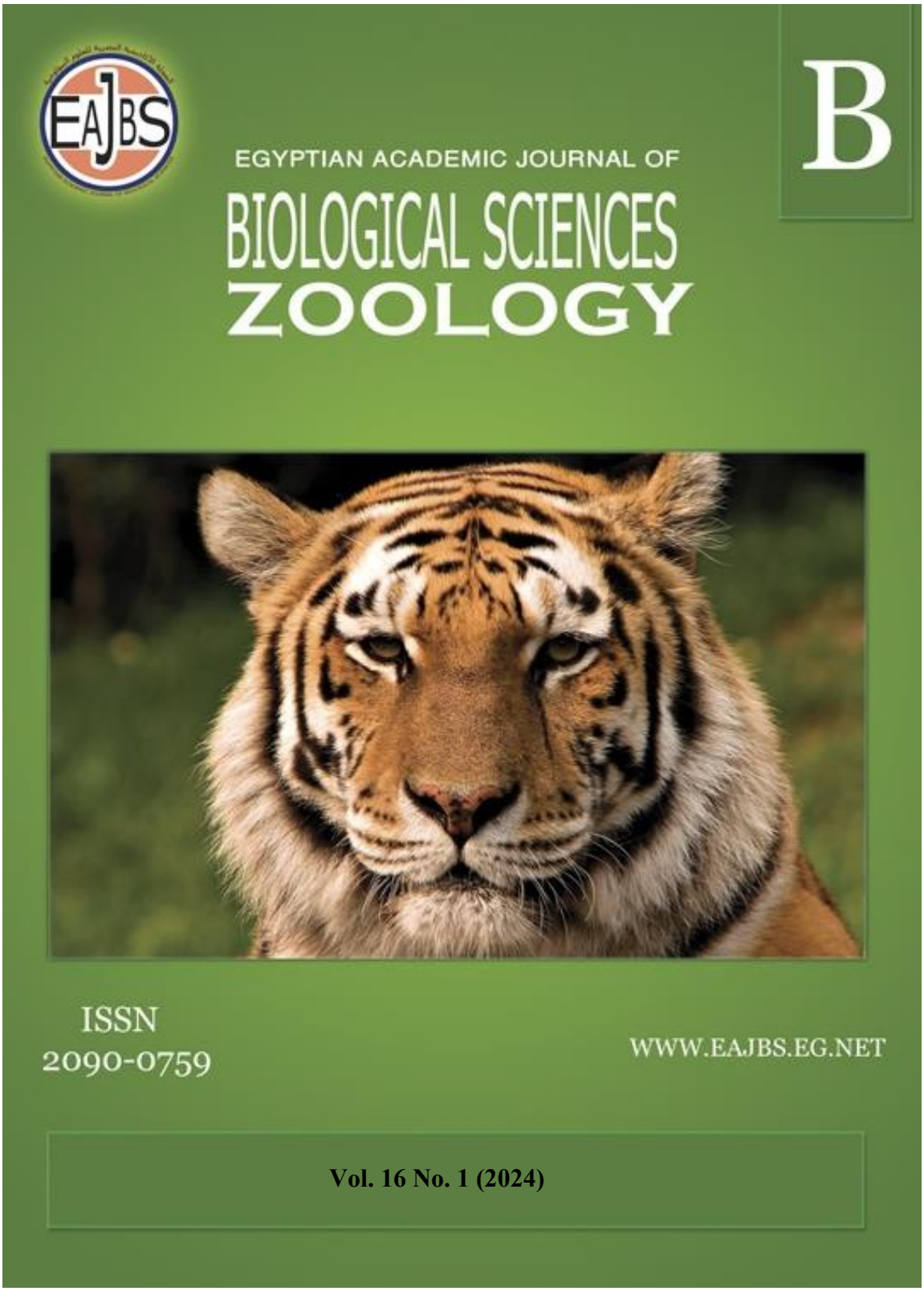
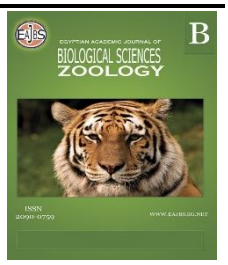


Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 16(1) pp:201-208 (2024)*
DOI: 10.21608/EAJBSZ.2024.356328

Egypt. Acad. J. Biolog. Sci., 16(1):201-208(2024)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



Geographic Genetic Diversity of *Gallus gallus* Using Large Mitochondrial rRNA Sequences

Mary Welson[1]; Nancy Abo Fandoud Radwan[2] and Mohammad Allam[3*]

[1]Zoology Department, Faculty of Science, Suez University, Egypt.
[2]Zoology Department, Faculty of Science, Arish University, Egypt.
[3]Zoology Department, Faculty of Science, Luxor University, Egypt.

*** E-mail** : mohammadallam2021@gmail.com

ARTICLE INFO
Article History
Received:27/4/2024
Accepted:27/5/2024
Available:31/5/2024

Keywords:
16S rRNA;
Gallus gallus;
Genetic distance;
Sequencing.

ABSTRACT

In the current work, the genetic variety of *Gallus gallus* from four sites was evaluated using large mitochondrial rRNA (*16S rRNA*). Samples were gathered from four different places within the Egyptian Governorate of Qena; Naga Hammadi City, Qena City, El-Taramsa Village and Qus City. The nucleotide lengths obtained from sequencing *16S rRNA* gene of the mitochondria in the four sites varied from 547 base pairs (bp) to 565 bp. The *16S rRNA* partial nucleotide sequences were entered into GenBank with accession numbers (PP422403 - PP422410). The findings show that the 547 bp nucleotide sequences found in Naga Hammadi City are the shortest. 569 bp made up the final alignments; of them, 566, three, and two were conserved sites, variable sites, and parsimony informative sites, respectively. Compared to the C+G content, the average A+T content was higher at 52.98%. Low genetic similarity was observed between Naga Hammadi and Qena Cities, this may be due to many factors like geographical and ecological conditions.

INTRODUCTION

Chicken is one of the most economically valuable domesticated animals in the world (Lawal and Hanotte, 2021). Four species make up the genus *Gallus* (family Phasianidae, order Galliformes); Ceylon Jungle Fowl (*G. lafayettei*), Green Jungle Fowl (*G. varius*), Grey Jungle Fowl (*G. sonneratii*) and Red Jungle Fowl (*Gallus gallus*) (Peters, 1976).

As DNA polymorphism and molecular studies have grown more widely available, so too have the techniques for measuring this polymorphism (Ould Ahmed *et al*., 2010). Partial or comprehensive mtDNA sequence variation analysis can give important insights into the population structure, demographic history, and human-mediated spread outside of the domestication core (Di Lorenzo *et al*., 2015).

A great deal of research has been done on mitochondrial sequences to determine the ancestry of domestic chickens. It has been proposed that domestic chickens have a monophyletic origin, deriving mostly from the Red Jungle Fowl (RJF) subspecies found in Southeast Asia (Fumihito *et al*., 1996 and Eltanany and Hemeda, 2016).

16S rRNA gene is widely used in phylogenetic, evolutionary, and taxonomic research and is regarded as the perfect molecular clock. Clear classification resulted from the abundance of appropriate primers and the abundance of incomplete sequences of the *16S* gene in the various databases (Sokefun, 2017). This work's main goal was to utilize *16S rRNA* sequences to provide a broad understanding of the spatially distributed genetic variability of *Gallus gallus* across four locations within the Egyptian Governorate of Qena.

MATERIAS AND METHODS

Collecting Samples And Extracting DNA:

Eight samples were gathered from four different places within the Egyptian Governorate of Qena; Naga Hammadi City, Qena City, El-Taramsa Village and Qus City. The specimens' tissues were kept at -20 °C till the genomic DNA was extracted. The manufacturer's instructions were followed in order to extract the genomic DNA from the frozen tissues using the QIAamp DNA Mini kit (Qiagen, Hidden, Germany) DNA extraction method.

