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 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 (Katoh and Standley, 2013) were used. This was followed by MSA using MUSCLE version1.3.8.31-1 (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 version3 (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.

sel=0	251 335
ostrich1 vip	DQGPIKR-HSDAVFTDNYTRLRKQMAVKKYLNSILNGKRSSEGESPDFLEGLEK
ostrich2 vip	DQGPIKR-HSDAVFTDNYTRLRKOMAVKKYLNSILNGKRSSEGESPDFLEGLEK
white tailed	GDIYIROSFFLAGFIGNFSPFCT
wild_turkey_vip	QDSPVKR-HSDAVFTDNYSRFRKOMAVKKYLNSVLTGKRSQEELNPAKLRDEAEILEPSFSENYDDVSVDELLSHLPLDL
saker_falcon_vip	QDSPVKR-HSDAVFTDNYSRFRKOMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDDLLSHVPSGPLK
peregrine_falcon_vip	QDSPVKR-HSDAVFTDNYSRFRKOMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDDLLSHVPSGPLK
annas_humming_bird_v	QDSPVKR-HSDAVFTDNYSRFRKOMAVKKYLNSVLTGKRSQEELNPAKLREEAELVEPSFSENYDSVDELLSHLPLDL
dalmatian_pelican_vi	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELVEPSFSENYDSVDELLSHLPLDL
stinkbird_vip	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYESVDELLSHLPLDL
collared_flycatcher_	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDELLSHLPLTVPL
emperor_penguin_vip	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLPDKAELLEPSFSENYDSVDELLSHLPLDF
rock_dove_vip	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDDLLSRLPLDL
southern_ostrich1_vi	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLEDEAELLEFSFSENYDDVSVDELLSRLPLDL
southern_ostrich2_vi	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDDVSVDELLSRLPLDL
gold_collared_manaki	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDELLSHLPLDL
medium_ground_finch_	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDELLSHLPLDL
white_throated_sparr	QDSPVKR-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPAKLRDEAELLEPSFSENYDSVDELLSHLPLDL
little_egret_vip	QDSPVKR-HSDAVFTDNYSRFRRQMAVKRYLNSVLTGRRSQEELNPAKLRDEAELLEPSFSENYDSVDELLSHLPLGL
wild_chicken2_vip	QDSPVKR-HSDAVFTDNYSRFRRQMAVKRYLNSVLTGRRSQEELNPAKLRGEAEILEPSFSENYDDVSVDELLSHLPLDL
wild_chicken1_vip	ODSPVKR-HSDAVFTDNYSRFRRQMAVKRYLNSVLTGRRSQEELNPARLRGEAEILEPSFSENYDDVSVDELLSHLPLDL
northern_fulmar_vip	QDSPVKH-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPTKLRDEAELLEPSFSENYDSVDELLSHLPLDL
swan_goose_vip	QDSPVRH-HSDAVFTDNYSRFRKQMAVKKYLNSVLTGKRSQEELNPSKLRDEAEILEPSFSENYDDVSVDELLSHLPLDL
rainbow_smelt_vip	DQVFVKR-HSDAIFTDNYSBFRKQMAVKKYLNSVLTGKRSLEDPPSVPEES-INDFTFQESYDDVNVDHLIN-FQLPL
japanese_rice_fish_v	MEEPVKR-HSDAIFIDNYSRFRKOMAVKKYLNSVLIGKRSLEDPGNSEPEEHRDEPNIFQESYDDINVDHLLSNFQLPL
blue_damselfish_vip	MDEPVKR-HTDAIFTDNYSRFRKQMAVKKYLNSVLTGKRSLEDPGNSDQEESRDEANTFQESYDDINVDHLLNNFQLPL
bicolor_damselfish_v	MDEPVKR-HTDAIFTDNYSBFRKQMAVKKYLNSVLTGKRSLEDPGTSDPEESRDEPNIFQESYDDINVDHLLNNFQLPL
olive_flounder_vip	NEEPVKK-HSDAIF IDNISK KKUNAVKYLNSVLIGKKSLEDPGISDAELSKOEPV
spotted_gar_vip	LUNPVKR-ROUALTIDNISKERKUNAVKKILNSVLIGKRSQEDINSERLQDEAATSEFSESSYDDVTVDELLNHLPLTL
zebra_fisn_vip	DUAPMEN-HSDAIFIDNISFFRUMAVERILDSVLIGHSOEDPPSMEESAGGETTYRESYDDVIVDRLLNHIPLPL
mexican_tetra_vip	DWIFVER-REDAIFIDRISHIERWMAVERILNSVLIGERSLEDPPSLQELSIGGEIIIERSIDDIIVNELLNHIPLPL

