



The Migratory Greater Flamingo of Iraqi Marshes Revealed by COI Gene Barcode

Mustafa S. F. Ziyadi¹, Najim M. Aziz², Rafid M. Karim^{2*}

¹Marine vertebrates Department, Marine Science Centre, University of Basrah, Basrah, Iraq

²Marine Biology Department, Marine Science Centre, University of Basrah, Basrah, Iraq

*Corresponding Author: rafid.karim@uobasrah.edu.iq

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ABSTRACT

The marshlands in the south and middle of Iraq are located along the migration route of immigrant birds. These marshes, characterized by warm weather and nutrition availability, provide an important habitat for these birds. The greater flamingo (*Phoenicopterus roseus*) (Aves: Phoenicopteridae) migrates between Eurasia and Africa. The flamingo population annually reaches the marshes of Iraq in winter. Due to morphological similarities between the Greater Flamingo *P. roseus* and another flamingo species, misidentification can occur. To identify the flamingo species and detect its migration route, the genetic barcode was used. The mitochondrial Cytochrome C Oxidase subunit I (COI) gene was amplified using universal primers. The amplification product was sequenced and analyzed as a DNA barcode. The sequence was checked on the Blast website of NCBI with the recorded the flamingo species from the surrounding area. Clustal Omega was utilized for sequence alignment. The study results confirmed that the population reaching the Iraqi marshes matches that of the European *P. roseus* population. The COI sequence of 675bp was deposited in the NCBI with the accession number of OM669759. The flamingo bird, which annually visits the Iraqi marshes, has been confirmed as *P. roseus*. The mitochondrial Cytochrome C Oxidase subunit I (COI) gene was proven as an efficient tool to discriminate among bird species. In addition, the Iraqi marshes are an important site for sustaining the migratory route of the greater flamingo and other aquatic birds.

INTRODUCTION

The greater flamingo *Phoenicopterus roseus* belongs to the family Phoenicopteridae. It is one of the immigrant wading birds (Al-Robaae, 2006; Habeeb *et al.*, 2018). The mesopotamian Iraqi marshes are located on the migration route in the winter season (Jawad *et al.*, 2021). At the same time, the nutrient availability and plant canopy in the Iraqi marshes would sustain a very rich diversity of living species of invertebrates and fish that support the immigrant birds (FAO, 2008; Habeeb *et al.*, 2019). Al-Robaae (2006) reported the breeding of some species of water birds in the Iraqi marshes, emphasizing their importance as a habitat for species conservation

(Kubba & Salim, 2010). Firstly, the avifauna checklist in Iraq was reported by Allouse (1953). After that, many revisions and additions were published (Allouse, 1960, 1961, 1962; Habeeb, 2008). The wading birds of the Iraqi marshes are redescribed and the ecology was studied by Habeeb *et al.* (2018). The greater flamingo (*Phoenicopterus roseus*) annually migrates from Eurasia to North Africa (Boucheker *et al.*, 2011). The immigration route of this bird is crossing the marshes of southern Iraq. The greater flamingo visits for several weeks of the winter season investing in areas and shallow wetlands immersed with salt and fresh water (Salim *et al.*, 2009). The flamingo population feeds on a wide range of food items. They filter out small shrimp, seeds, algae, microorganisms, invertebrates, and tiny insects (Stanford University, 2023). All studies on immigrant birds were descriptive using traditional methods for identification. The traditional protocol for knowing the immigration route is the tag rings (Balkiz *et al.*, 2015). While, the best method used for investigating the migration track of the migratory bird was the satellite telemetry (Koppen *et al.*, 2010; Guo-Gang *et al.*, 2014). This method is expensive and needs transmitters loading on birds and satellite service. For the same purpose, the genetic barcoding can be useful. The DNA barcoding method is cheap, reliable, and accurate (Hebert *et al.*, 2004). Recently, DNA barcoding has become a useful tool for species identification. In the last decades, DNA barcoding of selected genes particularly of mitochondrial DNA genes were used as genetic markers for biodiversity studies. For animals, the Cytochrome C Oxidase (CO1) gene was the standard barcode (Hebert *et al.*, 2003). Whereas, COI is the preferred for DNA barcodes. An informative segment of the COI gene sequence was chosen to solve the ambiguous or confused taxonomy (Saitoh *et al.*, 2015). Using COI as a differentiation tool reveals high interspecies genetic variation and low intraspecies differences (Stoeckle & Thaler, 2014). It is often used in biodiversity to detect living species accurately in a certain ecosystem (Folmer *et al.*, 1994). COI would be amplified, sequenced, and compared with the reference database of the National Center of Biotechnology Information (NCBI, 2023). It is important to join DNA barcoding studies on biodiversity with conventional field observation and identification. Geraci *et al.* (2012a) reported the greater flamingo breeding locations around the Mediterranean Sea. In biodiversity studies, it is important to join DNA barcoding with conventional field observation and identification methods (DeSalle *et al.*, 2005; Farooq *et al.*, 2020). The present study aimed to confirm the identification of the greater flamingo (*Phoenicopterus roseus*) and other common flamingo species such as the lesser flamingo (*P. minor*) and the chilean flamingo (*P. chilensis*), document the DNA barcode of the greater flamingo that crosses the marshes in southern Iraq, and detect the immigration route according to the DNA barcode similarity in NCBI.

