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First Genetically Differentiation between *Upupa epops major* and *Upupa epops epops* (Family: Upupidae)

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HIGHLIGHTS

- Hoopoe has been traditionally treated as a single species.
- RAPD PCR and COI gene barcodes were used to separate hoopoes.
- This study suggests the separation of *Upupa epops major* into a new species.

Abstract: Hoopoe has been traditionally treated as a single species within the order Coraciiformes. Presently, however, various authors have suggested separating the hoopoe into two or more species and even its order, Bucerotiformes. So, this work aimed to use the RAPD PCR and DNA sequences of the COI gene barcodes to confirm and to assess whether the Egyptian hoopoe is a different species named *Upupa epops major* from the European hoopoe called *Upupa epops epops*, and to determine the relationships among them. Five primers were used in this technique. Two hoopoes were taken in this work as studying birds, migratory and resident one. The results showed the highest genetic distance between them using different random primers while genetic identity was in general low, overall primers. DNA fingerprinting detected greater genetic distance between *Upupa epops major* and *Upupa epops epops* and low genetic identity, this may indicate that both hoopoes fall into two separate species. Furthermore, using mitochondrial cytochrome oxidase subunit I (COI) sequences in this study suggests the separation of *Upupa epops major* into a new species.

Keywords: Bucerotiformes; DNA fingerprinting; genetic identity; Hoopoe; sequences.

INTRODUCTION

Random amplified polymorphic DNA (RAPD) developed by Welsh and McClelland [1] and Williams and coauthors [2], the methodology proved to be a powerful tool in different genetic analyses. This approach detects DNA polymorphisms based on amplification using a single primer of arbitrary nucleotide sequence

of genomic DNA fragments. RAPD markers are attractive because they are specific and quick, nanograms of DNA are required, automation is feasible, and there is no requirement for previous DNA sequence information Williams et al. [2], modest coast and ability to detect relatively small amounts of genetic variation [3]. DNA (RAPD) or arbitrarily primed PCR fingerprinting gave an advantage in which molecular preliminary information of the species studied is not necessary and polymorphism pattern obtained usually varies among the species [4].

DNA barcode is a short sequence of standardized genomic region of mtDNA that is specific to a species. For the identification of most animal species a certain fragment of the mitochondrial gene COI, coding for a subunit of the enzyme cytochrome oxidase, has become widely known and used as ‘‘the DNA barcode’’ [5-9]. It has succeeded in the identification of the phylogeny of many animal groups, including birds [6, 10, 11]. Also, it has perfected successfully in the identification of the phylogeny of many animal groups, including birds [10-15]. Genetic studies on the mitochondrial gene can be used to resolve the phylogenetic relationships of high-level category [16], and also has a better resolution on the genetic relationship of subfamily, genus, inter-species and even the genetic structure of populations [17]. Rather surprisingly, as useful as these studies are, to date there has been no research regarding the mitochondrial gene sequencing and phylogenetic studies of *Upupa epops major* and *Upupa epops epops* to confirm the validity of the hypothesis that the *Upupa epops major* is the Egyptian resident form of the European hoopoe, or they are two different species.

Hoopoes are one of the most distinctive birds in the world. A migratory species, they are found in season throughout most of Europe, Asia and Africa and cannot be mistaken for anything else within that range. The hoopoe has been traditionally treated as a single species within the order Coraciiformes. Recently, however, various authors have suggested separating the hoopoe into two or more species and even its order, Bucerotiformes [18, 19].

The study aimed to use the RAPD PCR and DNA sequences of the COI gene barcodes: to confirm and to assess whether the Egyptian Hoopoe is a different species named *Upupa epops major* from the European Hoopoe called *Upupa epops epops*, and to determine the relationships among them.

MATERIAL AND METHODS

Sample and extraction of DNA

Fresh tissue samples of European Hoopoe *Upupa epops epops* (migratory Hoopoe) and Egyptian Hoopoe *Upupa epops major* (resident Hoopoe) (Figure 1) will be preserved in 100% ethanol for further molecular studies. DNA will be extracted from these samples using a GeneJET™ kit Genomic DNA Kit#K0721 following manufacturer’s protocol. All animal experiments involved in this study were approved by the Laboratory Animal Welfare and Animal Experimental Ethical Committee of IACUC Cairo University. We followed the guidelines of the Committee for experimental animals during this study.

