PMAS
Vol. 64 No 01(249) 2024
DOI - https://doi.org/10.5564/pmas.v64i01.3547

ARTICLE

A phylogenetic study of the Mongolian Tree Pipit (Anthus trivialis, Linnaeus, 1758) population based on mitochondrial DNA (mtDNA) genes

Ulziisaikhan Tumendemberel[1], Sod-erdene Byambadash[1], Gabor Sramko[2,3] and Tserendulam Batsukh[1]*

[1] *Laboratory of Genetics, Institute of Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia*
[2] *Evolutionary Genomics Research Group, Department of Botany, University of Debrecen, Debrecen, Hungary*
[3] *HUN-REN–UD Conservation Biology Research Group, Egyetem tér, Debrecen, Hungary*

ARTICLE INFO: Received: 27 Nov, 2023; Accepted: 09 March, 2024

Abstract: Our aim was to identify nucleotide polymorphisms, assess their distribution in haplotype diversity, and construct a phylogenetic tree by analyzing mt DNA markers of the Mongolian Tree Pipits(Anthus trivialis). We conducted this study using partial gene sequences of mitochondrial marker genes, such as COI, Cyt-b, D-loop, and ND2, to determine the genetic diversity of Mongolian Tree Pipits. We successfully amplified 2307 bp of the mitochondrial DNA fragments, including 469bp of COI, 435bp of Cyt-b, 554bp of D-loop and 846bp of ND2 from total 27 individuals of Mongolian (21) and Hungarian (6) populations of Anthus trivialis.The Hd value was the highest for ND2 (0.96) as compared with the other gene fragments in all populations, while it was 0.94 in the Mongolian population. Moreover, the nucleotide diversity (Pi) ranged from 0.00234 to 0.004 in all population, it was observed that the Pi was between 0.00183 and 0.00376 in the Mongolian population. The phylogenetic tree based on combined mt DNA sequences revealed two mean clades. The probability value of the node supporting the posterior between these clades is 0.65,which suggests an indicative support in relationship between the two clades. Furthermore, phylogenetic analysis showed that Mongolian Tree Pipits do share common genetic characteristics with other populations and do not form distinct clusters.

Keywords: *Population genetics, haplotype, Anthus trivialis, Phylogenetics, Cyt-b, ND2, D-loop, COI;*

INTRODUCTION

The Tree Pipit (*Anthus trivialis, Linnaeus, 1758*) is a migratory bird belonging to the *Motacillidae* family within *Passeriformes*. This species faces significant challenges, particularly in Southeast Asian countries, where it has become a target of (Source).

Corresponding author, email**: **tserendulamb@mas.ac.mn** **https://orcid.org/0000-0002-8409-0968

Vol. 64 No 01(249) 2024
DOI - https://doi.org/10.5564/pmas.v64i01.3547

In recent years, the population of the Tree Pipit in Central Asia has experienced a notable decline, leading to its inclusion in the category of Globally Decreasing Populations according to the International Union for Conservation of Nature (IUCN) assessment for the year 2023 [1]. The genus *Anthus*, to which the Tree Pipit belongs, is a diverse group within the *Motacillidae* family, comprising 43 species. The *Motacillidae* family is recognized as one of the most widespread and species-rich families within *Passeriformes*, a testament to its members' ecological diversity and adaptability [2, 3]. The challenges associated with determining the exact genetic relationships between birds in genus *Anthus*, such as the Tree Pipit *(Anthus trivialis)*, due to inconsistencies in appearance and plumage, are indeed significant. The genus *Anthus* encompasses a diverse group of bird species, and the variations in their external characteristics can make it challenging to establish a precise genetic relationship [4].

A comprehensive classification involved identifying four major groups: the Small-Bodied African Group are predominantly found in the African regions and are characterized by smaller body sizes; the Old Continental Group, reflecting the historical continuity of relatively large species, are distributed across Asia, Africa, and Europe; the Palearctic Migratory Group consist of species undertaking migration within the Palearctic region encompassing Europe, Asia, and North Africa; and the New Continental Group comprises of species found in North, Central, and South America and represent the *Anthus* species that have colonized the Americas [5].The Tree Pipit *(Anthus trivialis)* predominantly inhabits the temperate regions of Eurasia.

The first study of the phylogeography of birds to determine the genetic basis of geographic location in birds established amethod based on mitochondrial DNA (mtDNA) [6], which initiated a method that explores the genealogical relationship of birds based on their geographical locations. The use of mtDNA in genetic and phylogeographic studies is widespread because of its high evolutionary intensity and minimal DNA degradation [7].Tree Pipit is a migratory egg-laying bird that is listed as a rare species in the Red Book of Mongolia. Therefore, it is necessary to conduct field and genetic research to determine the subspecies that are distributed in Mongolia.

In this study, we focused on elucidating the nucleotide sequence of specific genes within the mtDNA of Tree Pipit. Proceeding from this premise, we focused on conducting a comparative genetic analysis of Mongolian Tree Pipits in relation to other global populations to determine if any differences, exist at the subspecies level, thus contributing valuable insights into the population genetics of Tree Pipit.

