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Full Length Research

Structural comparison of Mx1 protein of three strains of Nigerian Indigenous chicken and exotic chicken

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ABSTRACT: This research was conducted on three strains of Nigerian indigenous chicken with nucleotide sequences retrieved from the National Centre for Biotechnology Information (NCBI). The translated sequences were also used to model the possible tertiary structure of the proteins they encode for; this was done on the Swiss model server. Multiple sequence alignment analyses and evolutionary relatedness among the sequences identify the shared patterns that may determine their structure and functionality. Having determined the physicochemical properties of the proteins, their structure and their phylogenetic relatedness, the subcellular location was further predicted as this identifies the site in which the protein functions. The Mx1 (Myxovirus resistance 1) protein is pivotal in the defense against viral infection. The structures of observed similarities and variants in the structure of the Mx1 protein were obtained, and similarities and variants in structure, and to fully understand the factors responsible for the variant, secondary structures prediction was carried out. Variation among the Mx1 protein may have been a result of environmental factors owing to how different strains are exposed to environmental conditions. Variation in structure may also result from environmental factors owing to the fact that these strains of chickens may have been exposed to different environmental conditions throughout their phylogenic history, which may have influenced the structure of the protein. These variations could be because of mutations and adaption over the years. The phylogenetic tree showing genetic relatedness in the Mx1 gene among chickens studied revealed low genetic distance, indicating that the four strains are closely related. The variations in genetic differentiation were not significantly observed across poultry breeds; this state states the genetic relatedness of Mx1 protein in the four strains. Genetic difference in the Mx1 gene among strains used in this study ranges from 0.574-0.751. Normal feather had the highest value of 0.751, followed by Noiler (0.744), Naked neck (0.601) and Frizzle feather (0.574) respectively. The amino acid sequence also shows that the sequence of the strains was been has been maintained by natural selection and is conserved to maintain the structure or function of a protein. The findings of this study reveal a strong grasp of the genetic resemblance of the Mx1 protein among the four. Breeders should incorporate knowledge of genetic variation in the Mx1 protein among chicken strains into breeding strategies, taking the full potential of the strains studied.

Keywords: Mx1 (Myxovirus resistance 1) protein, Nigerian Indigenous Chicken, Noiler.

INTRODUCTION

The indigenous chicken is one of the most common and widely spread domestic animal species with an estimated population in 2010 of more than 1.6 billion in Africa (FAOSTAT, 2012). The Nigerian Indigenous chickens provide the backbone for the modern-day poultry sector (Yakubu *et al*., 2009) and also contribute substantially to household food security, diversify incomes, and make provision for quality food, energy, manure, and renewable assets in over 80% of rural households in the developing world. Despite their importance, information on their genetic variability and genetic relationships using blood protein makers is scarce. Humans raise chickens primarily as a source of food, consuming both their meat and their eggs and raise chickens as a source of income. The existing traditional poultry farming system, which is backyard farming, is unable to meet the ever-increasing

demand for poultry meat and eggs due to the rapidly growing population of the region. Low productivity of the Indigenous chicken as a result of small-scale backyard farming is attributed to the poor production potential of existing indigenous chicken breeds. Therefore, to increase productivity, the improved varieties, which are similar to the indigenous chickens or phenotypic replicas of indigenous fowl, are now massively introduced and raised in the region (Singh *et al*., 2002) for increased production potential. Indigenous chicken represents an important valuable animal genetic resource, and conservation, sustainable exploitation and improvement of local breeds are therefore important issues.

According to Sonaiya and Swan (2004), poultry birds are known to possess a high degree of efficiency in feed utilization with little or no socio-religious taboo in their consumption. Poultry meat and egg production accounts for more than 30% of all animal protein.

A disease is one major problem however, an important factor that adversely affects chickens in the topics is disease, which is one major problem in the poultry industry. Resistance to different disease changes among the breeds, types, and sometimes, location of the poultry. This is influenced greatly by both the genotype and the presence of a specific allele. Importantly, studies have suggested the strong influence of genetic factors in controlling resistance and susceptibility of poultry to infections.

The Mx1 proteins are interferon-induced guanosine triphosphate enzymes and show antiviral activity for AIV (Avian influenza virus) in humans and mice and poultry inclusive, which inhibits the proliferation of single-stranded negative-sense RNA viruses (Haller and Kochs, 2002). *Mx1* protein is found in the cytoplasm of cells and has a GTPase activity. *Mx1* also has an antiviral effect, dependent on the GTPase activity (Samuel, 2001).

Due to the rapid spread of infections among chickens, breeders have begun to focus on genes associated with the innate immune system, such as Myxovirus resistance protein gene with birds showing different susceptibilities (Hagiwara *et al*., 2020**)**. These genes play an active role in mitigating the incidence of infections. Myxovirus resistance (Mx) gene has been shown to have an inhibitory effect on influenza virus. It has a direct antiviral activity that inhibits a wide range of viruses by blocking an early stage of the viral replication cycle (Tamura *et al.,* 2013).

The Mx protein was reported to have intrinsic antiviral activity and be responsible for influenza virus resistance in mammals (Arnheiter *et al*., 1990). In contrast to mammals, there is no consistent conclusion regarding the antiviral activity of the chicken *Mx1* gene in either in vivo or in vitro assays (Ewald *et al*., 2011). Chickens only have one *Mx1* gene originally reported lacking antiviral activity (Bernasconi et al., 1995). The Mx1 gene encodes chicken Mx protein. It comprises 705 amino acids in which a tripartite guanosine triphosphate-binding motif and a leucine zipper motif are conserved among different species (Watanabe, 2007). Myxovirus resistance gene (Mx1 protein) is an interferon-induced gene that inhibits the proliferation of single-stranded negative sense RNA viruses. Ko *et al*. (2002) reported the antiviral activity of Mx1 protein in poultry. The chicken Mx protein is predominantly cytoplasmic and consists of 705 amino acids; also, the chicken Mx1 protein has 21289 bp linear DNA on chromosome number 1. It has 14 exotic regions, and the coding sequence is 2118 bp long (Bernasconi *et al*., 1995). Studying the structural differences of Mx1 protein in different strains of Nigerian indigenous chicken can provide valuable insight into the genetic diversity of the immune response to viral disease in these strains of chickens.

MATERIALS AND METHODS

Experimental animal

The Nigerian Indigenous chicken, namely, Frizzle feather, Naked neck, Normal feather, and the exotic chicken (Noiler), were used in this experiment.

Nucleotide sequence retrieval

Nucleotide sequences were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov) and were translated to amino acid sequences on the EMBOSS webserver (https://www.ebi.ac.uk/jdispatcher/st/emboss_transeq).

Modelling of 3D structure

The translated sequences were also used to model the possible tertiary structure of the proteins they encode for; this was done on the Swiss model server (www.swissmodel.expasy.org). Predicting the tertiary structure provides a model that can be used to understand the functionality of a protein, such as revealing the possible active sites and binding domains as 3D models of proteins are predicted and modelled from structures of native proteins with similar amino acid sequences.