MATERIALS AND METHODS

Three bird samples of the greater flamingo were obtained from the market of Chibayish town, near the central marshes in the province of ThiQar. Genomic DNA was extracted from the liver pieces using a DNA extraction Kit (Genomic DNA Mini Kit, Geneaid Biotech. Ltd Kit). They were stored at -20°C until amplification experiments. Amplification of Cytochrome C. Oxidase subunit 1 (COI) was done using a couple of primers [16] Bird F1 5' TTCTCCAACCACAAAGACATTGGCAC 3', and Bird R1 5' ACGTGGGAGATAATTCCAAATCCTG 3'. The PCR program consisted of the following steps: initial denaturation for 5min at 95°C , followed by 30 cycles of denaturation for 1 min at 95°C , annealing for 1min at 58°C , and elongation for 1min at 72°C . The program concluded with a final elongation for 6min at 72°C . The amplification products were analyzed for COI sequence. Chromas software was used to process the bird sequences, then they were checked in the National Center for Biotechnology Information /Blast [20]. Clustal W was used for the multiple sequence alignment (McWilliam *et al.*, 2013).

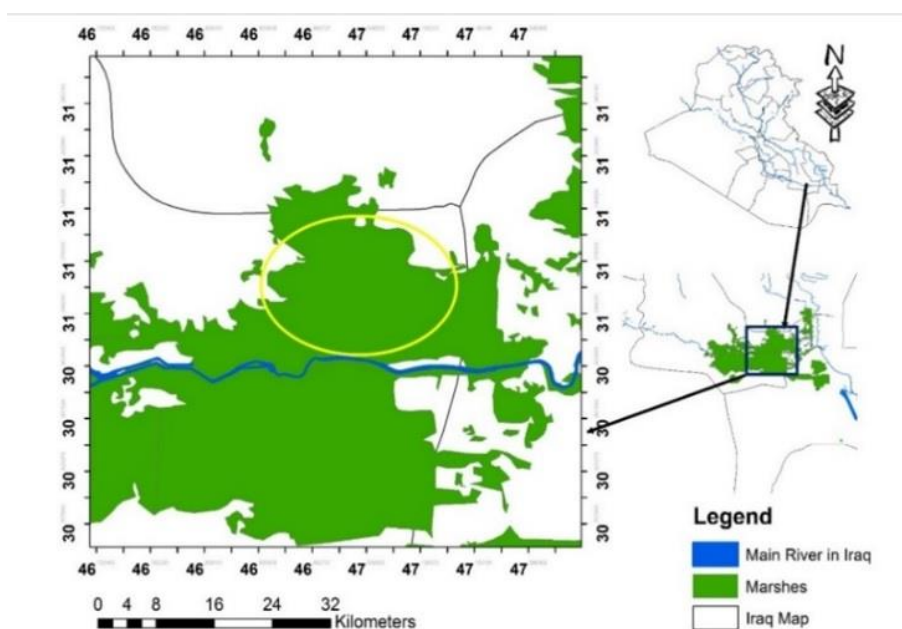


Fig. 1. Map of marshes in southern Iraq, bird hunting area is assigned

RESULTS

The result of the sequencing was a 675bp segment of the COI gene. When compared with NCBI BLAST data, this segment was found to match with the *P. roseus* (Scheme 1). The phylogenetic tree of COI gene sequences of the greater flamingo (*P. roseus*) from the Iraqi marshes (accession number OM669759.1), along with sequences from the European and African countries, and other species, *P. ruber* (NC_027934.1) and *P. chilensis* (FJ028028.1), was constructed using Clustal Omega. The analysis revealed

that the Iraqi flamingos clustered with the European and African greater flamingo sequences obtained from NCBI. At the same time, the lesser flamingo (*P. ruber*) correlated with the chilean flamingo (*P. Chilensis*) (Fig. 2).

