Genetic diversity among two native Indian chicken populations using cytochrome c oxidase subunit I and cytochrome b DNA barcodes

Ankit R. Dave1, Dhaval F. Chaudhary1, Pooja M. Mankad1, Prakash G. Koringa2 and D. N. Rank1

Background and Aim: India has large varieties (recognized, unrecognized) of native chickens (Desi) scattered throughout the country, managed under scavenging system different from commercial chicken breeds. However, they are less investigated for genetic diversity they harbor. The present study was planned to evaluate genetic diversity among two native chicken populations of North Gujarat (proposed Aravali breed) and South Gujarat (Ankleshwar breed). Aravali chicken, a distinct population with unique characters different from the registered chicken breeds of India is under process to be registered as a new chicken breed of Gujarat, India.

Materials and Methods: Two mitochondrial markers, namely, cytochrome c oxidase subunit I (COX I) and cytochrome b (Cyt b) genes were studied across 10 birds from each population. Methodology included sample collection (blood), DNA isolation (manual), polymerase chain reaction amplification of mitochondrial genes, Sanger sequencing, and purification followed by data analysis using various softwares.

Results: Haplotype analysis of the COX I gene unveiled a total eight and three haplotypes from the Aravali and Ankleshwar populations, respectively, with haplotype diversity (Hd) of 92.70 % for the Aravali and 34.50% for the Ankleshwar breed. Haplotype analysis of the Cyt b gene revealed a total of four haplotypes from the Aravali population with 60% Hd and no polymorphism in Ankleshwar breed. The phylogenetic analysis uncovered Red Jungle Fowl and Gray Jungle Fowl as prime roots for both populations and all domestic chicken breeds.

Conclusion: Study findings indicated high genetic variability in Aravali chicken populations with COX I mitochondrial marker being more informative for evaluating genetic diversity in chickens.

Keywords: Cytochrome b, Cytochrome oxidase, genetic diversity, mitochondrial DNA, phylogenetics, poultry.

Introduction

Biodiversity is the variation of life on Earth that is most important for several healthy ecosystems. Species diversity and genetic diversity within species play a key role in stable functioning of ecosystems. Genetic diversity is the variation of alleles and genotypes within the genome [1]. Genetic diversity among the domestic livestock is commonly measured through molecular markers such as microsatellites, mitochondrial DNA sequences, single-nucleotide polymorphisms (SNPs) available on commercial chips, and the complete metagenome sequences. Mitochondria are the powerhouse of a cell that produces energy for the cell. The animal mtDNA is ~ 17 kb of circular, coiled, multiple copied, extra-nuclear genome with high mutation rate and evolution rate of about 5-10 times faster than nuclear DNA, making it highly diverse within a species and fundamental material for phylogenetic and genetic diversity studies [2]. It has 37 genes, 13 protein-coding, two ribosomal RNA, and 22 transfer RNA genes [3], among which cytochrome b (Cyt b) and cytochrome c oxidase subunit I (COX I) are important for phylogenetic and genetic diversity studies.

Chicken is an iconic model to study domestic birds which have enormous population with hundreds of breeds and strains that can be grown in a controlled environment. A huge chicken population of 851.81 million with tremendous growth in chicken industry contributes significantly to the Indian economy (Anonymous, 2019). A total of 20 chicken breeds are registered in India (NBAGR, 2019) to which Gujarat contributes Ankleshwar and Busra. Indigenous chicken plays an important role in rural and/or tribal areas due to the number of different qualities, namely, disease resistance, efficient adaptability-survivability-mothering ability, and natural scavenging-nesting habit [4]. However, tribal areas of the eastern fringe of Gujarat...
have a poor reach of technologies and are less explored with respect to animal genetic resources. Moreover, the ancestors of the domestic chicken (*Gallus gallus domesticus*) are two wild avian species, namely, Red Jungle Fowl (*G. gallus*) and Gray Jungle Fowl (*Gallus sonneratii*) [5]. Domestication changes several traits in wild ancestor species. Hence, well-planned scientific study needs to be focused on such aspects.