Multiple sequence alignment and plotting of phylogenic tree

All the translated sequences were aligned in a multiple sequence alignment fashion on the COBALT Multiple Sequence Alignment tool (https://www.ncbi.nlm.nih.gov /tools/cobalt/re_cobalt.cgi) as described by Damas *et al.* (2020). Multiple sequence alignment analyses the evolutionary relatedness among the sequences and identifies the shared patterns that may determine their

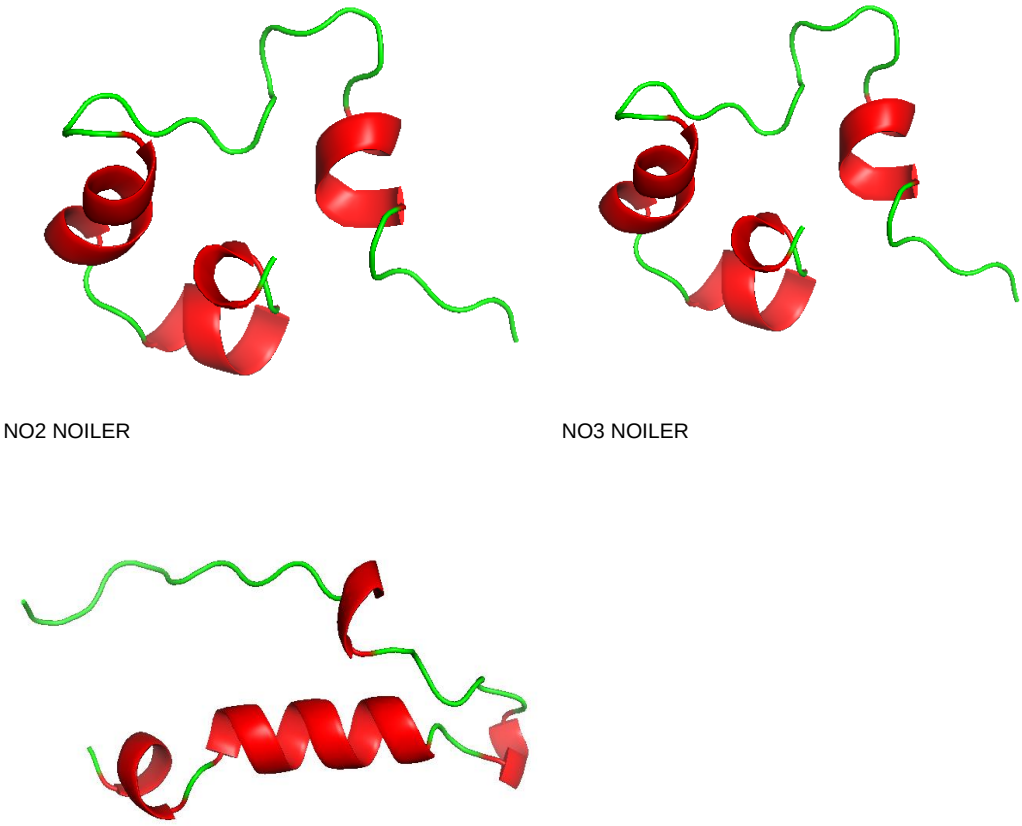


Figure 1a. Models of the protein structures.

structure and functionality. The aligned sequences were viewed with the **Conservation** and **MBLOSUM80** settings respectively.

Identification of the subcellular location

Having determined the physicochemical properties of our proteins, their structure, and also their phylogenetic relatedness, we went further to predict their subcellular location as this identifies the site in which the protein functions; since protein location controls availability to different types of biomolecules, thus its location determines the function of the protein.

Statistical analysis

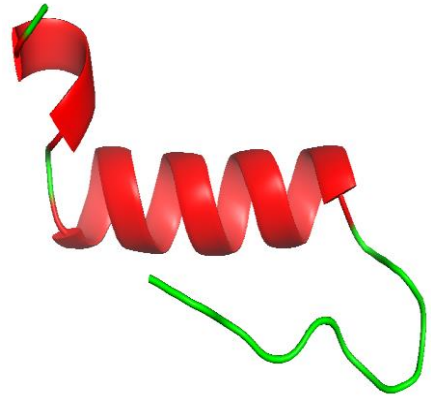
Analysis was subjected to Swiss model server (www.swissmodel.expasy.org), COBALT Multiple Sequence Alignment tool (https://www.ncbi.nlm.nih.gov /tools/cobalt/re_cobalt.cgi) as described by Damas et al. (2020) and the subcellular locations of our proteins were identified on the DeepLoc-1.0 online server (www.cbs.dtu.dk/services/DeepLoc/) as described by Armenteros *et al.* (2017) in their publication.

RESULTS

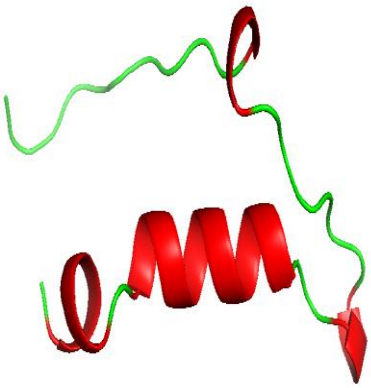
Structure alignment of the Mx1 protein of the Nigerian Indigenous chicken (Frizzle feather, Naked neck, and Normal feather) and the exotic strain (Noiler) reveals the similarities between the three strains of the Nigerian Indigenous chicken and the variance when compared with the exotic strain as modelled using the Swiss model (Figure 1).

Amino acid Sequence alignment revealed the similarity between the sequences. Conserved rejoins are indicated in red, low conserved regions are in blue, and non-conserved regions are gray. For better visualization, conserved rejoins are indicated in red, low conserved regions are in blue, and non-conserved regions are gray. To better visualize the alignment, the sequence alignment was viewed using the Conservation and Blosum80 methods (Figure 2).

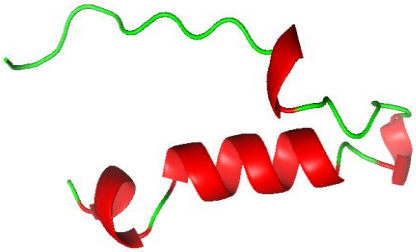
Amino acid sequence alignment using the Conservation method highlights highly conserved and less conserved



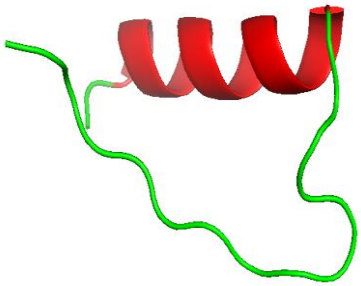
FR1 FF



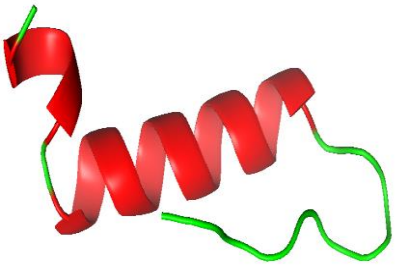
FF GALLUS GALLUS



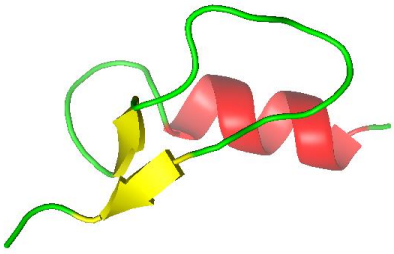
NM1



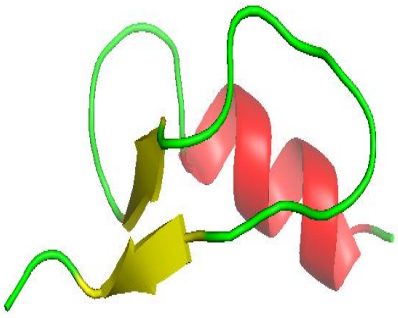
NM2



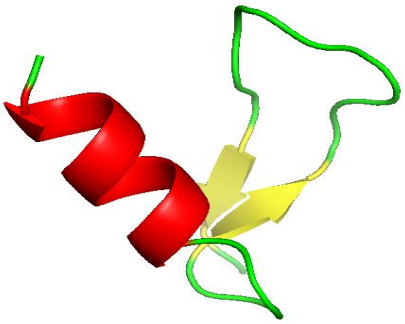
NM3



NM4



NM5



NM6

Figure 1. Contd.

