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GENETIC DISTANCES AMONG EIGHT ORNAMENTAL CHICKENS BY MOLECULAR TECHNIQUE

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ABSTRACT

The aim present study is to determine genetic distances among three local ornamentals with five commercial ornamental chickens using ten RAPD primer and specific mitochondrial DNA locus. Twenty RAPD primers were used; ten out of them were selected based on their number of bands (NB) and polymorphic characteristics. A total of 66 bands observed, 55 of them are polymorphic bands among chicken breeds, whereas overall bans 13 of them are unique bands whose highest unique band was obtained in primer OPA-20 and OPM-06. RAPD-PCR dendrograms show that the distance among ornamental chicken arrived 50.29% and phylogenetic dendrograms showed that two clusters, the first cluster branch consisted black and black-white local ornamental chicken, Silver Polish and Germany Phoenix Bantam with 25.59% distance between them while the second cluster also including local black-barred, American white brahman, Sultan Belgian, and American Lamborghini with 17.83 % genetic distance between them. The mitochondrial DNA dendrograms also show two main clusters among local and commercial ornamental chickens, the local black and black-white are in the first cluster while the local black-barred located at other clusters. It was concluded that the black, black-white was closer to each other. The high genetic distance 55.3% among breeds and variation in phenotypic shapes with different colors for ornamental chickens indicates that these local ornamental chickens have a good amount of genetic resources to made genetically improvement in further and it means the three local ornamental chickens are independent breeds.

Disciplinary: Multidisciplinary (Animal Sciences (Poultry Science), Biological Sciences (Genetics Science)).

1. INTRODUCTION

The fowl date of domestication of fowl is about 2000 B.C in history and, in this during a long

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time, modifying breeds of fowl were expended to requirement the multipurpose need of meat and eggs. Depend on many studies carried out, it is found that all commercial chicken is descendent of the red jungle fowl, Gallus gallus [1-3]. The biotechnology technique developed from RAPD analyses has previously been used to make molecular diversity for zebrafish and honeybees and a Z chromosome map of chickens[4]. In recent years most of the native chicken in eradication by crossing with ecotypes this issue has been caused to more attention to saving biodiversity among poultry breeds [5]. The molecular marker of RAPD is used as fingerprint technique patterns for chicken, duck, turkey, and goose meats. The chicken meat was tested by the RAPD method to establish the effectiveness and specificity each species of chicken's DNA samples [6, 7]. For finding out of biodiversity between Saudi chicken strains and other species of Gallus gallus has been used Mitochondrial Cytochrome b (Cyt b) which is efficient tool, not only the cytochrome c oxidase I but also, for separating among species has been used the short region of Cyt b gene was successfully accepted as a standard region in DNA barcoding, which would be supported efforts to construct a new library beside COI library [8, 9]. Cyt b has been considered one of the most useful genes for phylogenetic work and is used as a poultry DNA barcode tool, the evolutionary dynamics and Cyt b levels of genetic divergence typically associated with sister species, congeners, and confamilial genera usually are in a range in which the Cyt b gene is phylogenetically instructional and unlikely to be severely compromised by impregnation influence involving layover nucleotide shifts [8, 10, 11]. The aim this study is regional patterns of genetic diversity of ornamental chicken populations from Iraqi Kurdistan region, America and Europe were assessed using 10 oligonucleotide primers and partial gene of mitochondrial Cyt b.

Figure 1: Image of all ornamental chickens includes; 1: Local Black; 2: Local Black and White; 3: Local Black Barred;4: American White Brahman;5: Silver Polish; 6: Germany Phoenix Bantam; 7:Sultan Belgian and 8: American Lamborghini.

2. MATERIALS AND METHODS

2.1 SAMPLE COLLECTION AND DNA EXTRACTION

Collection of blood samples and genomic DNA isolation 40 blood samples from each eight breeds in which the first 3rd breeds of them are native Iraqi Kurdistan region were collected, five last other breeds are related to different country ornamental Gallus gallus [12, 13], Figure 1. Then, the genomic DNA was isolated from each blood sample using QIAamp® DNA Blood Mini Kit, QIAGEN GmbH Qiagenstr.140724 Hilden Germany were examined using spectrophotometric analysis based on 260 and 280 nm absorbance and agarose gel electrophoresis analysis. DNA samples were stored at -22 C for use [7, 14].