NC_027934.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 JF824506.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 JF824507.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 KT962872.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
OM669759.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 KJ400311.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 JF824492.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 NC_010089.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC
 KT962873.1 CAACCTGGAACTCTCCTAGGAGACGACCAAATCTATAACGTAATCGTTACCGCCCATGCC

FJ028028.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 NC_027934.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 JF824506.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 JF824507.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 KT962872.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
OM669759.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 KJ400311.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 JF824492.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 NC_010089.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG
 KT962873.1 TTCGTAATAATCTTCTTCATAGTTATACCAATCATAATTGGAGGTTTCGGAAACTGACTG

FJ028028.1 GTTCCCTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 NC_027934.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 JF824506.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 JF824507.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 KT962872.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
OM669759.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 KJ400311.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 JF824492.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 NC_010089.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC
 KT962873.1 GTTCCACTCATAAATTGGTGCTCCTGACATAGCATTCCCCGCATAAACAACATAAGCTTC

FJ028028.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGCCTCCTCCACAGTAGAAGCTGGAGCA
 NC_027934.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 JF824506.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 JF824507.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 KT962872.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
OM669759.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 KJ400311.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 JF824492.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 NC_010089.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA
 KT962873.1 TGACTACTACCCCATCCTTCTTACTCCTCCTGGCCTCCTCCACAGTAGAAGCTGGAGCA

FJ028028.1 GGCACAGGATGAACTGTATACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
 NC_027934.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
 JF824506.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
 JF824507.1 GGACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
 KT962872.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
OM669759.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
 KJ400311.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA

JF824492.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
NC_010089.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA
KT962873.1 GGCACAGGATGAACTGTGTACCCACCACTAGCTGGCAACATAGCCCATGCCGGAGCCTCA

FJ028028.1 GTAGACCTAGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
NC_027934.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
JF824506.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
JF824507.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
KT962872.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
OM669759.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
KJ400311.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
JF824492.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
NC_010089.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC
KT962873.1 GTAGACCTGGCTATCTTCTCCCTCCACCTAGCAGGTGTATCATCTATTCTCGGAGCAATC

FJ028028.1 AACTTTATCACTACTGCTATCAACATAAAACCACCGCCCTCTCACAATACCAAACCCCC
NC_027934.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
JF824506.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
JF824507.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
KT962872.1 AACTTTATCACACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
OM669759.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
KJ400311.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
JF824492.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
NC_010089.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC
KT962873.1 AACTTTATCACTACTGCTATCAACATAAAACCACCTGCCCTCTCACAATACCAAACCCCC

FJ028028.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
NC_027934.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
JF824506.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
JF824507.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
KT962872.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
OM669759.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
KJ400311.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
JF824492.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
NC_010089.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT
KT962873.1 CTATTCGTATGATCTGTCCTCATCACCGCTGTCCTATTCTACTCTCACTTCCAGTCCTT

FJ028028.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCG
NC_027934.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCG
JF824506.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA
JF824507.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA
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OM669759.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA
KJ400311.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA
JF824492.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA
NC_010089.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA
KT962873.1 GCCGCCGGCATTACCATACTACTAACAGACCGAAACCTAAACACCACATTCTTCGATCCA

FJ028028.1 GCCGGAGGAGGCGACCCAGTCCTATACCAACACCTCTTCTGATTCTTCGGTCACCCAGAA
NC_027934.1 GCCGGAGGAGGCGACCCAGTCCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
JF824506.1 GCCGGAGGAGGCGACCCGGTCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
JF824507.1 GCCGGAGGAGGCGACCCGGTCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
KT962872.1 GCCGGAGGAGGCGACCCGGTCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
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KJ400311.1 GCCGGAGGAGGCGACCCGGTCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
JF824492.1 GCCGGAGGAGGCGACCCGGTCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
NC_010089.1 GCCGGAGGAGGCGACCCGGTCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA

