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Molecular profiling of growth hormone in the juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂)

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Many factors contribute to the underdevelopment of oceanic aquaculture. The most significant factor of the development of hybrid grouper culture is the supply of satisfactory quantities of fast growing juveniles to stock grow-out systems at a minimized cost. In order to evaluate the growth hormone status of hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂), cDNA encoding growth hormone from the liver of juvenile fish was cloned and characterized. qPCR method was used to determine the expression of the growth hormone in the tissues. Relative expression of growth hormone in the brain was found to be highest (290.91 folds) and lowest in the stomach (1.35 folds). Bioinformatics tools were used to analyze the growth hormone proteins and prediction of its physicochemical properties, and amino acid compositions, as well as Gene Ontology, GO term predictions. The cloned hybrid grouper growth hormone gene sequence was found to be clustered monophyletically with solid bootstrap backing. It was found to be close to the *Cromileptes altivelis* growth hormone gene sequence and is positioned in the identical clade and consequential from the identical family. The expression pattern is comparable to that comprehended in other fishes and provides extra data for molecular biological studies on hybrid grouper fish.

Key words: Growth hormone, bioinformatics analysis, tissue expression, gene ontology, hybrid grouper

INTRODUCTION

Growth rate is a factor of commercial significance in the field of aquaculture for directly impacting the production, and hence income. A number of studies have reported on the significance of the growth hormone playing primary roles in reproduction, growth, immunity, cellular differentiation, and metabolism (in liver and brain majorly), by means of its receptors (Brooks and Waters, 2010; Mingarro et al., 2002). Growth hormone activities are "initiated by binding to a specific receptor (GHR), and assumed as localized on the cell membrane of target tissues, which induces a phosphorylation cascade for signalling and gene expression" (Herrington and Carter-

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Su, 2001). Growth hormone plays a number of roles in cellular immune system directly or with the help of the insulin growth factor-1 and work through mediators for its actions and the induction of insulin-like growth factor 1 (Brooks and Waters, 2010; Li et al., 1998; Brooks et al., 2014). Growth hormone receptor activation often refers to an outcome of the effects of growth hormone. How be it, the malfunctioning of its receptor is likely to give rise to several consequences like retarded growth and undue delay in puberty (Brooks and Waters, 2010). Growth hormone has been reported as having "an anabolic effect on bone, cartilage, and muscle tissue in the course of binding to the growth hormone receptors in skeletal muscle" (Brahm et al., 1997). Both "Insulin-like growth factor-I (IGF-I) and II (IGF-II) are released during binding to the receptors in liver as reported by some other studies" (McArdle et al., 2010), which lead to the stimulation for the increase in bone growth, cell development inside the muscle tissue and protein synthesis (Casanueva, 1992; Godfrey et al., 2003).

Cultured hybrid grouper owns quite a high market value in China. Nevertheless, one of the key issues reported as inhibiting its commercial bases is the more retarded growth in comparison with the other grouper species (Sekar et al., 2014; James et al., 1999; Kohno, 1997; Wang, 1997; Amenyogbe et al., 2019). Interestingly, hybrid grouper grows as a female from the juvenile stages, meanwhile changing into a male at adulthood. This incident is conflicting to other grouper species that are well-known that sexually, they mature from male to female and capable of altering sex at the sexual maturity aged between 3 and 5 years as discussed by Amenyogbe et al. (2019).

A single possible tool for the evaluation of a growth status in hybrid grouper involves accessing the expression of a controlling hormone in the hybrid grouper somatic growth. Currently, there is little or no substantial information available regarding the growth hormone cloning and qPCR expression profile of hybrid grouper; accordingly, the current research work aimed at cloning the full length of the juvenile hybrid grouper growth hormone, in addition to also studying its expression in various tissues and predict the possible roles played by the growth hormone in the development of the *Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂ fish since the major problem reported to be associated with the hybrid grouper is the retarded growth. We hope that the data in the current study provide the bases for any future revisions, which are targeted at evolving the fast growth and development of hybrid grouper.