MATERIALS AND METHODS

Sampling and laboratory work

As Tree Pipit is listed in the Mongolian Red Book, all sampling permissions were obtained from the Ministry of Environment and Tourism of Mongolia. During our field study at the Khovd Bird Ringing Station in Khovd aimag (province) in west Mongolia, we collected tissue samples from 21 *Anthus trivialis* individuals. The extraction of total genomic DNA from muscle tissue was performed using the DNA isolationkit (Zanaspex), following the manufacturer's protocol. We employed Polymerase Chain Reaction (PCR) amplification to obtain partial sequences of the mitochondrial *Cytochrome b* (*Cyt-b*), *NADH dehydrogenase subunit 2(ND2), D-loop*, and *Cytochrome oxidase subunit* 1 (*COI*) genes. For *COI*, we used primer pairs forward 5'-GCATGAGCAGGGATAGTGGGTA-3' and reverse 5'-GTGACCGAAGAATCAGAAGAGGT-3'. The primer pairs for *Cyt-b*were L15086 (5'-CTCCGTAGCTCACATATGCCGAG-3') and H15853 (5'-

GGCGGAAGGTTATTGATCGCAG-3'). For *ND2*, the primer pairs were 5'-GCAAAACTAATCTTCGTCACC-3' and 5'-ATAGGGGCGATTGGTAGAAG-3', while for *D-loop*, we used 5'-TAACTATGCATTACACTCTCTGCC-3' and 5'-GAAGGGCTTATTGAAGAGACGC-3'.The PCR conditions and annealing temperatures were the same for all genes, with the PCR program consisting of a pre-denaturation at 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 60°C for 1 min, and 72°C for 1 min, plus a final extension at 72°C for 6 min by using Thermal Cycler, BIOER. Following the PCR, the products underwent analysis through 1% agarose gel electrophoresis, and nucleotide sequencing was carried out using ABI3730XL (Macrogen Inc.).

The obtained sequences were organized using Seqman II (DNASTAR), and the resulting consensus sequences were aligned with ClustalW [8] in MEGA-X [9]. To ensure the absence of pseudogenes, we meticulously examined the mitochondrial genes for stop codons and indels, adhering to the vertebrate mitochondrial coding table. Additionally, for comparative analysis, we downloaded partial sequences from GenBank for *COI* (GU571734, GU571733, GU571261, GU571260, GQ481368, GQ481370, GQ481369, GQ481367, KF946594) and for *ND2* (GU816850, KU859174, KU859173, KU859172, KU859171, KU859170, KU859169, KU859167, KU859166, KU859165, KU859164, KP671568), as well as for *D-loop* (OQ344431, OQ344432) and *Cyt-b* (U46775, AY228048).

Nucleotide polymorphism

We estimated the number of segregating sites (S), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (Pi) for both Mongolian and other populations of Tree Pipits. These calculations were performed using DnaSP 5.0 [10] based on all partial genes of mtDNA. Additionally, we utilized Fu and Li ' s D[11]and Tajima's D [12] parameters, which were calculated in DnaSP 5.0, to assess evolutionary neutrality. We analyzed haplotype relationship within each species. Haplotype network maps were constructed by the TCS, using Popart v.1.7 [13].

Phylogenetic analysis of Tree Pipit mtDNA

We used BEASTv 1.10.4 [14] to elucidate the phylogenetic relationship among populations. Initially, the most appropriate models of molecular evolution were determined using MrModelTest2 [15], with the selection based on the Akaike information criterion (AIC). The optimal model identified for the combined partial genes of mitochondrial DNA (mDNA) was Hasegawa – Kishino – Yano + invariable sites + Gamma (HKY + I + G). Subsequently, Bayesian inference (BI) was applied in BEASTv 1.10.4 to the mtDNA dataset, utilizing the constant size coalescent tree prior for phylogenetic reconstruction. The Markov Chain Monte Carlo (MCMC) was run for 100 million generations, with sampling occurring every 1,000 generations. To summarize trees using the "Mean height" criterion, we used TREEANNOTATOR 1.8 from the BEAST package [16]. The initial 10% of trees were discarded as "burn-in" after confirming the stationarity of the chain likelihood values. Evaluation of convergence to posterior distributions of parameter estimates was performed by monitoring the effective sample size (ESS>200) and trace plots in TRACER 1.7 [17]. The consensus trees were visualized and displayed using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

RESULTS AND DISCUSSION

Genetic variation

We obtained 2307 bp of the mitochondrial DNA fragments, including 469bp of *COI*, 435bp of *Cyt-b*, 554bp of *D-loop* and 846bp of *ND2* from totally 27 individuals of Mongolian (21) and Hungarian (6) populations of *Anthus*

trivialis.The haplotype diversity (*H*d) was the highest for *ND2* (0.96) in all populations than other genes, while in Mongolian population it was 0.94. The nucleotide diversity (Pi) ranged from 0.00234 to 0.004 in all populations, while it was observed to be between 0.00183 and 0.00376 in the Mongolian population (Table-1).