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KT962873.1  GCCGGAGGAGGCGACCCGGTCCTATACCAGCACCTCTTCTGATTCTTCGGTCACCCAGAA
*****
FJ028028.1  GTCTACATCCTAATC
NC_027934.1  GTCTACATCCTAATC
JF824506.1  GTCTACATCCTAATC
JF824507.1  GTCTACATCCTAATC
KT962872.1  GTCTACATCCTAATC
OM669759.1  GTCTACATCCTAATC
KJ400311.1  GTCTACATCCTAATC
JF824492.1  GTCTACATCCTAATC
NC_010089.1  GTCTACATCCTAATC
KT962873.1  GTCTACATCCTAATC
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Scheme 1. Sequence alignment of the greater flamingos (*Phoenicopterus roseus*) OM669759.1 from Iraq, along seven Mediterranean regions, *P. ruber* NC_027934.1, *P. chilian* FJ028028.1. [created by Clustal Omega]

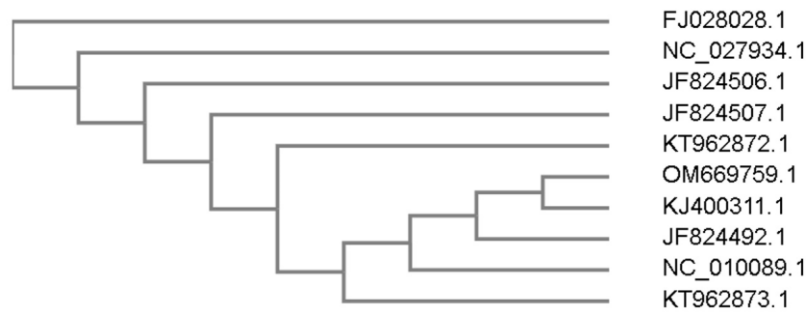


Fig. 2. Phylogenetic tree of the greater flamingo's (*P. roseus*) COI gene sequences from the Iraqi marshes OM669759.1, along the European and African countries, with *P. ruber* NC_027934.1 and *P. chilensis* FJ028028.1 created by Clustal Omega

On the other hand, the diversity is shown in Table (1). The intra-species similarity among *P. roseus* COI sequences was 99.852- 100%. At the same time, the inter-species variation among the three species: *P. roseus*, *P. ruber*, and *P. Chilensis* was 98.963- 97.778%. Simultaneously,, the sequence alignment of the greater flamingos (*Phoenicopterus roseus*) OM669759.1 from Iraq, along seven Mediterranean regions, *P. ruber* NC_027934.1, and *P. chilensis* FJ028028.1 created by Clustal Omega (Fig. 2). This alignment highlights the variant nucleotides among these species.

Table 1. Alignment comparison among *Phoenicopterus roseus* COI gene sequence and chosen sequences from various Mediterranean Sea basin countries, along with *P. ruber* and *P. chilensis*

No.	subject acc. Ver.	% identity	alignment length	mismatches	Scientific name	s. start	s. end	e-value	bit score	Country
1	OM669759.1	100.000	675	0	<i>P. roseus</i>	1	675	0.0	1247	This study
2	KJ400311.1	100.000	675	0	<i>P. roseus</i>	19	693	0.0	1247	(Torres <i>et al.</i> , 2014)
3	JF824492.1	100.000	675	0	<i>P. roseus</i>	29	703	0.0	1247	(Geraci <i>et al.</i> , 2012a)
4	NC_010089.1	100.000	675	0	<i>P. roseus</i>	5469	6143	0.0	1247	Morgan-Richards <i>et al.</i> , 2008)
5	KT962873.1	99.852	675	1	<i>P. roseus</i>	29	703	0.0	1242	Wattier <i>et al.</i> , 2015

6	KT962872.1	99.852	675	1	<i>P. roseus</i>	29	703	0.0	1242	Wattier <i>et al.</i> , 2015
7	JF824507.1	99.852	675	1	<i>P. roseus</i>	29	703	0.0	1242	(Geraci <i>et al.</i> , 2012a)
8	JF824506.1	99.852	675	1	<i>P. roseus</i>	29	703	0.0	1242	(Geraci <i>et al.</i> , 2012a)
9	NC_027934.1	98.963	675	7	<i>P. ruber</i>	5470	6144	0.0	1208	(Luo <i>et al.</i> , 2016)
10	FJ028028.1	97.778	675	15	<i>P. chilensis</i>	17	691	0.0	1164	(Kerr <i>et al.</i> , 2009)