Hence, the molecular investigation was undertaken for the determination of genetic diversity of unique and native chicken populations of North (proposed Aravali breed) as well as South Gujarat (Ankleshwar breed) through mitochondrial markers (COX I; and Cyt b).

**Materials and Methods**

**Ethical approval**

This study was approved by the Institutional Animal Ethics Committee of College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India.

**Study period and location**

The study was carried out from October 2018 to September 2019. The sampling was conducted from October to November 2018 in Aravali, Banaskantha, Sabarkantha and Bharuch district of Gujarat, India. The wet laboratory research work was done January to September 2019 at the Department of Animal Genetics and Breeding, Anand Agricultural University, Anand, Gujarat, India.

**Sample collection and DNA isolation**

The present study included two native populations of Aravali and Ankleshwar chicken (10 birds from each population). For sampling, we selected 10-12 villages from core breeding tracts of phenotypically “true to breed” of both populations. The blood samples were collected from Aravali (3-5 birds/village) and Ankleshwar (5-10 birds/village) breeds reared as backyard poultry farming across three districts Aravali, Banaskantha, and Sabarkantha of the North Gujarat (Aravali) and Bharuch district of South Gujarat (Ankleshwar). DNA extractions from whole blood samples were carried out using manual method [6]. Quality and purity of DNA were checked by agarose gel electrophoresis (0.8%) at 80 V for 45-60 min. The amplified products were visualized as compact bands of the expected size (~ 746 bp for COX I and ~ 415 bp for Cyt b) under ultraviolet light and documented by gel documentation system (Syngene, Gene Genius Bio-Imaging, USA). All PCR products were purified using a QIAquick gel extraction kit to obtain accurate sequence information.

**Sequencing, purification, and data analysis**

Cycle sequencing of all samples was carried out in a total reaction volume of 20 μL using BigDye® Terminator v3.1 Cycle Sequencing Ready Reaction-100 mix (Thermo Fisher Scientific, Applied Biosystems), BigDye® Terminator v1.1 and v3.1 ×5 Sequencing Buffer (Thermo Fisher Scientific, Applied Biosystems), forward/reverse primers, and 50-70 ng/μL purified PCR product (Gel extracted DNA) on 2720 thermal cycler (Applied Biosystems). Cycle sequencing conditions consisted 95°C for 5 min, followed by 32 cycles of 95°C for 20 s, at 55°C for 1 min, and at 72°C for 1 min, and a final extension step at 72°C for 5 min. PCR cycling conditions for the amplification of Cyt b mitochondrial gene consisted 96°C for 5 min, followed by 40 cycles of 95°C for 45 s, at 56°C for 1 min, at 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR amplification was confirmed by agarose gel electrophoresis (2 %) with 100 bp DNA ladder (Thermo Scientific, USA) at 80 V for 45-60 min. The amplified products were visualized as compact bands of the expected size (~ 746 bp for COX I and ~ 415 bp for Cyt b) under ultraviolet light and documented by gel documentation system (Syngene, Gene Genius Bio-Imaging, USA). All PCR products were purified using a QIAquick gel extraction kit to obtain accurate sequence information.