2.2 RANDOM AMPLIFIED POLYMORPHIC DNA – POLYMERASE CHAIN REACTION (RAPD-PCR)

For RAPD assay, a total of eight samples were investigated by 10 sets of RAPD primers (Table 1) were acquired from a study and were used to initiate the RAPD-PCR amplification[7, 15]. The primers have chosen, depend on the basis of GC content and base-pairing temperature for RAPD-PCR amplification. Amplifications were performed using a thermal cycler (MJ RESEARCH-PTC-200 Gradient Peltier Thermal Cycler ® 60- Well) with the final reaction volume of 20 µL. Two µL sample DNA was added to each tube to make the final volume (20 µL). Each reaction contained: 11 µL of Red Master Mix (AMPLIQON A/S Stenhuggervej 22-Germany), 25 Units/mL Taq polymerase, each dNTPs is 200 µM and MgCl2 was 1.5 mM), 2 µL of RAPD primer (197.13 µM–599.26 µM), 2 µL (30 ng) of DNA template and 5 µL of DNase free water. Many protocols were used but only one protocol gives as clearly bands. The primers (OPA-04, OPA-20, OPB-01, OPM-06, OPM-20, OPN-16, OPP-04, OPQ-03, OPQ-07 and 10 MER): programmed for 35 cycles of denaturation at 95 ℃ for 1 min, annealing at 37- 40 ℃ for 1 min and extension at 72 ℃ for 1.5 min. An initial denaturation step of 5 min at 95 ℃ and a final extension step of 7 min at 72 ℃ were included in the first and last cycles, respectively. The PCR amplification products were run in a 1.5% agarose gel (Staining with Ethidium bromide in Tris-borate EDTA buffer) and visualized under UV transillumination. The control reactions were set up without genomic DNA to avoid any DNA contamination (Figure 2) [12, 15, 16].

2.3 RAPD PHYLOGENETIC ANALYSIS

The presence and absence of bands within each RAPD pattern were scored as 1 and 0, which were used to assess the genetic distance among populations. The presence or absence of bands in each RAPD pattern was recorded using RAPD molecular technique [1, 15]. The genetic distances and genetic similarities among different varieties of chickens between local strain (Black local chicken, black white local chicken, Barred local chicken) and foreign strain (American White Brahman, Silver polish chicken, Germany Phoenix Bantam Black, Sultan Belgian chicken, American Lamborghini chicken). The Jaccard's similarity coefficient for genetic diversity among isolates and evaluated for pairwise comparisons based on the proportion of shared bands produced by the primers. The similarity matrix was subjected to cluster analysis by an unweighted pair group method for arithmetic mean (UPGMA) and a dendrogram was generated [17].

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2.4 MITOCHONDRIAL DNA(CYTOCHROME B GENE) POLYMERASE CHAIN REACTION AND GEL ELECTROPHORESIS

A pair of universal primers were used to amplify the partial sequence of the mitochondrial cytochrome b gene. Primers' sequences were as follows: L14816 (5´-CCA TCC AAC ATC TCAGCA TGA TGA AA-3´), H15173 (5´-CCC CTC AGA ATG ATA TTT GTC CTC A-3´)which amplified 340 bp [18]. Reactions of PCR amplification have performed in a whole volume of 25 µL. Each reaction mixture contained: 12.5 µL AMPLIQON ® Master mix (A/S Stenhuggervej 22-Germany), 1 µL of each primer (10 µM), 8.5 µL RNAse/DNase free and 2 µL of DNA. PCR was carried out in a professional thermal cycler (MJ RESEARCH-PTC-200 Gradient Peltier Thermal Cycler ® 60- Well). The cycling conditions included a single initial denaturation at 95°C for 5 min followed by 35 cycles of 94°C for 30s (denaturation), 53.6°C for 45 s (annealing), 72°C for during 45 s (extension) and a final extension step at 72°C for during 7 min. PCR products (10 µl) were separated by 2% agarose gel electrophoresis at 80 V for 45 min. A 100 bp plus DNA ladder (Fermentas, Thermo Fisher Scientific, USA) have used to determine sizes of the band products. The resulting DNA fragments were visualized by UV transillumination (Figure 3) [1, 8, 10, 18]

