Pakistan J. Zool., vol. 58(2), pp 989-991, 2026 **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230811163442

Short Communication

Characterization and Phylogenetic Analysis of the Mitochondrial Genome of *Clangula hyemalis* (Linaeus, 1758)

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Article Information
Received 11 August 2023
Revised 05 June 2024
Accepted 19 June 2024
Available online 23 June 2025 (early access)
Published 25 February 2026

Authors' Contribution
HT and XS collected the sample. HT and MM designed the study. HT, LB, YF, LL and SZ analyzed the data. HT and MM wrote the paper. All authors read, edited, and approved the final manuscript.

Key words
***Clangula hyemalis*, Phylogenetic, Beijing, Mitochondrial genome, Population, Climate**

ABSTRACT

The long-tailed duck (*Clangula hyemalis*) is a migratory bird and enduring rapid population declines, as extreme climatic alteration, and other reasons.It is also rare passage migrant into China. The mitochondrial genome (mitogenome) can provide information for phylogenetic analyses and evolutionary biology. In the present investigation, we generated complete mitogenome of the long-tailed duck using sanger sequencing. The mtDNA comprised 16,639 base pairs (bp), and contained 13 protein coding genes, 22 transfer RNAs and 2 ribosomal RNAs. Remarkably, this is the second and most complete published mitogenome for the *Clangula hyemalis* species. Our findings revealed that control region and ND4L would be expected to be as diversity fragments for identifying population-specific marker in *Clangula hyemalis*.

The long-tailed duck (*Clangula hyemalis*) is one of the unique migratory birds which is the only living member of its genus *Clangula*. The wintering population of this sea duck is undergoing rapid population declines across Europe, as oil or other pollution and extreme climatic change, etc., (Dickson and Smith, 2013; Day *et al*., 2015). Thus, it is classified as vulnerable species on the IUCN global red list of threatened species long-tailed ducks breed in the circumpolar Arctic and migrate to cold, temperate waters in the non-breeding period (Robertson and Savard, 2002). The distribution of scarce long-tailed duck has been found in Central and Southwest of China for years, driven by climatic factors over the past years (Wu and Shi, 2016). Despite the low diversity for breeding Long-tailed Duck, the wild individuals from China were not published. Mitochondrial complete genome still be needed to provide more evidence in order to study genetic diversity and migratory connectivity in this species (Mackenzie *et al*., 2022). Here, we described the mitogenome of wild-collected Long-tailed duck from China to provide basic genetic information about this species.

Material and methods

In 2020, we collected a wild migrating long-tailed duck from Beijing, China, (116°18′12″N and 40°10′11″E). A specimen was deposited at Beijing Wildlife Rescue and Rehabilitation Central Lab (contact person: Hengjiu Tian, hengjiutian@126.com, Beijing, China) under the voucher

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0030-9923/2026/0002-0989 $ 9.00/0

number BWRR-Ch-2020-001.The genomic DNA of each sample was extracted from the feature with TIANamp Genomic DNA Kit.

To get the complete mitochondrial genome, we selected custom-developed primers and minor gaps were resolving by overlapping primer-based PCR for DNA sequence walking (Table 1). All the purified PCR products were sequenced with sanger sequencing mode on an ABI 3730 XL machine. The mitochondrial genome was assembled following Xue *et al*. (2013), and then annotated using MitoAnnotator (Bernt *et al*., 2013). The map of mitochondrial genome was drawing in https://irscope.shinyapps.io/Chloroplot/.

Results and discussion

The complete mitogenome of *Clangula hyemalis* resulted 16,639 bp in length, including 13 protein-coding genes (PCGs), 22 transfer RNAs, 2 ribosomal RNAs, and a putative control region (D-loop region), shown in Figure 1. Rearrangement and of duplication mitochondrial genome were not observed in this species. The nucleotide composition of mitogenome of *Clangula hyemalis*is 29.1% for A, 33.2% for C, 15.8% for G, and 21.9% for T, with a slightly higher A+T content (51%). Only ND6 gene is encoded on the light strand, while other genes are distributed in the heavy strand. Among the 13 PCGs, only COX1, COX2, and ND5 uses GTG as the start codon and the rest genes initiate with the start codon of ATG.