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

sel=0	136 220
western clawed frog	YIHSLSSEMFNEFDERFTPSRRFLAKSVMSCHTSSLNTPEDREQAQQIQHEDLLNLVMRVLRSWNDPLLHMVAEVQDIREAPDTI
african_clawed_frog_	YIHSLSSEMFNEFDERFTPSRRFLAKSIMSCHTSSLNTPEDKEQAQQIQHEDLLNLVMRVLKSWNDPLLHMVAEVQDIREAPDTI:
duck_billed_platypus	YIHSLSSEMFTEFDERYAQGRGFITKAINSCHTASLATPEDKEQAQKIHHEDLLNLVLGVLRSWNEPLYHLVTEVRAMEEAPDTI:
streamside_salamande	YIHSLSSEMFNEFEEQYAQGRGFIMKSINTCHTSSLNTPEDKEQAQQIHHDDLLNLVLSVLRSWNDPLGHLVTEVHGIKEAPDTI
annas_hummingbird_pr	YIHFLSSEMFNEFDERYSQGRDFITKAVNGCHTSSLTTPEDKEQTQQIHHGDLLNLIVGVLRSWNDPLIHLASEVQRIKEAPATI
zebra_finch_prolacti	YIHFLSSEMFNEFDERYAQGRGFIAKAVNSCHTASLTTPEDKEQAQQIHHEDLLNLILGVLRSWNDPLIHLASEVQRIKEAPETI
collared_flycatcher_	YIHFLSSEIFNEFEERYAQGRGFIAKAVNSCHTASLTTPEDKEQAQQIHHEDLLNLILGVLRSWNDPLVHLASEVQRIKEAPETI
white_throated_sparr	YIHYLSSEMFNEFDERYAQGRGFIAKAVNSCHTASLTTPEDKEQAQQIHHEDLLNLILGVLRSWNDPLIHLASEVQRIKEAPETI
jungle_crow_prolacti	YIHFLSSEMFNEFDERYAQGRGFVAKAVNSCHTASLTTPEDKEQAQQIHHEDLLNLILGVLRSWNDPLIHLASEVQRIKEAPETI
ground_tit_prolactin	YIHFLSSEMFNEFDERYAQGRGFIAKAVNSCHTASLTTPEDKEQAQQIHHEDLLNLILGVLRSWNDPLVHLASEVQRMKEAPETI
wild_turkey_prolacti	_YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQTQQIHHEELLNLILGVLRSWNDPLIHLASEVQRIKEAPDTI
common_pheasant_prol	SLHFLSSEIFNEFDERYAQGRGFIAKAVNGCHTASLTTPEDKEQTQQIHHEELLNLILGVLRSWNDPLIHLASEVQRIKEAPDTI
ostrich_prolactin	_YIHFLSSEIFDEFDEFYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI
wild_chicken_prolact	YIHYLSSEIFNEFDERYAQGRGFITKAVNGCHISSLTTPEDKEQAQQIHHEDLLNLVVGVLRSWNDPLIHLASEVQRIKEAPDTI:
gold_collared_manaki	YIHFLSSEMFNEFDERYAQGRGFIAKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPETI
peregrine_falcon_pro	YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQTHHEDLLNLILGVLRSWNDPLIHLASEVQRIKEAPETI
rock_dove_prolactin	YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHISSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI:
white-tailed_eagle1_	YIHFLSSEIFNEFDERYAQGRGFIPKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLILAVLRSWNDPLIHLASEVQRIKEAPDTI
white_tailed_eagle2_	YIHFLSSEIFNEFDERYAQGRGFIPKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLILAVLRSWNDPLIHLASEVQRIKEAPDTI
greylag goose prolac	YIHFLSSEMFNEFDERYAQGRGFITKAVNGCHISSLITPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDII
knob-billed_duck_pro	YIHFLSSEMFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI:
wild_duck_prolactin	YIHFLSSEMFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI
the_great_cormorant_	YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLAVLRSWNDPLIHLASEVQRIKEAPDTI
arctic_fulmar_prolac	YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI
emperor penguin prol	YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHISSLTTPEDKEQAQQIHHEELLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI
dalmatian pelican pr	YIHFLSSEIFNEFDERYAQGRGFITKAVNGCHTSSLTTPEDKEQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI
japanese_quail_prola	YIHYLSSEIFNEFDERYAQGRGFITRAVNGCHTSSLTTPEDREQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIREAPDTI
common_quail_prolact	YIHYLSSEIFNEFDERYAQGRGFITRAVNGCHTSSLTTPEDREQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIREAPDTI
common peafowl prola	YIHFLSSEMFNEFDERYAQGRGFITRAVNGCHTASLTTPEDREQAQQIHHEDLLNLVLGVLRSWNDPLIHLASEVQRIKEAPDTI
helmeted guinea	YIHFLSSEMFNEFDERYAOGRGFITRAVNGCHTSSLTTPEDREOACOIHHEDLLNLVLGVLRSWNDPLIHLASEVOTIKEAPDTI

