn-A, trn-N, trn-C, trn-Y, trn-P, trn-E, and trn-S2), one PCGs gene: nad6, are on the main chain (J chain); and the remaining 14 tRNA genes are trn-F, trn-V, trn-L2, trn-I, trn-M, trn-W, trn-D, trn-K, trn-G, trn-R, trn-H, trn-S1, trn-L1, trn-T; Two rRNA genes: rrn-S, rrn-L; And 12 PCGs genes encoding: nad1, nad2, nad3, nad4, nad4L, nad5, atp6, atp8, cox1, cox2, cox3, cytb on the secondary (N) chain. There was no gene rearrangement (Fig. 1). The specific annotation results of each gene are shown in Table 2.

Phylogenetic analysis

In this study, both ML-tree and BI-tree show the same tree topology with good support, and it can be seen from the tree that Strigidae and Tytonidae are two distinct lineages under the owl shape. *Ciccaba nigrolineata* is nested in the Strix, and *Sceloglaux albifacies* is nested in the genus *Ninox*. *S. aluco* in this study is a sister group of *S. uralensis* (Kang et al. 2018), *Strix aluco* MN122823+ (*Strix aluco* OP850567+*Strix uralensis*) was formed with *S. aluco*; *Athene noctua* is a sister group of *Athene brama*, *Aegolius funereus* is a sister group of *Glaucidium cuculoides*, *Glaucidium brasilianum*; *Glaucidium brodiei brodiei*, *G. cuculoides*, *G. brasilianum*, *Athene noctua*, *A. brama*, *A. funereus* constitute the same group; *Strix* and *Bubo* are a sister group. And it forms an *Asio*+ (*Strix*+*Bubo*) monophyletic group with *Asio*; And a higher monophyletic group with *Otus*+ [*Asio*+ (*Strix*+*Bubo*)], this monophyly simultaneously with (*Sceloglaux albifacies*+*Ninox*) monophyly exhibited as dyadic taxa. (Fig. 2)

Divergence time evaluation

The divergence time tree based on 37 genomes shows that the time interval between Strigidae and Tytonidae from the common ancestor of Strigiformes was 8.69~13.76Ma. However, in the out-group, *Tyto alba*, *Tyto longimembris* and *Phodilus badius* diverged from the common ancestor about 4.57~7.24Ma. The divergence of Strigidae began about 6.81~10.79 Ma, and the earliest divergence of Surniinae occurred in Strigidae, and *Aegolius*, *Glaucidium*, and *Athene* occurred about 6.03-9.55 Ma. In this study, we found the divergence time between *S. aluco*(OP850567) and *S. aluco* of Margaryan. A (MN122823) was about 1.59~2.51Ma, and the divergence time between *S. aluco* and *S. uralensis* in China was about 1.38~2.19Ma. (Fig. 3)

Discussion

The mitochondrial genome structure of birds is a covalent double-chain loop structure, with a total of 37 genes, including 22 tRNAs, 2 rRNAs, 13 protein-coding genes (PCGs) and 1-2 non-coding control regions (D-loop). Where *nad6* and 8 tRNA encoding genes (*trnQ*, *trnA*, *trnN*, *trnC*, *trnY*, *trnS2*, *trnP* and *trnE*) are on the J chain (light chain), The remaining 14 tRNAs, 2 rRNAs, 12 protein-coding genes and 1-2 non-coding control regions (D-loop) are all on the N chain (heavy chain) (Wolstenholme 1992, Boore 1999), and the complete mitochondrial genome structure of all birds was consistent (Hanna et al. 2017). The complete mitochondrial genome sequence of *S. aluco* obtained in this study was circular, with a total length of 18,632 bp and a GC content of 46.76%. Its composition was as follows: the proportion of Adenine bases in the total base column (A%) was 29.57%; The ratio of Guanine base to total base (G%) was 14.09%. The ratio of Cytosine base to total base (C%) was 32.67%. The ratio of Thymidine to the total base column (T%) was 23.66%. The start codon of all 13 PCGs is ATG, and the transcription stop codon is AGG, TAG and TAA. The content of A+T (53.23%) was higher than that of G+C (46.76%), which was

consistent with the AT tendency of base bias in the vertebrate mitochondrial genome (Broughton et al. 2001, Ma et al. 2015), which is also consistent with the mitochondrial genome of other owls in Strigidae (Sun et al. 2020, Kang et al. 2018).

