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Author(s): Khaled Salem Etayeb , Jaber Yahia , Ali Berbash , Remi Wattier and Anne-Laure Brochet

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# First Breeding Evidence of Marbled Duck (*Marmaronetta angustirostris*) in Libya

KHALED SALEM ETAYEB<sup>1,\*</sup>, JABER YAHIA<sup>2</sup>, ALI BERBASH<sup>2</sup>, REMI WATTIER<sup>3</sup> AND ANNE-LAURE BROCHET<sup>4</sup>

<sup>1</sup>Tripoli University, Zoology Department, P.O. Box 13227, Tripoli, Libya

<sup>2</sup>Environment General Authority, Janzor Algheran, P.O. Box 13793, Tripoli, Libya

<sup>3</sup>Université de Bourgogne, UMR-CNRS 6282 Biogéosciences, 21000 Dijon, France

<sup>4</sup>Centre de Recherche de la Tour du Valat, Le Sambuc, 13200 Arles, France

\*Corresponding author; E-mail: khaledetayeb@yahoo.com

**Abstract.**—The first evidence of breeding of Marbled Duck (*Marmaronetta angustirostris*) in Libya, North Africa, is reported. In June 2012, at Mallaha wetland, in Tripoli, Libya, two dead ducklings were found that were later genetically identified as Marbled Duck. This breeding evidence suggests that this and other duck species may breed elsewhere in Libya. Better knowledge on Libyan, and more generally on north African, waterbirds is needed to document the population size and the status of these species. Such information will be important to secure the future of threatened species, such as the Marbled Duck. Received 10 October 2013, accepted 4 December 2013.

**Key words.**—breeding distribution, conservation status, Libya, Marbled Duck, *Marmaronetta angustirostris*, North Africa, wetlands.

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The Marbled Duck (*Marmaronetta angustirostris*) has an estimated world population of 50,000-55,000 birds with a fragmented distribution in the Mediterranean and western and southern Asia (Wetlands International 2006). It is classified as “Vulnerable” by the International Union for Conservation of Nature due to a long-term decline and a widespread loss of habitat (International Union for Conservation of Nature 2013). The Marbled Duck appears to be adapted to exploit shallow, temporary, unpredictable Mediterranean-type wetlands whose availability fluctuates markedly in both space and time (Sebastián-González *et al.* 2013).

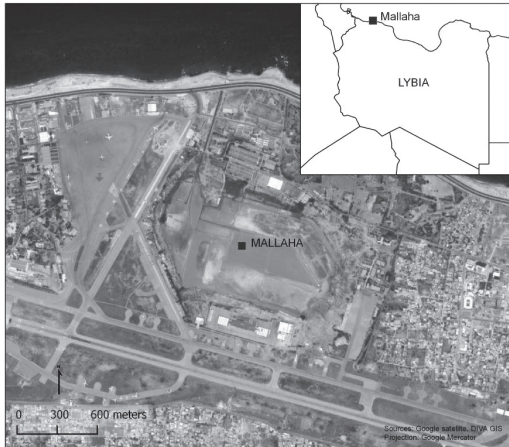
Studies of the ecology of the Marbled Duck have been carried out in several Mediterranean countries (e.g., Spain, Morocco, Turkey, Algeria and Tunisia) (Green 2005). The main purpose of these studies was to identify habitat management and other conservation measures required to prevent further population declines of this species in the Mediterranean (Green 2000). Unfortunately, the flooding cycle of many important Marbled Duck breeding sites has been modified by anthropogenic changes (e.g., dam construction), and this makes it less likely for them to breed successfully in these wetlands (Green 2000). The Marbled Duck has

been negatively affected by the extensive loss of natural, temporary wetlands in the Mediterranean (Green *et al.* 2002). The number of nesting sites of Marbled Duck is limited in the region.

In Libya, the Marbled Duck is considered to be a winter visitor. A few individuals were recently recorded between 2005 and 2011 during mid-winter counts on three different wetlands in Libya: Mallaha, Ayn Tawurgha and Wadi Zaret Dam (Etayeb *et al.* 2007; Hamza *et al.* 2008; Environment General Authority and Regional Activity Centre for Specially Protected Areas (EGA-RAC/SPA) 2012; Bourass *et al.* 2013). The Ferruginous Duck (*Aythya nyroca*) is the only duck species known to breed in Libya (P. Isenmann, pers. commun.). No previous evidence of attempted reproduction by Marbled Duck has been reported, although breeding occurs in neighboring Tunisia and Egypt (Green and Hilton 1993). Here, we report on the first breeding evidence for Marbled Duck in Libya.