Polymerase Chain Reaction (PCR) Conditions:

Primers forward and reverse were employed to amplify (*16S rRNA*) gene, as previously reported by (Simon *et al*., 1991). 1 µL of each forward and reverse primer, 25 µL of PCR master mix, and 1 µL of genomic DNA are included in a total reaction volume of 50 µL for the PCR reactions. The PCR program was made up of the following steps: (I) a four-minute initial denaturation at 94°C; (II) 35 cycles of denaturation, annealing, and extension for 60 seconds at 94°C, 49°C, and 72°C, respectively; (III) a final extension lasting seven minutes at 72°C. For the purpose of separating the amplified products, ethidium bromide-containing agarose gel (1.5%) was utilized.

PCR Product Sequencing and Phylogenetic Tree:

Macrogen completed all DNA sequencing (Seoul, South Korea). In order to receive accession numbers, the sequences were submitted to (GenBank/NCBI). BLAST/N at the NCBI was used to sequence the large mitochondrial rRNA (*16S rRNA*) gene for the four sites. MUSCLE (Edgar, 2004) was used to align the sequences using its default settings. Phylogenetic tree studies were carried out using MEGA version 11.0.11 (Tamura *et al*., 2021), 1000 bootstrap iterations (Felsenstein, 1985), and the Minimum-Evolution (ME) and Neighbor-Joining (NJ) techniques of tree construction. Kimura two-parameter distances were used to calculate sequence divergences (Kimura, 1980). Paleontological statistics (PAST) software version 2.17c (Hammer *et al*., 2001) was used to calculate the genetic similarity and dendrogram.

RESULTS

The nucleotide lengths obtained from sequencing the *16S rRNA* gene of the mitochondria in the four places varied from 547 base pairs (bp) to 565 bp. The *16S rRNA* partial nucleotide sequences have been inserted into GenBank with accession numbers (PP422403 - PP422410). The findings show that the 547 bp nucleotide sequences found in the Naga Hammadi City are the shortest. 569 bp made up the final alignments; of them, 566, three, and two were conserved sites, variable sites, and parsimony informative sites, respectively. Adenine (A), guanine (G), cytosine (C), and thymine (T) had average nucleotide frequencies of 31.36, 20,09, 26.93, and 21.62%, respectively. Compared to the C+G content, the average A+T content was higher at 52.98%. Additional information regarding the averages of the nucleotide frequencies and A+T contents was provided in (Table 1).

Table 1. Nucleotide rates and its average of *16S rRNA* sequences in the four places.

No.	Site	Base pair length	Nucleotide Number %				A+T Content (%)
			T%	C %	A%	G%	
1-	Naga Hammadi City -1	547	21.57	26.87	31.81	19.74	53.38
2-	Naga Hammadi City -2	547	21.57	26.87	31.81	19.74	53.38
3-	Qena City -1	555	21.80	26.85	31.17	20.18	52.97
4-	Qena City -2	555	21.80	26.85	31.17	20.18	52.97
5-	El-Taramsa Village -1	565	21.77	26.90	31.33	20.00	53.1
6-	El-Taramsa Village -2	565	21.59	26.90	31.33	20.18	52.92
7-	Qus City -1	565	21.42	27.08	31.15	20.35	52.57
8-	Qus City -2	565	21.42	27.08	31.15	20.35	52.57
	Average %	-	21.62	26.93	31.36	20.09	52.98

The four places' *16S rRNA* sequencing data were used to account for the genetic similarities. Using the genetic similarity shown in (Table 2) and Dendrogram (Fig. 1 and 2), the genetic relationship amongst the four places was established and showed that there was little genetic resemblance within Naga Hammadi and Qena Cities. The dendrogram included two primary clusters; (A) contained the samples from Naga Hammadi site, and (B) contained the rest samples. The (B) cluster contains two branches; the first contains samples from Qena City and the second contains samples from El-Taramsa Village and Qus City.