2.5 MITOCHONDRIAL DNA (CYTOCHROME B GENE) PHYLOGENETIC ANALYSIS

The DNA sequence was analyzed by Molecular Evolutionary Genetics Analysis (MEGA) software version X. [19]for sequence similarity among of eight sets of cytochrome b gene sequences of G. gallus to be find genetic distance between three sets of cytochrome b gene sequences ornamental Gallus native strains with other five-set of cytochrome b gene sequences ornamental Gallus foreign stains. Then for more emphasis, imported eight sets of partial cytochrome b gene sequences into nucleotide BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to comparative alignment and phylogenetic analyses to retrieve similar and dissimilarity among of eight sets of cytochrome b gene sequences [20, 21].

2.6 DNA PURIFICATION AND DNA SEQUENCING

Eight DNA fragment was excised from the gel using sterile, sharp cutter and purified using Zymoclean Gel DNA Recovery Kits (ZYMO RESEARCH, USA), according to the manufacturer's instructions. Purified products were directly sequenced using both the forward and reverse primers of PCR amplification. The sequencing process was performed by the Korean Macrogen Company.

3. RESULTS

3.1 RAPD-PCR RESULTS

The study results included the RAPD-PCR amplification and molecular distance analysis for ten short oligonucleotides primers in the RAPD technique in all chicken samples under study.

3.1.1 NUMBER (NB) AND SIZE OF BANDS (BP)

The total of primers amplified is appeared clear bands and was to investigate the genetic variations among the eight ornamental chicken breeds. A total of primers was polymorphic overall chicken samples (Figure 2). The overall NB for the 10 oligonucleotides primes were 66 bands, ranged from 4 in primer OPP-4 to 12 bands in OPA-20 (Table 1). Results in this study were higher than reported by [14] have found 187 bands from 12 RAPD primers.

In Table 1, the bands' size range over all the chicken started from 150 bp and ended at 1500 bp.

The smallest size of bands has recorded for OPP-04 (150 bp) in all chickens, while the highest size bands range was recorded for primer OPQ-03 locus (1500 bp) in Sultan Belgian and American Lamborghini chicken. Similar results were reported by [22], (2001) in [17] were the size range of band ranging from 237 to 3240/ bp.

3.1.2 NUMBER OF POLYMORPHIC BANDS (NPB)

The overall % polymorphism band for 66 bands in this study was 79.46 and 55 of 66 bands are polymorphic bands; among the primers, the OPA-20 have higher band with higher polymorphic bands has arrived 100% (Table 1). Depended on above results, there is a possibility to recommended that these loci can be used to define genetic distances among the present native and commercial ornamental chickens. The results have agreed with than reported by [14] in Saudi and [16] in Bangladeshi chickens.

3.1.3 UNIQUE BANDS

From ten oligonucleotides short primers seven of them have given a unique band. Overall unique bands from 66 bands got 13 bands for all ten RAPD primers. The highest numbers have obtained by primer OPA-20 (From 12 bands 3 of them are unique bands, ranging from 400 to 950 bp) and primer OPM-06 (From 8 bands 3 of them are unique bands, ranging from 350 to 750 bp) in Local Black Barred and American White Brahman ornamental chickens breed.