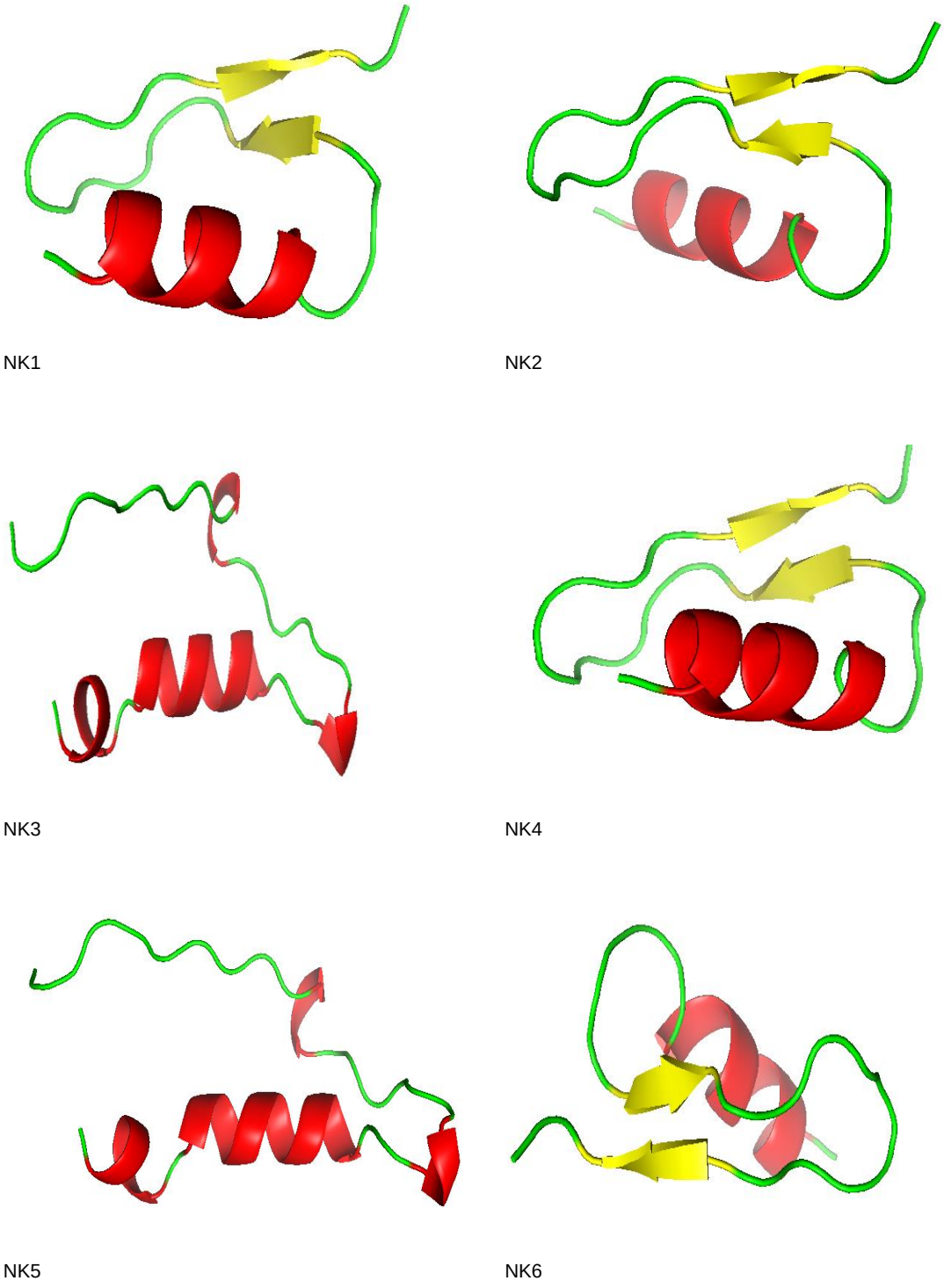


Figure 1d. Models of the protein structures.

amino acid positions based on the relative entropy threshold of the residue. Only alignment positions with no gaps were coloured. Red indicates highly conserved regions, blue indicates less conservation regions, and grey indicates non-conserved regions (Figure 3).

Amino acid further sequence alignment using Blosum80 methods displays the degree of match of residues relative to each alignment position/column. Blue represents a better match, while Green represents a worse match. The colour reflects the average match over all the other

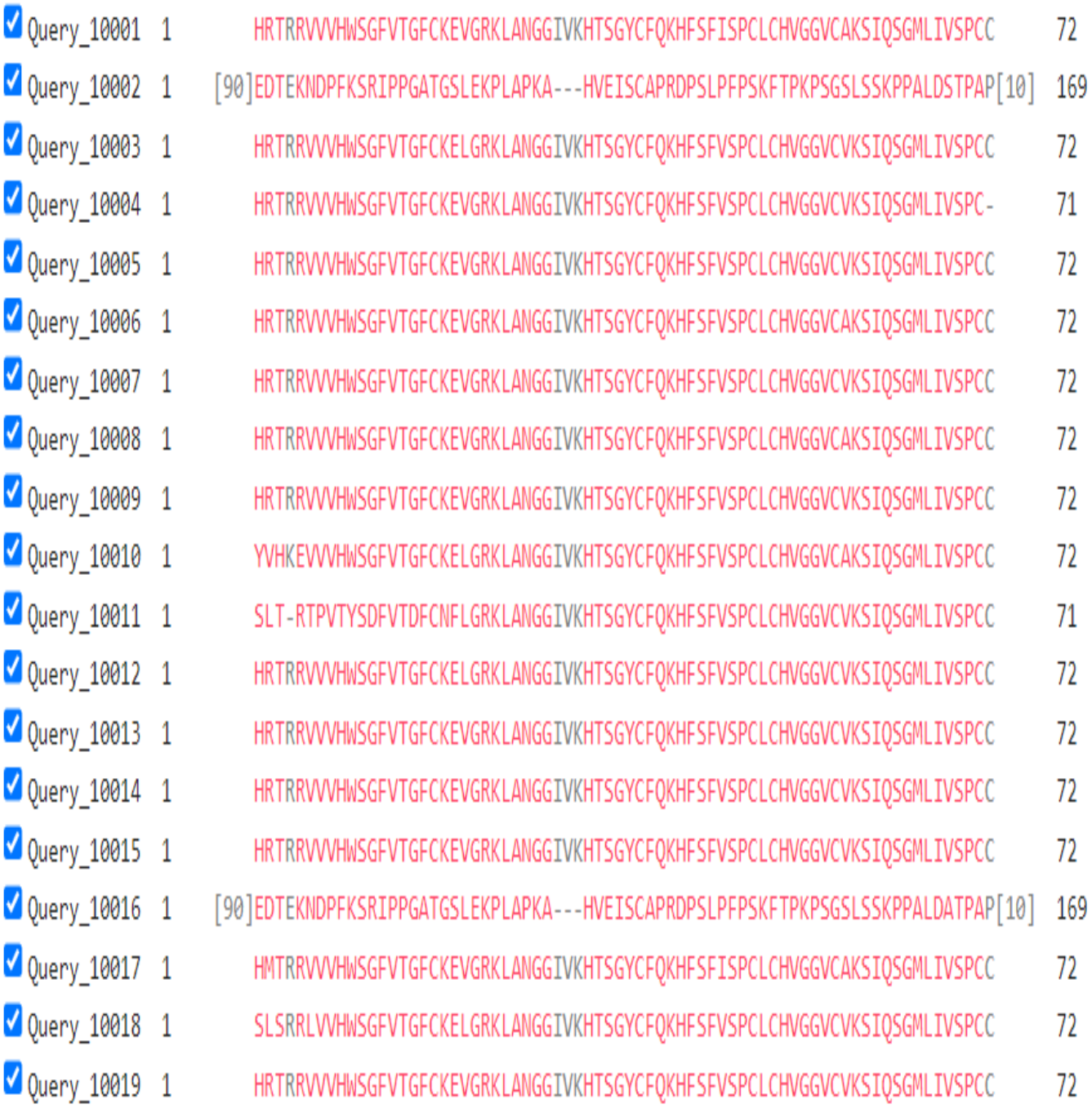


Figure 2. Amino acid sequence alignment of the protein.

residues in the column (Figure 4).