Table 2. Genetic similarity amongst the four places.

No.	Site	1	2	3	4	5	6	7	8
1	Naga Hammadi City -1	1	1	0.93673	0.93673	0.96637	0.96814	0.96637	0.96637
2	Naga Hammadi City -2	1	1	0.93673	0.93673	0.96637	0.96814	0.96637	0.96637
3	Qena City -1	0.93673	0.93673	1	1	0.97007	0.96837	0.97007	0.97007
4	Qena City -2	0.93673	0.93673	1	1	0.97007	0.96837	0.97007	0.97007
5	El-Taramsa Village -1	0.96637	0.96637	0.97007	0.97007	1	0.99823	0.99646	0.99646
6	El-Taramsa Village -2	0.96814	0.96814	0.96837	0.96837	0.99823	1	0.99823	0.99823
7	Qus City -1	0.96637	0.96637	0.97007	0.97007	0.99646	0.99823	1	1
8	Qus City -2	0.96637	0.96637	0.97007	0.97007	0.99646	0.99823	1	1

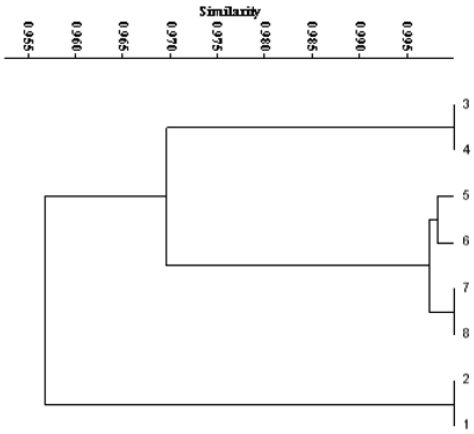


Fig. 1. Dendrogram revealing links amongst the four places depending on the sequenced regions of *16S rRNA*. Where, 1 and 2 were the samples of Naga Hammadi City, 3 and 4 were the samples of Qena City, 5 and 6 were the samples of El-Taramsa Village and 7 and 8 were the samples of Qus City.

	1	2	3	4	5	6	7	8
1	1	1	0.93673	0.93673	0.96637	0.96814	0.96637	0.96637
2	1	1	0.93673	0.93673	0.96637	0.96814	0.96637	0.96637
3	0.93673	0.93673	1	1	0.97007	0.96837	0.97007	0.97007
4	0.93673	0.93673	1	1	0.97007	0.96837	0.97007	0.97007
5	0.96637	0.96637	0.97007	0.97007	1	0.99823	0.99646	0.99646
6	0.96814	0.96814	0.96837	0.96837	0.99823	1	0.99823	0.99823
7	0.96637	0.96637	0.97007	0.97007	0.99646	0.99823	1	1
8	0.96637	0.96637	0.97007	0.97007	0.99646	0.99823	1	1

Fig. 2. Heatmap visualization of the genetic similarity amongst the four places based on the sequenced regions of *16S rRNA*. Where, 1 and 2 were the samples of Naga Hammadi City, 3 and 4 were the samples of Qena City, 5 and 6 were the samples of El-Taramsa Village, and 7 and 8 were the samples of Qus City.

Phylogenetic Analysis:

Using phylogenetic reconstruction, two approaches; Minimum-Evolution and Neighbor-Joining, were used to compare places' harmony. Similar results were obtained by the two phylogenetic approaches with minor adjustments to the support parameters.

Monophyly and Genetic Similarity In The Four Places:

According to the findings of the (*16S rRNA*) sequencing, samples from each site were categorized by site (Fig. 3 and 4). Additionally, every site—aside from site three-had comparable genetic similarities.

