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**Research Article**

# Connectivity and genetic diversity of wintering Common Pochards (*Aythya ferina*; Linnaeus, 1758) in Iran

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## Abstract

Genetic studies provide the cornerstone for understanding the genetic adaptations and population dynamics of the common pochard (*Aythya ferina*) and, consequently, for designing effective conservation strategies to ensure its long-term survival. This study investigated the genetic diversity of the common pochard in Iran using mitochondrial DNA (mtDNA) sequences (cytb and D-loop) from two wintering sites: the southern Caspian Sea and the Hor al-Azim wetland. Analysis of 30 D-loop sequences revealed 6 distinct haplotypes, with 5 found in Iran and 1 from China. Analysis of 30 cytb sequences identified 3 haplotypes, with 2 found in northern Iran and 1 shared by southern Iran and other regions. Low genetic diversity was observed within the Iranian population, with an average haplotype diversity of 0.109 for both genes. Tajima's D and Fu's FS tests did not show significant deviations from neutrality, suggesting past population expansion. Phylogenetic analysis revealed that Iranian pochards are closely related to populations from Europe and East Asia, indicating that these regions contribute to the Iranian wintering population. The low genetic distance and high gene flow observed among the studied samples, while indicative of connectivity between populations, likely reflects the mixing of individuals from diverse breeding origins rather than active gene exchange within the Iranian wintering sites, given the limited evidence of breeding in Iran. These findings highlight the importance of conserving Iran's wetlands as habitats for the common pochard, alongside preventing illegal hunting, to maintain connectivity among populations and ensure the conservation of gene flow and genetic diversity for the long-term survival of this species.

**Keywords**: Biodiversity, Caspian Sea, Hor al-Azim wetland, mtDNA, gene flow

## Introduction

The family Anatidae, part of the order Anseriformes, Anatinae has 8 groups: Tadornini, Tachyerini, Cariani, Merganettini, Anatini, Aythyini, Mergini, and Oxyurini (Livezey, 1997; Gonzalez et al., 2009; Broyer & Bourguemestre, 2020). Among these, the group Aythyini consists of medium-sized waterfowl with sexually dimorphic plumage. These birds predominantly inhabit lakes and coastal regions. The common pochard (*Aythya ferina* Linnaeus, 1758) is one of 12 migratory species within this group, with a global population estimated at 760,000–790,000 mature pairs and a European breeding population of 89,700–151,000 pairs (Fox et al., 2025). According to (Cramp & Simmons, 1977) this species was first recorded in northwestern Europe in the mid-19th century and was subsequently observed in Lithuania, Sweden, and Finland by the late 19th century (Zalakevicius, 1995). According to Sruoga et al., (2009) these findings suggest that the common pochard has established itself as a breeding species in northwestern Europe only within the last 150 years. Studies have reported low genetic diversity among populations of this species, likely reflecting their relatively recent establishment in northwestern Europe (Sruoga et al., 2009; Fox et al., 2016; Folliot et al., 2017). The findings indicate that populations in the Baltic and northwestern Russia are relatively young and genetically homogeneous (Mischenko et al., 2022; Homolková et al., 2024).

Common pochards breed in northern and Eastern Europe and migrate in autumn (September to November) to western, central, and southern Europe, as well as North Africa. Eastern populations also winter in southeastern and eastern Asia, including the Indian subcontinent, as far as Japan (Livezey, 1997). In spring, typically around March, they return to their breeding sites (Kharitonov et al., 2024; Zalakevicius, 1995). In Iran, common pochards are widespread during migration and winter along the southern Caspian Sea and in the Hor al-Azim wetland. These skilled diving birds primarily feed on aquatic plants, seeds, and small invertebrates (Zalakevicius, 1995). However, their dependence on healthy wetland ecosystems renders them vulnerable to habitat destruction caused by agricultural expansion, urbanization, and climate change. Overhunting and viral infections have further exacerbated population declines. As a result, the species was classified as Vulnerable (VU) by the IUCN in 2015 (Folliot et al., 2018; Mischenko et al., 2022; Homolková et al., 2024). Recent study has documented sharp population declines in Europe, Siberian wetlands, and wintering populations in Iran (Chavoshi et al., 2023). Conservation and breeding initiatives to mitigate these declines require robust data on genetic diversity (Gonzalez et al., 2009).