MATERIALS AND METHODS

Experimental fish

An aggregate of 3 female hybrid juvenile groupers (3-4 months) with the average body weights of 82.3 ± 4.32 g and length of 13.73 ± 0.13 cm obtained from Guangdong Hengxing Group Co. LTD., Guangdong Province, China was put to use for the purpose of experiment. They were kept in 500 L plastic that contained 400 L of water with the constant aeration for approximately 2 weeks with 288 h of day light and 288 h of darkness until the start of the experiment. The use of the live fishes for the experiment adhered to the guidelines of Institutional Animal Care and Fisheries and Aquaculture College, Laboratory of fish breeding, Guangdong Ocean University, China.

Ethics approval and consent to participate

This experiment was carried out in accordance with the rules and regulation of Guangdong Ocean University Animal Care and Use Committee (Guangdong Province, China).

The only anaesthetic approved by the College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, China for the general use for fishes is MS-222. The fish was placed in a solution of MS222 dissolved in water concentration amounting to 250 mg/L until the death is attained. Verification was performed through the observation of the absence of the opercular movement for a period of approximately 3 min in order to ensure that the fish was dead prior to decapitation. This was performed for the purpose of alleviating the suffering of the fish put to use for the study.

The tissue samples from "brain, heart, intestine, muscle, head kidney, liver, stomach, gill, and spleen were dissected with the use of sterilized instruments, followed by getting immediately frozen in liquid nitrogen, and storing at the temperature of -80°C until use" (Edens and Talamantes, 1998).

RNA isolation

The RNA isolation was done following (Amenyogbe et al., 2019) method. The procedure was carried out in accordance with the instructions of the manufacturer. The examination of the superiority of total RNA" was carried out using 1% agarose gel electrophoresis and UV spectrophotometry (NanoDrop, Thermo Scientific, USA).

Cloning of growth hormone (GH)

Aimed at cloning a partial cDNA fragment of GH, primers, as presented in Table 1, there were designed on the basis of the growth hormone (GH) sequences of *Epinephelus bruenus* (*GU138644.1*), *Epinephelus altivelis* (*EU003991.1*), *and Coho_salmon* (*M24768.1*) from NCBI. "First-strand cDNA was synthesized with the use of TRANSgen First-strand cDNA synthesize kit in accordance with the manufacturer's instructions. The partial cDNA fragment of growth hormone (GH) was amplified from the first-strand cDNA from the liver and brain tissues. We performed the PCR amplification in a volume of 50 µl that included forward and reverse primers (Table 1) 2.5 µl each, cDNA 2.5 µl, Premix Taq (Takara Taq Version 2.0 plus dye) 25 µl, in addition to double distilled water 17.5 µl. The amplification was carried out with the use of the following reaction conditions: 94°C for 5 min, followed by 35 cycles for 30 s at 94°C, for 30 s at 58°C, for 35 s at 72°C and 10 min at 72°C. We segregated the PCR products through the use of electrophoresis, and the DNA Bands were recycled and purified by making use of the SMART RACE cDNA purification Kit (Clontech, Palo Alto, CA). Subsequent to that, the purified DNA portion was subcloned into the pMD18-T vector (Takara, Japan), followed by transforming into the competent *Escherichia coli* DH5a cells. Five different individual positive clones were picked, followed by sending to Sangon Biological engineering (Guangzhou) LTD. for the purpose of sequencing. Cloning of 3′ and 5′ untranslated region

Table 1. Polymerase chain reaction (PCR) primers used in this study.

Primers Sequence	
M13 : CGCCAGGGTTTTCCCAGTCACGAC RV : GAGCGGATAACAATTTCACACAGGA	Vector (Pmd-18)
UPM-long: CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT UPM-short: CTAATACGACTCACTATAGGGC	Universal race primers
MGH-F1-CCATCGCCGTCAGCAGAGTTCAA MGH-R3-GCCTCAGGAGAGAGTCGACATTT	Partial
KG-GH-F1-CTGCGACGAACCTACGAACTGCTGG	3UTR
KG-GH-F2-TACGAACTGCTGGCGTGTTTCAAGA	3UTR
KG-GH-R2-ACTCCCAGGACTCCACCAGCCGATA	5UTR
KG-GH-R4-ATGTTGAACTCTGCTGACGGCGATG	5UTR
GP-βactin(F) –TACGAGCTGCCTGACGGACA GP-βactin(R)- GGCTGTGATCTCCTTTTGCA KG-GH-F1-CGACAAGCACGAGACGCAG KG-GH-R1-AGTTCCCATAAGGAGCCAA	RT-qPCR