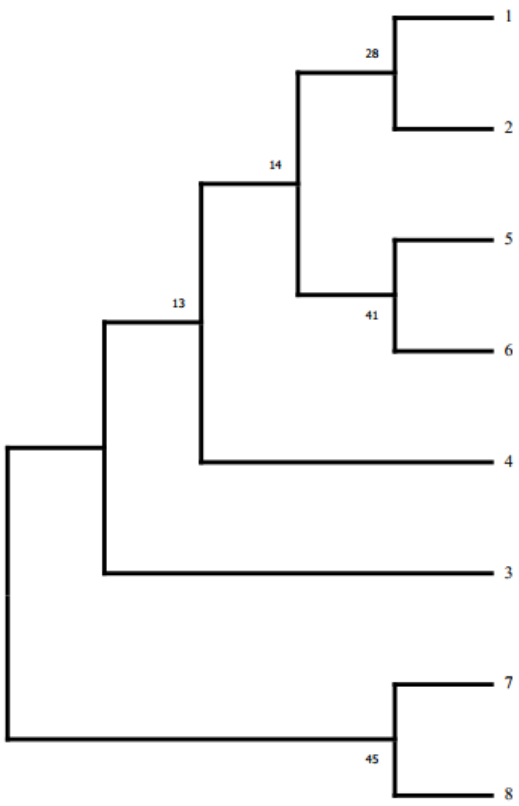


Fig. 3. Minimum-Evolution tree amongst the four places. Where, 1 and 2 were the samples of Naga Hammadi City, 3 and 4 were the samples of Qena City, 5 and 6 were the samples of El-Taramsa Village, and 7 and 8 were the samples of Qus City.

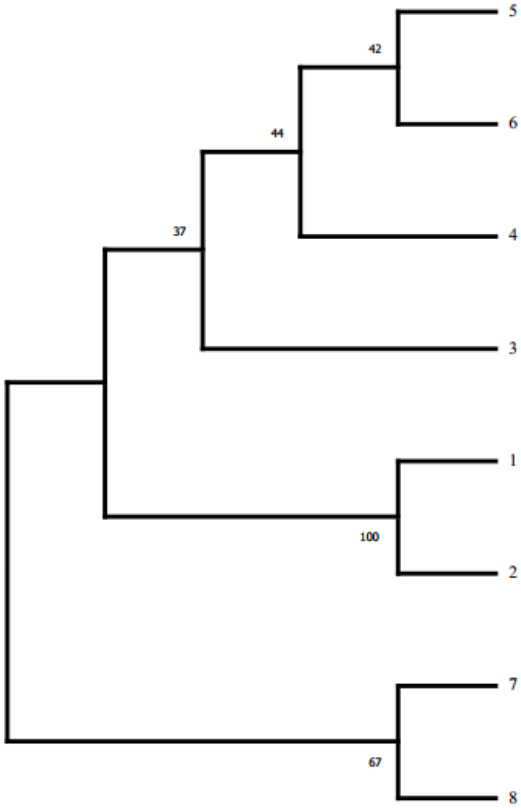


Fig. 4. Neighbor-Joining tree amongst the four places. Where, 1 and 2 were the samples of Naga Hammadi City, 3 and 4 were the samples of Qena City, 5 and 6 were the samples of El-Taramsa Village, and 7 and 8 were the samples of Qus City

DISCUSSION

Native chicken breeds are vital to the lifestyle and culture of rural households in underdeveloped and developing nations, primarily in Asia and Africa. They are a valuable source of animal protein, a source of cash income, and an integral part of the sociocultural life of the rural community (Padhi, 2016).

Information from genomic studies of livestock domestication may be used to support the preservation of the genetic variety of farm animals (Bruford *et al*. 2003). Genetic variety within a species can enhance its capacity to adapt to severe conditions and fend off disease (Gao *et al*., 2017). The two main metrics used to assess genetic variation are haplotype and nucleotide diversities, where larger values reflect increased genetic diversity (Chen and Zhang, 2006).