Figure 5 describes the phylogenetic tree of the three Nigerian indigenous chickens and the exotic strain (Noiler). It reveals that the subcellular location of the Mx1 protein of the NM, NK, and NO is genetically more related than the FR, i.e., the Normal feather (NM) and naked neck (NK) of the Nigerian indigenous chicken are related phylogenetically to the Noiler (Exotic strain) than the fizzle feather of the Nigerian indigenous chicken.

DISCUSSION

From the structures obtained, we observed nine (9) coil variants of the Mx1. To understand the factor(s) responsible for the variants, we carried out secondary structure prediction. This was to help us view the percentages of helix, sheet, and coils, as these determine the tertiary structure of a protein and its stability.

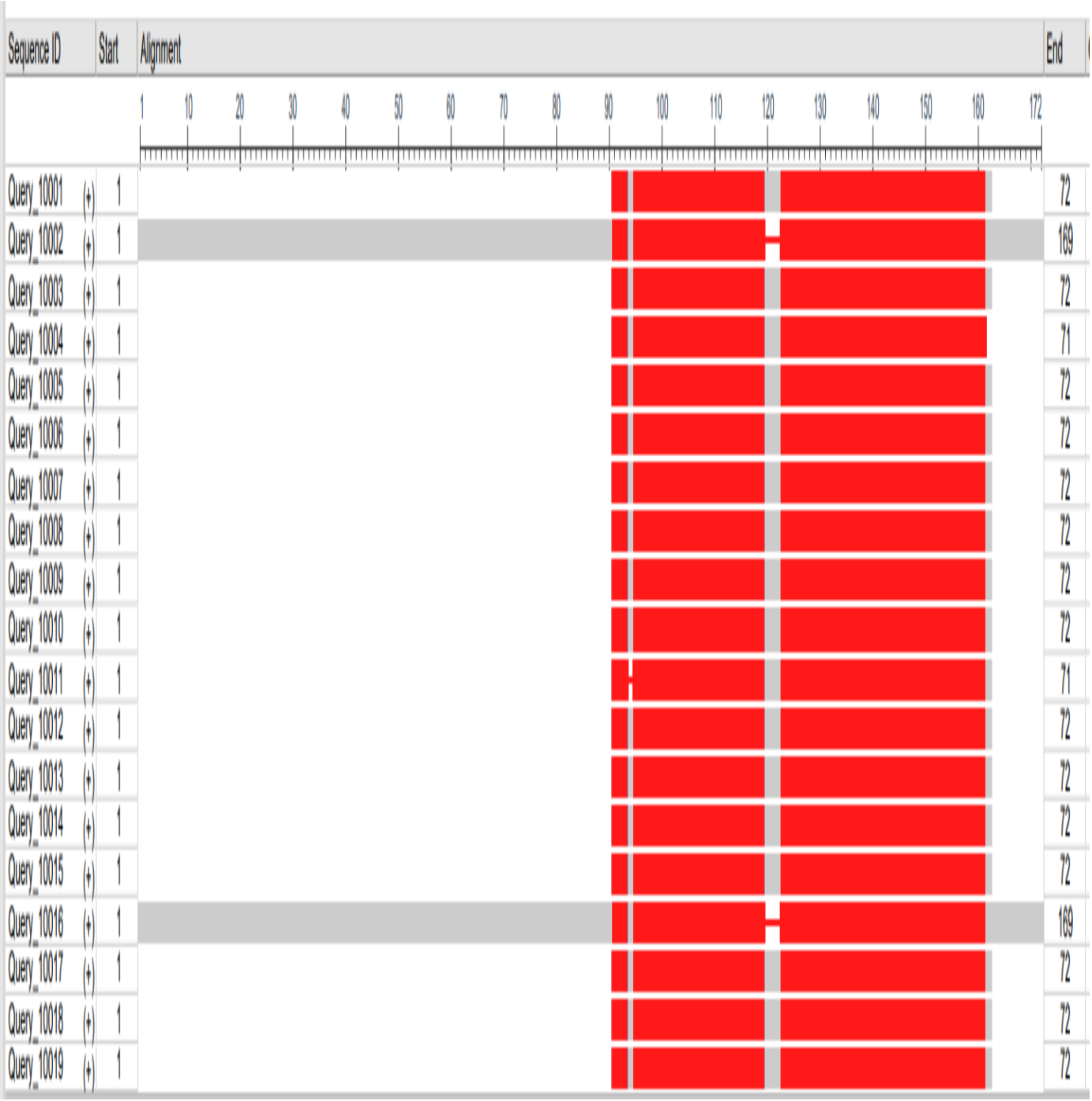


Figure 3. Amino acid sequence alignment viewed using the conservation setting.

The results obtained in this study showed some variations across the parameters studied. Variation in structure may also result from environmental history factors owing that these strains of birds may have been exposed to different environmental condition down their phylogenic history factors, owing that these strains of birds may have been exposed to different environmental conditions throughout their phylogenic history, which may have influenced the structure of the protein. These variations could be a result of mutations and adaption-adaptation over the years. Genetic differences in the myxovirus resistance 1 (Mx1) protein gene among strains of chicken range from 0.574-0.751.

Normal feather had the highest value of 0.751, Noiler had the value value, which was 0.744, Naked neck had the value of 0.601, and Frizzle feather had a value of 0.574,