Phylogenetic analysis of *s. aluco*

The BI and the ML tree have a consistent topology and each node has a high posterior probability. The phylogenetic tree of Strigiformes obtained by the mitochondrial genome in this study is consistent with the phylogenetic tree obtained by Li et al. (2022) through morphology. Our phylogenetic tree shows that Wink et al. (2009) compared Surnini (with Surnia, *Glaucidium* and *Taenioglaux*), Athenini (with *Athene*) and Aegolini (with *Aegolius*) under the Surniinae are feasible. In the phylogenetic tree constructed by Yu et al. (2021), *C. nigrolineata* was also nested in *Strix*. *S. albifacies* has been largely extinct on the island of New Zealand, Wood et al. (2017) extracted its mitochondrial genome from museum specimens and suggested changing its name to *Ninox albifacies* because it has the same morphological structure and phylogenetic position as the genus *Ninox*. *S. aluco* in this study is a sister group of *S. uralensis* uploaded to GenBank by Kang et al. (2018). While *S. aluco* uploaded with Margaryan.A forms a monophyly of *Strix aluco* MN122823+ (*Strix aluco* OP850567+*Strix uralensis*). Compared with the mitochondrial genome of *S. aluco* obtained by Margaryan. A, *S. aluco* in China is more closely related to the *S. uralensis*, extant eurasian birds communicated through woodland corridors during the Pleistocene interglacial, and combined with divergence-time tree analysis, such communication may have existed in the common ancestor of *S. aluco* MN122823 and *S. aluco* OP850567 (Voelker 2010), probably because at the beginning of the Pleistocene, the common ancestor of *S. aluco* MN122823 and *S. aluco* OP850567 had already been geographically isolated. The isolation of the Pleistocene refugium led to the divergence of the whole genome of the common ancestor of the forest owl at home and abroad. Foreign studies have shown that the Quaternary Period is characterized by a series of glacial-interglacial cycles (Woodruff 2010), with the ancestors of modern species seeking refuge in a suitable environment. The existing species on the Qinghai-Tibet Plateau may be the result of rapid population expansion in relatively warm refugia during the Pleistocene glaciation and interglacial period, forming the current distribution pattern and genetic diversity (Gao et al. 2015). Leigong Mountain in Guizhou Province just played the role of refugia for *S. aluco* during the Pleistocene glaciation period. Mitochondrial phylogeographic studies (BRITO 2005) show that the origin of *S. aluco* in Western Europe supports the "glacial refuge hypothesis", and that the species survived in three allopatric refuges in the Iberian Peninsula, Italy and the Balkans, becoming the main source of *S. aluco* in Europe during the late glacial period. DNA barcoding technology also proved that the geographical barrier of the Strait of Gibraltar played an extremely important role in the phylogenetic history of *S. aluco* (Doña et al. 2016). The phylogenetic relationship of Strigidae forms a phylogenetic relationship of Surniinae+[*Ninox*+ (*Otus*+ (*Asio*+ (*Strix*+*Bubo*)))]), The conclusion of *Otus*+ (*Asio*+ (*Strix*+*Bubo*)) is consistent with the conclusion of Kang et al. (2018). According to the genome analysis of Strigidae birds in Madagascar, *Strix* is most closely related to *Bubo*, followed by *Otus* (Fuchs et al. 2008).