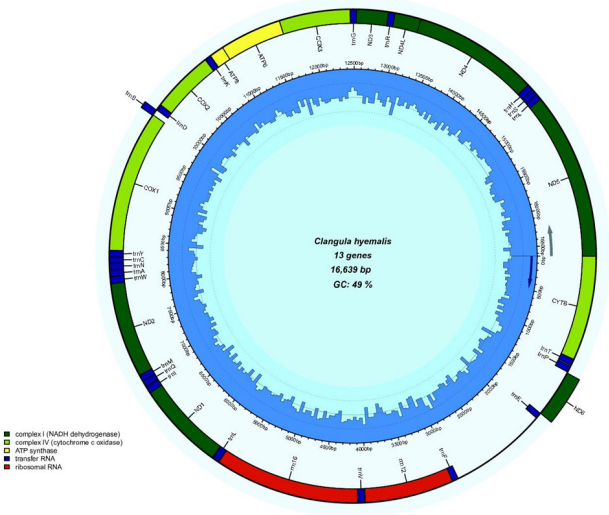

Fig. 1. The complete mitogenome structure of *Clangula hyemalis.*

A maximum likelihood analysis was carried out using MEGA X package (Kumar *et al*., 2016) with GTR+G model and 1,000 bootstrap replicates. We took two *Clangula hyemalis* mtgenomes (one is published with NCBI accession number MW077850.2 and MW849278.1) and the other eighteen birds from GenBank. The phylogenetic analysis confirms that the beijing's long-tailed duck close phylogenetic position to North American's one (Fig. 2).

Table 1. Primers used for amplification of *Clangula hyemalis* mitogeno

Primers name	Sequence
co1	F= AATGTAATCGTCACCGCCCA
	R= TACAGGATTGGGTCTCCCCC
cytb	F= ATCCTGACAGGTCTCCTGCT
	R= TACGAGGAGGTTGGCCACTA
dloop	F= GCTCCTACTCATACTACCCTGC
	R= CAGTGTCAAGGTGATTCCCCA
nd2	F= CGCATTGGGCACAACAATCA
	R= ATAGTGAGGCGAGGATTGCG
nd3-nd4	F= CCCCCGTACGAATGTGGATT
	R= GGAGGCAGATTGAGCTGGTT
nd1-3	F= AATCTGTGAAAGGAACTCGGC
	R= ATCATAGGATTGAGTAGACGGC
16S	F= GTGATAGCTGGTT(A/G)CC(C/T)G
	R= AAG(C/T)TCCACAGGGTCTTCTCG
CO3	F= ATGGCCCACCAAGCACACTC
	R= CAATATCAGGCTGCTGCTTC
ND3	F= AAACT(A/C/G)TCCCC(A/G/C/T)TACGAATG
	R= ATTCTGCTCATTCTAGGC
ND4	F= TAGCAAGCCAAAACCACCT
	R= GATCAGTGGGTTTGGATTAT
1	F= CAAAGTGGCATCTGTGGAAT
	R= CTGGCACAAGATTTACCGAC
2	F= AAAGAGCCCATTCGACC(C/T)TC
	R= GTTGGGTGGTTGAGCTGTGT
3	F= GACTCATCATCCAAGAGCTA
	R= GGATATACGGTTCAGCCTGT
4	F= AAACCTCCCGCACTCTCACA
	R= GACAACATCCCGTCATCATT
5	F= GATTCCACGGACTCCACGTA
	R= GGTTCAGGCGAGGGTTAGTA
6	F= AGA(A/G)CCAAACCCCCTCATTC
	R= AAAATAGCGCTAGTTCTGTG
7	F= CGCTCAATCCCAAACAAACT
	R= ATGGTATGTCCGCTTGTTAT

Compared with mitogenome of *Clangula hyemalis*from North American, we found 99.95% identify to the one from Beijing of China. In further, we found 14 SNPs sites and two indels sites between these

two mitogenomes (Table II). In a conclusion,the genetic diversity of *Clangula hyemalis* was lowest, and control region and ND4L would be expected to be as diversity fragments for identifying population-specific marker in *Clangula hyemalis*.