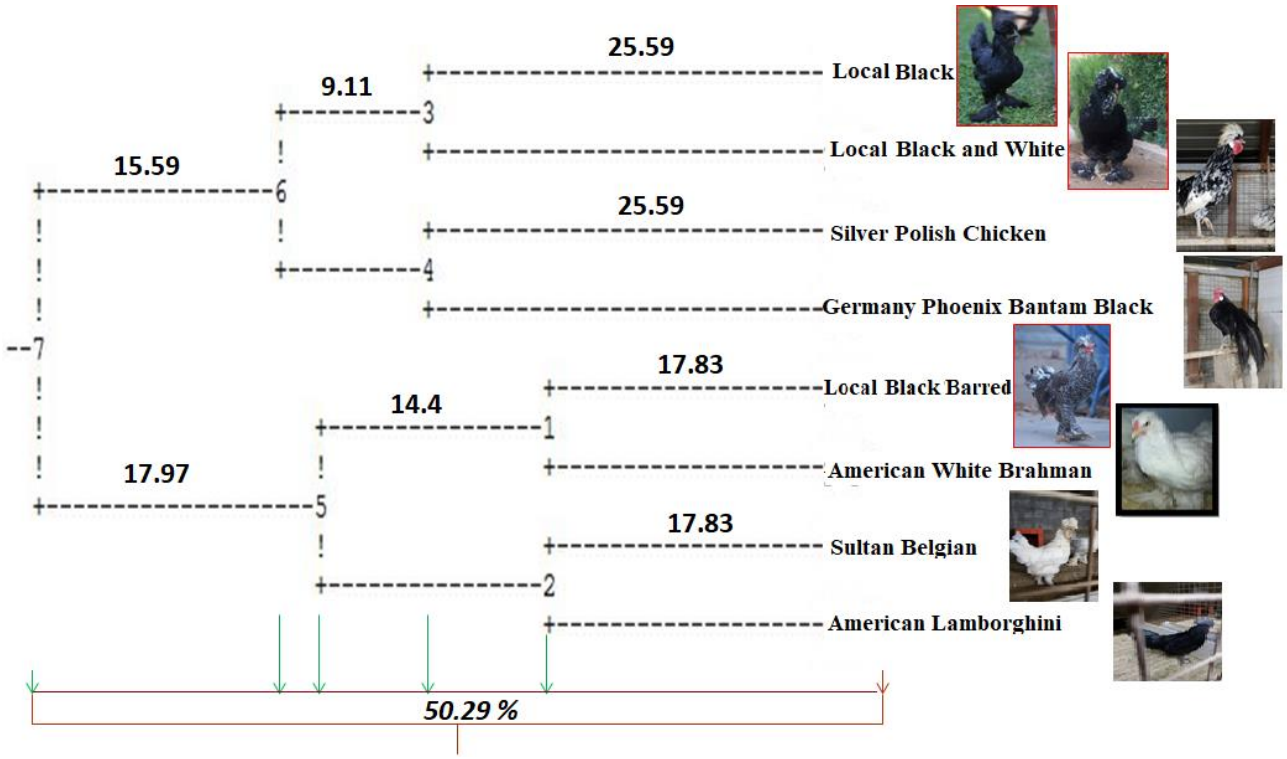

Figure 2: UPGMA dendrogram clarifies the differentiation between the eight chicken breeds.

3.2 PHYLOGENETIC TREE

As in the dendrograms (Figure 2), the overall molecular genetic distance among local and commercial ornamental chicken breeds arrived 50.29% and two clusters were found, the 1st cluster branch consisted of two sub-cluster, the first subcluster including black and black and white local ornamental chicken with 25.59% distance between them, while the second sub-cluster including the Silver Polish and Germany Phoenix Bantam with 25.59% distance between them, the overall

genetic distance between this two sub-clusters arrived 9.11%.