RAPD-PCR Procedures

Five primers generated clear and reproducible bands: primer 13-16 (CAG GCC CCG AAC AAT), primer 27-16 (GCA CGC ATG GTT TGC), 28-16 (CCC CGA GAA GCC TGA), primer 29-16 (CCC GCG GCC TAT GAG) and primer 30-16 (CCA GGG TGA GCG GCT).

PCR technique was carried out in 0.2 µL microfuge tubes. The total volume consisting of reaction mixture was 25 µL consisting of 19.8 µL sterile distilled water, 2.5 µL 10x PCR optimize buffer, 0.5 µL 25 mM deoxyribonucleotide phosphate, 10 pmol primers, 0.2 µL of 0.5 units *Taq* DNA polymerase. The solution mixture was placed in the thermal cycler and subjected to 45 cycles. The cycling conditions were as follows; predenaturation at 94°C for 3 minutes, denaturation at 94°C for 1 minute, annealing at 36°C for 1 minute, and extension at 72°C for 5 minutes, with a final extension at 72°C for 7 minutes at the end of 45 cycles. The amplified products were electrophoresed in1% agarose gel with 0.5x TBE buffer. After the gel had been stained with ethidium bromide, the fragment sizes were estimated using a 100-bp ladder and band patterns were visualized with a UV transilluminator.

PCR amplification and sequencing

Amplification of the COI gene fragments was carried out using the primer, BF1 (5’ TTC TCC AAC CAC AAA GAC ATT GGC AC 3’) and BR1 (5’ACG TGG GAG ATA ATT CCA AAT CCT G 3’). The 20 µL PCR reaction mix included 50 ng of genomic DNA template; 13.44 µl sterile ultrapure water, 2.0 µL of 10X buffer,

1.0 µL of MgCl, 0.8 units of Taq DNA polymerase, 0.4 µl of each forward and reverse primer and 2.0 µL of DNA template. The PCR amplification program consisted of 3 min at 94°C followed by five cycles of 35 sec at 94° C, 40 sec at 56° C and 35 sec at 72° C, followed by another 30 cycles of 35 sec at 94° C, 40 sec at 58° C, and 35 sec at 72° C, and finally 7 min at 72° C. The PCR products were visualized in 1.0% agarose gels and staining with ethidium bromide to visualize bands and viewed with an ultraviolet light source. A GeneJET™ kit (Thermo K0701) was used to purification the amplified PCR products according to the manufacturer's protocol. An ABI 3730xl DNA sequencer was used to perform sequencing of amplified PCR products.

Data analysis

RAPD patterns were analyzed and scored from photographs. For the analysis and comparison of the patterns, a set of distinct, well separated bands were selected. Band sharing (BS) between European hoopoe, *Upupa epops epops* and Egyptian hoopoe, *Upupa epops major* was calculated according to the formula given by Nei and Li [20]: $Bs_{ij} = 2\ N_{ij} / (N_i + N_j)$, where N_{ij} is the number of common bands observed in individuals i and j, and N_i and N_j are the total numbers of bands scored in individuals i and j respectively, with regard to all assay units. Thus, BS reflects the proportion of bands shared between two individuals and ranges from zero (no common bands) to one (all bands identical). Genetic distance (GD) was calculated as: GD = 1- Bs [21]. Genetic identity computed according to the equation [22]:

$$I = 1/N \sum_{i=1}^{N} 2V_i^{(1)}.\ V_i^{(2)} / (V_i)^2 + (V_i)^2$$

Where, *N* is the number of different bands in two given breeds and $V_i^{(1)}$ and $V_i^{(2)}$ are the frequencies of band *i* in the two breeds, respectively.

All mtDNA nucleotide sequences obtained in this work were aligned by using the Clustal W software and identical sequences were considered as the same haplotype. The Maximum Likelihood phylogenetic tree was constructed by calculating the distance matrix of different studied species through MEGA v.5.05 software [23]. Bootstrap values were used to estimating the support for tree nodes with 500 replicates.

RESULTS

RAPD analysis

In the present study, five oligonucleotide primers of random sequences were used to amplify the DNA from 20 specimens and a total of 230 scorable RAPD bands were obtained. The DNA fragments amplified in 20 specimens using primer 13-16, 27-16, 28-16, 29-16 and 30-16 have been presented in (Figure 2). The number of bands varied among species from 25 to 60 bands. The number size ranged between 184 bp and 1680 bp length. Primer 30-16 gave the maximum number of bands 60, while the minimum number of bands 25 was recorded with primer 27-16 in the studied species (Table 1).