DISCUSSION

DNA barcoding provides more specific information that confirms the initial morphological identification. Correctly, the flamingo population in the Iraqi marshes consists of the greater flamingo (*Phoenicopterus roseus*). Indeed, the flamingo population inhabiting the Iraqi marshes comprises the greater flamingo species, *Phoenicopterus roseus*. The migration route of this large bird was revealed by checking the COI sequence of different records recovered from the Genbank which were recorded from many Mediterranean regions. The results showed that this species visiting Iraqi marshes match that of Southwest Europe, mainly France, Spain, Italy, and Anatolia (Scheme 1). Mesopotamia is one of the important sites for immigrant birds to reach the South bank of the Mediterranean Sea, such as Algeria and Libya, and return to the same habitat (Geraci *et al.*, 2012b). At the same time, the greater flamingo was recorded in Mauritania (Diawara *et al.*, 2009) and Tunisia (Smart *et al.*, 2009). The analysis of the sequences of different specimens was around the Mediterranean Sea region (Scheme 1). Studies using DNA coding have shown that the greater flamingos have a high level of genetic diversity among their populations, with individuals from different regions showing significant genetic differences (Geraci *et al.*, 2012a). This suggests that the greater flamingos may have multiple variant breeding groups that are geographically separated (Bouchecker *et al.*, 2011). The phylogenetic tree, constructed using the Iraqi flamingo sequence and seven selected sequences via Clustal Omega, provides confirmation that the population from North-West Europe migrates to the marshes located in southern Iraq (Fig. 2). Description statistics among the five populations revealed the similarity of *P. roseus* sequences and the genetic distance with the *P. ruber* and *P. chilensis* species (Table 1). The DNA barcoding strand might be used to identify the birds and follow their migrations in Iraq and other parts of the world in the instance of the great flamingo sequenced. Geraci *et al.* (2012a) stated that the greater flamingo population started migrating from the south-western of Europe to the southern areas of the Mediterranean Sea. They identified the biodiversity and the location within the monitoring region. Bouchecker *et al.* (2011) indicated the connectivity of the Algerian flamingos with the population in the surrounding Mediterranean countries. They have been observed nesting in North Africa, specifically in Mauritania (Diawara *et al.*, 2009; Samraoui *et al.*, 2009) and Tunisia (Smart *et al.*, 2009). In Eurasia, nesting locations include Turkey (Balkiz *et al.*, 2007), Italy (Baccetti *et al.*, 2008), France (Béchet & Johnson, 2008; Béchet *et al.*, 2009), and Spain (Curcó *et al.*, 2009). The phylogenetic tree (Fig. 2) of

COI gene sequences of the greater flamingos (*P. roseus*) from the Iraqi marshes OM669759.1, which is mating with the European and African countries, means that the *P. roseus* migrates from the Eurasian region throughout the cold season for wintering. While it is not clustered with *P. ruber* NC_027934.1 and *P. chilensis*. That confirms that the flamingos of Iraq did not match those from other regions. This result proves the immigrant flamingo reached the Iraqi wetlands from the Mediterranean Sea region to conduct its manual trip to North-West Africa for breeding (**Bouchekeker *et al.*, 2011**). The intraspecific similarity and interspecific variation in Table (1) correctly prove that *P. roseus* of the Iraqi marshes manually migrated from the Eurasian cold regions to the African coastlands. Therefore, the results confirm that the Iraqi flamingo is not *P. ruber* or *P. Chilensis*. The mtDNA COI sequence of the greater flamingo (*Phoenicopterus roseus*) caught in the central marshes in the province of ThiQar was recorded in NCBI. The existence of the Iraqi marshes on the route of the immigrant birds from Europe to North Africa represents a resting, feeding, and reproduction time. Therefore, the sustainability of marshes is significant for the immigrant bird life (**Cherkaoui *et al.*, 2018**).

CONCLUSION

The study accurately resolves the identification of the greater flamingo crossing the Iraqi marshlands as *Phoenicopterus roseus*. It also proved that the greater flamingo visits the Iraqi marshes annually from the Eurasia regions to reach North-west Africa. Additionally, the COI gene is suitable to utilize as a DNA barcode for the immigration routes of the Iraqi avifauna. The findings underscore the significance of the Iraqi marshes as wintering sites not only for the immigrant greater flamingo (*P. roseus*) but also for other visiting and domestic water birds.

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