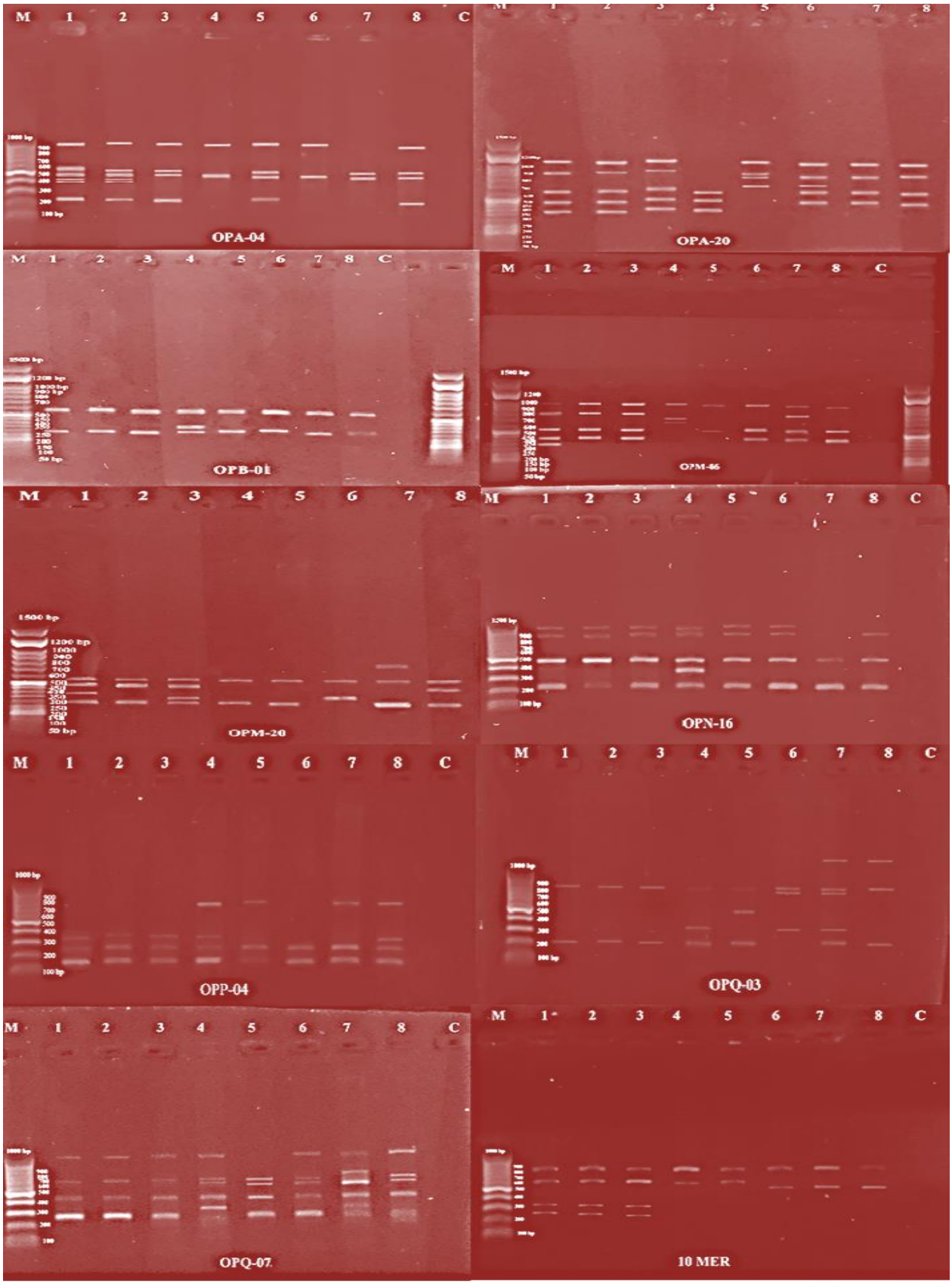

Figure 3: Gel electrophoresis for 10 RAPD- Primers ornamental chickens. Where: 1: Local Black; 2: Local Black and White; 3: Local Black Barred;4: American White Brahman;5: Silver Polish; 6: Germany Phoenix Bantam; 7:Sultan Belgian and 8: American Lamborghini and C is the control.

The second cluster also has two sub-clusters, the first subcluster including Local Black Barred

and American White Brahman ornamental chicken with 17.83% distance between them, while the second sub-cluster including the Sultan Belgian and American Lamborghini with 17.83 % distance between them, the overall genetic distance between this two sub-clusters arrived 14.4%.

These results indicated that the black and black and white local ornamental chicken breed is most genetically distant from the Local Black Barred breeds (50.29 %), and the results indicated that the Local Black Barred breed was closer to White Brahman ornamental chicken (17.83 %) than to the local breeds and Silver Polish with Germany Phoenix Bantam. Similar results were reported by [23] in Comparison of Egyptian and Saudi Local Chickens chicken.