Mitochondrial DNA (mtDNA) is a crucial genetic marker for studying animal populations, providing insights into genetic structure, phylogeography, and evolutionary history (Avise et al., 1987; Zhou et al., 2016). It is particularly useful for reconstructing phylogenetic relationships and conducting population genetics studies in ducks (Johnson & Sorenson 1998; Nabholz et al., 2008). Despite ongoing population declines, studies on the genetic diversity of the common pochard remain limited, with no reports including samples from Iran (Lui et al., 2012). This study aims to analyze the genetic diversity of common pochard populations using mitochondrial genes (cytb and D-loop) from samples collected along the southern Caspian Sea and the Hor al-Azim wetland in southwestern Iran. We integrated our data with the limited datasets available from other regions of the species' range to:

• Determine phylogenetic relationships among northern and southern Iranian populations.
• Assess levels of genetic diversity
• Compare genetic and haplotype diversity in Iran with other regions.
• Investigate potential genetic admixture between northern and southern Iranian populations.

## Martial and methods

### Sampling

Sampling was accomplished in the southern regions of the Caspian Sea (41.93° N, 50.66° E) in northern Iran and Khuzestan Province (Hor al-Azim wetland) (31.43° N, 49.04° E) in southwestern Iran (total samples = 30) from November 2021 to February 2023. Sampling was executed by considering sanitary conditions. Sampling was performed so as to adhere to the protocol of conserving fauna by taking samples from their muscles and feather samples of the hunted or confiscated ducks due to illicit hunting. The muscle samples were placed in 96% ethanol and conveyed to the laboratory. Genomic DNA was then extracted by means of the standard phenol-chloroform protocol (Sambrook 1989). Population samples were collected from the southern areas of the Caspian Sea in northern Iran (N1-N15) and Hor al-Azim wetland (S15-S30).

### DNA extraction, PCR settings and sequencing

DNA extraction was performed using the Viragen kit at the Genetics Laboratory of Tehran (zist fanavary pishgam company) in Iran. The mitochondrial cytochrome b (cytb) gene was sequenced for 30 samples as detailed according to Chavoshi et al., (2023). Furthermore, 30 samples were sequenced for the mitochondrial D-loop, using primers, with amplification conditions as described

by Lui. et al., (2011). All sequences were edited and aligned using SeqScape (Weichun et al., 2012). A total of 1564 nucleotides were obtained for the three-gene set (1008 bp for the cytb gene, 556 bp for the D-loop).

**Phylogenetic analyses**

We considered specimens from Iran to be from two populations based on previous findings of distinct morphometry and morphology (Lui, et al., 2011). We used the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (Pi), and the number of polymorphic sites (PS) to describe genetic diversity in each population using DnaSP v.6.12 (Rozas et al., 2018). We also calculated Fst in DnaSP to assess genetic variation between Caspian Sea and Hor al-Azim. Finally, we combined our samples from Iran (n = 30) with all published sequences of the species (n=10) from the GenBank database (Table 1) to provide a broader understanding of the how genetic diversity of *A. ferina* is spread across its distribution range.

A phylogenetic tree for 30 samples was constructed using BEAST v2.7 with cytb and D-loop data partitions, employing substitution rates from Lui et al., (2011) and a Calibrated Yule model. Haplotype networks for genus *Aythya* were generated in PopART using the minimum-spanning algorithm for mitochondrial genes cyt*b* and D-loop (Gonzalez et al., 2009; Liu et al., 2011).