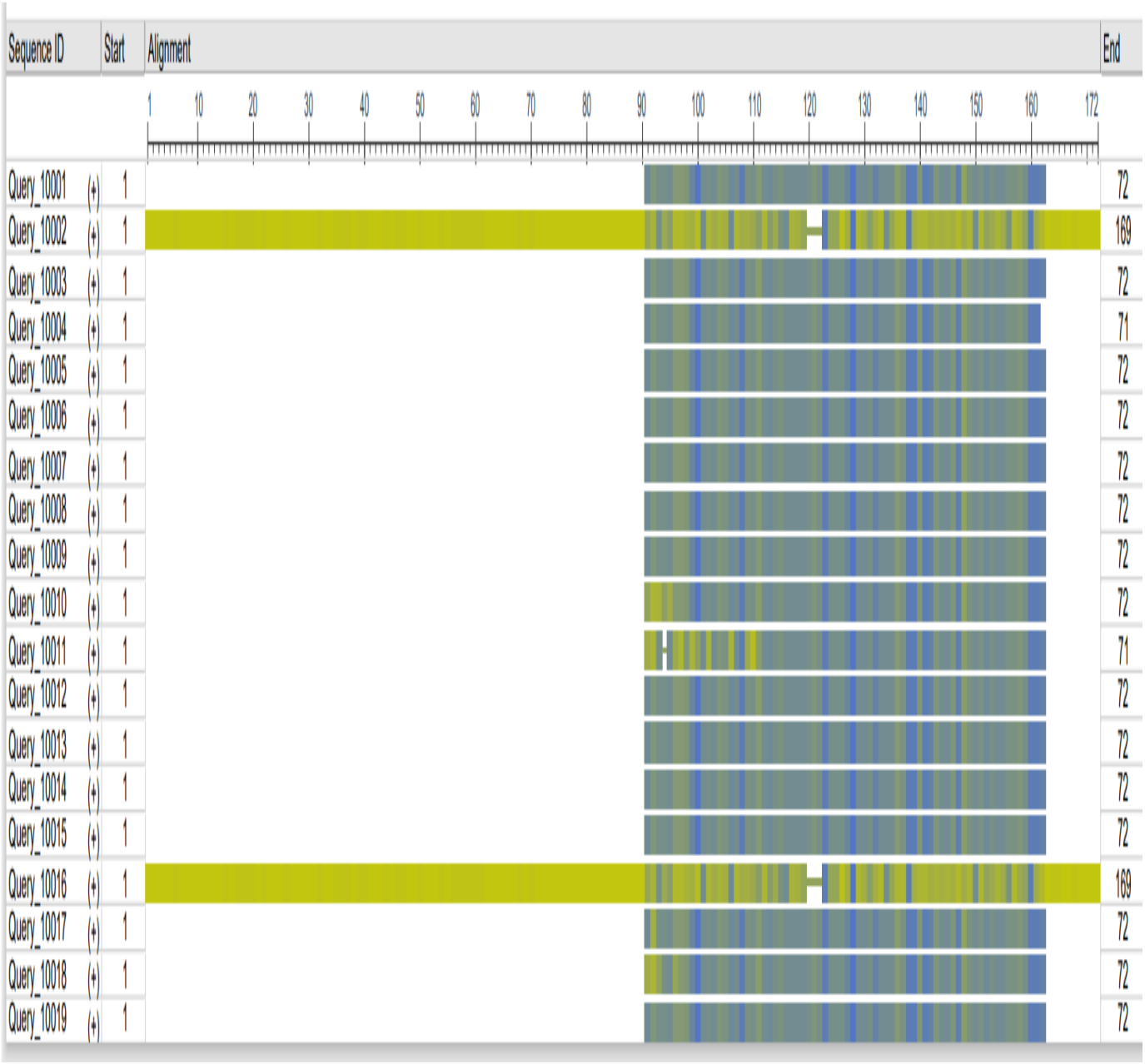
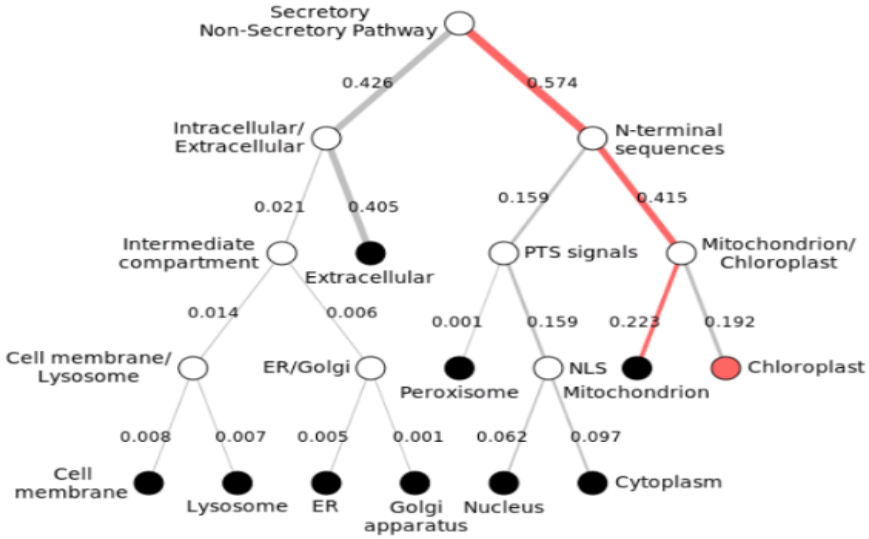
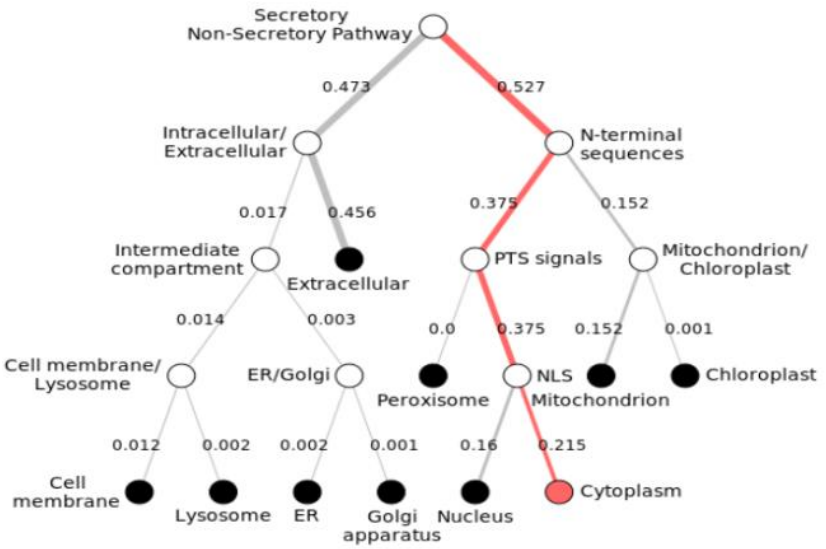


Figure 4. Amino acid sequence alignment viewed using BLOSUM80 setting. **Key:** Query_10001: **FF GALLUS GALLUS**; Query_10006: **FF Zyxin**; Query_100011: **FR1 FF**; Query_10002: **NK1**; Query10007:**NK2;** Query_100012: **NK3**; Query_10003: **NK4;** Query_10008: **NK5;** Query_100013: **NK6**; Query_10004: **NM1 NF**; Query_10009: **NM2 NF**; Query_100014: **NM3 NF**; Query_10005: **NM4;** Query_100010:**NM5 NF**; Query_100015: **NM6 NF**; Query_100016: **NN Zyxin;** Query_100017:**NO1 NOILER**; Query_100018:**NO2 NOILER;** Query_100019:**NO3 NOILER. Where:** FF = Frizzle feathered; NF = Normal feathered; NN = Naked neck; NM = Normal feathered; NK = Naked neck; NO = NOILER.

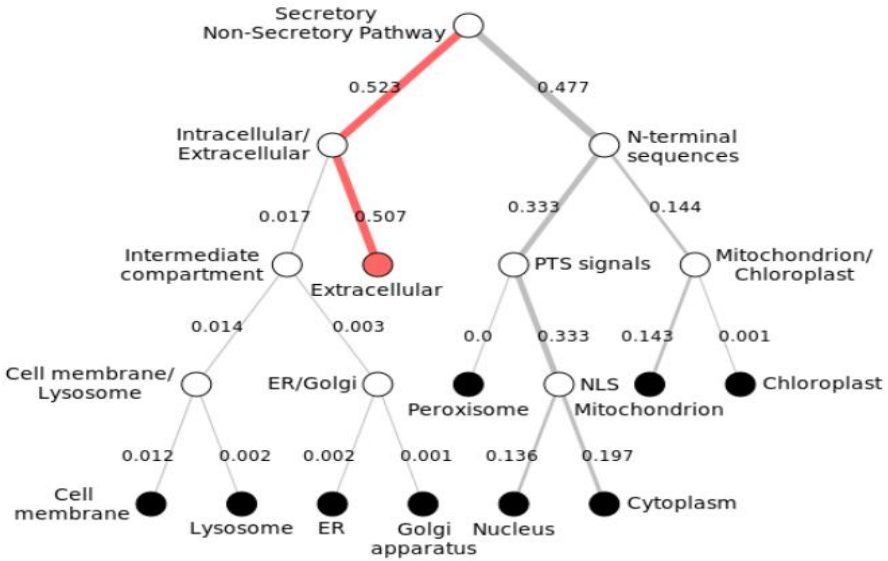
which was the lowest recorded value. The phylogenetic tree showing genetic relatedness in the myxo virus resistance protein gene among chickens studied revealed a low genetic distance, indicating that the four strains are closely related. The variations in genetic differentiation were not significantly observed across poultry breeds. These slight variations may have occurred due to a series of changes due to exposure and management undergone by the different strains over the years. These variations were seen in areas such as a number of segregating sites which were within the range of 0 - 26 as reported by Pandey *et al.* (2002) but were not by the report by Agaviezor and Chukwuemeka (2020), who recorded several number segregating sites which ranged from 41 – 174.



