

COMPUTATIONAL ANALYSIS OF MOLECULAR SIGNATURES OF SELECTION AT PRODUCTION GENES FOR EGG AND MEAT IN LIVESTOCK AND WILD RELATIVES

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Abstract

In Kenya, indigenous chicken form the majority of poultry. Their egg and meat production is low compared to commercial chicken. However there is variation among the indigenous chicken caused by evolutionary forces such as Natural Selection. The aim of this study was to model evolution and subsequent detection positive selection on genes for egg and meat production. Genes for egg production were prolactin, vasoactive intestinal peptide (vip) and intestinal peptide receptor (vipr) while genes for meat production were growth hormone (gh), growth hormone receptor (ghr), insulin growth factor 1(igf1) and insulin growth factor 1 receptor (igf1r). This was achieved by data mining of the sequences of these genes from databases followed by performing reciprocal BLASTp and BLOSUM62 substitution matrix using chicken sequence for each gene as the query. Homologues with an expectation value greater than 1e-10 were selected for each gene. Thereafter, Multiple Sequence Alignment was done using MUSCLE which uses an iterative algorithm. The alignments were edited using Seaview. MEGA6 was used to test for heterogeneity in substitution rate and for determining evolutionary model using lowest Akaike Information Criterion. Finally, phylogenetic trees were constructed using Nearest Neighbour Interchange with subtree pruning and regrafting of FastME followed by analysis for signatures of selection using PAML4. This led to inferred phylogenetic trees that modeled evolution of the genes in the different species and identification of positive selection on one amino acid site on igf1r. This is an advanced genetic technology that may be used to improve egg and meat production through artificial selection.

Key words: poultry; growth hormone; natural selection; insulin growth factor1 receptor; codon-substitution models; prolactin; vipr1

1.0 Introduction

Chickens, quails and guinea fowls are poultry which are domesticated by humans for production of eggs, meat and feathers. In Kenya, as in other countries worldwide, chickens account for largest livestock species reared by man. In Kenya, there are approximately 30 million domesticated birds. Indigenous chicken form 70%, layers, broilers and the breeding stock form 25% while the rest of the domestic birds like quails, guinea fowls, ducks, geese, turkeys and pigeons make up 5% of the poultry population (Olwande *et al.*, 2010). The eggs and meat from the poultry are a source of protein and a source of income when they are sold. Eggs and meat from Indigenous poultry are highly favored because of their taste. Indigenous chickens lay an average of 40-60 eggs per year while commercial chickens lay more than 300 eggs per year (FAO, 2010a). Their eggs are small weighing about 25-49g. Indigenous chicken are also slow in maturing and they have small bodies. Broilers are 2kg live weight at 5 weeks of age while indigenous cocks merely weigh more than 1kg in 20 weeks (FAO, 2010a). The government and some NGO poultry programs have attempted to improve indigenous poultry through crossbreeding with commercial genotypes which has not been successful.

Indigenous poultry are highly variable phenotypically in size, skin colour, live weights and egg production among other traits (Kingori *et al.*, 2010). This variation is as a result of individuals of a population changing to adapt to their environment through the process of Natural Selection. There are three forms of Natural Selection: (1) Purifying Selection which eliminates deleterious mutations (2) Positive Selection which refers to mutations that are advantageous (3) Neutral Selection which does not confer any fitness advantage (Oleksyk *et al.*, 2010).

One reliable way to detect positive selection is by comparing the rate of non-synonymous substitution (dN) to the rate of synonymous substitution (dS). Values of $dN/dS < 1$ indicate purifying selection while values of $dN/dS > 1$ indicate positive selection. When the $dN/dS = 1$, it indicates neutral selection (Yu *et al.*, 2011). There have been several methods that have been proposed to estimate the dN/dS rate (Nei, 2005). Codon-based maximum likelihood models by Nielsen and Yang are the most accurate for detecting positive selection among lineages and amino acid sites as they account for variability in selection pressure among sites (O'Brien and Suchard, 2009). These models have been used to detect positive selection in various genes like growth hormone gene (Yuri *et al.*, 2008), Leptin gene (Yu *et al.*, 2011) and Hyperglycemic hormone (Padhi *et al.*, 2007). In this study, we constructed phylogenetic trees to understand the evolution of the production genes and analysis for signatures of selection. In Kenya and most developing countries, improvement of indigenous poultry is not advanced (Dana *et al.*, 2010). Current technologies are not accurate as there is no evidence of the influence of genes on the phenotype. In this study, we used codon-based models to detect positive selection in genes for egg production and growth. Application of selection of genes in improving production is more accurate, economical and reliable (Cheng, 2010).

2.0 Methods

2.1 Retrieval of Sequences from Biological Databases

The full coding sequence or mRNA sequences of chicken Prolactin, Vasoactive Intestinal Peptide and Vasoactive Intestinal Peptide receptor genes implicated for egg production and Growth Hormone, Growth Hormone Receptor, Insulin-like Growth Factor I, Insulin-like Growth Factor I Receptor implicated for growth were retrieved using cross-database ENTREZ searches in GenBank. The chicken genome available at the annotated ENSEMBL Database was used as the reference genome.

2.2 Retrieval and Selection of Homologues to the Candidate Genes using Reciprocal BLAST

A reciprocal BLAST was done using the algorithm BLASTp which is available at NCBI to infer homology. The amino acid sequence of each candidate gene in the fasta format was used as the query in performing pairwise sequence alignments in non-redundant (nr) databases. The substitution matrix used was BLOSUM62. For egg production, homologues selected were birds with an E value greater than $1e-10$. For meat production, the homologues selected were animals that are commonly eaten by man with an E value of greater than $1e-10$. Amino acid sequences of the homologues were converted to their corresponding coding sequences and the stop codons manually removed to prevent interference with later analysis.

2.3 Multiple Sequence Alignment of Homologous Sequences

ClustalX2 (Larkin *et al.*, 2007) which is a MSA software that uses a progressive algorithm that is heuristic in nature was used to align the different homologues for each candidate gene to confirm homology and as a preceding step in phylogenetic analysis. PRANK that also uses the progressive algorithm and MAFFT (Kato and Standley, 2013) were used. This was followed by MSA using MUSCLE version 1.3.8.31-1 (Edgar, 2004). Jalview version 2.8 (Waterhouse *et al.*, 2009) and Seaview were used to view and edit the alignments.

A Comparison of the alignments from the three MSA softwares was done.