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

sel=0	379 463
wild turkey vipr1	RILVOKLHSPDVGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
wild duck vipr1	RILVOKLHSPDVGHNETSHYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKTEVKLVFELVVGSFÖGFVVAVLYCFLNGEVÖAELKR
wild chicken vipr1	RILVOKLHSPDVGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFINGEVQAELKR
japanese quail vipr1	RILVORLHSPDVGHNETSÖYSRLARSTLLLIPLFGIHYIMFAFFPDNFRAEVRLVFELVVGSFÖGFVVAVLYCFINGEVÖAELRR
southern ostrich vip	RILVOKLHSPDVGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
white tailed eagle1	RILVOKLHSPDIGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
white tailed eagle2	RILVOKLHSPDIGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
rock dove vipr1	RILVOKLHSPDVGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
saker falcon1 vipr1	RILVOKLHSPDVGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
saker falcon2 vipr1	RILVOKLHSPDVGHNETSQYSRLAKSTLLLIPLFGIHYIMFAFFPDNFKAEVKLVFELVVGSFQGFVVAVLYCFLNGEVQAELKR
peregrine falcon1 vi	RILVORLHSPDVGHNETSÖYSRLARSTLLLIPLFGIHYIMFAFFPDNFRAEVRLVFELVVGSFÖGFVVAVLYCFINGEVÖAELR
peregrine falcon2 vi	RILVORLHSPDVGHNETSÖYSRLARSTLLLIPLFGIHYIMFAFFPDNFRAEVRLVFELVVGSFÖGFVVAVLYCFINGEVÖAELRR
rainbow trout vipr1	RILRORVNCPDIGRNESNOYLRIAKSTLLLIPLFGINFIVFAFIPEOVNTEORIVFNLILGSFÖGFAVAVLYCFLNGEVÖSEMKR
blind cave fish vipr	RILROKINCPDIGRNESNOYSRIAKSTLLLIPLFGINFIIFAFIPEHIKTELRUVFDLILGSFOGFVVAVLYCFLNGEVOGEIKR
zebra fish viprl	RILROKINCPDIGRNESNOYSELAKSTLLLIPLFGINFIIFAFIPENIKTELRUVFDLILGSFOGFVVAVLYCFLNGEVOGEIKE
japanese_puffer1_vip	RILRONINCPDIGRNESNQYSRIAKSTLLLIPLFGINFIVFAFIPEQVKTELRIVFDLILGSFQGFVVAVLYCFLNGEVQGEIKR
japanese rice fish2	RILROKINCPDIGRNESNQYLRIAKSTLLLIPLFGINFIVFAFIPEQMKTELRIVFXXLFFSLQGFVVAVLYCFINGEVQGEIKR
orange_spotted_spine	RILRONINCPDIGRNESNQYLRIAKSTLLLIPLFGINFIVFAFIPEQVKTELRIVFDLILGSFQGFVVAVLYCFLNGEVQAEIKR
burtons mouth broode	RILRONINCPDIGRNDSNQYSRLAKSTLLLIPLFGINYIVFAFIPEQVKTELRUVFDLILGSFQGFVVAVLYCFLNGEVQGEIKR
burtons mouth broode	RILROKINCPDIGRNDSNOYSRIAKSTLLLIPLFGINYIVFAFIPEOVKTELRIVFDLILGSFOGFVVAVLYCFINGEVOGEIKR
zebra mbunal viprl	RILROKINCPDIGRNDSNOYSRIAKSTLLLIPLFGINYIVFAFIPEOVKTELRIVFDLILGSFOGFVVAVLYCFINGEVOGEIKR
fairy cichlid vipr1	RILROKINCPDIGRNDSNOYSRIAKSTLLLIPLFGINYIVFAFIPEOVKTELRIVFDLILGSFOGFVVAVLYCFINGEVOGEIKR
nile tilapia vipr1	RILROKINCPDIGRNDSNOYSRIAKSTLLLIPLFGINYIVFAFIPEOVKTELRIVFDLILGSFOGFVVAVLYCFINGEVOGEIKR
japanese puffer2 vip	RILROKMNCPDIGRRESHQYSRLAKSTLLLIPLFGINYIIFAFLPDHVQSKGRLVFDLILGSFQGFVVVVLYCFLNGEVQAEIKR
japanese rice fish1	RILLORMNCPDIGRRESNOYSELARSTLLLIPLFGINYILFAYIPENMHVQVRMVFDLVLGSFQGFVVAILYCFLNGEVQSEIRR
zebra mbuna2 vipr1	RILRORMNCPDIGRKESNOYSELAKSTLLLIPLFGINYIIFAFIPDDIHPOVRMVFDLILGSFOGFVVAVLYCFLNGEVOSEIKR
burtons mouth broode	RILROKMNCPDIGRKESNOYSELAKSTLLLIPLFGINYIIFAFIPDDIHPOVRMVFDLILGSFOGFVVAVLYCFLNGEVOSEIKR
zebra_mbuna3_vipr1	RILRORMNCPDIGRKESNQYSRLARSTLLLIPLFGINYIIFAFIPDDIHPQVRMVFDLILGSFQGFVVAVLYCFLNGEVQSEIKR
amazon_moly_vipr1	·KMNCPDIGRKESNQYSRLTKSTLLLIPLFGINYIIFAFIPEHIHPQLRMVFDLVLGSFQGFVVAVLYCFLNGEVQSEVKR
damselfish_vipr1	RILROKMNCPDIGRKESNQYSRLAKSTLLLIPLFGINYIVFGFIPEHIHPÖVRMVFDLILGSFÖGFIVAVLYCFLNGEVÖAEIKR

Figure 3: Multiple Sequence Alignment of vipr1 showing conserved sites. MSA was done by by MUSCLE v1.3.8.31-1 (Edgar, 2004)