**Table 1.** List of samples used for Phylogeny analysis with voucher numbers, region, and coordinates of collection from Iran along with GenBank samples (KJ710708, MW337298, EU585623, JQ237545.1, JQ 237544.1, KT934876, JQ422463, NC_059801, AY112946.1)

| No | Voucher number | Country | Region | °N | °E | NO | Voucher number | Country | Region | °N | °E |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Aythya ferina* N1 | Iran | Caspian Sea | 41.93 | 50.66 | 22 | *A. ferina* S7 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 2 | *A. ferina* N2 | Iran | Caspian Sea | 41.93 | 50.66 | 23 | *A. ferina* S8 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 3 | *A. ferina* N3 | Iran | Caspian Sea | 41.93 | 50.66 | 24 | *A. ferina* S9 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 4 | *A. ferina* N4 | Iran | Caspian Sea | 41.93 | 50.66 | 25 | *A. ferina* S10 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 5 | *A. ferina* N5 | Iran | Caspian Sea | 41.93 | 50.66 | 26 | *A. ferina* S11 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 6 | *A. ferina* N6 | Iran | Caspian Sea | 41.93 | 50.66 | 27 | *A. ferina* S12 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 7 | *A. ferina* N7 | Iran | Caspian Sea | 41.93 | 50.66 | 28 | *A. ferina* S13 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 8 | *A. ferina* N8 | Iran | Caspian Sea | 41.93 | 50.66 | 29 | *A. ferina* S14 | Iran | Hor al-Azim | 31.43 | **49.04** |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | *A. ferina* N9 | Iran | Caspian Sea | 41.93 | 50.66 | 30 | *A. ferina* S15 | Iran | Hor al-Azim | 31.43 | **49.04** |
| 10 | *A. ferina* N10 | Iran | Caspian Sea | 41.93 | 50.66 | 31 | *A. ferina* | China | **KJ710708** | | |
| 11 | *A. ferina* N11 | Iran | Caspian Sea | 41.93 | 50.66 | 32 | *A. ferina* | China | **MW337298** | | |
| 12 | *A. ferina* N12 | Iran | Caspian Sea | 41.93 | 50.66 | 33 | *A. ferina* | Germany | **EU585623** | | |
| 13 | *A. ferina* N13 | Iran | Caspian Sea | 41.93 | 50.66 | 34 | *A. ferina* | Switzerland | **JQ237545.1** | | |
| 14 | *A. ferina* N14 | Iran | Caspian Sea | 41.93 | 50.66 | 35 | *A. ferina* | Switzerland | **JQ 237544.1** | | |
| 15 | *A. ferina* N15 | Iran | Caspian Sea | 41.93 | 50.66 | 36 | *A. affinis* | USA | **KT934876** | | |
| 16 | *A. ferina* S1 | Iran | Hor al-Azim | 31.43 | 49.04 | 37 | *A. fuligula* | Switzerland | **JQ422463** | | |
| 17 | *A. ferina* S2 | Iran | Hor al-Azim | 31.43 | 49.04 | 38 | *A. nyroca* | Switzerland | **NC_059801** | | |
| 18 | *A. ferina* S3 | Iran | Hor al-Azim | 31.43 | 49.04 | 39 | *A. baeri* | China | **NC_057048** | | |
| 19 | *A. ferina* S4 | Iran | Hor al-Azim | 31.43 | 49.04 | 40 | *A. americana* | France | **AY112946.1** | | |
| 20 | *A. ferina* S5 | Iran | Hor al-Azim | 31.43 | 49.04 | | | | | | |
| 21 | *A. ferina* S6 | Iran | Hor al-Azim | 31.43 | 49.04 | | | | | | |

## Results

### Genetic Diversity

To provide a broader understanding of the genetic diversity of the common pochard within its distribution range, Iranian samples (n=30) were combined with published sequences from the common pochard (n=10) obtained from the GenBank database (Table 1). Additionally, we illustrated the relationships between Iranian haplotypes and the generated intermediate haplotype network (Fig. 1 & 2).

In the analysis of samples collected from northern and southern Iran, 30 sequences from the mitochondrial D-loop region, comprising 556 nucleotide pairs, were identified. These sequences exhibited both conserved and mutated positions, including 10 mutation sites and six distinct haplotypes. Among these haplotypes, five belong to Iran, and one is from China (Fig.1). Furthermore, upon examining 30 sequences from the 1008 base pairs of the mitochondrial cytochrome b gene in the samples collected from northern and southern Iran, two mutated positions and three different haplotypes were identified. Of these, two haplotypes are found in northern Iran

samples, and one is from a sample collected in China. The core haplotype includes other samples from both northern and southern Iran, as well as European and Asian samples (Fig. 2).