2.4 Selection of Substitution Model

MEGA6 was used to test for heterogeneity in substitution rate and select the evolution model using the Akaike Information Criterion.

2.5 Phylogeny Construction

Phylogenetic trees were inferred using Nearest Neighbour Interchange with subtree pruning and regrafting of FastME2 (Lefort *et al.*, 2015). 1000 bootstraps were used to test for confidence of the inferred relationships. The inferred trees were saved in Newick format. Dendroscope version 3 (Yang, 2007) was used for graphical visualization of the trees.

2.6 Detection of Signatures of Selection

The PAML5 package (Yang, 2007) was then used for phylogenetic analysis using maximum likelihood.

3.0 Results

In the supplementary we find the orthologues for prolactin, vip, vipr, gh, ghr, igf1 and igf1r generated from a reciprocal BLAST approach. In Figure 1, the alignment from MUSCLE for vip gene is shown with the conserved domains. In Figure 2 and 3 respectively, the conserved domains are shown for prolactin and vipr1 genes.

Multiple Sequence Alignment of gh showing the different domains (Figure 4). Multiple Sequence Alignment of ghr with the different domains (Figure 5). Figure 6 and 7 shows the Multiple Sequence Alignment of igf1 and igf1r genes.

Phylogenetic tree in Figure 8 illustrates evolution of prolactin in the different species while Figure 9, 10, 11 respectively illustrate evolution of gh gene, igf1r gene and vipr1 gene. Figure 12 shows one positively selected amino acid site on igf1r gene.

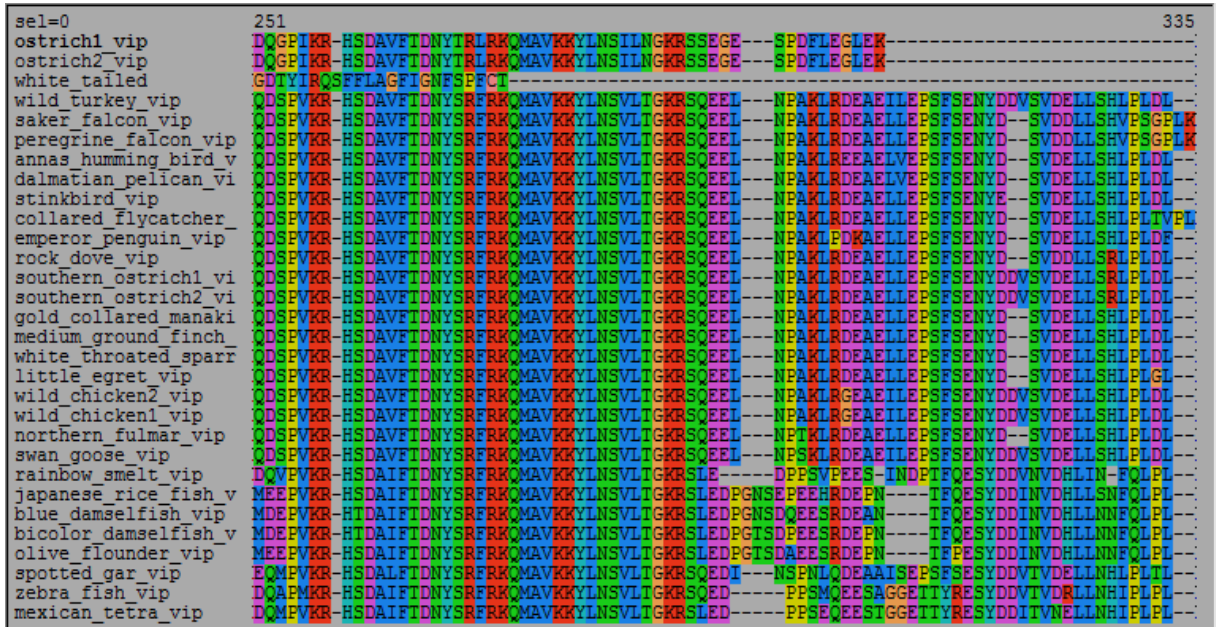


Figure 1: Multiple Sequence Alignment of vip showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

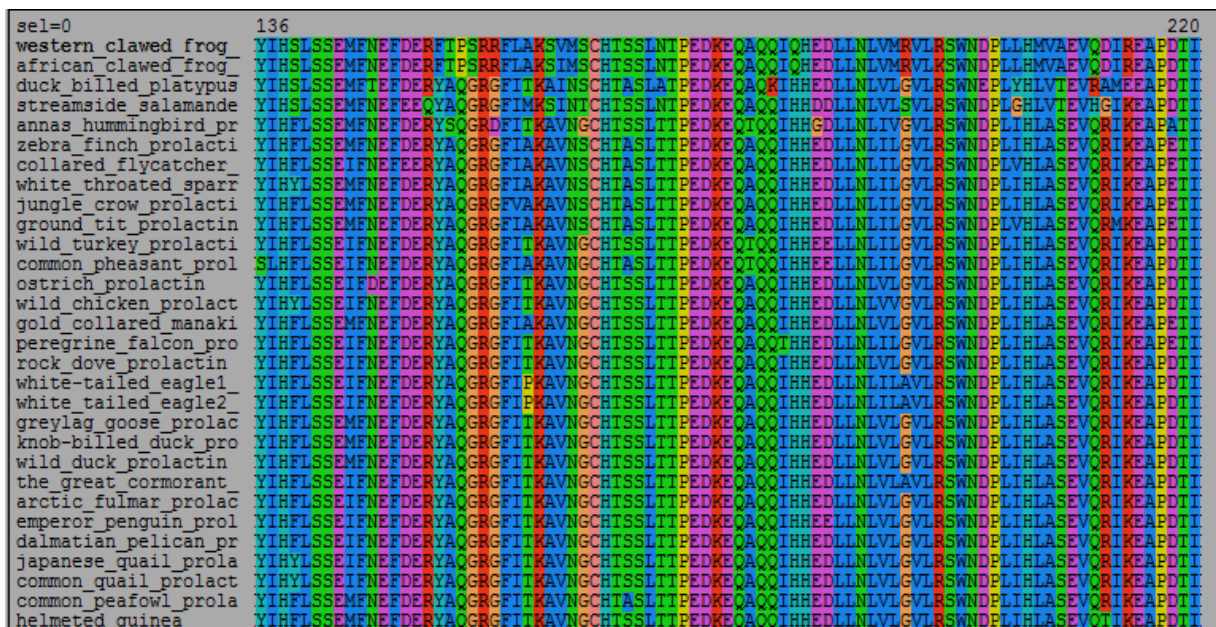


Figure 2: Multiple Sequence Alignment of prolactin showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