**Polymerase chain reaction (PCR) amplification of mitochondrial genes**

PCR amplification was carried out using two primer pairs, namely, BirdF1 and COIbirdR2 for COX I [7]; L15662 and H16065 for Cyt b [8]. PCR amplification was carried out in a total reaction volume of 25 μL using ×2 PCR Master Mix (Emerald, TaKara, Japan) primers and 50-100 ng DNA on 2720 thermal cycler (Applied Biosystems, Massachusetts, USA). PCR cycling conditions for the amplification of COX I mitochondrial gene consisted 94°C for 1 min, followed by five cycles (at 94°C for 1 min, at 45°C for 30 s, at 72°C for 1 min), 35 cycles (at 94°C for 1 min, at 54°C for 30 s, at 72°C for 1 min), and a final extension step at 72°C for 5 min. PCR cycling conditions for the amplification of Cyt b mitochondrial gene consisted 96°C for 5 min, followed by 40 cycles of 95°C for 45 s, at 56°C for 1 min, at 72°C for 1 min, and a final extension step at 72°C for 10 min. PCR amplification was confirmed by agarose gel electrophoresis (2 %) with 100 bp DNA ladder (Thermo Scientific, USA) at 80 V for 45-60 min. The amplified products were visualized as compact bands of the expected size (~ 746 bp for COX I and ~ 415 bp for Cyt b) under ultraviolet light and documented by gel documentation system (Syngene, Gene Genius Bio-Imaging, USA). All PCR products were purified using a QIAquick gel extraction kit to obtain accurate sequence information.

**Results**

**PCR amplification of mitochondrial genes**

DNA barcoding of 20 samples (10 each from Aravali and Ankleshwar breed) was done successfully by PCR amplification of COX I (~ 746 bp) and Cyt b (~ 415 bp) gene fragments and sequencing. Representative image of amplified PCR product is shown in Figure-1.

**Sequencing and data analysis**

Representative electropherogram images of COX I and Cyt b raw gene sequences are shown in
Table 2-1: Comparative haplotype analysis of COX I and Cyt b genes between Aravali and Ankleshwar chicken populations.

| Parameter/chicken population | COX I gene | Cyt b gene |
|------------------------------|------------|------------|
|                              | Aravali    | Ankleshwar | Aravali  | Ankleshwar |
| Selected region/number of sites | 1–653      | 1–682      | 1–345    | 1–327      |
| Number of total sites (excluding gaps/missing data) | 569 | 647 | 345 | 327 |
| Number of polymorphic sites (S) | 12 | 03 | 03 | - |
| Number of haplotype (h) | 08 | 03 | 04 | - |
| Haplotype/gene diversity (Hd) | 0.927 | 0.345 | 0.600 | - |
| Variance of Hd | 0.00442 | 0.02967 | 0.02369 | - |
| Standard deviation of Hd | 0.066 | 0.172 | 0.154 | - |
| Nucleotide diversity (Pi/π) Jukes and Cantor | 0.22857 | 0.00084 | 0.00233 | - |
| Average number of nucleotide difference (k) | 3.6 | 0.545 | 0.800 | - |

Table 2-2: Molecular definitions of eight COX I haplotype from Aravali chicken population.

| Polymorphic site location | 45 | 46 | 353 | 355 | 368 | 418 | 453 | 484 | 508 | 520 | 624 | 632 |
|---------------------------|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Reference sequence Gallus gallus | G | C | A | T | A | A | C | T | C | G | G | G |
| NGXHp_1 (NG1, 3) | - | A | - | C | G | - | A | G | A | T | A | C |
| NGXHp_2 (NG 2) | A | G | C | G | T | - | A | G | A | C | - | C |
| NGXHp_3 (NG 4) | - | A | - | C | G | - | G | A | A | C | - | C |
| NGXHp_4 (NG 5) | - | A | - | C | G | - | A | G | A | C | A | C |
| NGXHp_5 (NG 6) | - | A | - | C | G | - | G | G | A | C | - | C |
| NGXHp_6 (NG 7, 8) | - | A | - | C | G | - | A | G | A | C | A | C |
| NGXHp_7 (NG 9) | - | A | - | C | G | T | A | G | T | T | A | - |
| NGXHp_8 (NG 10) | - | A | - | C | G | - | G | A | A | C | - | A |

Consensus sequences of COX I gene (608 bp to 756 bp) and Cyt b gene (378 bp to 496 bp) were subjected to BLASTN at NCBI (http://www.ncbi.nlm.nih.gov/blast). All the consensus sequences were matched with the complete mitochondrial genome of *G. gallus*. A complete mitochondrial genomes of Red Jungle Fowl (*Gallus gallus murghi*; GU261708.1) and Gray Jungle Fowl (*G. sonneratii*; AP003320.1) were downloaded from NCBI site.