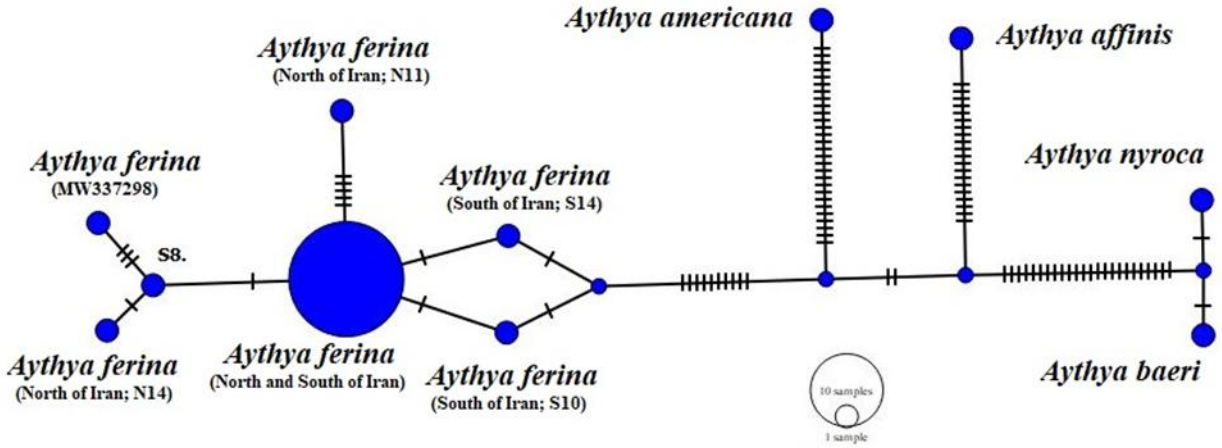


**Figure 1.** Using the D-loop sequences obtained from the northern and southern regions of the country and the samples available in the GenBank, the haplotype network was drawn by Popart software.

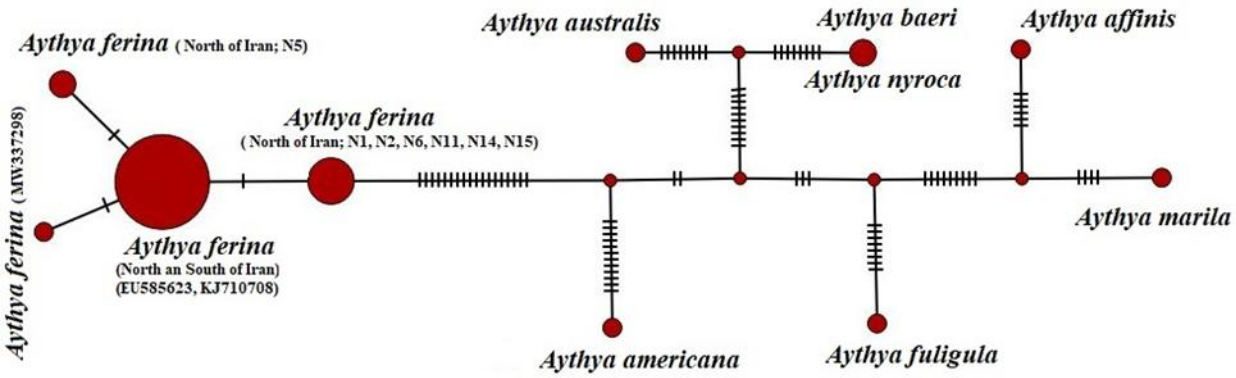


**Figure 2.** Using the cyt *b* sequences obtained from the northern and southern regions of the country and the samples available in the GenBank, the haplotype network was drawn by Popart software.

The analysis of the samples revealed low genetic diversity within the migratory pochard populations in Iran. The Rogers-Harpending index (r = 0.112) indicates a sudden expansion of the species. Gene flow (Nm) between the sampled populations in Iran and global populations was calculated at 1.381, suggesting relatively high gene flow and low genetic variation among the samples. In other words, gene flow between the populations is high.