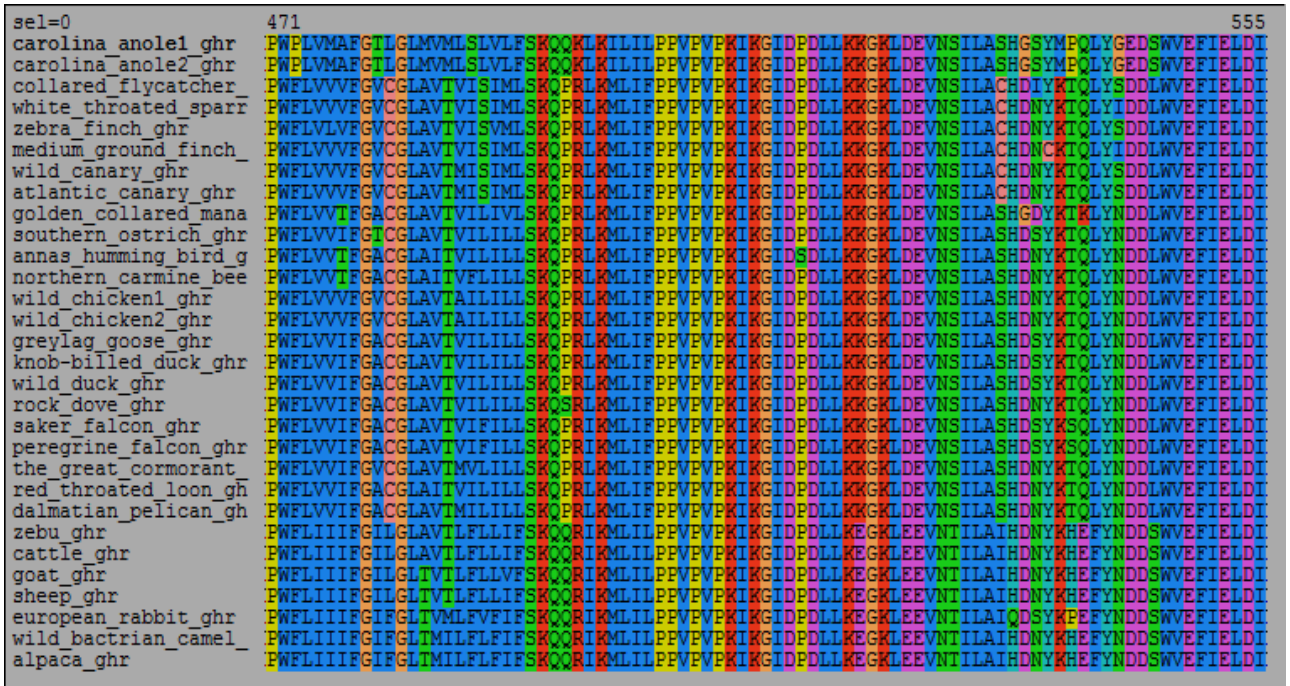


Figure 5: Multiple Sequence Alignment of ghr showing conserved sites. MSA was done by MUSCLE v1.3.8.31-1 (Edgar, 2004)

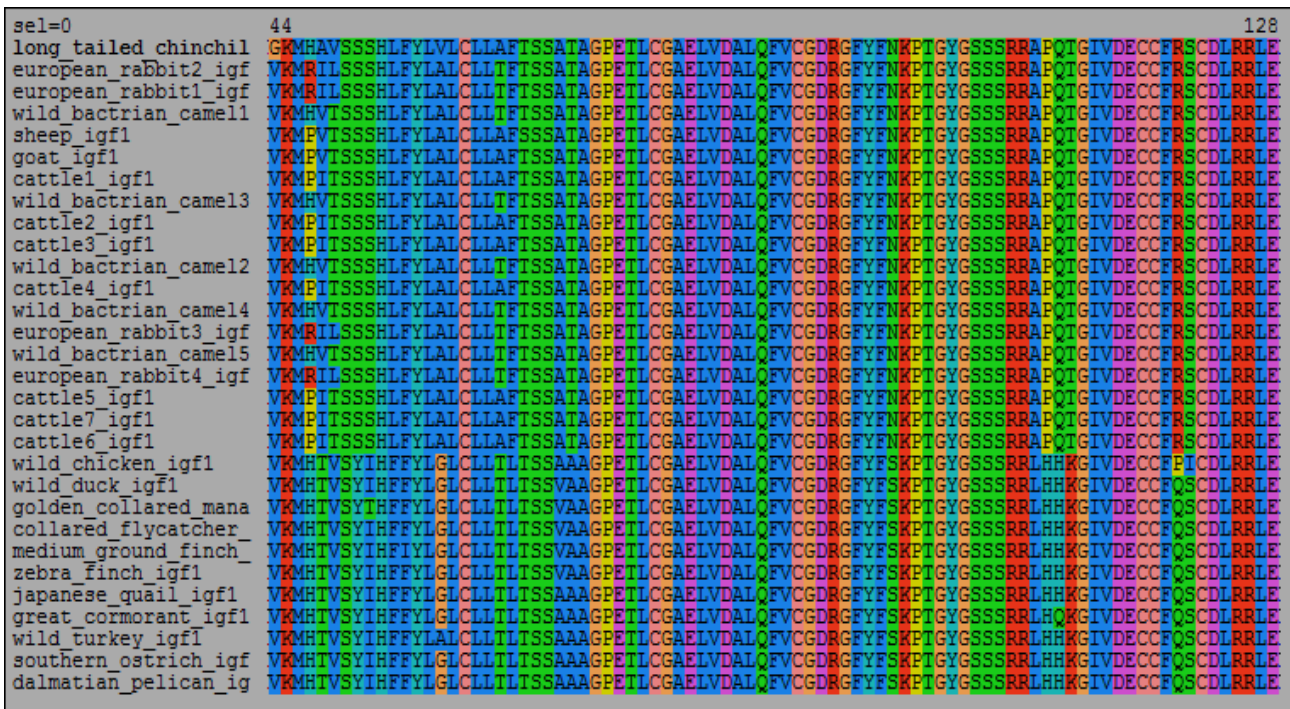


Figure 6: Multiple Sequence Alignment of igf1 showing conserved sites. MSA was done by MUSCLE v1.3.8.31-1 (Edgar, 2004)