Table1. ***Nucleotide polymorphism and results of neutrality tests for mtDNA genes of Tree Pipit.***

	COI		*Cyt-b*		*D-loop*		*ND2*	
	All	**MGL**	**All**	**MGL**	**All**	**MGL**	**All**	**MGL**
Length (bp)	469		435		554		849	
N	35	21	28	21	29	21	40	21
Nhap	9	6	13	8	14	11	26	14
Hd	0.644	0.5325	0.7196	0.5667	0.8571	0.8143	0.9679	0.9474
Pi	0.0026	0.00183	0.02879	0.00234	0.004	0.0041	0.00376	0.0035
s	9	6	67	9	13	12	35	18
Fu and Li's F	-2.588*	0.0018	0.70145	-2.99641	-2.567	-2.077	-3.99989	-2.62
Tajima's D	-1.505	-1.4851	-1.19957	-1.99058*	-1.294	-1.106	-2.13307**	-1.623

All-total population, MGL-Mongolian population. N, sample size; S, number of segregating sites; Nhap, number of haplotypes; Hd, haplotype diversity; Fu's FS, statistics of Fu's FS test (*P < 0.01); Fu and Li's D, statistics of Fu & Li's D-test (*P < 0.05); Tajima's D, statistics of Tajima's D-test (*P < 0.05)

In the haplotype network results, a total of 9 haplotypes were identified for the *COI* gene fragment. Haplotypes 1 and 2 were shared among all populations.Moreover, a total of 6 haplotypes were detected in the Mongolian population, among which haplotypes 6, 7, and 8 were unique. Haplotypes 4 and 9 were unique to the European population, and haplotype 3 was unique to the Russian-Kazakhstan populations(Figure-1). For the *Cyt-b* gene fragment, we identified a total of 13 haplotypes among the population, with 8 haplotypes detected in the Mongolian population. These haplotypes are divided into two main haplogroups corresponding to specific geographical areas. Haplotype1 is shared among all populations, while other haplotypes are specific to each haplogroup(Figure2). For the *D-loop* gene fragment, we identified a total of 14 haplotypes among the populations, with 11 haplotypes detected in the Mongolian population. Haplotype 12 and 14 are unique to the European population, and Haplotype 3 and 11 are unique to the Mongolian population, while haplotypes 1 and 2 were shared among all populations(Figure3).And for the *ND2* gene fragment, we identified more haplotypes compared to other genes, with a total of 26 haplotypes. As for the *ND2* gene haplotypes, no separate haplogroups were formed, and there were no specific differences in the overall distribution area (Figure 4). Additionally, we have identified and registered all investigated haplotype sequences in GenBank. The accession numbers are as follows: *COI* (OR787543-OR787558), *Cyt-b* (PP501875-PP501884), *ND2* (PP501885-PP501904), and *D-loop* (PP501905-PP501921). (See supplementary material Appendix1, Table1).

Vol. 64 No 01(249) 2024
DOI - https://doi.org/10.5564/pmas.v64i01.3547

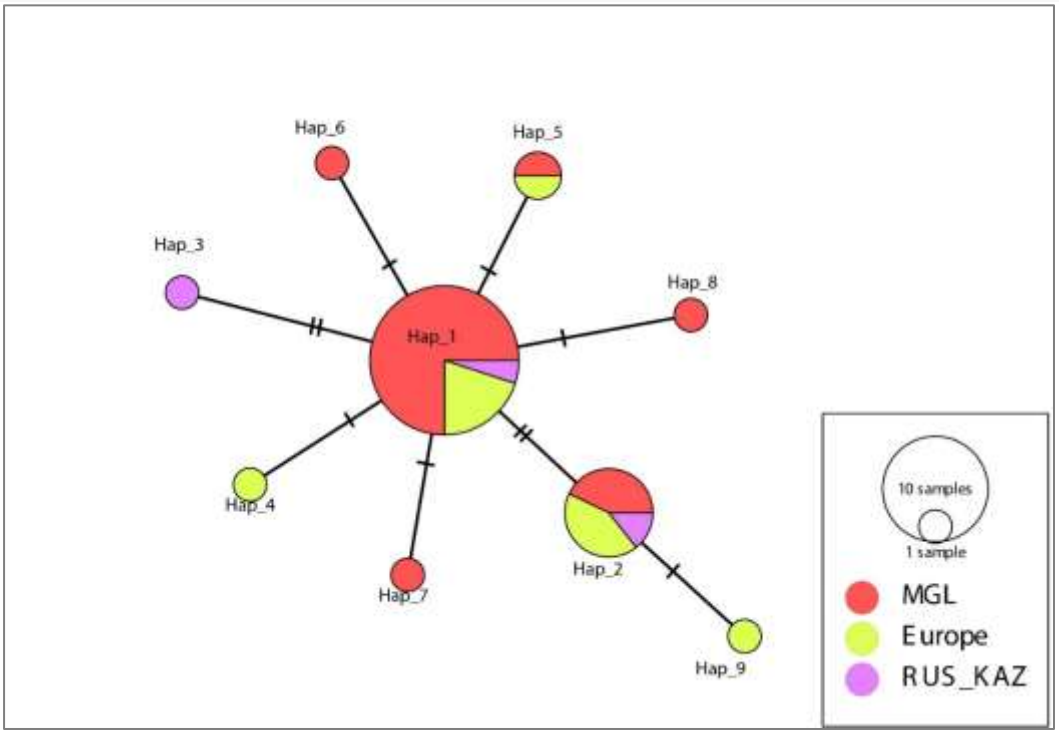

Figure 1. Haplotype TCS network result for *COI* gene fragment of Tree Pipit. Each colored circle represents a haplotype, and the area of a circle is proportional to the number of individuals with this haplotype