Divergence time evaluation of *s. aluco*

On the Qinghai-Tibet Plateau, the impact of mountain uplift on the formation of modern species (<2.0 Ma) is limited, and researchers are more willing to believe that climate fluctuations played a key role in the formation of species in the Middle Pleistocene (Wang et al. 2018, Renner 2016). During the Quaternary Period and Pleistocene (1.6~2.7Ma), there were severe climate shocks (Lisiecki and Raymo 2005), which played a positive role in promoting the formation of species (RULL 2008, Rull 2015, Rull 2011). The Pleistocene began 2.58 million years ago (2.58 Ma). Climate fluctuations during this period, especially during the ice age, affected the distribution of forests in the Northern Hemisphere and the evolution of species living in forests (Song et al. 2021). This series of climate fluctuations in the Pleistocene promoted species variation. This has led to species differentiation (Leonard et al. 2015). Glaciation has played an important role in influencing the population size, species and community genetic structure of today's species (Svendsen et al. 2004, Hewitt 2000, Hewitt 2004), the glacial-interglacial gyrations of the same period also affected the distribution of species (Kozma et al. 2018, Zhao et al. 2013, Hung et al. 2014, Mays et al. 2018), Glacial-interglacial cycles led to periodic shifts in glacial refuges for Pleistocene birds (Nadachowska-Brzyska et al. 2015), the isolation of glacier refugia will lead to the divergence of the whole genome of species, thus forming different species (Provost et al. 2022), this should be the reason why the common ancestor of *S. aluco* MN122823 and *S. aluco* OP850567 (this study) diverged at 1.59~2.51Ma, genetic divergence of the same lineage due to the isolation of refugees leads to lineage divergence. All kinds of species generally begin to migrate to the best habitat during the warm climate period (Claramunt and Cracraft 2015), in particular, species adapted to low altitudes in the early stage of climate change will move to high altitudes at this time, resulting in the reproductive isolation of species in the two places (Wiens 2004). During the Pleistocene-Holocene (1.10~0.60 Ma), the Qinghai-Tibet Plateau experienced three stages of rapid uplift, with mountains forming, climate-changing from moist and warm to dry and cold, and forests retreating to the edge of the plateau (Wang et al. 2008). The Quaternary Period climate shock led to the initial formation of the existing forest and mountain distribution pattern in the Northern Hemisphere. Birds began to be widely distributed after leaving the glacier refuge at the end of the glacier and initially formed the existing distribution pattern (Pujolar et al. 2022). The long-tailed forest owl may have moved north at this time and thus diverged from *S. aluco*. In addition, the rapid uplift of the Qinling Mountains from the end of the Early Pleistocene to the Middle Pleistocene may have made the Qinling Mountains a barrier to north-south bird communication (Li et al. 2019). The rapid uplift of the Qinling Mountains prevented communication between *S. aluco* and the common ancestor of *S.uralensis*, which was originally distributed on the north and south sides.

Conclusions

In summary, by sequencing the complete mitochondrial genome of *S. aluco*, and mapping its phylogenetic tree and divergence time tree, the phylogenetic relationship of Strigiformes (Tytoninae + Phodilinae) + (Striginae+Ninoxinae+Surniinae) is summarized, with Tytonidae including Tytoninae (with *Tyto*) and Phodilinae (with *Phodilus*) as the out-group, Strigidae comprises Striginae (with *Asio*, *Bubo*, *Strix*, *Ciccaba* and *Otus*) +Ninoxinae+Surniinae (with *Athenini*, *Aegolini* and *Glaucidium*). The divergence time tree showed that the divergence time between *S. aluco* of China and *S. aluco* of other countries was about 1.59~2.51Ma, suggesting that the common ancestor of *S. aluco* at home and abroad was separated by geographical isolation at the beginning of the Pleistocene. The divergence between *S. aluco* and *S. uralensis* in China was about 1.38~2.19Ma. During this time, the rapid uplift of the Qinling Mountains led to the divergence of the ancestors of *Strix* on the north and south sides of the Chinese mainland. At the same time, due to Climatic oscillation in the Pleistocene, the existing *S. aluco* population on the Qinghai-Tibet Plateau may have rapidly expanded in relatively warm shelters such as Leigong Mountain to form the current distribution pattern.