## METHODS

On 6 June 2012, we visited Mallaha wetland (32° 53' 58" N, 13° 17' 15" E) as part of our field work counting birds in wetlands in and around Tripoli, Libya. Mallaha is a wetland located in northeastern Tripoli (Fig. 1).



**Figure 1.** Map of study area of Mallaha, Tripoli, Libya.

Mallaha is a salt marsh, fed by a canal from the sea year-round and by rainfall during winter. It has a surface area of about 3.75 ha. The northern part of Mallaha is dominated by dry sandy areas, grasses, dry and wet streams, canals, and brackish and salty pools. It also contains trees and shrubs, dumping yards for garbage and waste materials, the ruins of old residential and military buildings, and gravel and dust roads. There are salt marshes in the southwestern portion that provide food and shelter for a variety of aquatic birds (Etayeb *et al.* 2012). During this survey, we found two dead ducklings that were not fully fledged and, therefore, had undoubtedly hatched in the area.

The ducklings were partially decomposed and no morphological or anatomical features were available for a positive identification of the species. Therefore, one whole leg of each duckling was sent to the Université de Bourgogne (France) for genetic analyses to identify the species. DNA was phenol-chloroform extracted from a small piece of dried flesh. We amplified a *circa* 320 bp portion of the NADH dehydrogenase subunit 2 (ND2) gene using PR-ND2B-F: 5'-CCACTATAGC-CATCGCTTCA-3' (Geraci *et al.* 2012) and H6031: 5'-CACTTTGGTATAAACCTGT-3' (Donne-Goussé *et al.* 2002). This target was selected because ND2 has been shown to be discriminating between Anseriformes species (Johnson and Sorenson 1998; Gonzalez *et al.* 2009). These primers were chosen as they would amplify small Polymerase Chain Reaction (PCR) products (*circa* 300 bp) compatible with the limited DNA source. Amplification was carried out in 30  $\mu$ l volume including 3  $\mu$ l DNA template, 200  $\mu$ M each dNTP, 200 nM each primer, 1x reaction buffer and 0.25 U HotMaster DNA polymerase (5-PRIME). A T3 thermocycler (Biometra) was used beginning with an initial denaturation at 94 °C for 1.5 min, followed by 34 cycles consisting of 30 sec at 94 °C, 45 sec at 46 °C and 45 sec at 65 °C, and a final extension step at 65 °C for 10 min. PCR were ExoSAP purified according to the supplier's recommendations and sequenced by Macrogen Inc., using the Big

Dye Sequencing protocol (Applied Biosystems 2012). Sequences were edited and aligned manually using Molecular Evolutionary Genetics Analysis (MEGA; Tamura *et al.* 2011). Taxonomic identification was based on: 1) Basic Local Alignment Search Tool (BLAST; Altschul *et al.* 1990) results against any nucleotide sequence available in Genbank (April 2013); and 2) k2p genetic distance between duckling sequences and the available sequence in Genbank for six species for which reproduction in Libya seems possible: Mallard (*Anas platyrhynchos*, Genbank accession number AF059142), Ferruginous Duck (EU585689), Common Shelduck (*Tadorna tadorna*, AF059173), Ruddy Shelduck (*T. ferruginea*, EU585727), White-headed Duck (*Oxyura leucocephala*, AY747868) and Marbled Duck (AF059164). In addition, a tree based on the Neighbor Joining method (NJ; Saitou and Nei 1987) was constructed using MEGA with 1,000 bootstraps to help in visualizing the results.

## RESULTS AND DISCUSSION

Two sequences of 255 nucleotides were produced. Both gave a perfect match with the Marbled Duck sequence with a BLAST (Altschul *et al.* 1990) total and maximum score of 473 (E value  $2e^{-130}$ ) and nil k2p distance value. In comparison, BLAST identities and k2p distances were 87-91% and 0.094-0.122 with other possible targets. These results are illustrated by a NJ k2p phylogenetic tree (Fig. 2) confirming that the ducklings were Marbled Ducks. The primer pair used in the present study might be useful for field degraded material from other bird species as both Marbled Duck and Greater Flamingo (*Phoenicopterus roseus*, used as positive control) amplified, and these species are taxonomically unrelated.