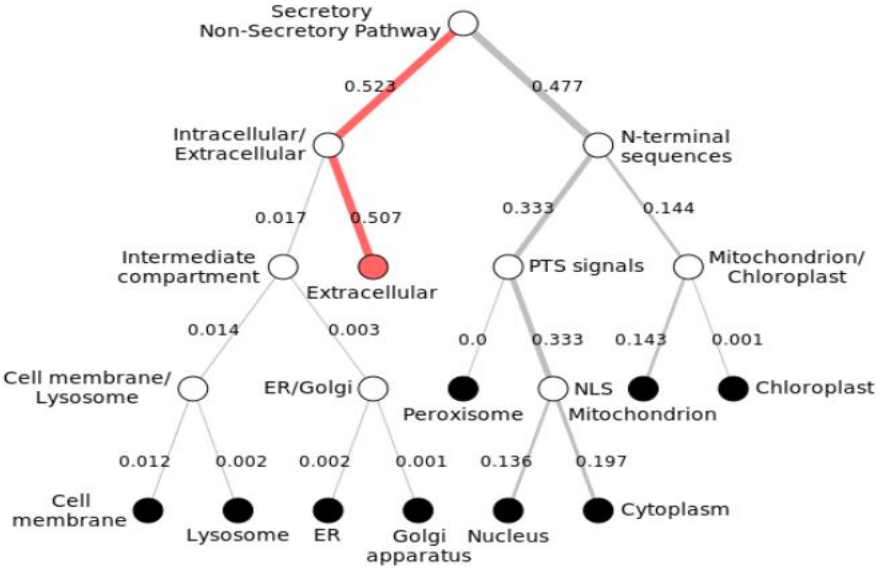
FF GALLUS GALLUS (Mx1)



FR1 FF (Mx1)

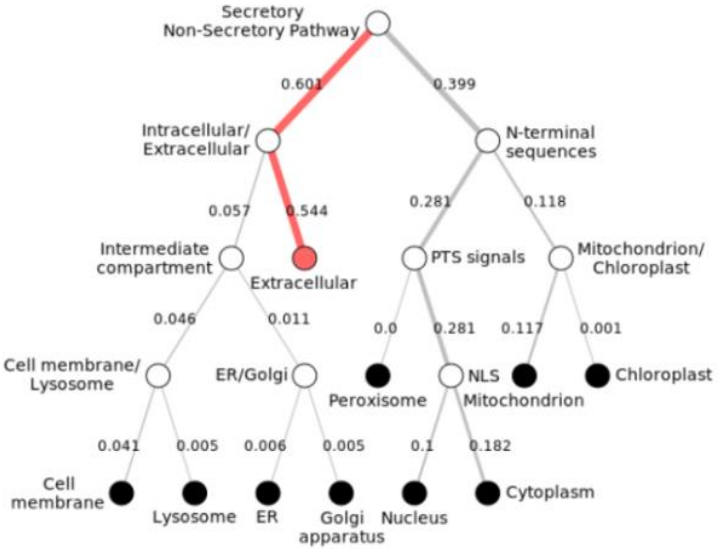


NK1 (Mx1)

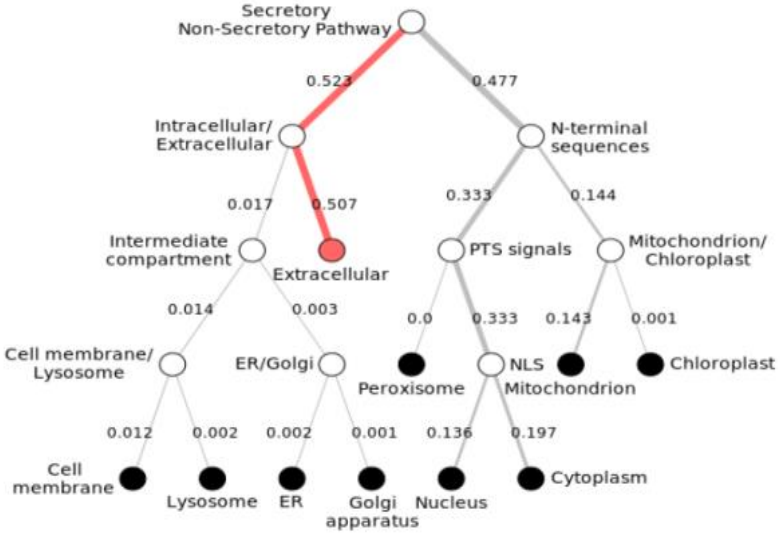


NK2 (Mx1).

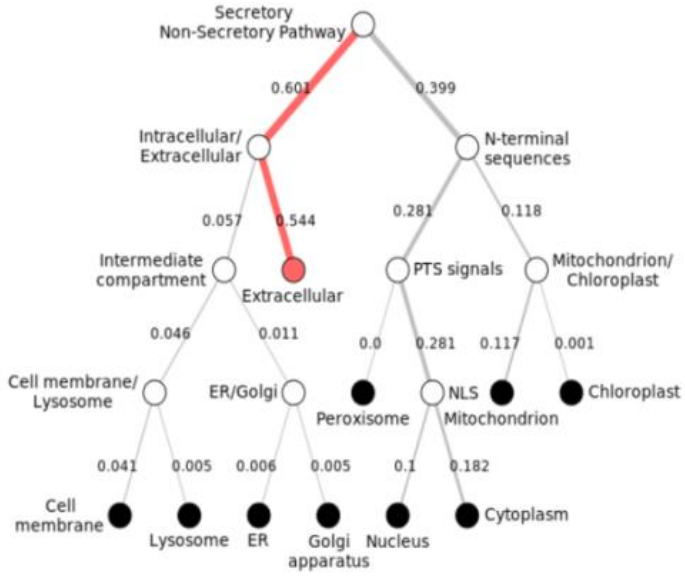
Figure 5. Representations of the subcellular location of the Mx1 protein, the graph showed that Mx1 proteins are extracellular and soluble.



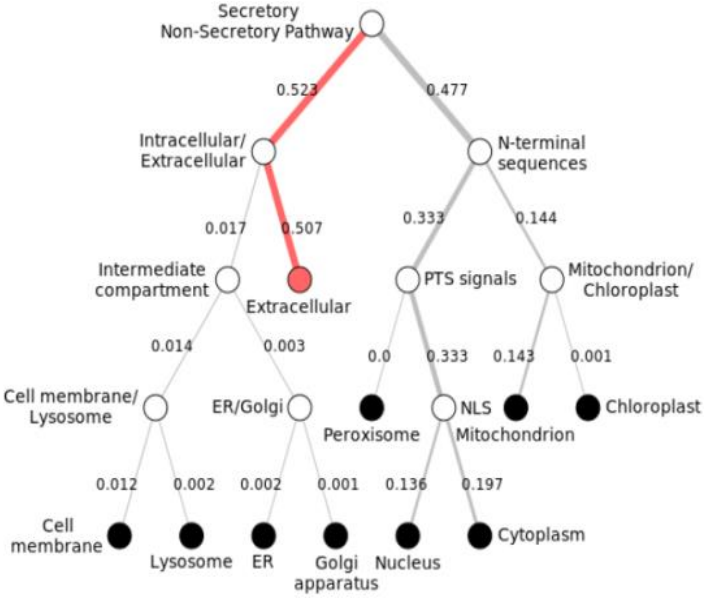
NK3 (Mx1)