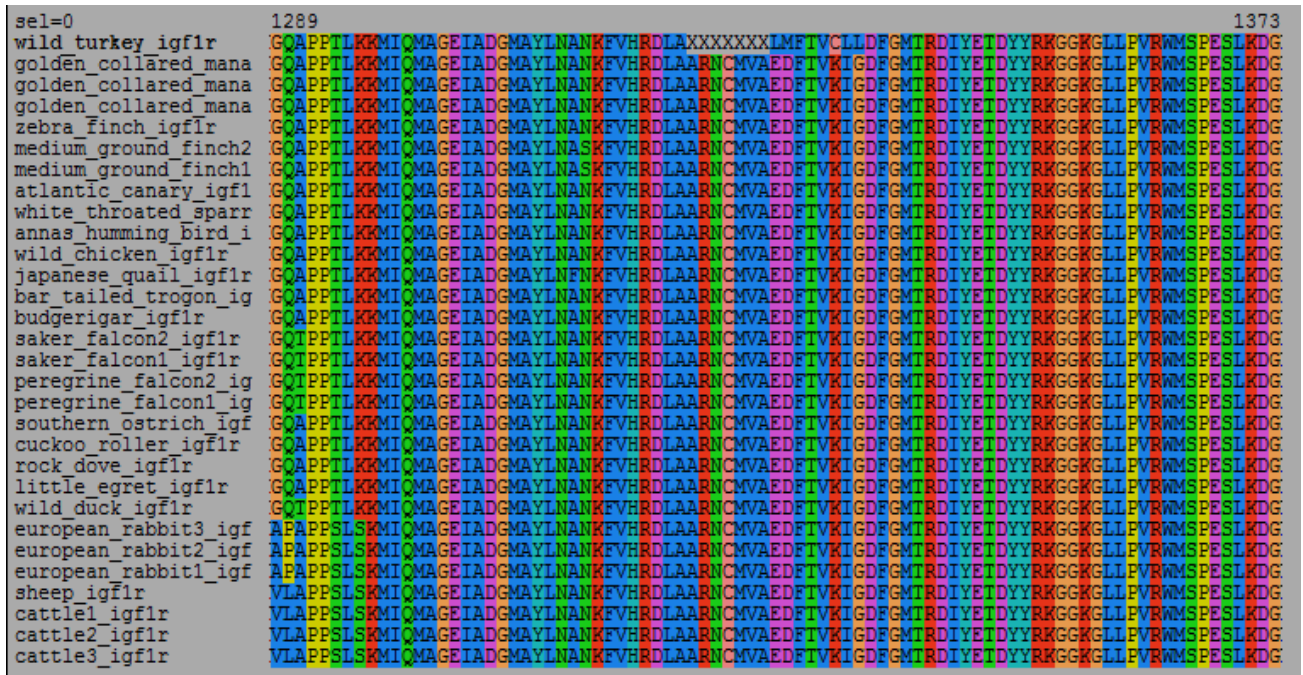


Figure 7: Multiple Sequence Alignment of *igf1r* showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

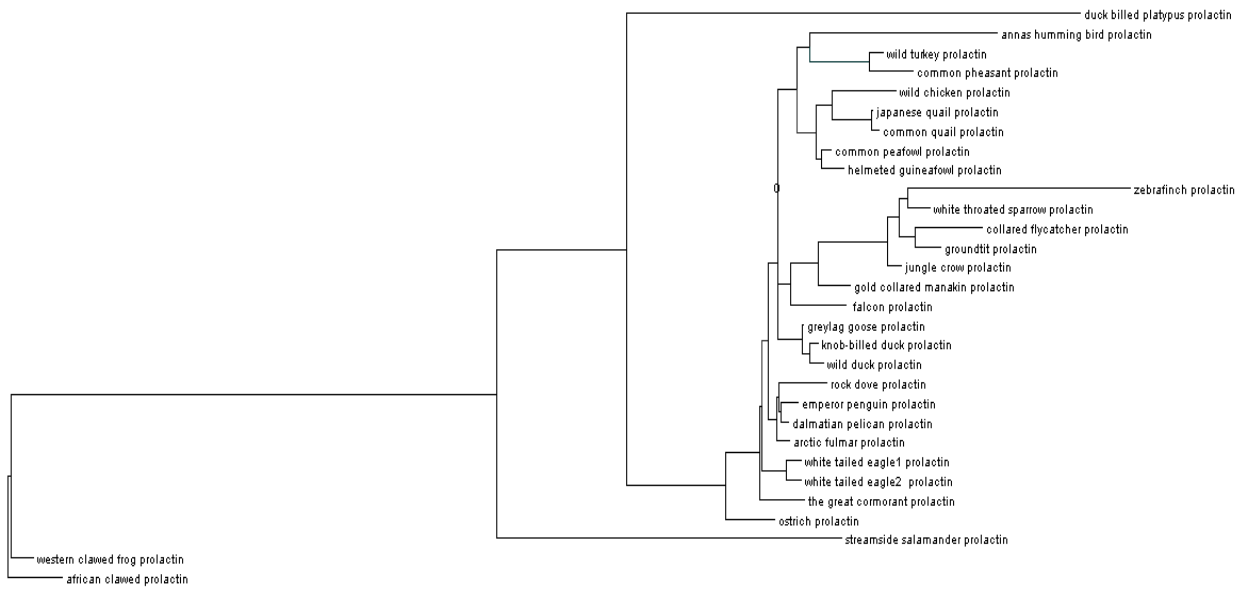


Figure 8: Phylogeny illustrating evolution of prolactin. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