The Random amplified polymorphic DNA (RAPD) fingerprint was used for detection of the genetic diversity between *Upupa epops major* and *Upupa epops epops*. The results showed a high genetic distance range (0.17 to 1.00) with an average of 0.600 using different random primers. Band sharing indices between studied species using different primers are presented in Table 1. A high level of band sharing was found between *Upupa epops major* and *Upupa epops epops* with the primer 30-16. A low level of band sharing was found between studied species with both of the primers 27-16 and 28-16. The average of band sharing was in general low, with overall primers of 0.400. The results showed a genetic identity range (0 to 0.821) with an average of 0.428 (Table 1).

Sequencing analysis

COI barcodes were recovered for a total of twenty specimens (Seven from *Upupa epops epops* and thirteen from *Upupa epops major*) from the family Upupidae. No insertions/deletions, heterozygous sites or stop codons were discovered, accepting the view that all of the amplified sequences form functional mitochondrial COI sequences.

To investigate and recognize created sequences, each was blast searched as a request through NCBI (National Center for Biotechnology Information) Blastn tool (www.ncbi.nlm.nih.gov/BLAST/). Sequences of *Upupa epops epops* with better hits were recovered and used for further comparison to COI gene sequences

from the current study. On the other hand, there is a lack of sequences for *Upupa epops major*. The resulted COI gene sequences of Upupidae were submitted to the GenBank (NCBI) and the accession numbers of *Upupa epops epops* were GQ482885, JF498815, KP252253, KP252254 and KY661879 and were used for constriction of phylogram and genetic distance detection.

The average nucleotide frequencies of *Upupa epops epops* are 26.3% (A), 25.1% (T/U), 31.7% (C) and 16.9% (G). The percent composition of nucleotide varied from 26.0 to 26.4% % (A), 24.8 to 25.6% (T), 31.3 to 31.9% (C), and 16.6 to 17.2% (G), which indicate that the COI gene sequence of these species, are C rich and poor in T, A and G. While the average nucleotide frequencies of *Upupa epops major* are 26.4% (A), 25.2% (T/U), 32.2% (C) and 16.2% (G), which indicate that the COI gene sequence of these species, are C rich and poor in T, A and G.

Genetic distance was calculated between the species belonging to Upupidae. Distances calculated between specimens of *Upupa epops epops* showed that very smallest differences (from 0.000 to 0.022) and within specimens of *Upupa epops major* are 0.000 whereas the highest genetic distance detected between *Upupa epops epops* and *Upupa epops major* amounted to 0.508 (Table 2).

The phylogenetic tree of the studied species of Upupidae was constructed using the Maximum Likelihood method, based on COI sequences (Figure 3). In the mitochondrial tree, a total of five unique haplotypes were identified in sequences from the COI gene of twenty specimens of Upupidae. The haplotypes of *Upupa epops epops* and *Upupa epops major* were deposited together in the phylogram and appeared as a sister group. Phylogenetic tree based on COI sequences data, two main clades were produced: in the first clade, *Upupa epops epops*. In the second branch, *Upupa epops major* was laid.

Table 1. Total number of band, polymorphic bands, genetic distance, band sharing and genetic identity between *Upupa epops epops and Upupa epops major* using different RAPD primers.

Primer No.	Total bands	Polymorphic bands	Genetic distance	Band sharing	Genetic identity
13 - 16	45.00	15.00	0.33	0.67	0.717
27 - 16	25.00	25.00	1.00	0.00	0.000
28 - 16	40.00	40.00	1.00	0.00	0.000
29 - 16	60.00	30.00	0.50	0.50	0.606
30 - 16	60.00	10.00	0.17	0.83	0.821
Average	———	———	**0.600**	**0.400**	**0.428**