sel=0	135 22
chinese sturgeon gro	VFEKLKDLEEGIVALMRDLGEGGFGSSTLLKLTYDKFDVNLRNDDALFKNYGLLSCFKKDMHKVETYLKVMKCRRFVESNCTL
russian sturgeon gro	VFEKLKDLEEGIVALMRDLGEGGFGSSTLLKLTYDKFDVNLRNDDALFKNYGLLSCFKKDMHKVETYLKVMKCRRFVESNCTL
gold collared manaki	VYEKLKDLEEGIQALMRELEDRSPRGPQILRPTYEKFDVLVRSEDALLHNYGLLGCFKKDLHRVETYLKVMKCRRYGDGNCAV
white throated sparr	VYEKLKDLEEGIQALMRELEERSPRGPQVLKATYEKFELHLRGEDALVKNYGLLSCFKKDLHKVETYLKVMRCRRYGEGNCAL
zebra finch1 growth	VYEKLKDLEEGIQALMRELEERSPRGPQVLKFTYEKFELHLRGEEALVKNYGLLSCFKKDLHKVETYLKVMRCRRYGEGNCAL
wild canary1 growth	VYEKLKDLEEGIQALMRELEERSSRGPQVLKPTYEKFELHLRGEDALVKNYGLLSCFKKDLHKVETYLKVMRCRRYGEGNCAL
medium ground finch2	VYEKLKDLEEGIQALMRELEERSPRGLQVLKPTYEKFELHLRGEDALVKNYGLLSCFKKDLHKVETYLKVMRCRRYGEGNCAL
collared flycatcher	VYEKLKDLEEGIQALMRELEERSPRGAQLLKLTYEKFEPHLRGEDALLKNYGLLSCFKKDLHKVETYLKVMRCRRYGEGNCAL
ground tit growth ho	VYEKLKDLEEGIÕALMRELEERSPRGAÕLLKATYEKFELHLRGEDALLKNYGLLSCFKKDLHKVETYLKVMRCRRYGEGNCAL
zebra finch2 growth	VYEKLKDLEEGIÖALMRELODRSFRGFÖILKATYEKFDIHLRSEDALLONYGLLSCFKKDLHKVETYLKVMKCRRYGEGNCTI
wild canary2 growth	VYEKLKDLEEGILALMRELODSPRGPOILKAVYEKFDIHLRSEDALLONYGLLSCFKKDLHKVETYLKVMKCRRYGEGNCTI
white throated sparr	VYEKLKDLEEGIOALMRELODBGPRGPOILKATYEKFDIHLRSEDALLÖNYGLLSCFKKDLHKVETYLKVMKCRRYGEGNCTF
medium ground finch1	VYEKLKDLEEGIÕALMRELÕDRSPRGPÕILKATYEKFDIHLRSEDALLÕNYGLLSCFKKDLHKVETYLKVMKCRRYGEGNCTI
european pied flycat	VYEKLKDLEEGIÕALMRDRSPRGPÖLLRPTYDKFDIHLRNEDALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
wild chicken growth	VFEKLKDLEEGLOPIMRELEDRSPRGPOLLRFTYDKFDIHLRHGDALLNNYGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
southern ostrich gro	VYEKLKDLEEGIÕALMRELEDRSSRGPPLLRSTYDKFDIHLRNEEALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
common quail growth	VFEKLKDLEEGIÕALMREVEDRSPRGFOLLRPTYDKFDIHLRNEDALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
wild duck growth hor	VFEKLKDLEEGIÕALMRELEDRSPRGPÕLLKPTYDKFDIHLRNEDALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
greylag goose growth	VFEKLKDLEEGIÕALMRELEDRSPRGPÕILKFTYDKFDIHLRNEDALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGESNCTI
rock dove growth hor	VYEKLKDLEEGIŐALMRELEDRSPRGFŐILKPTYDKFDIHLRNEDALMKNYGLLACFKKDLHKVETYLKVMKCRRYGEGNCTV
arctic fulmar growth	VYEKLKDLEEGIÕALMRELEDRSPRGPÕILKPTYDKFDIHLRNEDALLKNYGLLSCFKKDLHKVETYLKVMKCRRFGEGNCTV
peregrine falcon gro	VYEKLKDLEEGIÕALMRELEDRGPRGFÖLLKPTYDKFDIHLRTEDALLKNYSLLSCFKKDLHKVETYLKVMKCRRYGEGNCTV
sheep2 growth hormon	VYEKLKDLEEGIIALMRELEDVIPRAGÕILKOTYDKFDINMRSDDALLKNYGLLSCFRKDLHKTETYLRVMKCRRFGEASCAF
sheep1 growth hormon	VYEKLKDLEEGILALMRELEDVTPRAGOILKOTYDKFDTNMRSDDALLKNYGLLSCFRKDLHKTETYLRVMKCRRFGEASCAF
zebu growth hormone	VYEKLKDLEEGILALMRELEDGTPRAGÖILKÖTYDKFDINMRSDDALLKNYGLLSCFRKDLHKTETYLRVMKCRRFGEASCAF
cattle growth hormon	VYEKLKDLEEGILALMRELEDGTPRAGÖILKÖTYDKFDTNMRSDDALLKNYGLLSCFRKDLHKTETYLRVMKCRRFGEASCAF
goat growth hormone	VYEKLKDLEEGILALMRELEDGTPRAGOILKOTYDKFDTNMRSDDALLKNYGLLSCFRKDLHKTETYLRVMKCRRFGEASCAF
european rabbit grow	VYEKLKDLEEGIOALMRELEDGSPRVGÖLLKÖTYDKFDTNLRGDDALLKNYGLLSCFKKDLHKAETYLRVMKCRPFVESSCVF
wild bactrian camel	VYEKLKDLEEGIÕALMRELEDGSPRAGÕILRÕTYDKFDTNLRSDDALLKNYGLLSCFKKDLHKAETYLRVMKCRRFVESSCAF

Figure 4: Multiple Sequence Alignment of gh showing conserved sites. MSA was done by MUSCLE v1.3.8.31-1 (Edgar, 2004)