Comparative haplotype analysis between Aravali and Ankleshwar chicken populations is shown in Table 1. Furthermore, the molecular definitions of haplotypes identified are shown in Tables 2-4.

**Haplotype analysis for COX I gene barcodes**

Total numbers of polymorphic sites were higher (12) in Aravali chicken population than in Ankleshwar chicken population (three). The total numbers of haplotypes found were also higher in Aravali chicken population (eight haplotypes with haplotype diversity [Hd] 92.70%) than Ankleshwar chicken population (three haplotypes with Hd 34.50%). In the Aravali population, except haplotypes NGXHp_1 and NGXHp_6, all other haplotypes were represented by single samples, whereas Ankleshwar chicken population had 80% frequency of the haplotype SGXHp_1 with other two haplotypes, namely, SGXHp_2 and SGXHp_3 represented by single samples. The nucleotide diversity in Aravali chicken population was significantly higher (22.85%) than Ankleshwar chicken population (0.08%). It is pertinent to mention that consensus sequence length was slightly shorter (653 bp) in Aravali than Ankleshwar (682 bp) chicken populations.

**Haplotype analysis for Cyt b gene barcodes**

After quality filtering, overall selected sequence length was 345 bp for Aravali chicken population.
Table 3: Molecular definition of three COX I haplotypes from Ankleshwar chicken population.

| Polymorphic site location | Reference sequence Gallus gallus | SGXHp_1 (SG 1, 4-10) | SGXHp_2 (SG 2) | SGXHp_3 (SG 3) |
|---------------------------|----------------------------------|----------------------|----------------|----------------|
|                           | C                                | G                    | T              | T              |

Table 4: Molecular definition of four Cyt b haplotypes from Aravali chicken population.

| Polymorphic site location | Reference sequence Gallus gallus | NGbHp_1 (NG 1) | NGbHp_2 (NG 2) | NGbHp_3 (NG 3-9) |
|---------------------------|----------------------------------|----------------|----------------|------------------|
|                           | T                                | C              | -              | -                |

Figure 2: Raw sequences (blue bars) and electropherogram (colored peaks) of COX I gene of representative samples of Aravali (a) and Ankleshwar (b) population. (a) NG05 (b) SG01.

and 327 bp for Ankleshwar chicken population. Total numbers of polymorphic sites in Aravali chicken population were three with 0.23% nucleotide diversity and total four haplotypes were found with Hd 60%, whereas Ankleshwar chicken population had 100% identical sequences, which indicated that the Aravali chicken population had higher genetic diversity compared to the Ankleshwar chicken population (with
more number of haplotypes and higher haplotype as well as nucleotide diversity).

The phylogenetic analysis

The phylogenetic analysis was performed using bioinformatics software, namely, MEGA-X v.10.0.5 (Pennsylvania State University, Pennsylvania, USA) (Molecular Evolutionary Genetics Analysis). For comparative phylogeny, COX I and Cyt b gene sequences of various domestic chicken breeds were downloaded from the NCBI site (http://www.ncbi.nlm.nih.gov), namely, Aseel (KP211418.1), Kadaknath (KP211425.1), White Leghorn (AP003317.1), White Plymouth Rock (AP003318.1), Minorea (AF354171.1), and wild ancestor of domestic chicken breeds, namely, Red Jungle Fowl (Gallus gallus; GU261708.1) and Gray Jungle Fowl (G. sonneratii; AP003320.1). Phylogenetic trees were constructed using Neighbor-Joining method (1000 bootstrap replications; Kimura 2-parameter model) and presented in Figure-4. The present findings supported that the Red Jungle Fowl and Gray Jungle Fowl form prime roots for all the existing domestic chicken breeds. Furthermore, analysis suggested that

Figure-3: Raw sequences (blue bars) and electropherogram (colored peaks) of Cyt b gene of representative samples of Aravali (a) and Ankleshwar (b) population. (a) NG10 (b) SG04.
Ankleshwar chicken population might have evolved earlier than Aravali chicken population.