Because the mitochondrial genome is easy to isolate from the nuclear genome, contains many copies within the cell, is small, and accumulates mutations quickly, it has gained popularity in evolutionary and population genetics research (Moritz *et al*., 1987; Sotelo *et al*., 1993 and Unseld *et al*., 1995). For phylogenetic, population genetic, and phylogeographic investigations, mitochondrial DNA is a commonly employed method (Bhuiyan *et al*., 2013).

In this study, different genetic variations were found amongst the four sites ranging from 0.93673 to 0.99823. The lowes genetic similarity was observed between

Naga Hammadi and Qena Cities This may be due to many factors like geographical and ecological conditions. Rahimi *et al*. (2005) reported that an essential requirement for a species' preservation and continued genetic advancement is the estimation of its genetic variability. Numerous factors, including geographical and ecological ones that might lead to population variance and division, contribute to the genetic diversity of a population. Furthermore, population genetic structure might also be the product of gene flow, genetic drift, and balance (Abdel-Kafy *et al*., 2016 and Oseni and Oke, 2012).

Compared to other mtDNA genes, *16S rRNA* has a slower rate of mutation and lower rates of substitution, making it beneficial for research on species, populations, and families (Garland and Zimmer, 2002). When compared to G+C, the entire *16S rRNA* gene exhibits A+T richness (Bo *et al*., 2013). In this study, all understudied species display a greater proportion of A+T than C+G. This was in corroboration with many studies. The *16S rRNA* sequence alignment was 569 bp. Most of them 566 were conserved locations. This was in line with (Saikia e*t al*., 2016; van der Kuyl *et al*., 1995) who reported that highly conserved mitochondrial genes were found in a variety of animal species, which aided in the creation of universal primers for the amplification of mitochondrial genes.

Gallus gallus was the subject of numerous investigations employing a variety of molecular markers. Nishibori *et al*. (2005) conducted research using mtDNA and nuclear sequences to ascertain the genetic links between the four species of junglefowl and the position of the chicken in those relationships. For the chicken population, genetic variation and maternal origin have previously been investigated using partial mtDNA displacement-loop sequences (Berthouly-Salazar *et al*., 2010, Cuc *et al*., 2011, Do *et al*., 2019 and Nguyen *et al*., 2022). Siriwadee *et al*. (2023) conducted research to look at the molecular identification of various local Thai chicken breeds using mtDNA barcodes.

Conclusion:

Large mitochondrial rRNA sequences were utilized in this study to evaluate the geographic genetic diversity of *Gallus gallus* from four places. Low genetic similarity was observed between Naga Hammadi and Qena Cities, this may be due to geographical and ecological conditions. *16S rRNA* genes appear to be beneficial in revealing the geographic genetic relationships of *Gallus gallus*.

Declarations:

Ethical Approval: The Ethics of Animal Experiments Committee at South Valley University's Faculty of Science oversaw the experimental animal methods (Permit No.: 001/02/2024).

Conflicts of Interest: There is no conflict of interest.

Informed consent: All the authors of this manuscript accepted that the article is submitted for publication in the Egyptian Academic Journal of Biological Sciences, B. Zoology, and this article has not been published or accepted for publication in another journal, and it is not under consideration at another journal.

Authors Contributions: All authors are equal in contribution.

Funding: None.

Acknowledgements: Not applicable.

REFERENCES

Abdel-Kafy, E.; Ghaly, I. S.; Ben Larbi, M.; Ahmed, S. S.; Badawi, Y. . ., & Hassan, N. S. (2016). Genetic diversity and phenotype characterization of native rabbit in Middle-Egypt. *Journal of New Sciences, IABC*, (16)(January).

Berthouly-Salazar, C.; Rognon, X.; Van, T. N.; Gely, M.; Chi, C. V.; Tixier-Boichard, M.; Bed'Hom, B.; Bruneau, N.; Verrier, E.; Maillard, J. C. and Michaux, J. R. (2010). Vietnamese chickens: a gate towards Asian genetic diversity. *BMC*

Genetics,11(53): 1-11.