3.2.1 AMPLIFICATION OF CYTOCHROME B GENE

All fragments of 340 bp in size have successfully amplified from chicken belonged to each of eight breeds [23] (Figure 3).

Table 1: Nucleotide sequence of selected random primers.

No.	Primer Name	Sequence (5 -3')	GC Content %	No. of amplified bands	No. of polymorphic bands	% Polymorphism	Size range (bp)
1	OPA-04	AATCGGGCTG	60	7	6	85.57	200-1000
2	OPA-20	GTTGCGATCC	60	12	12	100	350-1200
3	OPB-01	GTTTCGCTCC	60	4	3	75	300-600
4	OPM-06	CTGGGCAACT	60	8	8	100	350-1000
5	OPM-20	AGGTCTTGGG	60	7	6	85.71	300-750
6	OPN-16	AAGCGACCTG	60	5	3	60	220-1300
7	OPP-04	GTGTCTCAGG	60	4	2	50	150-800
8	OPQ-03	GGTCACCTCA	60	6	5	83.33	210-1500
9	OPQ-07	CCCCGATGGT	70	8	6	75	250-1000
10	10 MER	AACGCGCAAC	60	5	4	80	250-850
Overall			61	66	55	79.46	150-1500

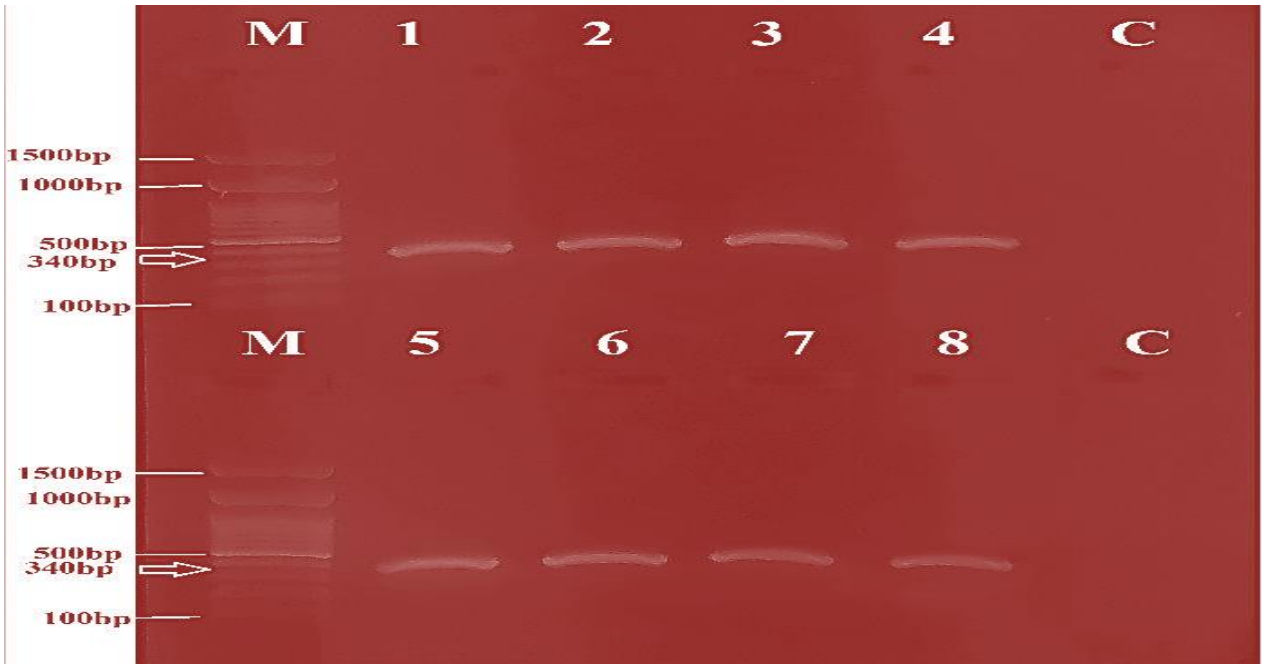

Figure 4: Electrophoretic analysis of PCR product amplified with mt Cytb universal primer. Lane M: 100 bp plus DNA marker, Lane 1: Local Black , Lane 2: Local Black and white, Lane 3: Local black Barred, Lane 4: American White Brahman, Lane 5: Silver Polish, Lane 6: Germany Phoenix Bantam, Lane 7: Sultan Belgian, Lane 8: American Lamborghini and Lane C: Control.