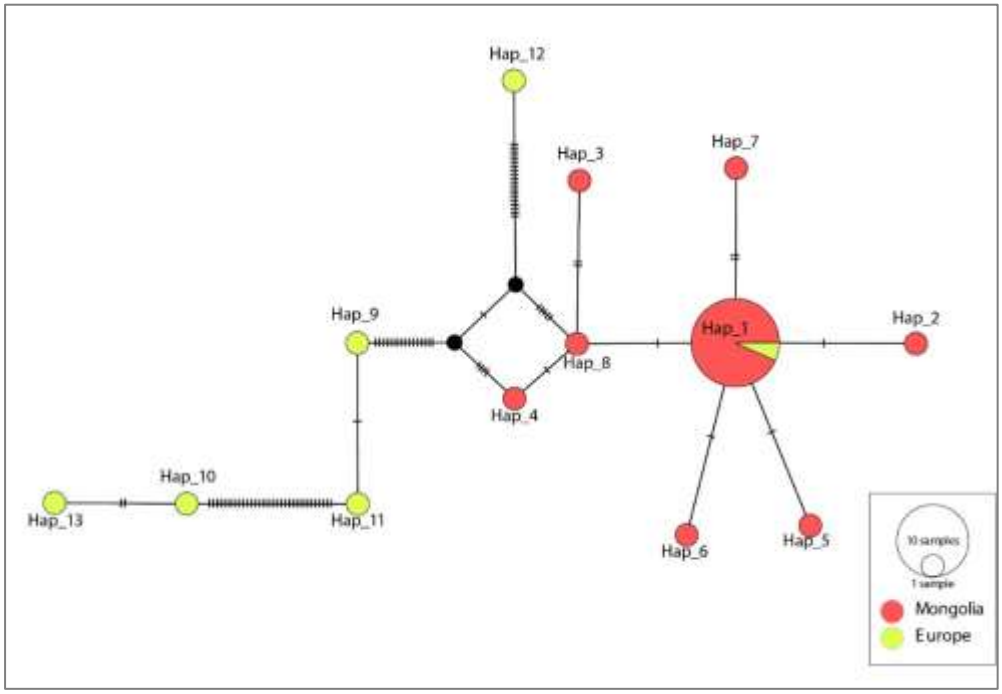

Figure 2. Haplotype TCS network result for *Cyt-b* gene fragment of Tree Pipit

Vol. 64 No 01(249) 2024
DOI - https://doi.org/10.5564/pmas.v64i01.3547

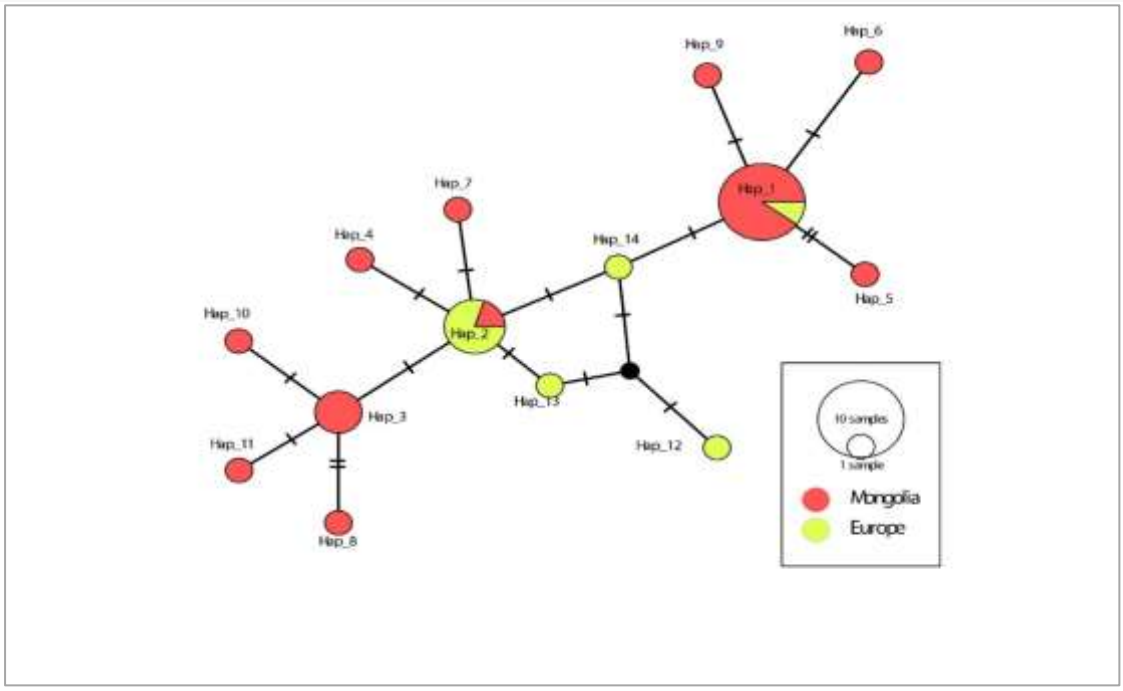

Figure 3. Haplotype TCS network result for *D-loop* gene fragment of Tree Pipit

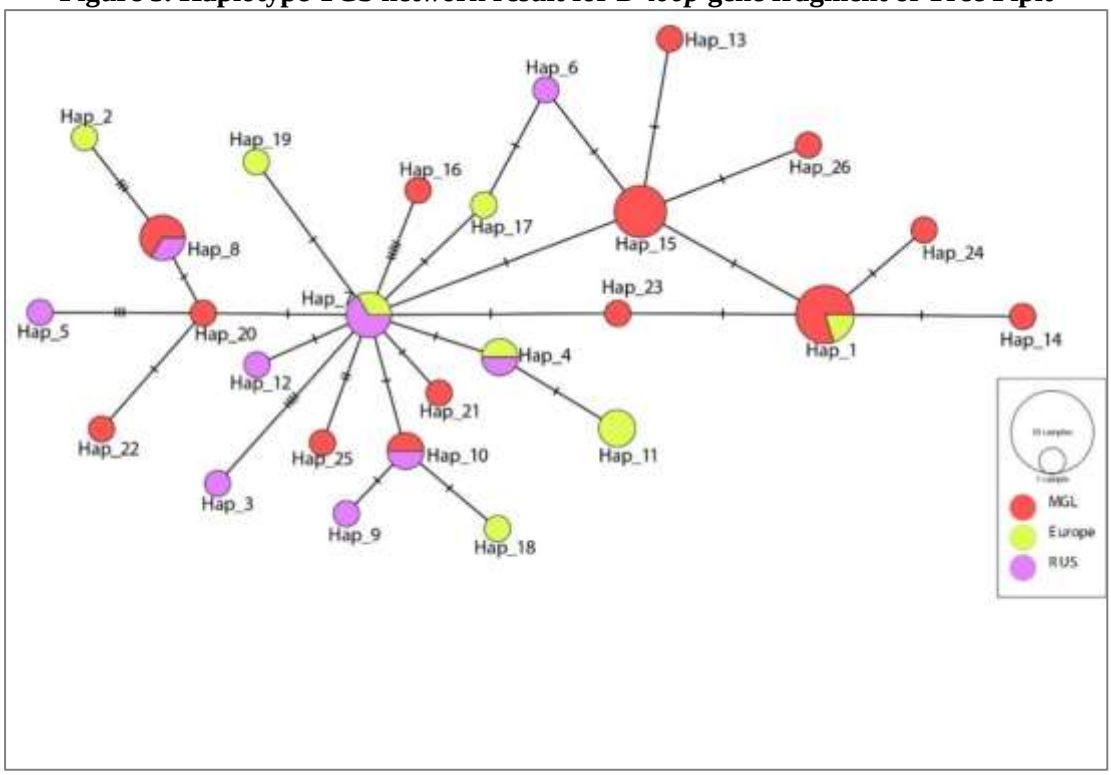

Figure 4. Haplotype TCS network result for *ND2* gene fragment of Tree pipit

Phylogenetic structure in mtDNA

Basing on the phylogenetic tree constructed from Tree Pipit mtDNA genes, the tree generated from concatenated mtDNA sequences revealed two primary supported clades in Bayesian Inference (BI) analyses.We selected closely related taxa using the outgroup *Anthus hodgsoni* (KX189345, KJ455323, KX189345, KX189345). The node supporting the posterior probability value between these clades is 0.65. This value suggested an indicative support for the relationship between the two clades.

Vol. 64 No 01(249) 2024
DOI - https://doi.org/10.5564/pmas.v64i01.3547

The Mongolian samples were included in the same cluster with Tree Pipit populations from Sweden, Russia, and Norway, while the Tree Pipit samples collected from Hungary, used in our study, formed a separate cluster from the Mongolian population, but clustered together withthe Russian, Norwegian, and Swedish populations forming a single cluster with these popluations (Fugure-5).

The objective of this research was to ascertain the subspecies of Tree Pipits migrating to Mongolia by examining the *COI*, *Cyt-b*, *D-Loop*, and *ND2* genes of the mtDNA in 27 samples, including with Hungarian population, comparing them with data from the global GeneBank.

The analysis yielded compelling results, indicating a 100% match with *Anthus trivialis trivialis* species (KF946594.1) and 96.4% match with *Anthus hodgsoni* species, unequivocally classifying the studied samples as belonging to the *Anthus trivialis trivialis* subspecies. This genetic identity is further corroborated by the congruence with the results of the phylogenetic tree. Our genetic study reveals that the migratory Tree Pipit population in Mongolia aligns distinctly with the *Anthus trivialis trivialis* subspecies cluster. This observed correlation may be attributed to various ecological factors, including geographical distribution and the species' known long-distance migration patterns, as previously suggested by researchers [18].