Bhuiyan, M. S.; Chen, S.; Faruque, S.; Bhuiyan, A. K. and Beja-Pereira, A. (2013). Genetic diversity and maternal origin of Bangladeshi chicken. *Mol. Biol. Rep*., 40: 4123–4128.

Bruford, M. W.; Bradley, D. G. and Luikart, G. (2003). DNA markers reveal the complexity of livestock domestication. *Nature Reviews Genetics*, 4: 900-910.

Bo, Z., Xu, T., Wang, R., Jin, X., & Sun, Y. (2013). Complete mitochondrial genome of the Bombay duck *Harpodon nehereus* (Aulopiformes, Synodontidae). *Mitochondrial DNA*, 24(6): 660–662. https://doi.org/10.3109/19401736.2013.772988.

Chen, S.-Y. and Zhang, Y.-P. (2006). Genetic methods and applications in the study of the origin of domestic animals. *Chinese Science Bulletin*, 51: 2469-2475.

Cuc, N. T. K.; Simianer, H.; Groeneveld, L. F. and Weigend, S. (2011). Multiple maternal lineages of Vietnamese local chickens inferred by mitochondrial DNA D-loop sequences. Asian-Australasian Journal of Animal Sciences, 24(2): 155-161.

Di Lorenzo, P.; Ceccobelli, S.; Panella, F.; Attard, G. and Lasagna, E. (2015). The role of mitochondrial DNA to determine the origin of domestic chicken. *World's Poultry Science Journal*,71:311–8.

Do, S. Q.; Nguyen, L. T. P.; Nguyen, T. H. and Nguyen, T. Q. (2019). Genomic characterization of three Vietnamese indigenous chicken varieties using mitochondrial D-loop sequences. *Canadian Journal of Animal Science*, 99(4):833-839.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, *32*(5), 1792–1797. https://doi.org/10.1093/nar/gkh340.

Eltanany, M. A. and Hemeda, S. A. (2016). Deeper insight into maternal genetic assessments and demographic history for Egyptian indigenous chicken populations using mtDNA analysis. *Journal of Advanced Research*, 7: 615-623.

Felsenstein, J. (1985). Confidence Limits On Phylogenies: An Approach Using The Bootstrap. *Evolution; International Journal of Organic Evolution*, 39(4), 783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x.

Fumihito, A.; Miyake, T.; Takada, M.; Shingu, R.; Endo, T. Gojobori, T., et al. (1996). Monophyletic origin and unique dispersal patterns of domestic fowls. *Proceedings of the National Academy of Sciences, USA*, 93:6792–5.

Gao, Y.S.; Jia, X.X.; Tang, X.J.; Fan, Y.F.; Lu, J.X.; Huang, S.H.; Tang, M.J. (2017). The genetic diversity of chicken breeds from Jiangxi, assessed with BCDO2 and the complete mitochondrial DNA D-loop region. *PLoS ONE*, 12, e0173192.

Garland, E. D. and Zimmer, C. A. (2002). Techniques for the identification of bivalve larvae. *Marine Ecology-Progress Series - Mar Ecol-Progr Ser*, 225: 299–310. https://doi.org/10.3354/meps225299.

Hammer, Ø.; Harper, D. A. T., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1): 1-9.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16(2), 111–120. https://doi.org/10.1007/BF01731581.

Lawal, R. A. and Hanotte, O. (2021). Domestic chicken diversity: Origin, distribution, and adaptation. *Animal Genetics*, 52: 385–394.

Moritz, C., Dowling, T. E., and Brown, W. M. (1987). Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics*, 18: 269–292. https://doi.org/10.1146/annurev.es.18. 110187. 001413.