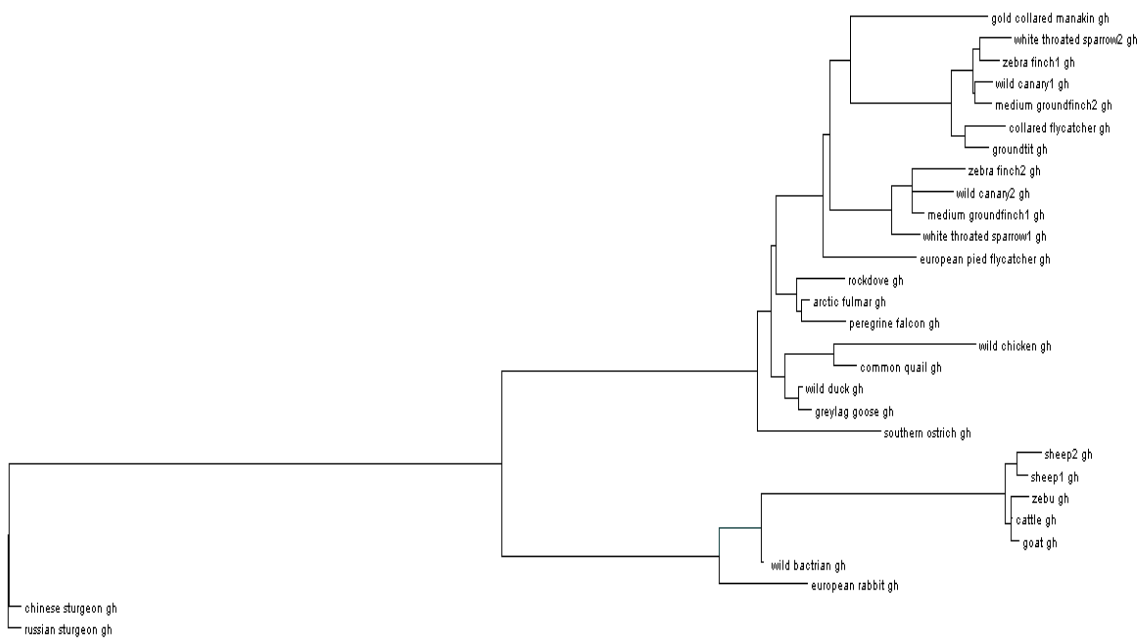


Figure 9: Phylogeny of growth hormone. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

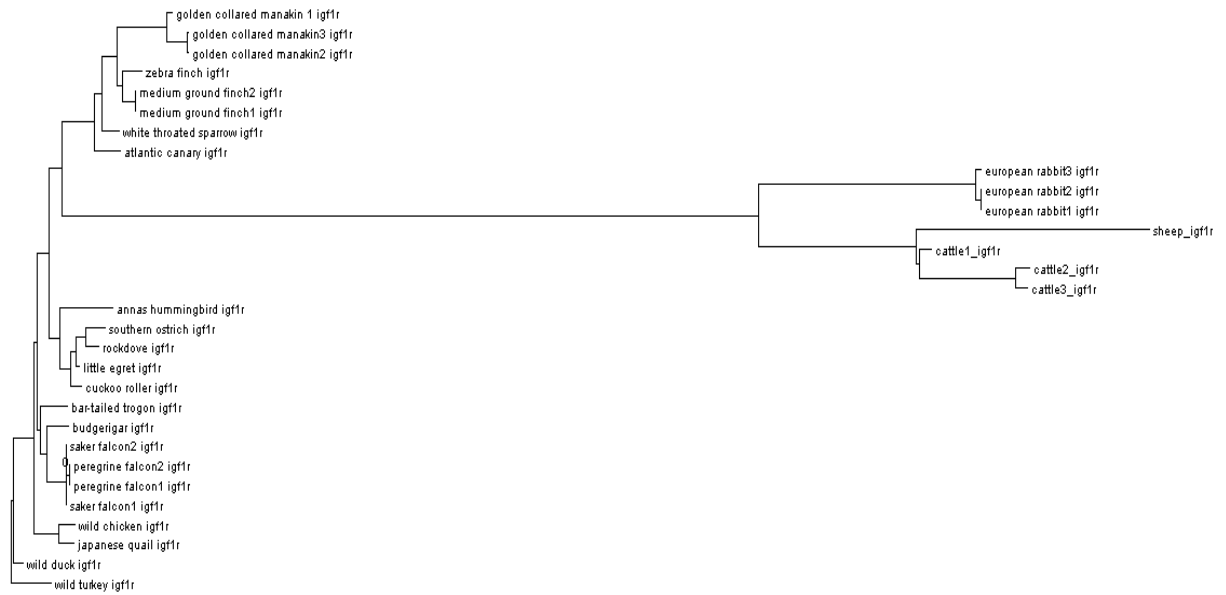


Figure 10: Phylogeny of *igf1r*. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

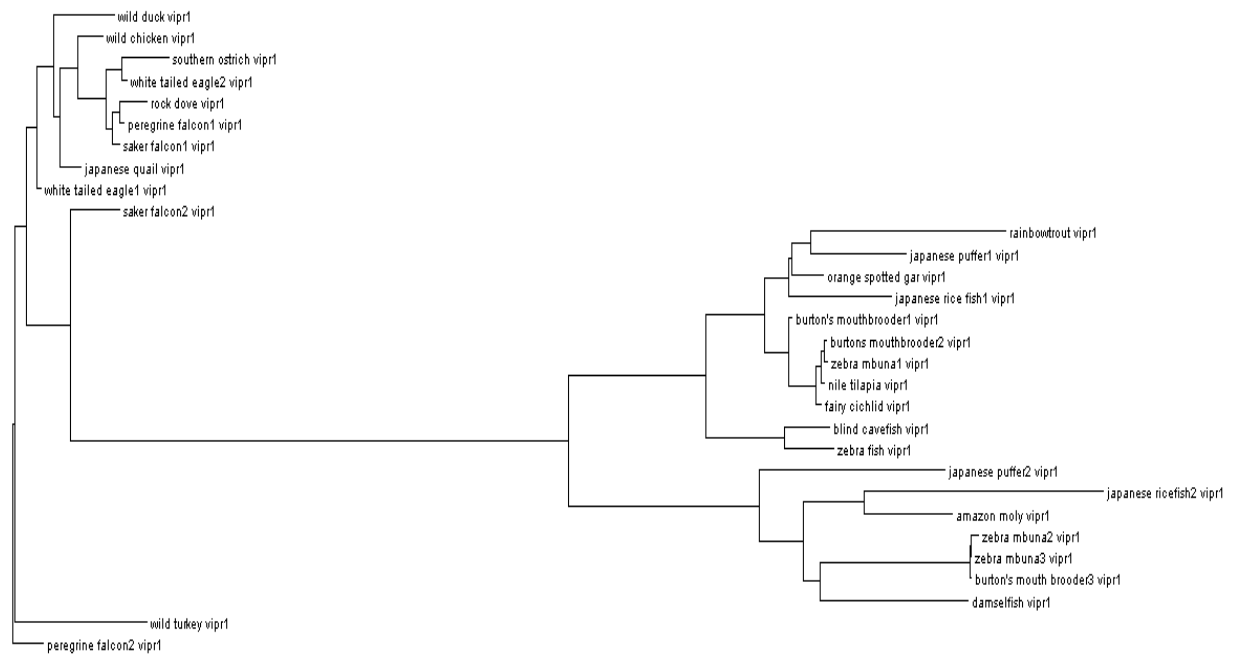


Figure 11: Phylogeny of *vipr1*. The phylogenetic tree was constructed using FastME which is distance-based. 1000 bootstrap replicates were performed for tree evaluation.