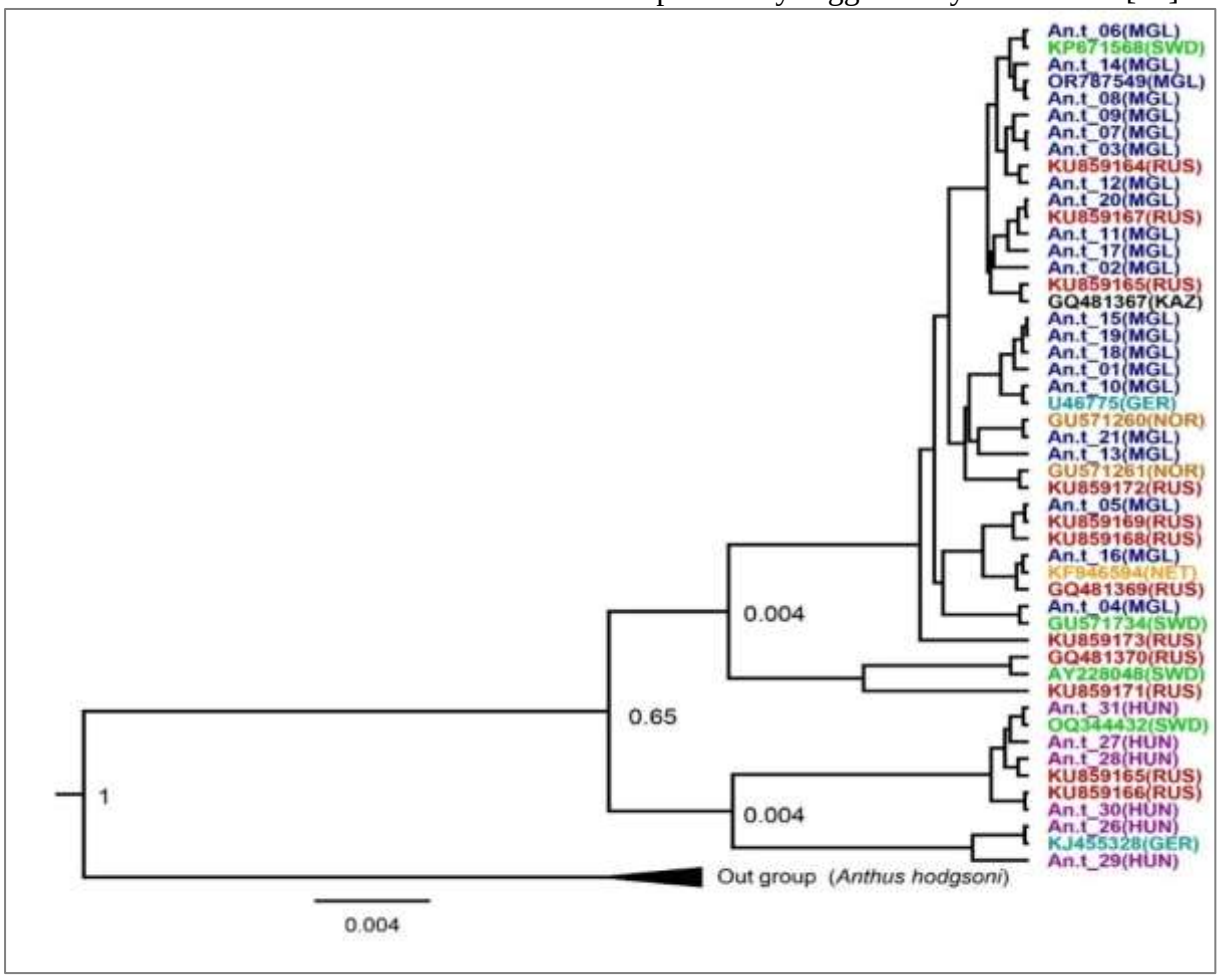

Figure 5. A phylogenetic tree based on combined genes (*COI, Cyt-b, D-loop, ND2*) of Tree pipit mtDNA. Note: An.t_1-21- Mongolian *Anthus trivialis* samples (specimen number), SWD-Sweden, KAZ-Kazakhstan, NOR-Norway, RUS-Russia, NED-Netherlands, GER-Germany.Node supporting value is 0.65 between mean clade

There are several evolutionary phylogenetic researches, which used mtDNA *Cyt-b* marker in order to distinguish among *Anthus* family of birds [5, 19]. *Anthus* family is quite similar in color and acoustic [20], which is why it makes it challenging to distinguish them by their appearance. Hence, the current study could analyze 21 samples from Mongolia and 6 samples from Hungary by combined four markers of mtDNA and created phylogenetic tree. The result showed that Mongolian populations of *Anthus trivialis* share the same clade with European populations. By comparing our findings with other previous studies, we confirmed that the Mongolian population of *Anthus trivialis* is classified as belonging to the Eastern Palearctic species and is representative of the broader Eurasian population [21, 22].

The advantage of our research lies also in the primary methodology employed, which involves conducting phylogenetic analysis by concatenating the mtDNA gene sequences utilized in our study. This approach has the potential to produce a relatively precise and robust results compared to analyzing genes individually in isolation [23]. Additionally, we applied the Bayesian inference (BI) phylogenetic method, developed in the BEAST program, which has been widely applied in population genetics studies of widespread bird species [19, 24, 25].