Data ability

The complete mitochondrial genome of *S.aluco* has been uploaded to NCBI, GenBank accession number: OP850567.

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Conflicts of interest

The authors declare no competing or financial interests.

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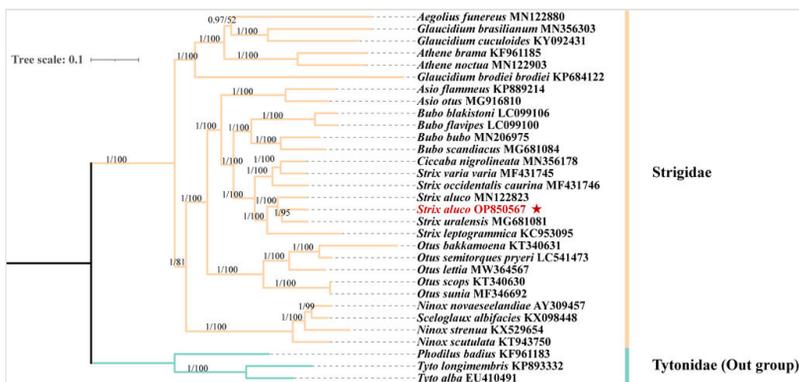


Figure 2.

BI/ML--tree, Bayesian phylogenetic tree of 37 genes (24 rRNAs, 13PCGs) from 31 species of Strigiformes. The node labels are BI/ML posterior probability and bootstrap support value respectively, and the scale indicates the probability of nucleotide change within each branch length. The GenBank of the sequences has been indicated next to the species name. Branches of different subfamilies are distinguished by different colors, with Tytoninae (with *Phodilus badius*, *Tyto longimembris* and *Tyto alba*) being the out-group. The *S. aluco* mitochondrial genome obtained by this sequencing has been marked by ★.

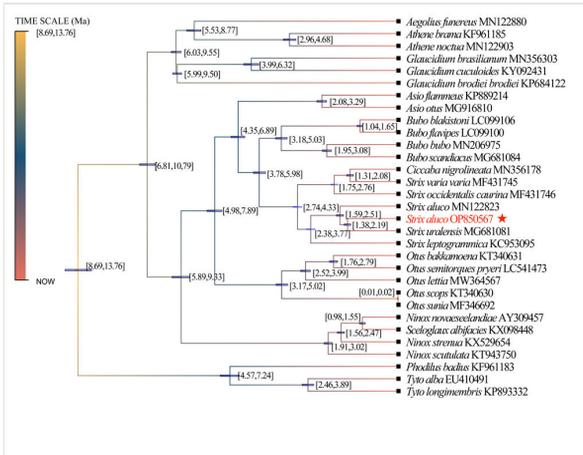


Figure 3.

Divergence time tree. Through the divergence time tree obtained by Beast2.6.7 based on the Bayesian method, the node horizontal bar indicates that the posterior probability of this age interval is 95%, and the divergence time has been marked at the node.

Table 1.

Mitochondrial genome sequences used in this study.