**Bold sequence accession numbers**

All sequences were submitted in BOLD SYSTEMS (Barcode of Life Data System; www.boldsystems.org) under the project name “ARD.” The unique BOLD sequence IDs for respective samples are indicated in Table-5.

**Discussion**

Originally 60 samples each from both breeds were collected from their respective breeding tracts and evaluated for genetic diversity using microsatellite markers (unpublished data). Both the breeds are reared by tribal community around 500 kilometers apart. Aravali is sparsely distributed across three districts (Aravali, Banaskantha, and Sabarkantha, approximately 10,000 square kilometers) of North Gujarat; while Ankleshwar is more concentrated in and around Ankleshwar town and is sparsely distributed in forested area in approximately 5000 square kilometers of Bharuch district of South Gujarat. Aravali is mostly reared in the group of 5-10 birds (including 1-2 males) per household, while Ankleshwar is reared in and around Ankleshwar town in the groups of 10-20 birds including 2-4 males and in a smaller group in surrounding areas. Accordingly, sampling was done from 10 to 12 villages of core breeding tracts, with phenotypically true to breed populations of both chicken breeds, 3-5 birds per villages for Aravali and 5-10 birds per villages for Ankleshwar. The 10×2 samples were the subset of original sample set. Sex ratio was 3 males:7 females.

Hd and nucleotide diversity (Pi) of populations are the main indexes for evaluating mtDNA variation and genetic diversity of a breed or a population. COX I and Cyt b haplotype analysis in this study revealed Aravali population to be genetically more variable harboring more polymorphic sites (12 for COX I and three for Cyt b) and haplotypes (eight for COX I and four for Cyt b) with higher haplotype (0.927 for COX I and 0.600 for Cyt b) and nucleotide diversity (0.228 for COX I and 0.0023 for Cyt b) than Ankleshwar breed. This supports our findings that Aravali are genetically more variable than Ankleshwar based on microsatellite profiling (unpublished data).

These markers (COX I and Cyt b) have been used by number of investigators to identify native chicken, to study genetic diversity, population structure, evolution, and origin of native chicken breeds. High genetic diversity as revealed by a higher number of polymorphic sites defining more number of haplotypes was reported in Chinese black bone chickens (22 and 24) [9], Chinese native chickens (5 and 10) [10], Tibetan chicken (4 and 6) [11], Chinese native chickens (24 and 24) [12] in COX I marker and in black boned chicken breeds (17 and 8) [13], and game chicken breed of China (7 and 6) [14] in Cyt b marker. However, these markers (COX I and Cyt b) are more commonly used in evaluation of genetic diversity among wild populations than domesticated populations. High genetic diversity with the higher number of polymorphic sites defining more number of haplotypes was reported in Mediterranean breeding colonies of Greater Flamingo (Phoenicopterus roseus) (15 and 16) [15] and Lesser Flamingos (Phoenicopterus minor) from Africa and Gujarat (11 and 14) [16]. Although, this is a small study including only two breeds, it

![Figure-4: Phylogenetic trees made from obtained sequences of COX I gene (a), Cyt b gene (b) of Aravali and Ankleshwar chicken populations with sequences of domestic chicken breeds and two wild ancestors (Red Jungle Fowl and Gray Jungle Fowl). Aravali and Ankleshwar chicken show divergence from classical layer (White Leghorn) and meat (White Plymouth Rock).](image-url)
supported the diversity estimation by microsatellite markers (data not shown).