M13, RV, UPM-Long and short are all universal primers, GP- βactin is Grouper βactin, MGH and KGH indicate growth hormone gene, and 3&5UTR stands for untranslated regions.

(UTR) end of growth hormone (GH), was done following (Amenyogbe et al., 2019) method.

We carried out 3′ RACE in both the first and second amplification. First PCR amplification was carried out with a 20 µl volume of the reaction mixture, including 1 µl of UPM long primer, in addition to sense primer 1 (Table 1), 1 µl cDNA, 10 ul of premix Taq (Takara Taq Version 2.0 plus dye) and sterile distilled water 7 µl. We carried out second PCR amplification with a 50 µl volume of the reaction mixture, consisting 2.5 µl UPM short primer, and sense primer 2 (Table 1), 2.5 µl of the first reaction as template, 25 µl of premix Taq (Takara Taq Version 2.0 plus dye) and sterile distilled water 17.5 µl. In addition, 5′ RACE was carried out using Amenyogbe et al. (2019) procedures and methodologies.

Gene analysis

We assembled the sequences of partial, 3′ and 5′ UTR to form the full-length cDNA of the target gene by the use of DNAman8 software (*https://*dnaman.software*.informer.com*). The "Gene translation, predictions of the amino acid sequence, and location of domains were done using EXPASY (http://expasy.org/tools) and SMART (htt://smart.emblheidelberg.de) web tool. "Cysteines and tyrosines residues site were predicted by using the http://cic.scu.edu.cn/bioinformatics/Predict_Cys.zip web tool". Multiple sequence analysis of amino acids was performed using ClustalX2 software (Larkin et al., 2007) and (https://www.softpedia.com/get/Science-CAD/GeneDoc.shtml to identify similarities. In order to establish genetic relationships, phylogenetic analysis was carried out, and a consensus tree builds using (https://www.megasoftware.net/). The physical and chemical possessions of the protein were analyzed using the PROTEAN program (DNASTAR Inc.: Madison, WI, USA, 200) to see their impact of the growth. The Subcellular localization was performed using PSORT ((http://psort.hgc.jp/form2.html). SoftBerry Psite software(http://linux1.softberry.com/berry.phtml?topic=psite&group=programs&subgroup=proloc) was also used to predict the potential phosphorylation sites and glycosylation sites. Karplus and Schulz Flexibility method (Karplus and Schulz, 1985), Kolaskar and Tongaonkar Antigenicity method (Kolaskar and Tongaonkar, 1990) and Parker Hydrophobicity Prediction method (Parker et al., 1986) were used to predict the flexibility, antigenicity, and hydrophobicity of GH respectively. The COFACTOR sever (Zhang et al., 2017) method was used for Gene Ontology (GO) terms predictions.

Tissue mRNA expression of GH by qRT-PCR

Total RNA from the brain, gill, liver, muscle, intestine, spleen, stomach, head kidney and heart were used as the template for the first strand cDNA synthesis using TRANSgen First-strand cDNA synthesizes kit. The mRNA levels of GH in tissues were determined by Real-time qPCR using a Roche Light Cycler®96 SW1.1 with a 10 µl reaction consisting, 5 µl Transtart Tip Green qPCR Supermix (TransGen Biotech, China), and 0.4 µl of each sense and antisense primer, 3.6 µl of H_2O and 0.6 µl of cDNA. *β-actin* was used as an internal control to normalize gene expressions (Table 1). A melting curve was performed to detect the specificity. Amenyogbe et al. (2019) procedures were followed for the reaction conditions for the Real-time qPCR. The $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to calculate the results.