Table 2. Total genetic distance between hoopoes' species.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	P. d. (Out Group)	0.000															
2	U. e. e. 1	0.654	0.000														
3	U. e. e. 2	0.641	0.022	0.000													
4	U. e. e. 3	0.641	0.022	0.000	0.000												
5	U. e. e. 4	0.641	0.022	0.000	0.000	0.000											
6	U. e. e. GQ482885	0.641	0.022	0.000	0.000	0.000	0.000										
7	U. e. e. JF498815	0.643	0.020	0.002	0.002	0.002	0.002	0.000									
8	U. e. e. KP252253	0.641	0.022	0.000	0.000	0.000	0.000	0.002	0.000								
9	U. e. e. KP252254	0.641	0.022	0.000	0.000	0.000	0.000	0.002	0.000	0.000							
10	U. e. e. KY661879	0.641	0.022	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000						
11	U. e. e.	0.641	0.022	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000	0.000					
12	U. e. m. 1	0.588	0.508	0.508	0.508	0.508	0.508	0.509	0.508	0.508	0.508	0.508	0.000				
13	U. e. m. 2	0.588	0.508	0.508	0.508	0.508	0.508	0.509	0.508	0.508	0.508	0.508	0.000	0.000			
14	U. e. m. 3	0.588	0.508	0.508	0.508	0.508	0.508	0.509	0.508	0.508	0.508	0.508	0.000	0.000	0.000		
15	U. e. m. 4	0.588	0.508	0.508	0.508	0.508	0.508	0.509	0.508	0.508	0.508	0.508	0.000	0.000	0.000	0.000	
16	U. e. m.	0.588	0.508	0.508	0.508	0.508	0.508	0.509	0.508	0.508	0.508	0.508	0.000	0.000	0.000	0.000	0.000

U. e. e. refer to *Upupa epops epops* while U. e. m. is *Upupa epops major* and P. d. is *Passer domesticus*. Specimen's number denotes the accession number of NCBI database.

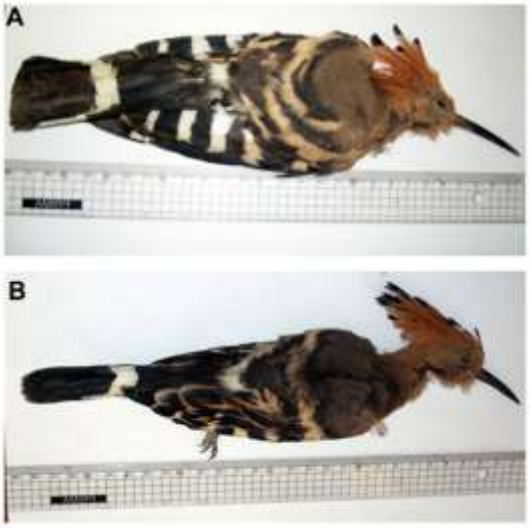

Figure 1. Hoopoe species, A: *Upupa epops epops* and B: *Upupa epops major*, specimens caught from Coast of Damietta, Egypt.

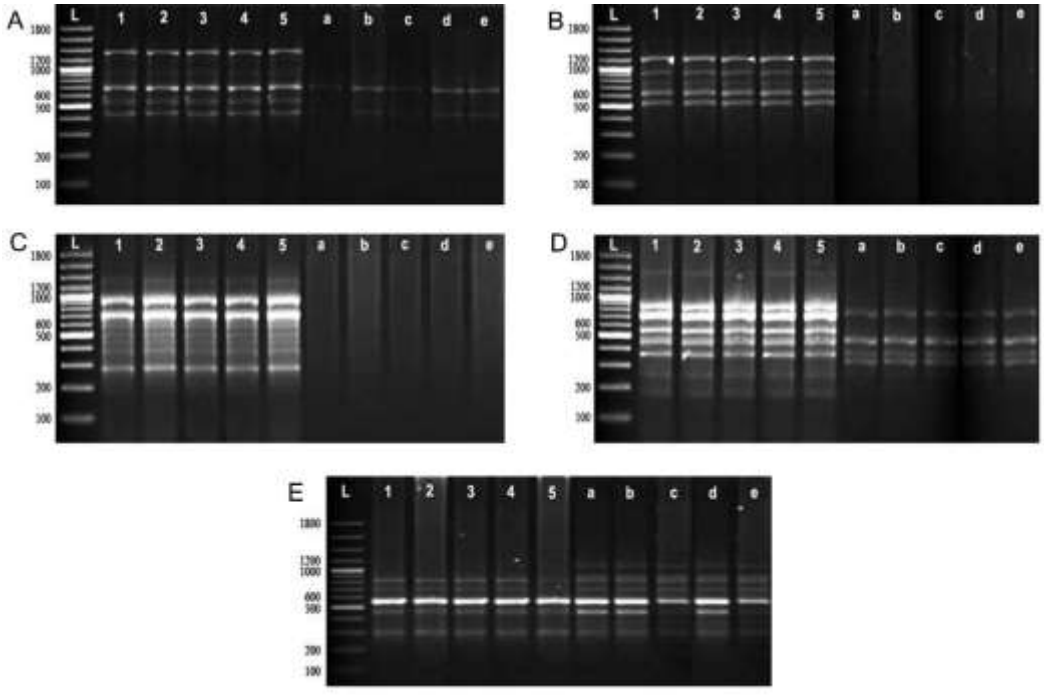

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Figure 2. RAPD amplification products; A: primer 13-16, B: primer 27-16, C: primer 28-16, D: primer 29-16 and E: primer 30-16. Lane L: DNA marker, lanes from 1 to 5 are *Upupa epops major* and lanes a, b, c, d and e are *Upupa epops epops*.