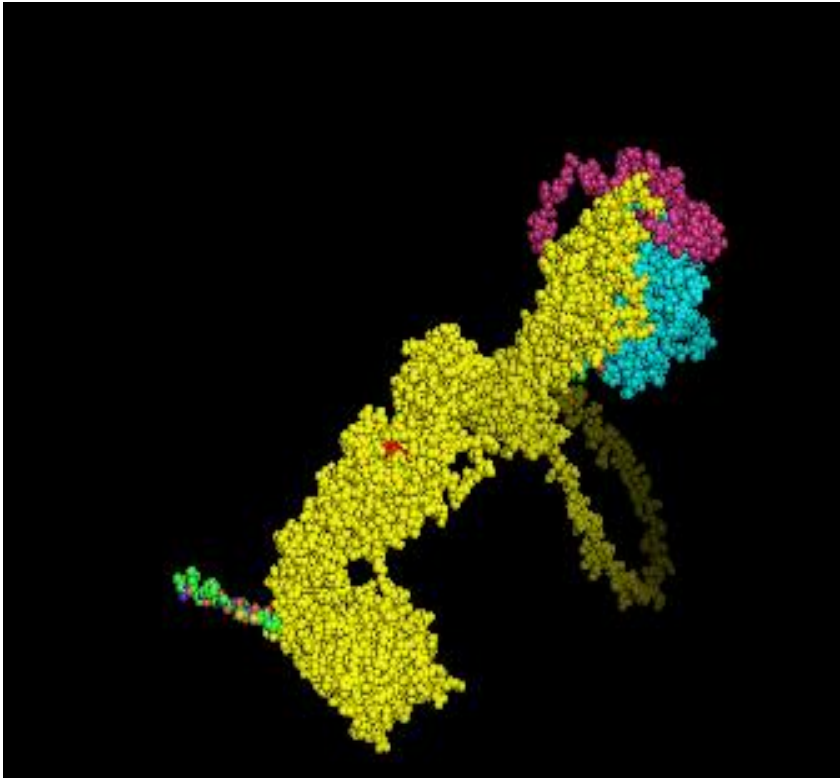


Figure 12: The 3D structure of igf1r showing the different domains. The site shown in red is under positive selection. The site is located in the L2 domain (shown in yellow) which is important for binding insulin. This structure was displayed by pymol v1.7.6 (DeLano, 2002).

4.0 Discussion and Conclusion

In this study, we successfully modeled evolution of the different production genes and performed analysis for positive selection. The computational method used is fast in modeling evolution compared to previous methods that took a long time.

Growth hormone is a polypeptide hormone which is present in all vertebrates (Kawauchi *et al.*, 2002). It has a crucial function in growth and promoting differentiation at different target sites. In birds, growth hormone has other secondary functions such as reproduction, egg production and aging (Zhao *et al.*, 2004).

According to the phylogenetic tree of gh (Figure 9), we find that birds are clustered together and ruminants are clustered together. It is believed that the rate of evolution for any particular protein is constant although the rates differ significantly from one protein to another. However, the evolution of gh shows a pattern of variable evolutionary rate which is unusual (Forsyth and Wallis, 2002). The evolution of gh is generally slow because of the important roles it plays and perhaps the constraints imposed by multiple functions.

The sequences are highly conserved in the birds as can be seen in Figure 4 while there are some substitutions in the ruminants. This is as a result of bursts of rapid change that occurred in some mammals. These bursts of rapid change occurred in two occasions: i) during primate evolution and ii) during evolution of artiodactyls. Most of the changes that have occurred during gh evolution occurred during these bursts.

An accepted explanation for the rapid evolution is adaptive natural selection although there lacks a well-defined associated functional change (Forsyth and Wallis, 2002).

This study is significant in understanding the process of evolution of the production genes. This is an important milestone in that *in vitro* and *in vivo* studies could be carried out to confirm the effect of positive selection of igf1r gene on growth. Subsequently, improvement of egg and meat production may be done using the positively selected genes as markers in molecular breeding.

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Supplementary Material*Table 1: Prolactin homologues*

| Specie | E value | Accession Number |
|------------------------|---------|------------------|
| Common quail | 7e-150 | BAD10927.1 |
| Wild turkey | 5e-147 | AAB60604.1 |
| White throated sparrow | 3e-148 | XP 005481833.1 |
| Gold collared manakin | 3e-148 | KFW 77597.1 |
| Peregrine falcon | 7e-145 | XP 005235773.1 |
| Arctic fulmar | 5e-163 | KFV 94966.1 |
| Greylag goose | 5e-145 | XP 007653890.0 |
| Ostrich | 4e-143 | BAF81528.1 |
| Wild chicken | 5e-162 | AAG01026.1 |
| Common peafowl | 1e-160 | BAG68293.1 |
| Japanese quail | 8e-151 | BAJ61717.1 |
| Helmeted guinea fowl | 2e-151 | BAG68294.1 |
| Dalmatian pelican | 2e-143 | KFQ 6004.1 |
| Wild duck | 3e-144 | BAD14942.1 |
| Emperor penguin | 2e-134 | KFM 11481.1 |
| Knob billed duck | 1e-143 | CAJ55836.1 |
| White tailed eagle 1 | 2e-139 | KFQ 01370.1 |
| Common pheasant | 2e-143 | BAG68292.1 |
| The great cormorant | 2e-143 | KFW 89232.1 |
| White tailed eagle 2 | 1e-139 | XP 007867890.0 |
| Rock dove | 2e-134 | ADK73557.1 |
| Jungle crow | 2e-139 | BAJ61712.1 |
| Ground tit | 4e-139 | XP 005525306.1 |
| Collared flycatcher | 1e-138 | XP 005041658.1 |
| Anna's humming bird | 2e-114 | XP 908765780.0 |
| Zebra finch | 5e-121 | XP 004186110.1 |
| Streamside salamander | 7e-123 | AP93863.1 |
| Duck billed platypus | 5e-114 | XP 007657220.1 |
| African clawed frog | 6e-114 | NP 001086486.1 |
| Western clawed frog | 5e-114 | NP 001093699.1 |