Statistical analysis

The data in this study were articulated as means±SD. We presented Significant differences in the data using one-way ANOVA followed by Duncan's post-hoc test and a probability level less than 0.05 ($P < 0.05$) was used to indicate significance. We performed all statistics using SPSS 16.0 (SPSS, Chicago, IL, USA).

RESULTS

Characterization of growth hormone

Sequence analysis of the cloned growth hormone (GH)

Figure 1. GH amino acid sequence was "compared with other similar sequences from other species including human and mouse. Conserved cysteine residues are indicated by Isosceles triangle. Down arrows indicate conserved tyrosine residues. Where *E. fuscogus* represent: *Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂ GH; *C. altiveli* represent: *Cromileptes altivelis* GH; E. coioides represent: *Epinephelus coioides* GH*; L. japonicus* represent: *Lateolabrax japonicas,* GH; *S. aurata* represent: *Sparus aurata* GH; *L. cyanellus* represent: *Lepomis cyanellus* GH; *H. sapiens* represent: *Homo sapien* GH and *M. musculus* represent: *Mus musculus* GH.

revealed it contained 899 base pair with an open reading frame of 588 base pair, 5′utr of 146 base pair and 3′ utr of 166 base pair. The GenBank accession number MK82763 was given after the sequence was submitted to NCBI. The sequence of deduced amino acid of GH was established to comprise of 156 amino acid residues and a putative Pfam Growth Hormone Binding Protein domain. Growth hormone comprises conserved cysteine residues of four and seven conserved tyrosine residues (Figure 1). The sequence of amino acid identities of hybrid grouper growth hormone (hgGH), results from the phylogenetic analysis consistent with growth hormone from other vertebrates (Table 2).

Multiple sequence alignment

The alignment of growth hormone (GH) amino acid sequence of hybrid grouper and similar sequences from other species was carried out using ClustalW online tool (Figure 1). The alignment results showed that the cysteines of growth hormone were conserved in all fish species which could be responsible for the stability of growth hormone (Figure 1). Growth hormone of hybrid grouper was found mainly to be single chain proteins. "A phylogenetic tree was built using the neighbor-joining method based on the deduced amino acid sequences of growth hormone (GH) protein sequence of the hybrid

Table 2. Percentage identity of the amino acid sequences.

1	2	3	4	5	6	7	8		Species
	86.0	86.0	85.4	84.5	84.3	34.1	6.7	1	*E.fuscoguttatus*× *E.polyphekadion*
		99.4	98.5	97.7	97.1	36.7	7.9	2	*C. altivelis*
			98.8	98.3	97.7	36.7	7.9	3	*E. coioides*
				97.7	97.4	37.0	8.2	4	*L. japonicus*
					96.8	36.2	8.2	5	*L. cyanellus*
						36.2	8.2	6	*S. aurata*
							5.2	7	*M. musculus*