Other mitochondrial markers such as D-loop (control region) have also been used by various investigators to explore diversity among different native chicken breeds across the world. Higher number of polymorphic sites defining more number of haplotypes were reported in Samar Philippines native chickens (17 and 5) [17], Hungarian native chickens (17 and 11) [18], Egyptian native chickens (28 and 18) [19], native chicken breeds of Jiangsu (33 and 19) [20], and chicken breeds of Korea (84 and 31) [21]. These studies showed high genetic diversity with higher haplotype and nucleotide diversity in Samar Philippines native chickens (0.92 and 0.0056) [22], Hungarian native chickens (0.626 and 0.0049) [18], Egyptian native chickens (0.81 and 0.0045) [19], native chicken breeds of Jiangsu (0.862 and 0.00591) [20], and chicken breeds of Korea (0.604 and 0.007) [21].

Today, newer markers such as SNPs and whole mitogenome sequencing are available for assessing the genetic diversity among domestic breeds. However, considering the costs involved and assess to technology, barcoding markers were used in the present study. SNPs have been used for genome-wide analysis to assess the conservation status and the genomic variability of Italian chicken breeds [22]. Hungarian native chickens (0.92 and 0.0056) [22], Hungarian native chickens (0.626 and 0.0049) [18], Egyptian native chickens (0.81 and 0.0045) [19], native chicken breeds of Jiangsu (0.862 and 0.00591) [20], and chicken breeds of Korea (0.604 and 0.007) [21].

Table-5: Unique BOLD sequence IDs of submitted sequences in BOLD SYSTEMS for respective samples.

| S. No. | Chicken breed                  | Institutional sample ID | BOLD sample ID | BOLD sequence ID |
|-------|--------------------------------|-------------------------|---------------|------------------|
| 1.    | North Gujarat/Aravali          | NG01                    | PB_NG01       | ARD001-19.COI-5P | ARD001-19.CYT B |
| 2.    |                                 | NG02                    | PB_NG02       | ARD002-19.COI-5P | ARD002-19.CYT B |
| 3.    |                                 | NG03                    | PB_NG03       | ARD003-19.COI-5P | ARD003-19.CYT B |
| 4.    |                                 | NG04                    | PB_NG04       | ARD004-19.COI-5P | ARD004-19.CYT B |
| 5.    |                                 | NG05                    | PB_NG05       | ARD005-19.COI-5P | ARD005-19.CYT B |
| 6.    |                                 | NG06                    | PB_NG06       | ARD006-19.COI-5P | ARD006-19.CYT B |
| 7.    |                                 | NG07                    | PB_NG07       | ARD007-19.COI-5P | ARD007-19.CYT B |
| 8.    |                                 | NG08                    | PB_NG08       | ARD008-19.COI-5P | ARD008-19.CYT B |
| 9.    |                                 | NG09                    | PB_NG09       | ARD009-19.COI-5P | ARD009-19.CYT B |
| 10.   |                                 | NG10                    | PB_NG10       | ARD101-19.COI-5P | ARD101-19.CYT B |
| 11.   | South Gujarat/Ankleshwar       | SG01                    | PB_SG01       | ARD011-19.COI-5P | ARD011-19.CYT B |
| 12.   |                                 | SG02                    | PB_SG02       | ARD012-19.COI-5P | ARD012-19.CYT B |
| 13.   |                                 | SG03                    | PB_SG03       | ARD013-19.COI-5P | ARD013-19.CYT B |
| 14.   |                                 | SG04                    | PB_SG04       | ARD014-19.COI-5P | ARD014-19.CYT B |
| 15.   |                                 | SG05                    | PB_SG05       | ARD015-19.COI-5P | ARD015-19.CYT B |
| 16.   |                                 | SG06                    | PB_SG06       | ARD016-19.COI-5P | ARD016-19.CYT B |
| 17.   |                                 | SG07                    | PB_SG07       | ARD017-19.COI-5P | ARD017-19.CYT B |
| 18.   |                                 | SG08                    | PB_SG08       | ARD018-19.COI-5P | ARD018-19.CYT B |
| 19.   |                                 | SG09                    | PB_SG09       | ARD019-19.COI-5P | ARD019-19.CYT B |
| 20.   |                                 | SG10                    | PB_SG10       | ARD020-19.COI-5P | ARD020-19.CYT B |