Tajima's neutrality test and the Fu's F statistic yielded values of -2.376 and -2.174, respectively, both of which were not statistically significant ($p$-value ≥ 0.10). The Popart software identified 10 mutation steps between the D-loop haplotypes. Haplotypes S8, S10, and S14 from southern Iran are connected to the core haplotype by a single mutation. Haplotype N14, with two mutations, and N11, with five mutations, are more distantly related to the core haplotype. Similarly, the Chinese sample, with four mutations, is separated from the core.

In the cytb haplotypes, two mutation steps separate them from the core. Haplotype N5 is separated by a single mutation, while the haplotype containing samples N1, N2, N6, N11, N14, and N15 is also separated by a single mutation from the core. All of these samples are from northern Iran. The Chinese sample's haplotype is also separated by one mutation from the core. The core haplotype includes all southern Iran samples, with remaining samples from northern Iran, Europe, and Asia associated with it.

Tajima's neutrality test and Fu's F statistic for the cytb haplotypes yielded values of -0.1828 and -0.101, respectively, and were not statistically significant ($p$-value ≥ 0.10). The average haplotype diversity in Iran for both mitochondrial genes (cytb & D-loop) was calculated to 0.109. Additional diversity measures, including total haplotype diversity (Hd), nucleotide diversity (Pi), polymorphism (s), total number of mutations (Et. a), and the standard deviation of nucleotide diversity (K), are provided in Table 2.

**Table 2.** Estimates of Genetic variability in 30 specimens of *A. ferina* samples from wintering population of Iran, analyzed for the mitochondrial control region and cytochrome *b*.

| Genes | N | S | Et. a | H | Hd | Sd. Hd | Pi | K | Fu Fs | Tajima`s D |
|---|---|---|---|---|---|---|---|---|---|---|
| **D-loop** | 30 | 10 | 10 | 6 | 0.31 | 0.109 | 0.001 | 0.791 | -2.376 | -2.174 |
| **Cytochrome *b*** | 30 | 2 | 2 | 3 | 0.432 | 0.109 | 0.00046 | 0.46 | -0.101 | -0.1828 |

The phylogenetic tree of common pochards from Iran, constructed using GenBank samples (KJ710708, MW337298, EU585623) and data from the current study, was generated with a bootstrap value of 1000. The tree reveals the distribution of samples from northern and southern Iran, along with those from Europe and Asia, suggesting that the wintering pochards in Iran likely migrates from regions in Asia (e.g., China) and Europe (e.g., Germany). The tree also clearly positions *A. ferina* in relation to other species within the *Aythya* genus. Notably, *A. ferina* and *A.*

*americana* are highly similar, and the presence of a common ancestor clearly accounts for this similarity (Figure 3).