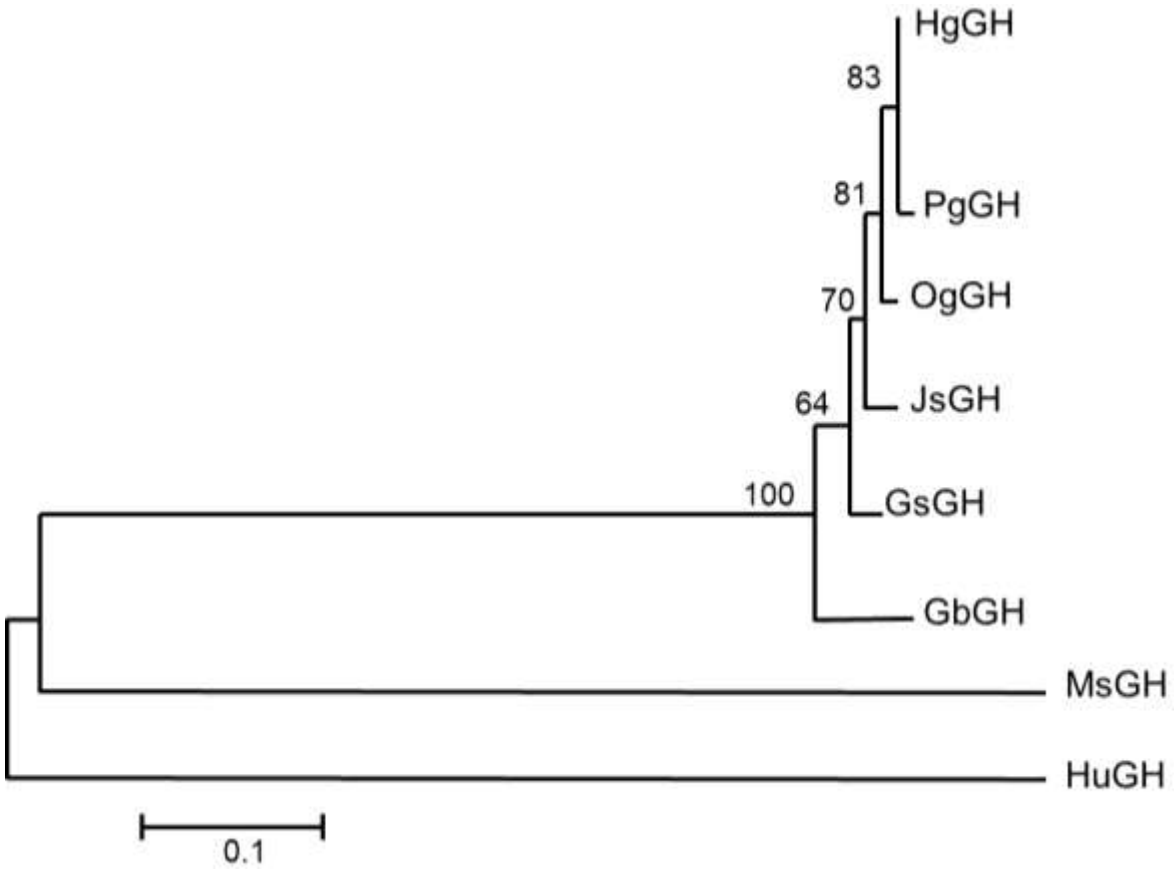


Figure 2. The neighbor-joining phylogenetic tree was built based on the deduced amino acid with growth hormone protein sequences of similar protein sequences of other species. The numbers at each branch designated the proportion of bootstrap values on 1000 duplicates. The phylogenetic space is 0.1 as untaken in the *scale bar.* The following are the species names and GenBank accession numbers; hybrid grouper (HgGH), *Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂ GH (*MK282763*); Panther grouper (PgGH), *Cromileptes altivelis* GH, (ABS19662); Orange-spotted grouper (OgGH), *Epinephelus coioides* GH (AAK57697)*;* Japanese seabass (JsGH), *Lateolabrax japonicas,* GH (AGD80842); Gilthead seabream (GbGH), *Sparus aurata* GH (AAA03329); Green Sunfish (GsGH), *Lepomis cyanellus* GH (AAS20461); Human (HuGH), *Homo sapien* GH (NM_014394); and Mouse (MsGH), *Mus musculus* GH (NM_028263).

grouper and other species, including human and mouse and the results showed that hybrid grouper" and *Cromileptes altivelis were* clustered together (Figure 2).