Table 2: Vip homologues

| Vip Gene | | |
|---------------------|------------------|----------------|
| Species | Accession Number | E value |
| Wild chicken 1 | 0.0 | NP 990697.2 |
| Swan goose | 0.0 | XP 008776654.0 |
| Collared flycatcher | 0.0 | XP 005643344.4 |
| Wild turkey | 0.0 | XP003204177.1 |
| Southern ostrich 1 | 0.0 | KFV78141.1 |
| Northern fulmar | 0.0 | AA99877.0 |

| | | |
|-------------------------|-----|----------------|
| Gold collared manakin | 0.0 | BAB98877 |
| Rock dove | 0.0 | BAB99879 |
| Saker falcon | 0.0 | AA98765 |
| Peregrine falcon | 0.0 | KFW56765.4 |
| Little egret | 0.0 | KFV87765.5 |
| Emperor penguin | 0.0 | XP 004543220.0 |
| Dalmatian pelican | 0.0 | XP 005465365.1 |
| Wild chicken 2 | 0.0 | NP 7866878.0 |
| Southern ostrich2 | 0.0 | NP 8979898.9 |
| Stinkbird | 0.0 | NP 64763764.8 |
| Medium groundfinch | 0.0 | KFW7878787 |
| Anna's hummingbird | 0.0 | KFQ2334434 |
| White throated sparrow2 | 0.0 | XP 008987487.0 |
| Spotted gar | 0.0 | XP 006574638.6 |
| Ostrich1 | 0.0 | BAB768567 |
| Ostrich2 | 0.0 | AA6758.0 |
| White tailed eagle | 0.0 | NP 8976565.0 |
| Zebra fish | 0.0 | BAB87765 |
| Mexican tetra | 0.0 | BAB56432 |
| Japanese ricefish | 0.0 | AA69870.0 |
| Bicolor damselfish | 0.0 | XP 005644322.3 |
| Olive flounder | 0.0 | XP 008887766.0 |
| Rainbow smelt | 0.0 | XP 003476654.1 |
| Blue damsel fish | 0.0 | XP 007876548.0 |

Table 3: *vipr1* homologues

| Vipr1 Gene | | |
|---------------------|------------------|---------|
| Specie | Accession Number | E value |
| Wild chicken | BAA95164.1 | 0.0 |
| Japanese quail | AED87510.1 | 0.0 |
| Wild duck | EOA98591.1 | 0.0 |
| Rock dove | EMC82014.1 | 0.0 |
| Southern ostrich | BAA76574.1 | 0.0 |
| Saker falcon1 | XP 005442369.1 | 0.0 |
| Peregrine falcon1 | XP 005229590.1 | 0.0 |
| White tailed eagle1 | XP 007856847.0 | 0.0 |
| Wild turkey | Q91085.2 | 0.0 |
| Saker falcon2 | XP 005442370.1 | 0.0 |
| Peregrine falcon2 | XP 005229591.1 | 0.0 |
| White tailed eagle2 | AAB67768.0 | 0.0 |
| Blind cavefish | XP 007249106.1 | 0.0 |
| Zebra fish | AAI162971 | 0.0 |

| | | |
|--------------------------|----------------|--------|
| Nile tilapia | XP 003439239.2 | 0.0 |
| Fairy cichlid | XP 006802769.1 | 0.0 |
| Burton's mouthbrooder1 | XP 005912343.1 | 0.0 |
| Zebra mbuna1 | XP 005463577.0 | 0.0 |
| Zebra mbuna2 | XP 006756847.8 | 0.0 |
| Damsel fish | XP 007876487.0 | 0.0 |
| Orange spotted spinefoot | ACC78770.1 | 0.0 |
| Japanese rice fish1 | AA787879.0 | 0.0 |
| Burton's mouthbrooder2 | XP 005933737.1 | 0.0 |
| Zebra mbuna3 | XP 007878780.0 | 0.0 |
| Japanese rice fish 2 | XP 004081326.1 | 0.0 |
| Japanese puffer1 | CAC82587.1 | 0.0 |
| Japanese puffer2 | XP 003977758.1 | 0.0 |
| Rainbow trout | AAU29499.1 | 0.0 |
| Burton's mouth brooder3 | XP 007457657.6 | 0.0 |
| Amazon moly | XP 007548620.1 | 2e-159 |

Table 4: gh homologues

| Gh Gene | | |
|--------------------------|------------------|---------|
| Specie | Accession Number | E value |
| Wild chicken | AHM95535.1 | 9e-35 |
| Arctic fulmar | EOA56765.0 | 8e-67 |
| Wild duck | EOA99704.1 | 5e-34 |
| Greylag goose | AAN37412.1 | 9e-34 |
| Common quail | ACJ73931.1 | 5e-34 |
| Peregrine falcon | XP 005238874.1 | 2e-30 |
| Rock dove | EMC85315.1 | 6e-32 |
| White throated sparrow1 | XP 005487217.1 | 2e-28 |
| Medium groundfinch1 | XP 005425878.1 | 1e-29 |
| South ostrich | EOA76764.0 | 4e-34 |
| Wild canary1 | ABB56767.0 | 5e-33 |
| Medium groundfinch2 | XP 005431290.1 | 8e-27 |
| European pied flycatcher | ABB70042.1 | 5e-33 |
| Zebra finch1 | XP 002196167.1 | 1e-27 |
| Gold collared manakin | XP 003686878.1 | 5e-33 |
| Wild canary2 | XP 002435767.0 | 1e-25 |
| Ground tit | XP 005524208.1 | 2e-29 |
| Collared flycatcher | XP 005059628.1 | 1e-25 |
| White throated sparrow2 | XP 006576477.8 | 4e-27 |
| Zebrafinch2 | XP 002187284.1 | 3e-28 |
| European rabbit | XP 007636368.7 | 2e-28 |
| Wild Bactrian camel | XP 006177464.1 | 4e-23 |

| | | |
|------------------|----------------|-------|
| Zebu | XP 001122366.7 | 2e-23 |
| Chinese sturgeon | XP 006356356.0 | 2e-23 |
| Russian sturgeon | ABK74674.6 | 9e-24 |
| Sheep1 | ABK59498.1 | 2e-23 |
| Sheep2 | ABO21737.1 | 5e-23 |
| Cattle | ABK67647.0 | 5e-23 |
| Goat | ADX66303.1 | 9e-24 |