Even though we included only one population of Tree Pipit samples from Mongolia, we could include European samples, which were collected from Hungary. Moreover, several other Asian and European Tree Pipit population representative genomes were downloaded from NCBI for a comparative analysis.

Most studies on the *Anthus* family focus on their appearance and migration patterns [5, 26], with only a limited number of phylogenetic studies specifically examining this family. Hence, the current research is the first phylogenetic study of Tree Pipit of Mongolia. To clarify whether or not other subspecies of *Anthus trivialis* migrate in Mongolia, further research is needed to incorporate more samples from other Tree Pipit populations of Mongolia according to its distribution area.

CONCLUSIONS

The Tree Pipit population in Mongolia exhibits significant haplotype diversity within each mitochondrial DNA genes without distinct separation leading to the formation of separate haplogroups. The phylogenetic tree analysis revealed no discernible segregation, implying that the Tree Pipit in Mongolia's Khovd region is part of the same subspecies as *Anthus trivialis trivialis*. Additionally, the study identified the presence of the 2 common haplotypes observed in both Mongolia and the other populations, suggesting a continuous and unrestricted gene flow among them.

Acknowledgement

Many thanks to the researchers at the Wildlife Science and Conservation Center of Mongolia, as well as Dr. Máté Havasi and Dr. Zsófia Tótha, for their invaluable support in facilitating sample collections for this study. We gratefully acknowledge the basic research funding (ШҮСс-2020/22) provided by the Mongolian Foundation for Science and Technology of as well as mobility grants (NKM2024-26/2024; C24/31) provided from the Hungarian and Mongolian Academy of Sciences, which gave us great opportunity to do the collaborative research.

REFERENCES

1. B. International, "BirdLife International (2023) IUCN Red List for birds," 2023.
2. S. Tyler, "Family Motacillidae (pipits and wagtails)," *Handbook of the birds of the world*, Vol. 9. pp. 686-786, 2004.
3. E. C. Dickinson, N. David, and M. A. Alonso-Zarazaga, "Some comments on Schodde & Bock (2016) on gender agreement," *Bulletin of the British Ornithologists' Club,* Vol. 137, No. 2, pp. 142-144, 2017. https://doi.org/10.25226/bboc.v137i2.2017.a2.
4. B. P. Hall, *The taxonomy and identification of pipits (genus Anthus)*. British Museum (Natural History), 1961. https://doi.org/10.5962/p.314161.
5. G. Voelker, "Molecular evolutionary relationships in the Avian genusAnthus (Pipits: Motacillidae)," *Molecular Phylogenetics and Evolution*, Vol. 11, No. 1. pp. 84-94, 1999. https://doi.org/10.1006/mpev.1998.0555.
6. J.Avise, "INTRASPECIFIC PHYLOGEOGRAPHY: The Mitochondrial DNA Bridge Between Population Genetics and Systematics," 1987. https://doi.org/10.1146/annurev.ecolsys.18.1.489.
7. S. V. Drovetski, M. Raković, G. Semenov, I. V. Fadeev, and Y. A. Red'kin, "Limited phylogeographic signal in sex-linked and autosomal loci despite geographically, ecologically, and phenotypically concordant structure of mtDNA variation in the Holarctic avian genus Eremophila," *PLoS One*, Vol. 9, No. 1, p. e87570, 2014.
8. J. D. Thompson, D. G. Higgins, and T. J. Gibson, "CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice," *Nucleic acids research*, Vol. 22, No. 22, pp. 4673-4680, 1994. https://doi.org/10.1093/nar/22.22.4673.
9. S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, "MEGA X: molecular evolutionary genetics analysis across computing platforms," *Molecular biology and evolution*, Vol. 35, No. 6, p. 1547, 2018. https://doi.org/10.1093/molbev/msy096.
10. P. Librado and J. Rozas, "DnaSP v5: a software for comprehensive analysis of DNA polymorphism data," *Bioinformatics*, Vol. 25, No. 11, pp. 1451-1452, 2009. https://doi.org/10.1093/bioinformatics/btp187.
11. Y.-X. Fu, "Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection," *Genetics*, Vol. 147, No. 2, pp. 915-925, 1997. https://doi.org/10.1093/genetics/147.2.915.
12. F. Tajima, "Statistical method for testing the neutral mutation hypothesis by DNA polymorphism," *Genetics*, Vol. 123, No. 3, pp. 585-595, 1989. https://doi.org/10.1093/genetics/123.3.585.
13. J. W. Leigh and D. Bryant, "POPART: full-feature software for haplotype network construction," *Methods in ecology and evolution*, Vol. 6, No. 9, pp. 1110-1116, 2015. https://doi.org/10.1111/2041-210X.12410.
14. M. A. Suchard, P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond, and A. Rambaut, "Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10," *Virus Evolution*, Vol. 4, No. 1, 2018. https://doi.org/10.1093/ve/vey016.
15. J. Nylander, "MrModeltest2 v2. 3," *Evolutionary Biology Center, Uppsala University. Uppsala, Sweden. Available from: http://www. abc. se/~ nylander*, 2004.
16. A. Rambaut and A. Drummond, "TreeAnnotator. Version 1.8, distributed as part of the BEAST Package," Ed, 2010.
17. A. Rambaut, A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard, "Posterior summarization in Bayesian phylogenetics using Tracer 1.7," *Systematic biology*,Vol. 67, No. 5, pp. 901-904, 2018. https://doi.org/10.1093/sysbio/syy032.
18. Moga, T. Hartel, and K. Öllerer, "Ancient oak wood-pasture as a habitat for the endangered tree pipit Anthus trivialis," *Biologia*, Vol. 64, No. 5, pp. 1011-1015,

2009. https://doi.org/10.2478/s11756-009-0167-7.