NK4 (Mx1)

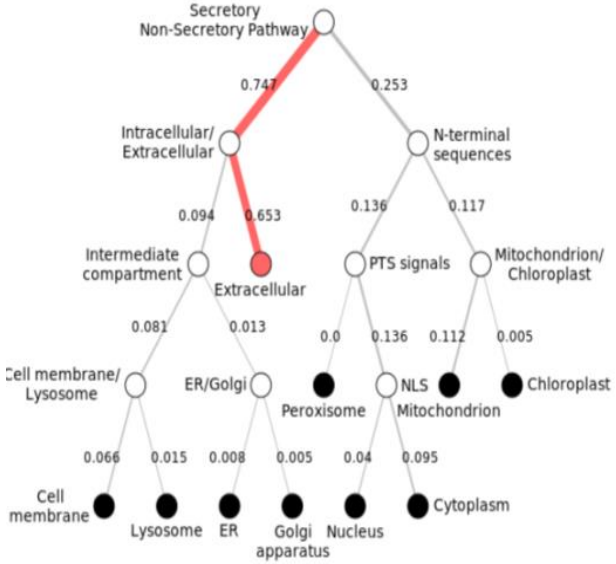


NK5 (Mx1)

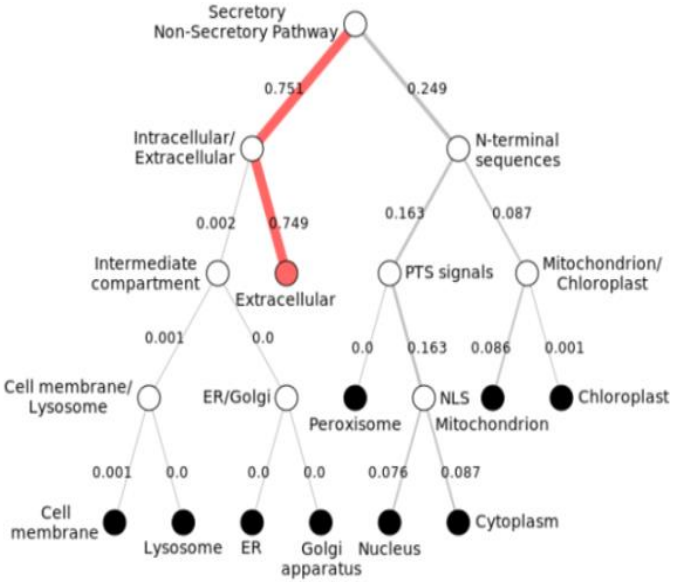


NK6 (Mx1)

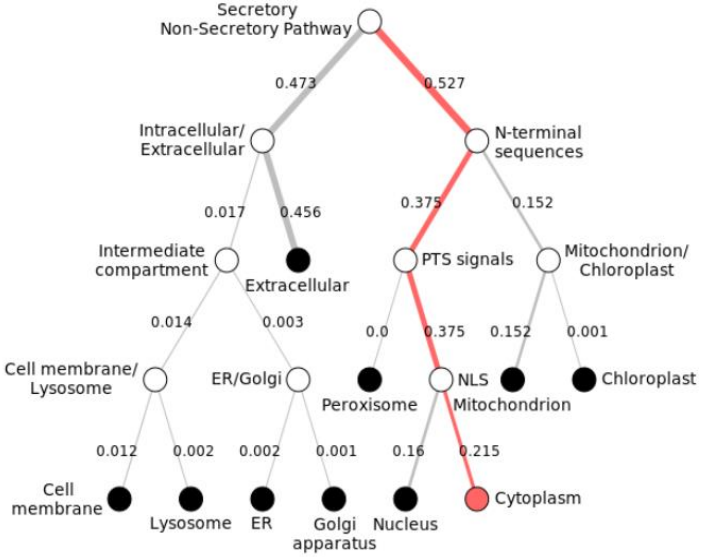
Figure 5. Contd.



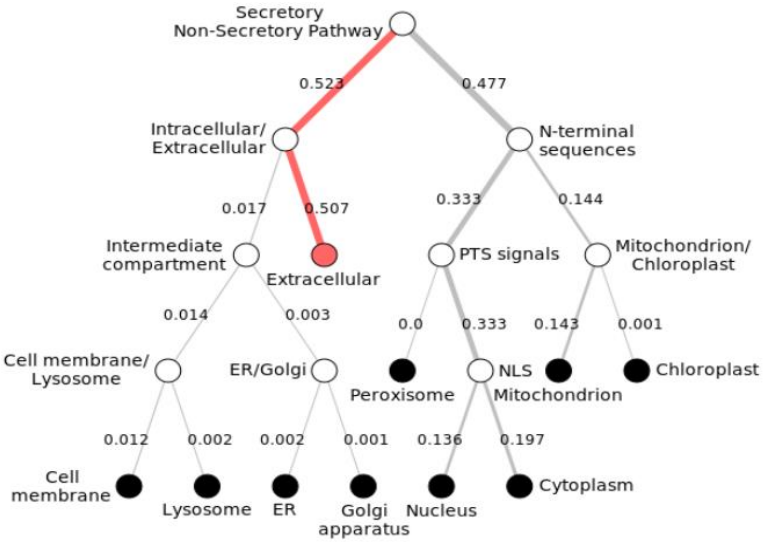
NM1 NF (Mx1)



NM2 NF (Mx1)

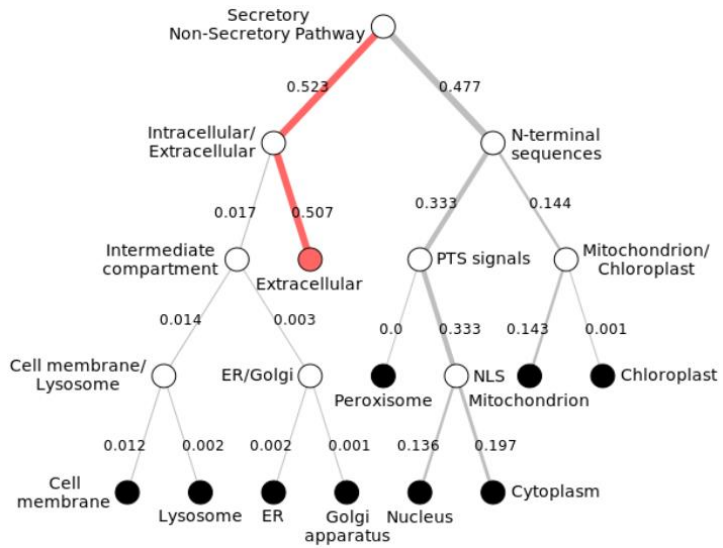


NM3 NF (MX1)

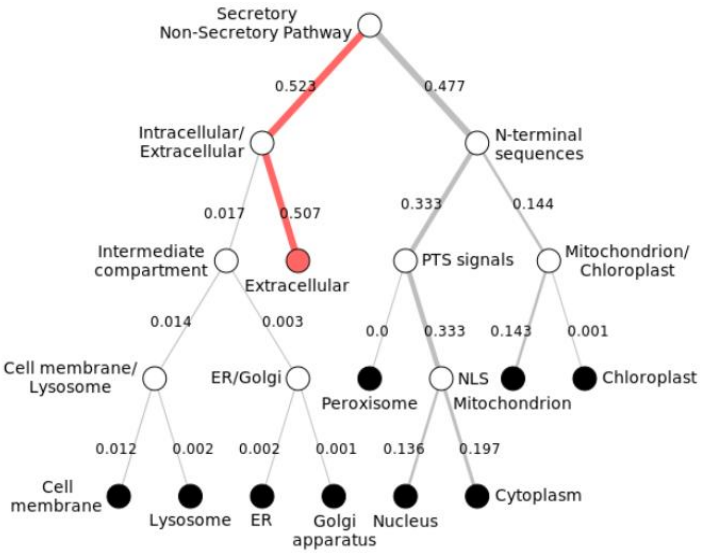


NM4 NF (Mx1)