Taxon	GenBank accession	Size (bp)	Notes	Reference
<i>Aegolius funereus</i>	MN122880	17166	Partial	Direct Submission
<i>Asio flammeus</i>	KP889214	18966	Complete	Zhang et al. 2016
<i>Asio otus</i>	MG916810	17555	Complete	Lee et al. 2018
<i>Athene brama</i>	KF961185	16194	Partial	Direct Submission
<i>Athene noctua</i>	MN122903	15776	Partial	Direct Submission
<i>Bubo blakistoni</i>	LC099106	19379	Partial	Direct Submission
<i>Bubo bubo</i>	MN206975	18956	Complete	Direct Submission
<i>Bubo flavipes</i>	LC099100	19447	Partial	Direct Submission
<i>Bubo scandiacus</i>	MG681084	18734	Complete	Kang et al. 2018
<i>Ciccaba nigrolineata</i>	MN356178	14875	Partial	Feng et al. 2020
<i>Glaucidium brasilianum</i>	MN356303	17717	Partial	Feng et al. 2020
<i>Glaucidium brodiei brodiei</i>	KP684122	17318	Complete	Sun et al. 2016
<i>Glaucidium cuculoides</i>	KY092431	17392	Complete	Liu et al. 2019
<i>Ninox novaeseelandiae</i>	AY309457	16223	Complete	Harrison et al. 2004
<i>Ninox scutulata</i>	KT943750	16208	Complete	Direct Submission
<i>Ninox strenua</i>	KX529654	16206	Complete	Sarker et al. 2016
<i>Otus bakkamoena</i>	KT340631	17389	Complete	Park et al. 2019a
<i>Otus lettia</i>	MW364567	16951	Complete	Yu et al. 2021
<i>Otus scops</i>	MW489467	17595	Complete	Direct Submission
<i>Otus semitorques</i>	LC541473	18834	Complete	Direct Submission
<i>Otus sunia</i>	KT340630	17413	Complete	Park et al. 2019b
<i>Sceloglaux albifacies</i>	KX098448	15565	Partial	Wood et al. 2017
<i>Strix aluco</i>	MN122823	16490	Partial	Feng et al. 2020
<i>Strix aluco</i>	OP850567	18832	Complete,	This study
<i>Strix leptogrammica</i>	KC953095	16307	Complete	Liu et al. 2014
<i>Strix occidentalis</i>	MF431746	19889	Complete	Hanna et al. 2017
<i>Strix uralensis</i>	MG681081	18708	Complete	Kang et al. 2018
<i>Strix varia</i>	MF431745	18975	Complete	Hanna et al. 2017
Out group				
<i>Phodilus badius</i>	KF961183	17086	Complete	Mahmood et al. 2014
<i>Tyto alba</i>	EU410491	16148	Partial	Pratt et al. 2009
<i>Tyto longimembris</i>	KP893332	18466	Partial	Xu et al. 2016

Table 2.

Analysis of mitochondrial genome feature.

Feature	Strand	Position	Length (bp)	Initiation Codon	Stop Codon	An
trnF	N	1-68	68			
rrnS	N	68-1050	983			
trnV	N	1050-1121	72			
rrnL	N	1134-2709	1576			
trnL2	N	2709-2783	75			
nad1	N	2798-3757	960	ATG	AGG	
trnI	N	3756-3827	72			
trnQ	J	3839-3909	71			
trnM	N	3909-3977	69			
nad2	N	3978-5018	1041	ATG	TAG	
trnW	N	5017-5091	75			
trnA	J	5093-5161	69			
trnN	J	5163-5236	74			
trnC	J	5239-5305	67			
trnY	J	5305-5376	72			
cox1	N	5378-6928	1551	ATG	AGG	
trnS2	J	6920-6991	72			
trnD	N	6995-7063	69			
cox2	N	7066-7749	684	ATG	TAA	
trnK	N	7759-7828	70			
atp8	N	7830-7997	168	ATG	TAA	
atp6	N	7988-8671	684	ATG	TAA	
cox3	N	8671-9454	784	ATG	T(AA)	
trnG	N	9455-9523	69			
nad3	N	9524-9875	352	ATG	TAA	
trnR	N	9878-9946	69			
nad4l	N	9948-10244	297	ATG	TAA	
nad4	N	10238-11615	1378	ATG	T(AA)	
trnH	N	11616-11685	70			
trnS1	N	11688-11753	66			
trnL1	N	11756-11826	71			
nad5	N	11812-13647	1836	ATG	TAA	
cob	N	13653-14795	1143	ATG	TAA	

trnT	N	14797-14866	70			
OH_0	N	15595-15792	198			
trnP	J	16409-16478	70			
nad6	J	16485-17006	522	ATG	TAG	
trnE	J	17010-17083	74			
OH_1	N	17668-17865	198			