19. H. V. Norambuena, P. Van Els, C. P. Muñoz-Ramírez, and P. F. Victoriano, "First steps towards assessing the evolutionary history and phylogeography of a widely distributed Neotropical grassland bird (Motacillidae: Anthus correndera)," *PeerJ*, Vol. 6, p. e5886, 2018. https://doi.org/10.7717/peerj.5886.
20. P. Doniol-Valcroze, P. Coiffard, P. Alström, M. Robb, P. Dufour, and P.-A. Crochet, "Molecular and acoustic evidence support the species status of Anthus rubescens rubescens and Anthus [rubescens] japonicus (Passeriformes: Motacillidae)," *Zootaxa*, Vol. 5343, No. 2, pp. 173-192, 2023. https://doi.org/10.11646/zootaxa.5343.2.4.
21. P. Arctander, O. Folmer, and J. FJELDSÅ, "The phylogenetic relationships of Berthelot's Pipit Anthus berthelotii illustrated by DNA sequence data, with remarks on the genetic distance between Rock and Water Pipits Anthus spinoletta," *Ibis*, Vol. 138, No. 2, pp. 263-272, 1996. https://doi.org/10.1111/j.1474-919X.1996.tb04338.x.
22. B. Finch *et al.*, "High levels of mitochondrial cytochrome b sequence diversity are present within the Anthus similis complex in sub-Saharan Africa," *Ostrich*, Vol. 84, No. 2, pp. 145-151, 2013. https://doi.org/10.2989/00306525.2013.822028.
23. S. R. Gadagkar, M. S. Rosenberg, and S. Kumar, "Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree," *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, Vol. 304, No. 1, pp. 64-74, 2005. https://doi.org/10.1002/jez.b.21026.
24. P. van Els, H. V. Norambuena, and R. S. Etienne, "From pampa to puna: Biogeography and diversification of a group of Neotropical obligate grassland birds (Anthus: Motacillidae)," Vol. 57, Ed: Wiley Online Library, 2019, pp. 485-496. https://doi.org/10.1111/jzs.12278.
25. G. Song *et al.*, "Complete taxon sampling of the avian genus Pica (magpies) reveals ancient relictual populations and synchronous Late-Pleistocene demographic expansion across the Northern Hemisphere," *Journal of Avian Biology*, Vol. 49, No. 2, pp. jav-01612, 2018. https://doi.org/10.1111/jav.01612.
26. P. Zduniak and R. Yosef, "Migration and staging patterns of the Red-throated (Anthus cervinus) and Tree Pipits (Anthus trivialis) at the migratory bottleneck of Eilat, Israel," *Ornis Fennica*, Vol. 88, No. 3, pp. 129–137-129–137, 2011. https://doi.org/10.51812/of.133775.

DOI - https://doi.org/10.5564/pmas.v64i01.3547

Table 2. Haplotypes of Tree pipit *(Anthus trivialis)* mtDNA genes

Haplotype	Genbank accession numbers	mtDNA genes
H1	OR787543	*COI*
H2	OR787544	
H3	OR787545	
H4	OR787546	
H5	OR787547	
H6	OR787548	
H7	OR787549	
H8	OR787550	
H9	OR787551	
H10	OR787552	
H11	OR787553	
H12	OR787554	
H13	OR787555	
H14	OR787556	
H15	OR787557	
H16	OR787558	
H1	PP501875	*Cyt-b*
H2	PP501876	
H3	PP501877	
H4	PP501878	
H5	PP501879	
H6	PP501880	
H7	PP501881	
H8	PP501882	
H9	PP501883	
H10	PP501884	
H1	PP501885	*ND2*
H2	PP501886	
H3	PP501887	
H4	PP501888	
H5	PP501889	
H6	PP501890	
H7	PP501891	
H8	PP501892	
H9	PP501893	
H10	PP501894	
H11	PP501895	
H12	PP501896	
H13	PP501897	
H14	PP501898	
H15	PP501899	
H16	PP501900	
H17	PP501901	
H18	PP501902	
H19	PP501903	

Vol. 64 No 01(249) 2024
DOI - https://doi.org/10.5564/pmas.v64i01.3547

H20	PP501904	
H1	PP501905	*D-Loop*
H2	PP501906	
H3	PP501907	
H4	PP501908	
H5	PP501909	
H6	PP501910	
H7	PP501911	
H8	PP501912	
H9	PP501913	
H10	PP501914	
H11	PP501915	
H12	PP501916	
H13	PP501917	
H14	PP501918	
H15	PP501919	
H16	PP501920	
H17	PP501921	