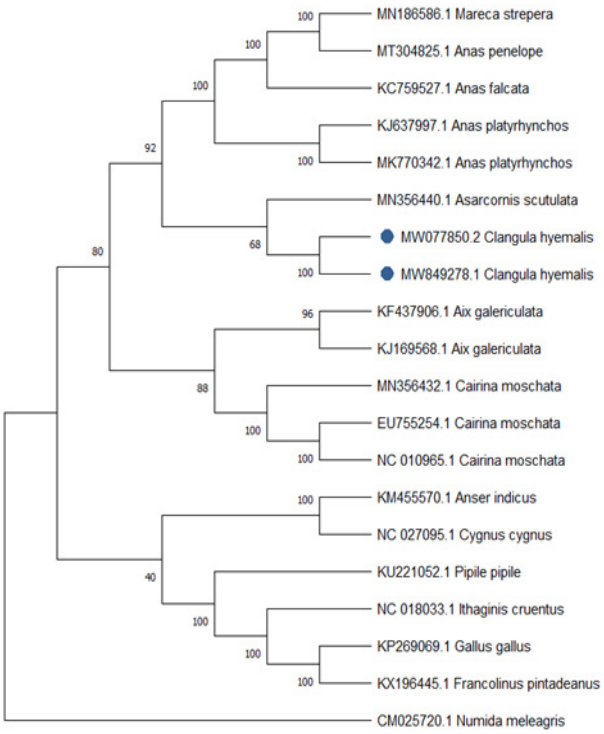

Fig. 2. A phylogenetic tree of *Clangula hyemalis* and the other eighteen species.

Table II. Genetic diversity of *Clangula hyemalis* mitogenome from China and American.

SNP/ InDel	No.	Gene name	Type
INDEL	1	Control region	34bp
SNP	4	Control region	C/T, C/T, G/A, T/C
SNP	2	Control region	A/G
SNP	1	*ND1*	G/A
SNP	1	*tRNA-Cys*	T/A
SNP	1	*COX1*	A/G
INDEL	1	*COX1*	C
SNP	1	*tRNA-Arg*	A/R
SNP	3	*ND4L*	T/C, T/C, A/G
SNP	1	*ND6*	A/G

DECLARATIONS

Acknowledgement

We thank Xiaoping Wang for picture providing throughout our project.

Funding

This work was supported by Beijing Financial Project, grant No. 23CB066.

Disclosure statement

No potential conflict of interest was reported by the author(s). The authors comply with the International Union for Conservation of Nature (IUCN) policies research involving species at risk of extinction, the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Data availability statement

The genome sequence data by Sangon sequencing that support the findings of this study are openly available in GenBank of NCBI at (https://www.ncbi.nlm.nih.gov) (https://www.ncbi.nlm.nih.gov/) under the accession no.MW077850.2.

Statement of conflict of interest

The authors have declared no conflict of interest.

References

Bernt, M, Donath, A. and Jühling F., 2013. Mol *Phylogenet Evol.,* **69**:313-319.

Bird Life International, 2014. *The IUCN red list of threatened species*. www.iucnredlist.org.

Day, R.H., Rose, J.R., Prichard, A.K. and Streever, B., 2015. *Island Arctic Alaska*, **68**: 367-379. https://doi.org/10.14430/arctic4507

Dickson, D.L. and Smith, P.A., 2013. *J. Wildl. Manage*., **77**: 777-790. https://doi.org/10.1002/jwmg.529

Counihan, K.L., Skerratt, L.F., Franson, J.C. and Hollmén, T.E., 2015. *Virology*, **485**: 393-401. https://doi.org/10.1016/j.virol.2015.07.026

Kumar, S., Stecher, G., Li, M., Knyaz, C. and Tamura, K., 2018. *Mol. Biol. Evol.,* **35**: 1547-1549.

Mackenzie, C.L., Kent-Flora, E.A., Baxter, J.M., Gormley, K.S.G., Cassidy, A.J., Sanderson, W.G. and Porter, J.S., 2022. *Front. Mar. Sci*., **9**. https://doi.org/10.3389/fmars.2022.772259

Robertson, G.J. and Savard, J.PL., 2002. Long-tailed duck (*Clangula hyemalis*). In: *The birds of North America Online A*. Poole, Ed. http://bna.birds.cornell.edu/bna/species/651 https://doi.org/10.2173/bna.651

Wu, J. and Shi, Y., 2016. *Ecol. Inf*., **31**: 147-155. https://doi.org/10.13001/1081-3810.3202

Xue W., Hou, G., Li, C., Kong, X., Zheng, X., Li, J. and Sun, X., 2013. *Mitochondrial DNA*, **24**: 339-341. https://doi.org/10.3109/19401736.2012.760081