Figure 3. Phylogenetic tree of Maximum Likelihood analyses based on the COI gene from *Upupa epops epops* and *Upupa epops major* using Kimura 2-Parameter distances and values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI database, rooted with *Passer domesticus*.

DISCUSSION

The main advantages of RAPD markers are the possibility of working with anonymous DNA and the relatively low expense, also fast and simple to produce RAPD marker [24-26]. Moreover, RAPD analysis might be useful for systematic investigation at the level of species and subspecies, and more sensitive and technically easier to perform and produced results with low statistical error.

Bader [27] reported that divergent populations or separate species tend to have genetic identities (I) < 0.8 and genetic distances (GD) > 0.2 whereas DNA fingerprinting detected greater genetic distance between European and Egyptian hoopoes was 0.60 and genetic identity was 0.428 which may indicate that both hoopoes were two separate species.

In the past, the systematic position of many monotypic genera of birds was dubitable. This is because the genera were not based on phylogenetic studies but on subjectively deduce evolutionary specialty, which in turn was based on phenotypic distinctiveness and commonly their discrete location. Indeed, some taxonomists have grouped large numbers of species in monotypic genera but the introduction of molecular phylogenetic methods in bird taxonomy exposed that many of these were nested within other genera [28-30]. DNA barcoding using mitochondrial COI sequences has enormous potential of discriminating closely related species across diverse phyla in the animal kingdom [5,6].

COI constructed Maximum Likelihood tree placed *Upupa epops epops*, *Upupa epops major* and *Passer domesticus* (as an outgroup of this study) in three different clades due to these are three distinct species. While *Upupa epops epops* and all deposited COI sequences from GenBank formed high bootstrap-supported clusters without any overlap between species and genetic distances among them were high as well supported with RAPD analyses. For this reason, the present results supported phylogenetic position of *Upupa epops major* outside the *Upupa epops epops* clade and give a new hypothesis that *Upupa epops major* and *Upupa epops epops* should be considered in two separate species.