sel=0	471 555
carolina anole1 ghr	.PWPLVMAFGTLGLMVMLSLVLFSKQQKLKILILPPVPVPKIKGIDPDLLKKGKLDEVNSILASHGSYMPQLYGEDSWVEFIELDI:
carolina_anole2_ghr	.PWPLVMAFGTLGLMVMLSLVLFSKQQKLKILILPPVPVPKIKGIDPDLLKKGKLDEVNSILASHGSYMPQLYGEDSWVEFIELDI:
collared flycatcher	.PWFLVVVFGVCGLAVTVISIMLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILACHDIYKTOLYSDDLWVEFIELDI
white throated sparr	.PWFLVVVFGVCGLAVTVISIMLSKOPRLKMLIFPFVFVFKIKGIDPDLLKKGKLDEVNSILACHDNYKTOLYIDDLWVEFIELDI
zebra finch ghr	.PWFLVLVFGVCGLAVTVISVMLSKOPRLKMLIFPFVFVFKIKGIDPDLLKKGKLDEVNSILACHDNYKTOLYSDDLWVEFIELDI
medium_ground_finch_	.PWFLVVVFGVCGLAVTVISIMLSKOPKLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILACHDNCKTOLYIDDLWVEFIELDI.
wild canary ghr	.PWFLVVVFGVCGLAVTMISIMLSKOPRLKMLIFPFVFVFKIKGIDPDLLKKGKLDEVNSILACHDNYKTOLYSDDLWVEFIELDI
atlantic canary ghr	.PWFLVVVFGVCGLAVTMISIMLSKOPRLKMLIFPFVFVFKIKGIDPDLLKKGKLDEVNSILACHDNYKTOLYSDDLWVEFIELDI
golden_collared_mana	.PWFLVVTFGACGLAVTVILIVLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHGDYKTKLYNDDLWVEFIELDI:
southern_ostrich_ghr	.PWFLVVIFGTCGLAVTVILILLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDSYKTOLYNDDLWVEFIELDI.
annas_humming_bird_g	.PWFLVVTFGACGLAITVILILLSKQPRLKMLIFPPVPVPKIKGIDSDLLKKGKLDEVNSILASHDNYKTQLYNDDLWVEFIELDI:
northern_carmine_bee	.PWFLVVTFGACGLAITVFLILLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTOLYNDDLWVEFIELDI.
wild_chicken1_ghr	.PWFLVVVFGVCGLAVTAILILLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTOLYNDDLWVEFIELDI.
wild_chicken2_ghr	.PWFLVVVFGVCGLAVTAILILLSKQPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTQLYNDDLWVEFIELDI.
greylag_goose_ghr	.PWFLVVIFGACGLAVTVILILLSKQPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDSYKTQLYNDDLWVEFIELDI.
knob-billed_duck_ghr	.PWFLVVIFGACGLAVTVILILLSKQPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDSYKTQLYNDDLWVEFIELDI.
wild_duck_ghr	.PWFLVVIFGACGLAVTVILILLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDSYKTOLYNDDLWVEFIELDI.
rock_dove_ghr	.PWFLVVIFGACGLAVTVILILLSKQSRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTQLYNDDLWVEFIELDI.
saker_falcon_ghr	.PWFLVVIFGACGLAVTVIFILLSKQPRIKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDSYKSQLYNDDLWVEFIELDI.
peregrine_falcon_ghr	.PWFLVVIFGACGLAVTVIFILLSKOPRIKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDSYKSOLYNDDLWVEFIELDI.
the great cormorant	.PWFLVVIFGVCGLAVTMVLILLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTOLYNDDLWVEFIELDI.
red_throated_loon_gh	.PWFLVVIFGACGLAITVILILLSKOPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTOLYNDDLWVEFIELDI.
dalmatian_pelican_gh	.PWFLVVIFGACGLAVTMILILLSKQPRLKMLIFPPVPVPKIKGIDPDLLKKGKLDEVNSILASHDNYKTQLYNDDLWVEFIELDI:
zebu_ghr	.PWFLIIIFGILGLAVTLFLLIFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIHDNYKHEFYNDDSWVEFIELDI.
cattle_ghr	.PWFLIIIFGILGLAVTLFLLIFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIHDNYKHEFYNDDSWVEFIELDI.
goat_ghr	.PWFLIIIFGILGLTVTLFLLVFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIHDNYKHEFYNDDSWVEFIELDI.
sheep_ghr	.PWFLIIIFGILGLTVTLFLLIFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNIILAIHDNYKHEFYNDDSWVEFIELDI:
european_rabbit_ghr	.PWFLIIIFGIFGLTVMLFVFIFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIQDSYKPEFYNDDSWVEFIELDI:
wild_bactrian_camel_	PWFLIIIFGIFGLTMILFLFIFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIHDNYKHEFYNDDSWVEFIELDI:
alpaca_ghr	PWFLIIIFGIFGLTMILFLFIFSKQQRIKMLILPPVPVPKIKGIDPDLLKEGKLEEVNTILAIHDNYKHEFYNDDSWVEFIELDI

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

sel=0	44 128
long tailed chinchil	GKMHAVSSSHLFYLVLCLLAFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
european_rabbit2_igf	VKMRILSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
european rabbit1 igf	VKMRILSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
wild bactrian camel1	VKMHVTSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
sheep igf1	VKMPVTSSSHLFYLALCLLAFSSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
goat igf1	VKMPVTSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
cattle1 igf1	VKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
wild bactrian camel3	VKMHVTSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
cattle2 igf1 -	VKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
cattle3 igf1	VKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
wild bactrian camel2	VKMHVTSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
cattle4 igf1	VKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
wild bactrian camel4	VKMHVTSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
european rabbit3 igf	VKMRILSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
wild bactrian camel5	VKMHVTSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
european rabbit4 igf	VKMRILSSSHLFYLALCLLTFTSSATAGPETLCGAELVDALQFVCGDRGFYFNKPTGYGSSSRRAPQTGIVDECCFRSCDLRRLE
cattle5 igf1	VMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
cattle7 igf1	VKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
cattle6 igf1	VKMPITSSSHLFYLALCLLAFTSSATAGPETLCGAELVDALOFVCGDRGFYFNKPTGYGSSSRRAPOTGIVDECCFRSCDLRRLE
wild chicken igf1	VKMHTVSYIHFFYLGLCLLTLTSSAAAGPETLCGAELVDALOFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFPICDLRRLE
wild duck igf1	VKMHTVSYIHFFYLGLCLLTLTSSVAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
golden collared mana	VKMHTVSYTHFFYLGLCLLTLTSSVAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
collared flycatcher	VKMHTVSYIHFFYLGLCLLTLTSSVAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
medium ground finch	VKMHTVSYIHFIYLGLCLLTLTSSVAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
zebra finch igf1	VKMHTVSYIHFFYLGLCLLTLTSSVAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
japanese quail igf1	VKMHTVSYIHFFYLGLCLLTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
great cormorant igf1	VMMHTVSYIHFFYLGLCLLTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHQKGIVDECCFQSCDLRRLE
wild turkey igf1	VKMHTVSYIHFFYLALCLLTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
southern_ostrich igf	VKMHTVSYIHFFYLGLCLLTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE
dalmatian pelican ig	VKMHTVSYIHFFYLGLCLLTLTSSAAAGPETLCGAELVDALQFVCGDRGFYFSKPTGYGSSSRRLHHKGIVDECCFQSCDLRRLE