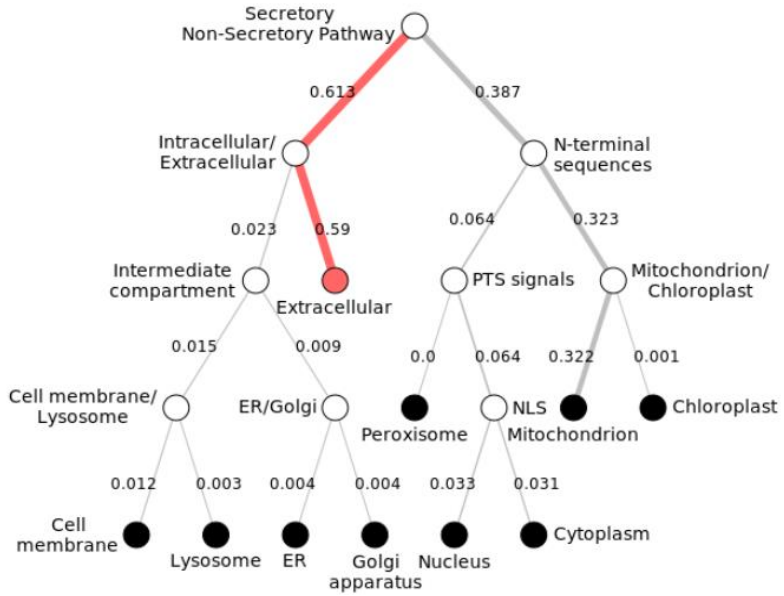
Figure 5. Contd.



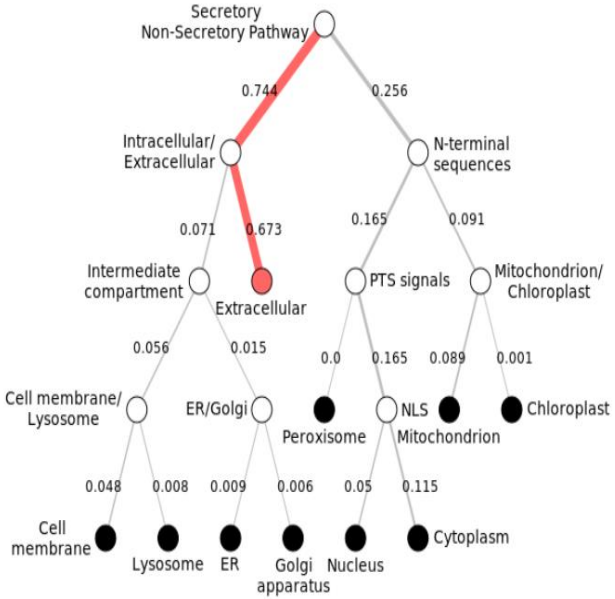
NM5 NF (Mx1)



NM6 NF (Mx1)



NO1 NOILER (Mx1)



NO2 NOILER (Mx1)

Figure 5. Contd.

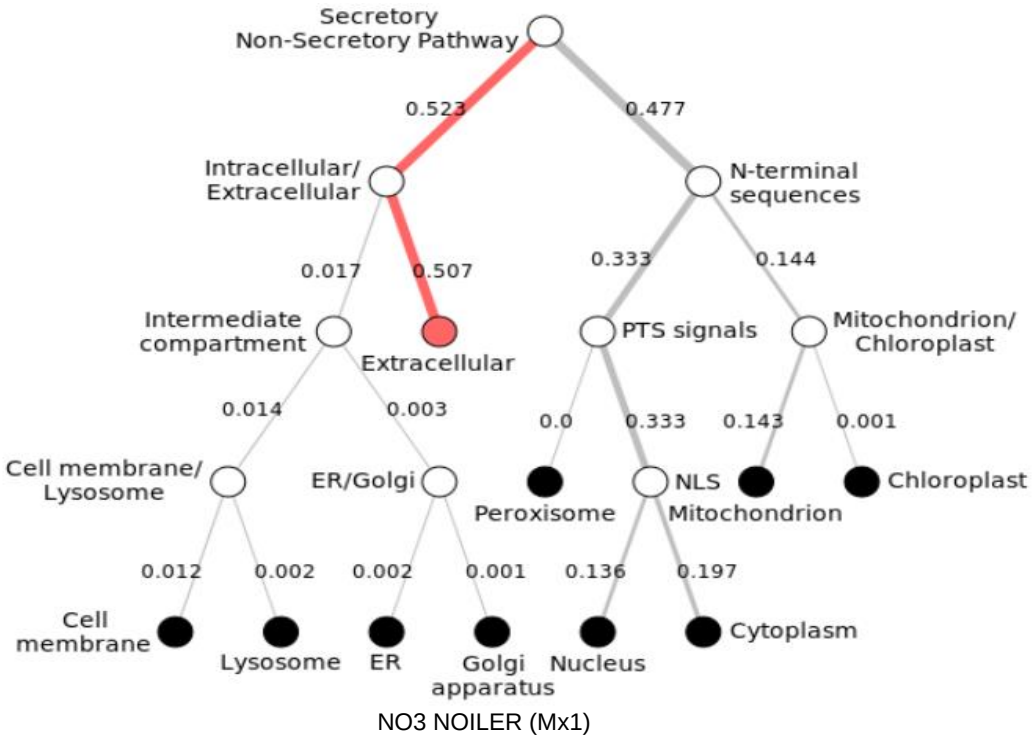


Figure 5. Contd.

The genetic distance between poultry breeds, as observed by Pandey *et al.* (2002), was within the range of 0.5609-0.8982 but was not in agreement with the values obtained in this study but was very close to that of this study. This states the genetic relatedness of Mx1 protein in the three strains of the Nigerian indigenous chicken and the exotic strain (Noiler).

The myxovirus resistance (*MX*) genes are evolutionarily conserved in nearly all vertebrates. MXgene expression is induced by type I or III interferon, and the corresponding gene products inhibit a wide range of viruses (Haller and Kocks, 2002). Over many generations, nucleic acid sequences in the genome of an evolutionary lineage have gradually changed due to mutations and deletions. Sequences may also recombine or be deleted due to chromosomal rearrangements. Conserved sequences persist in the genome despite such forces and are thought to have a functional value, as evidenced by Kimura and Ohta (1974). The amino acid sequence alignment shows that the sequence of the strains has been maintained by natural selection and is conserved to maintain the structure or function of a protein. This was in agreement with the findings on sequence genomic that shows agreement with the findings on sequence genomics that show a high sequence conservation of Mx1 protein in the three strains of Nigerian Indigenous chicken (Flaming *et al*., 1998). This demonstrates the protein's important role in antiviral defence, inhibiting the possibility of variations that could alter the protein's function.

Conclusion

The findings of this study reveal a strong grasp of the genetic resemblance of the Mx1 protein among the three strains of the Nigerian indigenous chickens and the exotic strain (Noiler). The limited diversity and significant genetic similarities observed are based on the likelihood that substantial variations induced by environmental factors and exposure to the environmental conditions is yet to occur in the genetic make-up of these strains that could be detected.

CONFLICT OF INTEREST

The authors declare that they have no conflict interest.

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