This study provides the first record of breeding Marbled Ducks in Libya. The nearest known breeding populations of Marbled Ducks are in Tunisia, where the species is a resident breeder, and also winters there in high numbers, especially on the edges of the Sahara (Isenmann *et al.* 2005). The Tunisia population is stable or slightly increasing. While Tunisian and Libyan wetlands are very similar, few Marbled Ducks have been found in Libya (Etayeb *et al.* 2007; Hamza *et al.* 2008; Environment General Authority and Regional Activity Centre for Specially Protected Areas 2012; Bourass *et al.* 2013). Libyan wetlands have not had comprehensive

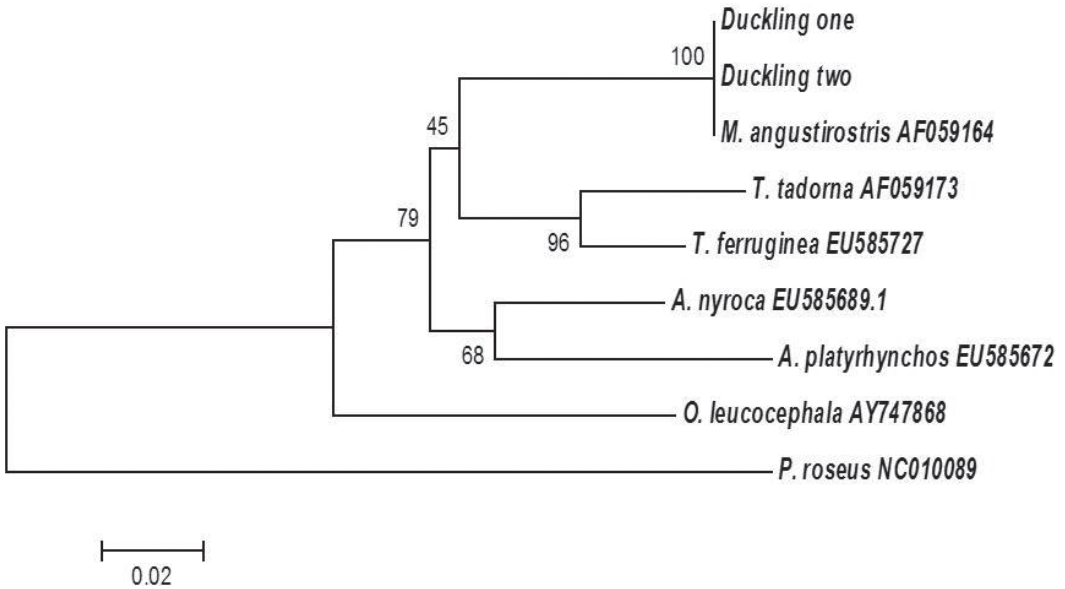


Figure 2. A neighbor joining tree of partial NADH dehydrogenase subunit 2 (ND2) sequences with 1,000 bootstraps showing the phylogenetic identity of the two ducklings found at Mallaha, Tripoli, Libya. Species compared were Marbled Duck (*Marmaronetta angustirostris*), Mallard (*Anas platyrhynchos*), Ferruginous Duck (*Aythya nyroca*), Common Shelduck (*Tadorna tadorna*), Ruddy Shelduck (*T. ferruginea*) and White-headed Duck (*Oxyura leucocephala*). The Greater Flamingo (*Phoenicopterus roseus*) was used as an out group. The scale at the bottom of the figure is representing the k2p distance. Values at nodes are the bootstrap support in percentage.

surveys and much more probably remains to be discovered; indeed, there are likely to be important unknown sites in Libya and overall in North Africa.

Over 50% of suitable Marbled Duck habitat may have been destroyed during the 20<sup>th</sup> century, and wetland drainage for agriculture occurs across its entire range. Hydrological work has severely affected breeding sites in Tunisia, Turkey, Morocco and Spain (Green and Hilton 1993). Many suitable habitats remain in Libya, whereas they have been widely destroyed in other places in the Mediterranean. The coastline of Libya has different types of Mediterranean wetlands such as lagoons, salt marshes, bays, lakes and islands (Environment General Authority and Regional Activity Centre for Specially Protected Areas 2012). Those wetlands provide shelter and foraging areas for wintering and migratory birds during their crossing from Europe and Asia to Africa (e.g., Etayeb *et al.* 2007, 2012). Bird community and feeding resources at the Mallaha site and on other Libyan wetlands match closely the

breeding habitat requirements of Marbled Duck. Several Libyan wetlands could, therefore, potentially become or could already be regular breeding sites for waterfowl.

This breeding evidence gives hope that Marbled Duck and maybe other duck species could be breeding in Libyan wetlands that have not been surveyed, especially desert oases. Therefore, surveys of Libyan wetlands and research are needed to improve knowledge of both duck breeding and wintering distributions and parameters in Libya and overall in North Africa (e.g., Hoffman *et al.* 1996; Bouchecker *et al.* 2009). Knowledge of North African waterbirds indeed needs to be improved to update information on population size and conservation status, notably to secure the future of threatened species, such as Marbled Duck.

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