3.3 PHYLOGENETIC MITOCHONDRIAL DNA

Phylogenetic analysis based on the cytochrome b gene sequences from chickens belonged to eight variety breeds revealed that the local black and local black and white breeds were clustered with breed American Lamborghini. In contrast, the black-barred breeds were clustered with the chicken breed from the outside of Iraq (Figure. 5). The nucleotide sequence distance between eight examined breeds ranged from 0.00 to 0.074. There were no nucleotide sequence differences between breeds Local Black Barred, Silver Polish and Germany Phoenix Bantam. The results obtained from genotyping based on cytochrome b was in agreement with the results from the rapid procedure.

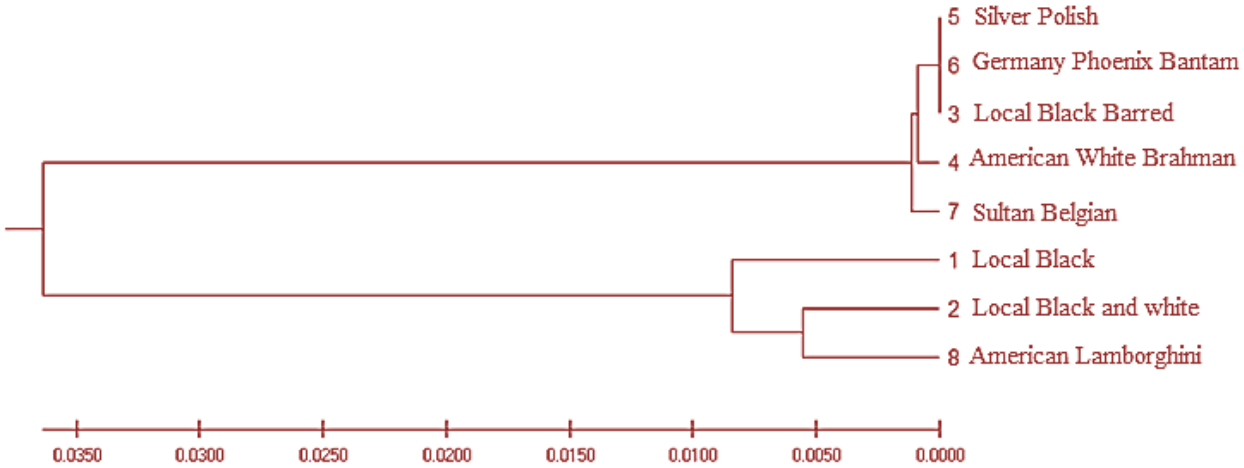

Figure 5: Mega X program has appeared phylogenetic positioning of ornamental *G. Gallus* of eight breeds of partial sequences of mtCyt b of 1: Local Black, 2: Local Black and white, 3: Local black Barred, 4: American White Brahman, 5: Silver Polish, 6: Germany Phoenix Bantam, 7: Sultan Belgian, and 8: American Lamborghini.

4. CONCLUSION

The high genetic distance (50.29 %) and variation in phenotypic and colors for all samples indicate that these local ornamental chickens have a good amount of variation to make genetically improvement in further and it means the three local ornamental chickens are independent breeds. Overall partial cytochrome b gene in the amount of 340 bp information, the molecular phylogenetic tree revealed that both local black color and local black and white color in same cluster are independent of others. The above results can be used by breeders to clarify the mapping of the genetic diversity of the local ornamental chickens and can depend on these results to make mating system or crossing among these chicken breeds or selection within/among ornamental chickens to speed up the performance in local ornamental chickens in Kurdistan.

5. AVAILABILITY OF DATA AND MATERIAL

Data can be made available by contacting the corresponding author.

6. ACKNOWLEDGEMENT

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