CONCLUSION

This is the first study to detect the genetic relationship between *Upupa epops epops* and *Upupa epops major*. Furthermore, using mitochondrial cytochrome oxidase subunit I (COI) sequences in this study suggests the separation of *Upupa epops major* into a new species. Moreover, the COI sequencing technique developed in this study was proved to be a simple, reliable and rapid method for differentiating closely related taxa and considered a useful source of phylogenetic data.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Welsh J, McClelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic. Acids Res. 1990;18(24):7213–8.
2. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic. Acids Res. 1990;18(22):6531–5.
3. Ragot M, Hoisington DA. Molecular markers for plant breeding: comparisons of RFLP and RAPD genotyping costs. Theor. Appl. Genet. 1993;86(8):975–84.
4. Zulkifli Y, Alitheen NB, Son R, Raha AR, Samuel L, Yeap SK, Nishibuchi M. Random amplified polymorphic DNA-PCR and ERIC PCR analysis on Vibrio parahaemolyticus isolated from cockles in Padang, Indonesia. Int. Food Res. J. 2009;16:141-50.
5. Hebert PD, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA barcodes. Proc. Biol. Sci. 2003a;270(1512):313–21.
6. Hebert PD, Ratnasingham S, deWaard JR. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proc. Biol. Sci. 2003b;270:S96–S99.
7. Hebert PD, Stoeckle MY, Zemlak TS, Francis CM. Identification of Birds through DNA Barcodes. PLoS Biol. 2004a;2(10):e312.
8. Cai Y, Yue B, Jiang W, Xie S, Li J, Zhou M. DNA barcoding on subsets of three families in Aves. Mitochondrial DNA 2010;21(3-4):132–7.
9. Breman F, Jordaens K, Sonet G, Nagy Z, Houdt JV, Louette M. DNA barcoding and evolutionary relationships in Accipiter Brisson, 1760 (Aves, Falconiformes: Accipitridae) with a focus on African and Eurasian representatives. J. Ornithol. 2012;154:265-87.
10. Kerr KC, Stoeckle MY, Dove CJ, Weigt LA, Francis CM, Hebert PD. Comprehensive DNA barcode coverage of North American birds. Mol. Ecol. Notes 2007;7(4):535–43.
11. Johnsen A, Rindal E, Ericson P, Zuccon D, Kerr K, Stoeckle M, Lifjeld J. DNA barcoding of Scandinavian birds reveals divergent lineages in trans-Atlantic species. J. Ornithol. 2009;151:565-78.
12. Hebert PD, Penton EH, Burns JM, Janzen DH, Hallwachs W. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. USA. 2004b;101(41):14812–7.
13. Kerr KC, Birks SM, Kalyakin MV, Red'kin YA, Koblik EA, Hebert PD. Filling the gap - COI barcode resolution in eastern Palearctic birds. Front. Zool. 2009;6:29.
14. Yang C, Xiao Z, Zou Y, Zhang X, Yang B, Hao Y, Moermond T, Yue B. DNA barcoding revises a misidentification on musk deer. Mitochondrial DNA. 2015;26(4):605–12.
15. Yoo HS, Eah JY, Kim JS, Kim YJ, Min MS, Paek WK, Lee H, Kim CB. DNA barcoding Korean birds. Mol. Cells. 2006;22(3):323-7.
16. Simon C, Buckley TR, Frati F, Stewart JB, Beckenbach A. Incorporating molecular evolution into phylogenetic analysis, and a new compilation of conserved polymerase chain reaction primers for animal mitochondrial DNA. Annu. Rev. Ecol. Evol. Syst. 2006;37:545-79.
17. Zhou Z, Ye H, Huang Y, Shi F. The phylogeny of Orthoptera inferred from mtDNA and description of *Elimaea cheni* (Tettigoniidae: Phaneropterinae) mitogenome. J. Genet. Genomics. 2010;37(5):315-24.
18. Yuri T, Kimball RT, Harshman J, Bowie RC, Braun MJ, Chojnowski JL, et al. Parsimony and model-based analyses of indels in avian nuclear genes reveal congruent and incongruent phylogenetic signals. Biology (Basel). 2013;2(1):419-44.
19. Jarvis ED, Mirarab S, Aberer AJ, Li B, Houde P, Li C, et al. Whole-genome analyses resolve early branches in the tree of life of modern birds. Science. 2014;346(6215):1320-31.

20. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 1979;76(10):5269–73.
21. Bártfai R, Egedi S, Yue G, Kovács B, Urbányi B, Tamás G, et al. Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. Aquaculture. 2003;219:157-67.
22. Kuhnlein U, Dawe Y, Zadworny D, Gavora JS. DNA fingerprinting: a tool for determining genetic distances between strains of poultry. Theor. Appl. Genet. 1989;77(5):669-72.
23. Amura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 2011;28(10):2731-9.
24. Jui-Jane LT, Jo-Chen C, Hsiang-Chi H, Der-Yuh L, Chein T. Analysis genetic similarity in inbred Tiawan native chicken by random amplified polymorphic DNA. J. Agric. Assoc. China. 1999;186: 89-98.
25. Kumar K, Kumar S, Ahlawat S, Kumar PR, Singh R, Bisht G. Randomly amplified polymorphic DNA markers in genetic analysis of relatedness in Japanese quail lines. Indian J. Anim. Sci. 2000;70:604-7.
26. Bednarczyk M, Siwek M, Mazanowski A, Czekalski P. DNA polymorphism in various goose lines by RAPD-PCR. Folia Biol. (Krakow). 2002;50(1-2):45-8.
27. Bader J. Measuring genetic variability in natural populations by allozyme electrophoresis. Case Western Reserve University, Ohio.1998.
28. Johnson KP, De kort S, Dinwoodey K, Mateman AC, Cate C, Lessells CM, et al. A molecular phylogeny of the dove genera Streptopelia and Columba. Auk. 2001;118:874-87.
29. Gibson R, Baker A. Multiple gene sequences resolve phylogenetic relationships in the shorebird suborder Scolopaci (Aves: Charadriiformes). Mol. Phylogenet. Evol. 2012;64(1):66-72.
30. Alström P, Jønsson KA, Fjeldså J, Ödeen A, Ericson PG, Irestedt M. Dramatic niche shifts and morphological change in two insular bird species. R. Soc. Open Sci. 2015;2(3):140364.