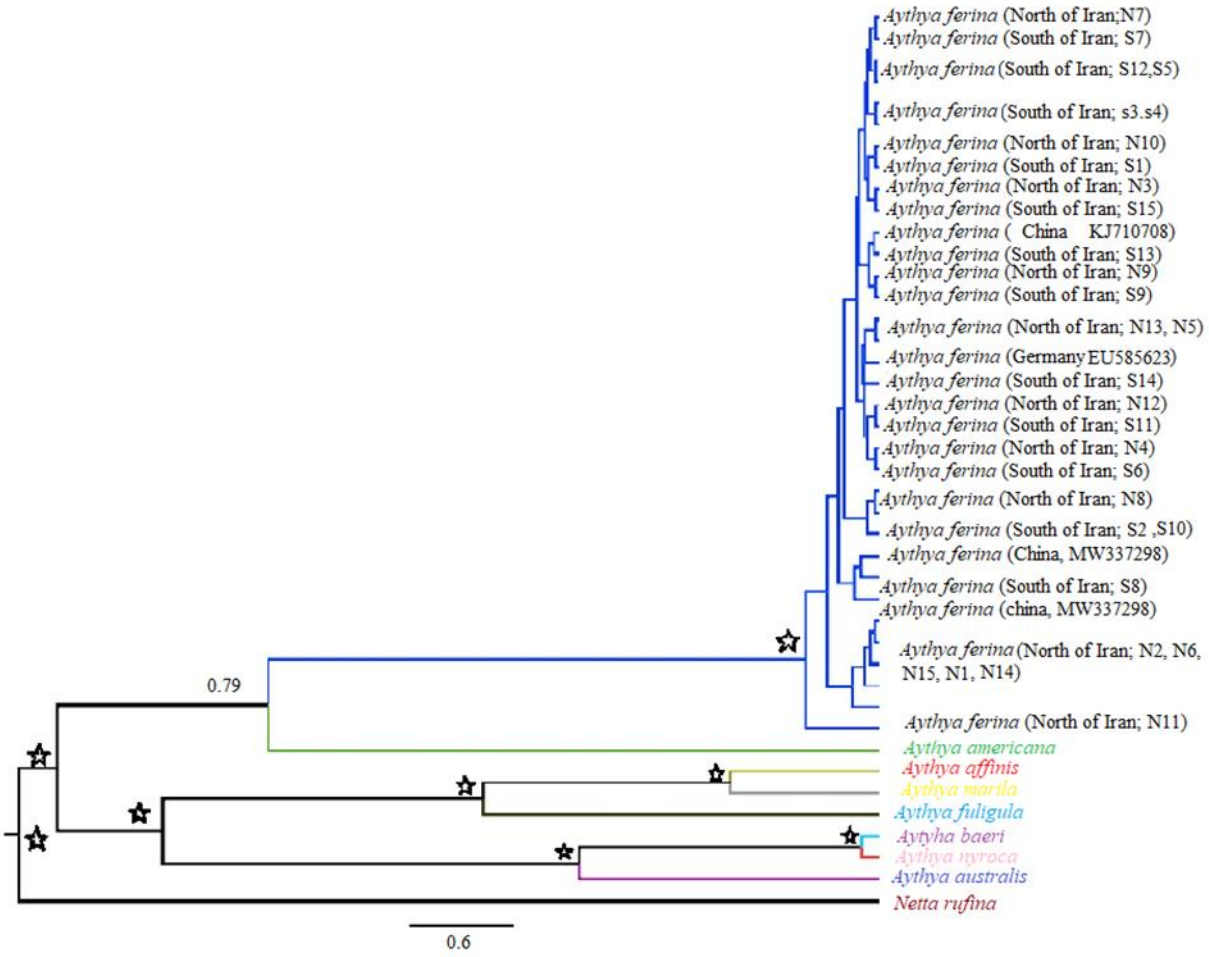


**Figure 3.** Phylogenetic tree based on the maximum likelihood method between *A. ferina* in North and South of Iran using fragments of 1564 pairs of mtDNA gene (D-loop and cytb).

## Discussion

Our study evaluates the genetic diversity of mitochondrial genes (D-loop & cyt*b*) in diving ducks (*A. ferina*) from northern and southern Iran. The cytb haplotype diversity was low, particularly for the southern Iran samples, which were all grouped within the core haplotype. In contrast, the D-loop haplotype diversity was higher and included samples from both northern and southern Iran. These haplotypes diversity suggest that the wintering populations in the Caspian Sea and Hor al-Azim wetland are likely formed by pochard populations from Europe and East Asia.

The results indicate that a broad range of migratory populations overwinter in various regions of northern and southern Iran. These populations may converge during migration and wintering, enabling individuals from different populations to return to sites other breeding sites. This gene flow introduces new genes into the population, enhancing genetic diversity and reducing inbreeding effects (Yadav and Ri, 2024; Liu et al., 2011). Studies on 201 samples of *A. ferina* from Western and Eastern Europe, the UK, and Russia showed that pochards tend to migrate due to climate change or food scarcity, which results in high gene flow among individuals of this species (Keller et al., 2009; Kazam et al., 2024). Furthermore, the current study also found that, due to the small genetic distance, gene flow between the Iranian populations and global populations is high, with no subspecific variations. The genetic differences between the populations in northern and southern Iran are within-population variations.