Table 5: *ghr* homologues

| Ghr Gene | | |
|----------------------------|------------------|---------|
| Specie | Accession Number | E value |
| Wild chicken1 | AGG38006.1 | 0.0 |
| Wild chicken2 | NP001001293.1 | 0.0 |
| Dalmatian pelican | XP 0876532.0 | 0.0 |
| Greylag goose | ACY38605.1 | 0.0 |
| Saker falcon | XP 005433804.1 | 0.0 |
| Peregrine falcon | XP 005242027.1 | 0.0 |
| Wild duck | ACT 20710.1 | 0.0 |
| Knob billed duck | ACT 20711.1 | 0.0 |
| Rock dove | EMC76968.1 | 0.0 |
| Southern ostrich | EMC9876.0 | 0.0 |
| Anna's humming bird | ACY2165.0 | 0.0 |
| Golden collared manakin | ACY3476.0 | 0.0 |
| Red throated loon | ACY5876.1 | 0.0 |
| White throated sparrow | XP 005493766.1 | 0.0 |
| Zebra finch | XP 002193695.2 | 0.0 |
| Medium groundfinch | XP 005422066.1 | 0.0 |
| Wild canary | XP 3454267876.0 | 0.0 |
| The great cormorant | NP6473676787.0 | 0.0 |
| Collared flycatcher | AA018173.1 | 0.0 |
| Northern carmine bee-eater | XP 6376387878.0 | 0.0 |
| Atlantic canary | XP 0087765409.0 | 0.0 |
| European rabbit | 1401239A | 0.0 |
| Wild Bactrian camel | AA987430.0 | 0.0 |
| Alpaca | AA897322.0 | 0.0 |
| Carolina anole1 | XP 008101043.1 | 0.0 |
| Carolina anole2 | XP 0008101044.1 | 0.0 |
| Goat | XP 0077863233.0 | 0.0 |
| Zebu | ABM92307.2 | 0.0 |
| Cattle | AAU94310.1 | 0.0 |
| sheep | NP001009323.1 | 0.0 |

Table 6: *igf1* homologues

| <i>Igf1</i> Gene | | |
|-------------------------|------------------|---------|
| Specie | Accession Number | E value |
| Wild chicken | AGG38005.1 | 2e-94 |
| Japanese quail | AAF67202.1 | 2e-94 |
| Great cormorant | XP 008766769.2 | 1e-64 |
| Wild turkey | XP 003202426.1 | 7e-95 |
| Wild duck | ABS76279.1 | 2e-94 |
| Zebra finch | XP 006754322.0 | 2e-62 |
| Collared flycatcher | XP 005040114.1 | 7e-94 |
| Golden collared manakin | XP 006921111.4 | 1e-63 |
| Medium ground finch | XP 005421104.1 | 1e-93 |
| Dalmatian pelican | AAF98765.0 | 2e-94 |
| Southern ostrich | AAF34222.0 | 3e-75 |
| Wild Bactrian camel1 | XP 006186100.1 | 1e-64 |
| European rabbit1 | XP 008254938.1 | 2e-62 |
| Cattle1 | AAF56222.1 | 2e-62 |
| Long tailed chinchilla | XP 005374627.1 | 7e-65 |
| Goat | BAB77524.1 | 3e-75 |
| Sheep | ACG49835.1 | 1e-72 |
| Wild Bactrian camel2 | XP 006186101.1 | 5e-52 |
| Wild Bactrian camel3 | XP 006186102.1 | 7e-66 |
| Wild Bactrian camel4 | XP 006186103.1 | 1e-64 |
| Wild Bactrian camel5 | XP 006186104.1 | 6e-79 |
| Cattle2 | AAF22156.2 | 1e-63 |
| European rabbit2 | XP 008254939.1 | 7e-50 |
| Cattle3 | AAF42111.0 | 7e-50 |
| Cattle4 | AAF11114.2 | 3e-75 |
| European rabbit3 | XP 008254940.1 | 2e-62 |
| European rabbit4 | XP 008254941.1 | 5e-77 |
| Cattle5 | AAF75333.2 | 3e-75 |
| Cattle6 | AAF73432.0 | 3e-75 |
| Cattle7 | AAF23407.1 | 2e-62 |

Table 7: *igf1r* homologues

| <i>Igf1r</i> Gene | | |
|-------------------|------------------|---------|
| Specie | Accession Number | E value |
| Wild chicken | AGG38009.1 | 0.0 |
| Japanese quail | BAF73401.1 | 0.0 |
| Saker falcon1 | XP 005436689.1 | 0.0 |
| Peregrine falcon1 | XP 005242493.1 | 0.0 |
| Saker falcon2 | XP 005436690.1 | 0.0 |

| | | |
|--------------------------|-----------------|-----|
| Peregrine falcon2 | XP 005242494.1 | 0.0 |
| Medium ground finch1 | XP 005424278.1 | 0.0 |
| Zebra finch | XP 002199843.1 | 0.0 |
| Medium ground finch2 | XP 005424279.1 | 0.0 |
| Golden collared manakin1 | XP 004687532.2 | 0.0 |
| Golden collared manakin2 | XP 000997654.8 | 0.0 |
| Golden collared manakin3 | EMC 77848.3 | 0.0 |
| Wild duck | EOB07472.1 | 0.0 |
| Little egret | EOB23699.0 | 0.0 |
| Rock dove | EMC 77329.1 | 0.0 |
| Southern ostrich | EMC 76589.5 | 0.0 |
| Wild turkey | XP 0032009598.1 | 0.0 |
| Atlantic canary | XP 0034509876.0 | 0.0 |
| White throated sparrow | XP 0012567876.5 | 0.0 |
| Anna's humming bird | XP 0035779654.0 | 0.0 |
| Budgerigar | XP 0045885434.3 | 0.0 |
| Cuckoo roller | XP 0056328797.1 | 0.0 |
| Bar tailed trogon | XP 0011187072.0 | 0.0 |
| Cattle1 | XP 0078656766.5 | 0.0 |
| European rabbit1 | XP 0066666988.2 | 0.0 |
| European rabbit2 | XP 0011765445.3 | 0.0 |
| European rabbit3 | XP 0044498885.2 | 0.0 |
| Sheep | XP 0040085983.1 | 0.0 |
| Cattle2 | XP 0067333333.3 | 0.0 |
| Cattle3 | XP 0078899906.2 | 0.0 |