Nguyen, T. T. B.; Duc, N. H.; Khoa, D. V. A.; Tuong, N. H.; Reyer, H.; Wimmers, K.; Thuy, D. T. N. and Thuy, N. T. D. (2022). Genetic diversity of Vietnamese native chicken breeds based on mitochondrial DNA D-loop sequence. *Journal of Animal & Plant Sciences*, 32(3):653-662.

Nishibori, M.; Shimogiri T.; Hayashi T. and Yasue, H. (2005). Molecular evidence for hybridization of species in the genus *Gallus* except for *Gallus varius*. *International Society for Animal Genetics*, *Animal Genetics*, 36, 367-375.

Oseni, S. and Oke, O. (2012). Morphological and Quantitative Characteristics of a Composite Rabbit Population in Backyard Systems in Southwestern Nigeria. *Proceedings 10th World Rabbit Congress SO, Oseni.* Sharm El-Sheikh–Egypt., p223-227.

Ould Ahmed, M.; Ben Salem, F.; Bedhiaf, S. and M'Naouer, D. (2010). Analyse moléculaire de la diversité génétique des dromadaires (Camelus dromedarius) en Tunisie. *Biotechnology, Agronomy, Society and Environment*, 14:399–408.

Padhi, M. K. (2016). Importance of indigenous breeds of chicken for rural economy and their improvements for higher production performance. *Scientifica*, 7; 2016: 9 p.

Peters, J. L. (1976). Order Galliformes In 'Check-list of birds of the World' Vol.II, pp.3-133. Harvard University Press, Cambridge.

Rahimi, G.; Khanahmadi, A.; Nejati-Javaremi, A. and Smailkhanian, S. (2005). Evaluation of genetic variability in a breeder flock of native chicken based on randomly amplified polymorphic DNA markers. *Iranian Journal of Biotechnology, 3*(4): 231–234.

Saikia, D. P.; Kalita, D. J.; Borah, P.; Sarma, S.; Dutta, R. and Rajkhowa, D. (2016). Molecular characterization of the mitochondrial 16S rRNA gene of cattle, buffalo and yak. *Veterinarski Arhiv*, 86(6): 777–785.

Simon, C.; Franke, A. and Martin, A. (1991). The polymerase chain reaction: DNA extraction and amplification. In Molecular Techniques in taxonomy. Eds. G. M. Hewitt, A. W. B. Johnston and J. P. W. Young. *NATO AS1 Series H* 57: 329- 355, 57, 329 355.

Siriwadee, P.; Wirot, L.; Thanapol, P. and Wirawan, N. (2023). Genetic diversity among five native Thai chickens and Khiew-Phalee chickens in lower-northern Thailand using mitochondrial DNA barcodes. *Biodiversitas*, 24(4): 1962-1970.

Sotelo, C. G.; Piñeiro, C.; Gallardo, J. M. and Pérez-Martin, R. I. (1993). Fish species identification in seafood products. *Trends in Food Science & Technology*, 4(12): 395–401. doi.org/10.1016/0924-2244(93)90043-A.

Sokefun, O. B. (2017). The cichlid 16S gene as a phylogenetic marker: Limits of its resolution for analyzing global relationship. *International Journal of Genetics and Molecular Biology*, 9(1), pp. 1–7.

Tamura, K.; Stecher, G. and Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7): 3022–3027. doi: 10.1093/molbev/msab120.

Unseld, M.; Beyermann, B.; Brandt, P. and Hiesel, R. (1995). Identification of the species origin of highly processed meat products by mitochondrial DNA sequences. *PCR Methods and Applications*, 4(4): 241–243. https://doi.org/10.1101/gr.4.4.241.

van der Kuyl, A. C.; Kuiken, C. L.; Dekker, J. T. and Goudsmit, J. (1995). Phylogeny of African monkeys based upon mitochondrial 12S rRNA sequences. *Journal of Molecular Evolution*, 40(2), 173–180. https://doi.org/10.1007/BF00167111.