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

sel=0	1289				1373
wild turkey igf1r	GOAPPTLKKMIOMAGEIADGMAYLN	ANKEVHRDLAXXXXXXXLMETV	CLLDFGMTRDIYET	OYYRKGGKGLLPV	RWMSPESLKDG
golden collared mana	GOAPPTLKKMIOMAGEIADGMAYLN	ANKFVHRDLAARNCMVAEDFTV	HIGDFGMTRDIYET	DYY <mark>RKGGKGLLP</mark> V	RWMSPESLKDG
golden collared mana	GOAPPTLKKMIOMAGEIADGMAYLN	AN <mark>K</mark> FVH <mark>RDLAAR</mark> NCMVAEDFTV	KIGDFGMTRDIYET	DYY <mark>RKGGKGLLP</mark> V	RWMSPESLKDG:
golden collared mana	GOAPPTLKKMIOMAGEIADGMAYIN	ANKEVHRDI.42 RNCMVAEDETV	KIGDFGMTRDIYET	NYY <mark>RKGGKGT.T.P</mark> V	RWMSPESTRDG
golden collared mana	GCE2PTLKKMLOMAGEIADGMAYLN		KIGDFGMTRDIYET	NYY <mark>RKGGKGT.T.PV</mark>	RWMSPEST.KDG.
Wolden collared mana	GCL2PILKKMIOMAGEIHDGMAYLN	THE REPORT OF THE PARTY OF THE P	RIGDFGMTRDIYET	NYY <mark>RKGGKGT.T.P</mark> P	RWMSPEST.KDG.
Wolden collared mana	GCL2PIL KKMIOMACEIHDGMAYL		RIGDFGMTRDIYET	NYY <mark>RKGGKGT.T.</mark> ₽₽	RWMSPEST.KDG.
Colden collared mana	GCL2PIL KNOL OMAGEIHDGMAYL		RIGDFGMTRDIYET	OYY <mark>RKGGKGLLB</mark>	REMSPEST. KDG
Rolden collared mana	GCLZPIL KROTOMACEIHDCMAYIN		HIGDFGMTRDIYET	OYY <mark>RKGGKGLLB</mark>	REMSPEST KDG.
Colden collered mana	GCLZPIL KROTOWACEIHDCMAYIN	annas-18666an bhuile	RIGDFGMTRDIYET	NYY <mark>RKGGKGT.L</mark> .RA	REMSPEST. KDG.
Colden collered mana	GCLZPIL KKMIRMAGEINDGMAŸIN		HIGDFGMTRDIYET	OYY <mark>RKGGKGT.L.</mark> RA	REMSPEST KDG.
Colden coldered mana	HGREZPTL <mark>KINCERNAGEINDGMAŸI</mark> N	TRANSPORTATION OF A DESCRIPTION	HIGDFGMTRDIYET	NYY <mark>RKGGKGT1338</mark>	REMSPEST KDG.
Colden coldered mana	GCL2PTL KMMICKACEIMDCMAYIN	anar handlad haaraan ing	RIGDFGMTRDIYET	NYY <mark>RKGGKGL1:</mark> 38	REMSPEST KDG.
Colden coldered mana	GAL 2PTL KIMU CHACE LADOMA VIN		RIGDFGMTRDIYET	NYY <mark>RKGGKGL1.</mark> 38	REMSPEST KDG.
Wolden coldered mana	GCL2PTL KINCLOWACE LADOMA ?!!		KILDEGMTRDIYET	NYVRKGKGKGT1.88	RUMSPEST. KDG
Wolden coldered mana	GAL 2PTU MILLENACE INDEMAT	AND AND ADDRESS OF A DREAM OF A D	KI DEGMTRDIYET	OVVERSCROTT NO	RUMSPERT.KDG.
welden coldered wana	GAL PTI MOUSHACE IN DOMAS	A CONTRACTOR OF A CONTRACT OF	HILDEGMTRDTYET	NYVERAGEGIT 38	RUMSPERT. MDG
welder woldered wana	GAL PTI MALENACE IN DOMESTIC	Sectors Charles and Barriel and	HILDEGMTRDTYET	DYVERSGEGITI 88	RUMSPERT. HDG
Webderwoldered wana	GAL PPTLEMACEIMDOM		RICOFOMTROTYET)PVERACECT 188	RIMS PRATING
Webderwoldered wana	-GAL PPTLEMACE LEDGER	anabas <u>aadilas hafib</u>	RICOFOMTROTYET	nghassecrett 38	RIMS PRAT KDG
Webderwoldered wana	-GAL PPTL COLONA CELLID CHARTE	anad <u>aa iniin</u>	RIADECMTRDIVET	NYVERACUCT 188	FILMS PRAT KDG
Welder woldered wana	- GAL PPTLEMICTONACE LEDGAR	aliantis sequent initio	RIGDECMERDIYET	ngassecrett 38	FORMS PRAT. KDG
Wolder woldered sana	CARL PPTL MILLONACE LADGE CONTRACT	an Resource Init In	HIRDERMERDIVET	DSABACROTT SA	FUMS PRAT KING
Wolder woldered suma:	SACE OF THE MALL CHARTER DOMAGE	ennagen i <u>n abbolik sin in</u>	RIGIINCORDIYET	1277 BRACKGTT AR	RIMS PROT KING
Wolder woldered same:	SACE PETERMACE IN DOMES	ennagen i nabb dis ini	RIGIINCORDIYET	D2ABBBCRCLUB	RIMS PRET KDG
Wolder woldered same:	SAAL SPILL FINAL SMALL SUBJECT STATE	ennegen in webbeide sig	EIEIIDEDEDIYET	N2WEERAGEGT1.88	RIMS PRET KDG
Wolder woldered ama:	SACE SPILL MOLT SWACE IN DOMAIN	strage to the	WHEIT DODDIVET	127 EXACTLAS	REAS PRET KOG
Wolder woldered atmax	SACEPTING (FRACEINDON)		CONTRACTOR DI VET	Democratic as	FORSPRET KDG
Wolder woldered atmax	SACEPTING (FORACEINDON)		WHEN TO CONTYRE	PRESCRIPTING	FORSPREE KING
Wedderse dered atman	2002 PTLEDUT CHACE INDOM:	ACCESSION OF A CONTRACT OF	WHHIP DOD OT YES	New Brack GILLAN	FOR SPREEDG.