Tajima's D index and Fu's FS index for the pochard populations revealed negative values (*p*-value < 0.01), but these were not statistically significant (Tajima, 1989; Tamura & Nei, 1993). The distribution of the populations, as indicated by the Rogers and Harpending index, was uniform, suggesting population expansion in the past (Rogers & Harpending, 1992). Results from two other indices support this finding, with Fu's and Tajima's indices providing stronger evidence for past population conditions (Ramos-Onsins & Rozas, 2002). The phylogenetic tree for the Iranian migratory pochards yielded results consistent with those from previous studies (Gonzalez et al., 2009; Liu et al., 2011).

Based on the identified haplotypes, the study suggests that the sampled individuals exhibit minimal differences from global geographic populations. There is very little genetic variation and high gene flow between these geographic populations, consistent with the findings of Lui et al. (2011). Pochards sampled in wintering areas in Europe, the Caspian Sea, and East Asia, despite their large geographical distances, showed minor genetic variation (Stanevičius et al., 2008). The genetic diversity associated with the previously defined wintering areas did not differ significantly from zero for mtDNA (Liu et al., 2011). Worldwide studies on duck populations, using movement patterns and genetic and phylogenetic methods, have highlighted intraspecific diversity in ducks, aligning with the findings of the current study (Liu et al., 2011; Kraus et al., 2013; Mischenko et al., 2020).

The results from the D-loop and cytb genes accurately positioned northern and southern Iranian samples on the phylogenetic tree, alongside Asian and European GenBank samples, indicating that

pochards migrate to Iran from these regions, in agreement with the findings of Liu et al., (2012). The use of mitochondrial markers for genetic diversity studies at the species level and for phylogenetic relationships has proven effective. Studies on the Anatidae family, particularly diving ducks, have shown that *A. ferina* is very similar to *A. americana*, with minor phylogenetic differences (Gonzalez et al., 2009; Zhai et al., 2021).

The phylogenetic tree for the populations from northern and southern Iran, along with global samples, highlights the similarity between *A. ferina* and *A. americana*, both of which share a common ancestor. Mitochondrial DNA studies (D-loop and cytochrome b) enabled the identification of distinct genetic populations of pochards in Iran and the assessment of genetic similarities at the individual level. Genetic analysis serves as a powerful tool for assessing genetic diversity, especially in the context of global climate change, which affects the distribution of different bird populations.

## Conclusion

This study investigated the genetic diversity of common pochards (*A. ferina*) wintering in Iran using mitochondrial DNA sequences (cyt*b* and D-loop) from 30 individuals sampled from two key wintering sites: the southern Caspian Sea and the Hor al-Azim wetland. Our findings reveal low genetic diversity within the Iranian populations, characterized by limited haplotype diversity (six D-loop and three cyt*b* haplotypes). Phylogenetic analysis demonstrated close genetic relationships between Iranian pochards and populations from Europe and East Asia, suggesting that these regions contribute significantly to the Iranian wintering population. Notably, high gene flow (Nm=1.381) was observed between Iranian and global populations, indicating strong connectivity among wintering sites. This high gene flow, despite the observed low genetic diversity, likely reflects recent population expansion, as suggested by neutrality tests.

These findings have significant implications for the conservation of the common pochard. The observed low genetic diversity, while potentially influenced by recent population expansion, underscores the importance of maintaining connectivity among wintering and breeding populations across the species' range to ensure long-term genetic viability and resilience. This is particularly crucial given the ongoing population declines observed in Europe, Siberian wetlands, and wintering populations in Iran. The low genetic distance and high gene flow among the studied samples, Showing Iran serves as a suitable location for the congregation of diving ducks during

the wintering period. The conservation of wetlands as habitats for the diving duck, along with the prevention of illegal hunting, plays a crucial role in ensuring the persistence of this species.

Our study contributes to the limited existing knowledge on the genetic diversity of common pochards in Iran. Further research, including larger sample sizes and the inclusion of nuclear markers, is necessary to further elucidate the genetic structure of Iranian populations and to refine conservation strategies for this vulnerable species.

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