Partridge [27], and Huangshan Black [28] chickens of China. However, microsatellites and DNA barcodes still accepted as markers of choice because they are technically less demanding and cost-effective in evaluation. Ample literature is available on use of microsatellite markers in domestic chicken [29-31]. Nonetheless, uses of SNP markers and whole mitogenome sequencing have recently been appeared in the publication, which can be used to evaluate genetic diversity of domestic chicken.

Conclusion

As far as the authors are aware, this is the first diversity study involving mitochondrial markers in domestic chicken in India. Aravali chicken population seems to be more genetically diverse, as reflected by higher number of COX I and Cyt b haplotypes than the Ankleshwar chicken population. Out of two mitochondrial markers, COX I stood out to be more informative than Cyt b for the use of genetic diversity study. Considering limited power of these markers, recent markers such as SNPs and whole mitogenome sequencing are suggested to evaluate genetic diversity of chicken with ideal sampling strategies involving more number of male samples.

Authors’ Contributions

ARD, PGK, and DNR: Designed the study. ARD and DFC: Collected the samples. ARD and PMM: Carried out research in the laboratory. ARD: Analyzed, wrote, and revised the manuscripts. DNR and PGK: Supervised the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

The authors would like to thank Dr. F. P. Savaliya, Research Scientist and Head, Poultry Research
Station (PRS), Anand Agricultural University, Anand, Gujarat, India, for helping in the selection of birds for the study. This study was funded by Anand Agricultural University Project titled ‘Study on correlated response to selection in the experimental flock of poultry, B.H. 6374’.

Competing Interests

The authors declare that they have no competing interests.

Publisher’s Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

1. Frankham, R., Ballou, J.D., Briscoe, D.A. and McInnes, K. (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge.
2. Bowang, C., Jusheng, H. and Xingbo, S. (2000) Study on genetic diversity of Cunninghamia lanceolata and Taiwania floussiana by using chloroplast microsatellites. Sci. Silvae Sinicae, 36(3): 46-51.
3. Taaman, J.W. (1999) The mitochondrial genome: Structure, transcription, translation and replication. Biochim. Biophys. Acta., 1410(2): 103-123.
4. Khan, A.G. (2008) Indigenous breeds, crosses and synthetic hybrids with modified genetic and economic profiles for rural family and small scale poultry farming in India. Worlds Poult. Sci. J., 64(3): 405-415.
5. Kanginakudru, S., Metta, M., Jakati, R.D. and Nagaraju, J. (2008) Genetic evidence from Indian Red Jungle Fowl corroborates multiple domestication of modern day chicken. BMC Evol. Biol., 8(1): 174.
6. John, S.W.M., Wetzner, G., Rozen, R. and Scriver, C. R. (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge.
30. Roh, H.J., Kim, S.C., Cho, C.Y., Lee, J., Jeon, D., Kim, D.K. and Manikku, L. (2020) Estimating genetic diversity and population structure of 22 chicken breeds in Asia using microsatellite markers. *Asian-Australas. J. Anim. Sci.*, 33(12): 1896.

31. Nxumalo, N., Ceccobelli, S., Cardinali, I., Lancioni, H., Lasagna, E. and Kunene, N.W. (2020) Genetic diversity, population structure and ancestral origin of KwaZulu-Natal native chicken ecotypes using microsatellite and mitochondrial DNA markers. *Ital. J. Anim. Sci.*, 19(1): 1277-1290.

**********