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 a*., 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

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 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 welldefined 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 makers 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 XP 004543220.0 Dalmatian pelican 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 KFW287765.4 Medium groundfinch 0.0 KFW2878787 Anna's hummingbird 0.0 KFQ2334434 White throated sparrow2 0.0 XP 006574638.6 Ostrich1 0.0 XP 008987487.0 Spotted gar 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 BAB87655 Mex			
Rock dove 0.0 BAB99879 Saker falcon 0.0 AA98755 Peregrine falcon 0.0 KFW56755.4 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 005987487.0 Spotted gar 0.0 XP 005974638.6 Ostrich1 0.0 BAB7655.0 Vihite tailed eagle 0.0 AA6758.0 Vihite tailed eagle 0.0 AA69870.0 Vihite tailed eagle 0.0 AA69870.0 Japanese ricefish 0.0 AA69870.0 Bicolor damselfish 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3	Gold collared manakin	0.0	BAB98877
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 KFW787877 Anna's hummingbird 0.0 KFQ2334434 White throated sparrow2 0.0 XP 008987487.0 Spotted gar 0.0 XP 00897487.0 Ostrich1 0.0 BAB768567 Ostrich2 0.0 AA6758.0 Vhite tailed eagle 0.0 NP 8976565.0 Zebra fish 0.0 BAB87765 Mexican tetra 0.0 AA69870.0 Bicolor damselfish 0.0 XP 008847766.0 Olive flounder 0.0 XP 008887766.0 Binobw smelt 0.0 XP 003476654.1	Rock dove	0.0	BAB99879
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 897898.9 Stinkbird 0.0 NP 64763764.8 Medium groundfinch 0.0 KFW238787 Anna's humningbird 0.0 KFQ2334434 White throated sparrow2 0.0 XP 008987487.0 Ostrich1 0.0 AA6758.0 Ostrich2 0.0 AA6758.0 Ostrich2 0.0 AA6758.0 Vhite tailed eagle 0.0 AA6758.0 Zebra fish 0.0 BAB87655.0 Mexican tetra 0.0 AA69870.0 Bicolor damselfish 0.0 XP 00564322.3 Olive flounder 0.0 XP 00887766.0 Biabow smelt 0.0 XP 003476654.1	Saker falcon	0.0	AA98765
Little egret 0.0 KFV87765.5 Emperor penguin 0.0 XP 004543220.0 Dalmatian pelican 0.0 XP 00545365.1 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 KFV877877 Anna's hummingbird 0.0 KFQ2334434 White throated sparrow2 0.0 XP 008987487.0 Spotted gar 0.0 XP 00897487.0 Ostrich1 0.0 BAB768567 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 NP 8976565.0 Vexican tetra 0.0 BAB57655 Mexican tetra 0.0 AA69870.0 Bicolor damselfish 0.0 XP 008887766.0 Olive flounder 0.0 XP 008887766.0 Biue damsel fish 0.0 XP 003476654.1	Peregrine falcon	0.0	KFW56765.4
Emperor penguin 0.0 XP 00454322.0.0 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 006574638.6 Spotted gar 0.0 XP 006574638.6 Ostrich1 0.0 BAB765567 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 NP 8976565.0 Weixcan tetra 0.0 BAB87765 Mexican tetra 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 008887766.0 Biue damsel fish 0.0 XP 003476654.1	Little egret	0.0	KFV87765.5
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 KFW787877 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 Vehican tetra 0.0 BAB87765 Mexican tetra 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 005644322.3 Bue damsel fish 0.0 XP 003476654.1	Emperor penguin	0.0	XP 004543220.0
Wild chicken 2 0.0 NP 786878.0 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 00574638.6 Ostrich1 0.0 BAB768567 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 AA6758.0 Verkican tetra 0.0 BAB87655.0 Japanese ricefish 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 00387766.0 Bue damsel fish 0.0 XP 003476654.1	Dalmatian pelican	0.0	XP 005465365.1
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 008987487.0 Ostrich1 0.0 XP 006574638.6 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 AA6758.0 White tailed eagle 0.0 NP 8976565.0 Mexican tetra 0.0 BAB87765 Japanese ricefish 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 00387766.0 Rainbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	Wild chicken 2	0.0	NP 7866878.0
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 005574638.6 Ostrich1 0.0 BAB768567 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 NP 8976565.0 Verkican tetra 0.0 BAB56432 Japanese ricefish 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 00887766.0 Rainbow smelt 0.0 XP 007876548.0	Southern ostrich2	0.0	NP 8979898.9
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 BAB56432 Japanese ricefish 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 003786548.0	Stinkbird	0.0	NP 64763764.8
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 00887766.0 Qive flounder 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 003875648.0	Medium groundfinch	0.0	KFW7878787
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 White tailed eagle 0.0 BAB87765 Zebra fish 0.0 BAB56432 Mexican tetra 0.0 AA69870.0 Bicolor damselfish 0.0 XP 005644322.3 Olive flounder 0.0 XP 003887766.0 Rainbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	Anna's hummingbird	0.0	KFQ2334434
Spotted gar 0.0 XP 006574638.6 Ostrich1 0.0 BAB768567 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 NP 897656.0 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 003887766.0 Rainbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	White throated sparrow2	0.0	XP 008987487.0
Ostrich1 0.0 BAB768567 Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 NP 897656.0 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 003887766.0 Blue damsel fish 0.0 XP 007876548.0	Spotted gar	0.0	XP 006574638.6
Ostrich2 0.0 AA6758.0 White tailed eagle 0.0 NP 897656.0 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 003887766.0 Bianbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	Ostrich1	0.0	BAB768567
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 Bainbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	Ostrich2	0.0	AA6758.0
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	White tailed eagle	0.0	NP 8976565.0
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	Zebra fish	0.0	BAB87765
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	Mexican tetra	0.0	BAB56432
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	Japanese ricefish	0.0	AA69870.0
Olive flounder 0.0 XP 008887766.0 Rainbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	Bicolor damselfish	0.0	XP 005644322.3
Rainbow smelt 0.0 XP 003476654.1 Blue damsel fish 0.0 XP 007876548.0	Olive flounder	0.0	XP 008887766.0
Blue damsel fish 0.0 XP 007876548.0	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 mouthbroooder1 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 00787880.0 0.0 Zebra mbuna3 XP 007877880.0 0.0 Japanese rice fish 2 XP 003977758.1 0.0 Japanese puffer1 CAC82587.1 0.0 Japanese puffer2 XP 003977758.1 0.0 Burton's mouth brooder3 XP 007457657.6 0.0 Burton's mouth brooder3 XP 007457657.6 0.0			
Fairy cichlidXP 006802769.10.0Burton's mouthbroooder1XP 005912343.10.0Zebra mbuna1XP 005463577.00.0Zebra mbuna2XP 006756847.80.0Damsel fishXP 007876487.00.0Orange spotted spinefootACC78770.10.0Japanese rice fish1AA7879.00.0Burton's mouthbroooder2XP 005933737.10.0Zebra mbuna3XP 007878780.00.0Japanese rice fish 2XP 004081326.10.0Japanese puffer1CAC82587.10.0Japanese puffer2XP 003977758.10.0Burton's mouth brooder3XP 007457657.60.0Burton's mouth brooder3XP 007548620.12e-159	Nile tilapia	XP 003439239.2	0.0
Burton's mouthbroooder1 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 mouthbroooder2 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 007548620.1 2e-159	Fairy cichlid	XP 006802769.1	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 mouthbroooder2 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	Burton's mouthbroooder1	XP 005912343.1	0.0
Zebra mbuna2XP 006756847.80.0Damsel fishXP 007876487.00.0Orange spotted spinefootACC78770.10.0Japanese rice fish1AA787879.00.0Burton's mouthbrooder2XP 005933737.10.0Zebra mbuna3XP 007878780.00.0Japanese rice fish 2XP 004081326.10.0Japanese puffer1CAC82587.10.0Japanese puffer2XP 00397758.10.0Rainbow troutAAU29499.10.0Burton's mouth brooder3XP 007548620.12e-159	Zebra mbuna1	XP 005463577.0	0.0
Damsel fishXP 007876487.00.0Orange spotted spinefootACC78770.10.0Japanese rice fish1AA787879.00.0Burton's mouthbrooder2XP 00593737.10.0Zebra mbuna3XP 007878780.00.0Japanese rice fish 2XP 004081326.10.0Japanese puffer1CAC82587.10.0Japanese puffer2XP 003977758.10.0Rainbow troutAAU29499.10.0Burton's mouth brooder3XP 007548620.12e-159	Zebra mbuna2	XP 006756847.8	0.0
Orange spotted spinefootACC78770.10.0Japanese rice fish1AA787879.00.0Burton's mouthbroooder2XP 005933737.10.0Zebra mbuna3XP 007878780.00.0Japanese rice fish 2XP 004081326.10.0Japanese puffer1CAC82587.10.0Japanese puffer2XP 003977758.10.0Rainbow troutAAU29499.10.0Burton's mouth brooder3XP 007548620.12e-159	Damsel fish	XP 007876487.0	0.0
Japanese rice fish1 AA787879.0 0.0 Burton's mouthbroooder2 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	Orange spotted spinefoot	ACC78770.1	0.0
Burton's mouthbroooder2 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 007548620.1 2e-159	Japanese rice fish1	AA787879.0	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	Burton's mouthbroooder2	XP 005933737.1	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	Zebra mbuna3	XP 007878780.0	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	Japanese rice fish 2	XP 004081326.1	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	Japanese puffer1	CAC82587.1	0.0
Rainbow trout AAU29499.1 0.0 Burton's mouth brooder3 XP 007457657.6 0.0 Amazon moly XP 007548620.1 2e-159	Japanese puffer2	XP 003977758.1	0.0
Burton's mouth brooder3 XP 007457657.6 0.0 Amazon moly XP 007548620.1 2e-159	Rainbow trout	AAU29499.1	0.0
Amazon moly XP 007548620.1 2e-159	Burton's mouth brooder3	XP 007457657.6	0.0
	Amazon moly	XP 007548620.1	2e-159

Table 4: gh homologues

Accession Number	E value
AHM95535.1	9e-35
EOA56765.0	8e-67
EOA99704.1	5e-34
AAN37412.1	9e-34
ACJ73931.1	5e-34
XP 005238874.1	2e-30
EMC85315.1	6e-32
XP 005487217.1	2e-28
XP 005425878.1	1e-29
EOA76764.0	4e-34
ABB56767.0	5e-33
XP 005431290.1	8e-27
ABB70042.1	5e-33
XP 002196167.1	1e-27
XP 003686878.1	5e-33
XP 002435767.0	1e-25
XP 005524208.1	2e-29
XP 005059628.1	1e-25
XP 006576477.8	4e-27
XP 002187284.1	3e-28
XP 007636368.7	2e-28
XP 006177464.1	4e-23
	Accession NumberAHM95535.1EOA56765.0EOA99704.1AAN37412.1ACJ73931.1XP 005238874.1EMC85315.1XP 005487217.1XP 005425878.1EOA76764.0ABB56767.0XP 005431290.1ABB70042.1XP 003686878.1XP 002196167.1XP 005524208.1XP 005559628.1XP 005059628.1XP 005059628.1XP 002187284.1XP 007636368.7XP 006177464.1

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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-

Table	6: igf1	homologues
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lgf1 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

lgf1r 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