Gene expression

Even though at a different level, hybrid grouper growth

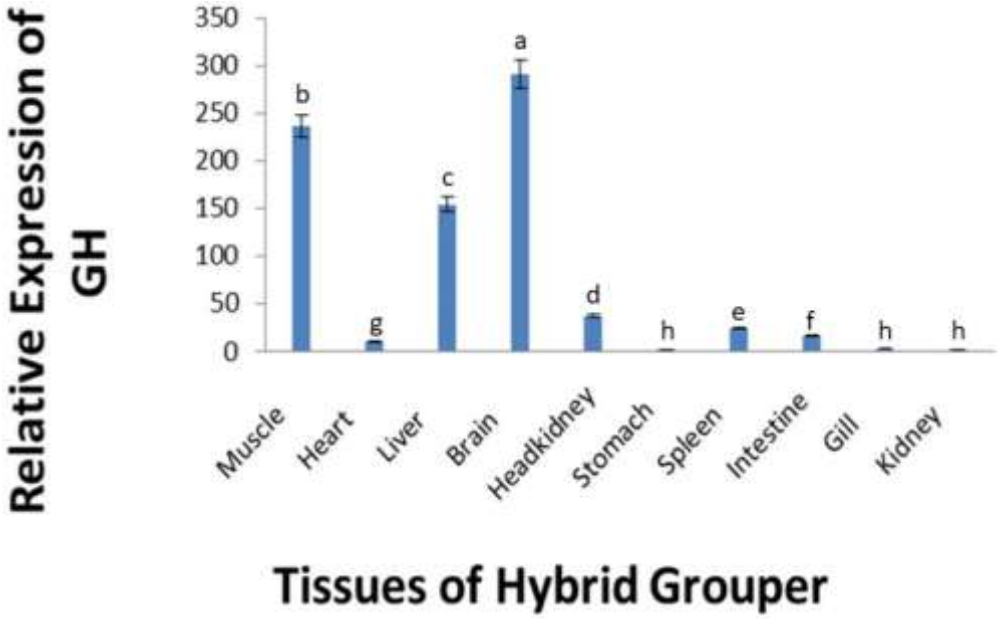


Figure 3. Expressions of hybrid grouper growth hormone were "detected by Real-time PCR from different tissues using β-actin as an internal control to normalize gene expressions. Relative expression of growth hormone in the brain was found to be highest, followed by the muscle, liver, head kidney, spleen, intestine, heart, gill, kidney and stomach respectively. Note： Statistics presented as the mean ± SD of triplicate experiments. Alphabets a, b,c,d,e,f,g,and h indicate statistical differences at $P < 0.05$.

hormone (HgGH) expressed in all the tissues that were examined, and relative expression of GH in the brain was found to be highest, followed by the muscle, liver, head kidney, spleen, intestine, heart, gill, kidney and stomach respectively as shown in Figure 3.

Sequence analysis and physiochemical properties

Scrutiny of the physical and chemical possessions of the hybrid grouper growth hormone sequence revealed the molecular structural formula of hybrid grouper growth hormone to be $C_{782}H_{1246}N_{212}O_{244}S_5$ and an aggregate atom numeral of 2489. Growth hormone has "a theoretical predicted ion isoelectric value of 6.38 and instability index of 65.43 classifying it as an unstable protein with the molecular weight of 17.68 kDa. The amino acid sequence of GH potentially contains one casein kinase II phosphorylation sites, four protein kinase C phosphorylation sites, one N-myristoylation site, and two microbodies C-terminal targeting signal".

Protein secondary structure and amino acid composition

The sequence examination of the amino acid of hybrid grouper growth hormone showed that "the protein contained 50 hydrophobic residues (30.91%), 19 acidic residues (13.32%), 18 basic residues (14.47%) and 53 polar amino acids (33.12%). Aliphatic catalogue and grand average of hydropath city (GRAVY) of growth were 90.71 and −0.4, respectively. The total quantity of negatively charged residues (Asp and Glu) was 19, and the total number of positively".

Antigenic, hydrophobic structure and flexibility regions of hybrid grouper GH

The antigenicity, hydrophobic regions and flexibility regions of hybrid grouper growth hormone (hgGH) were predicted using Kolaskar and Tongaonkar, Parker Hydrophilicity and Karplus and Sechulz Flexibility methods respectively (Figure 4A, B, and C). The property of being able to promote a specific immune response was predicted (Table 3), and the antigenic sites are pronounced as surface domains evolved from side chains of amino acid which might be detached in sequence however close in space.

"Hydrophobic residues forming the binding interface were directed under the threshold value and represented by the residues" D24, I26, V40, E52, W54, F56, P57, S58, R59, P73, S76, E94, L95, D98, S99, L102, Q103, S113, T123, Y124, F130, T139, L141, T142, A144, K145 and C146. The transmembrane residues were directed

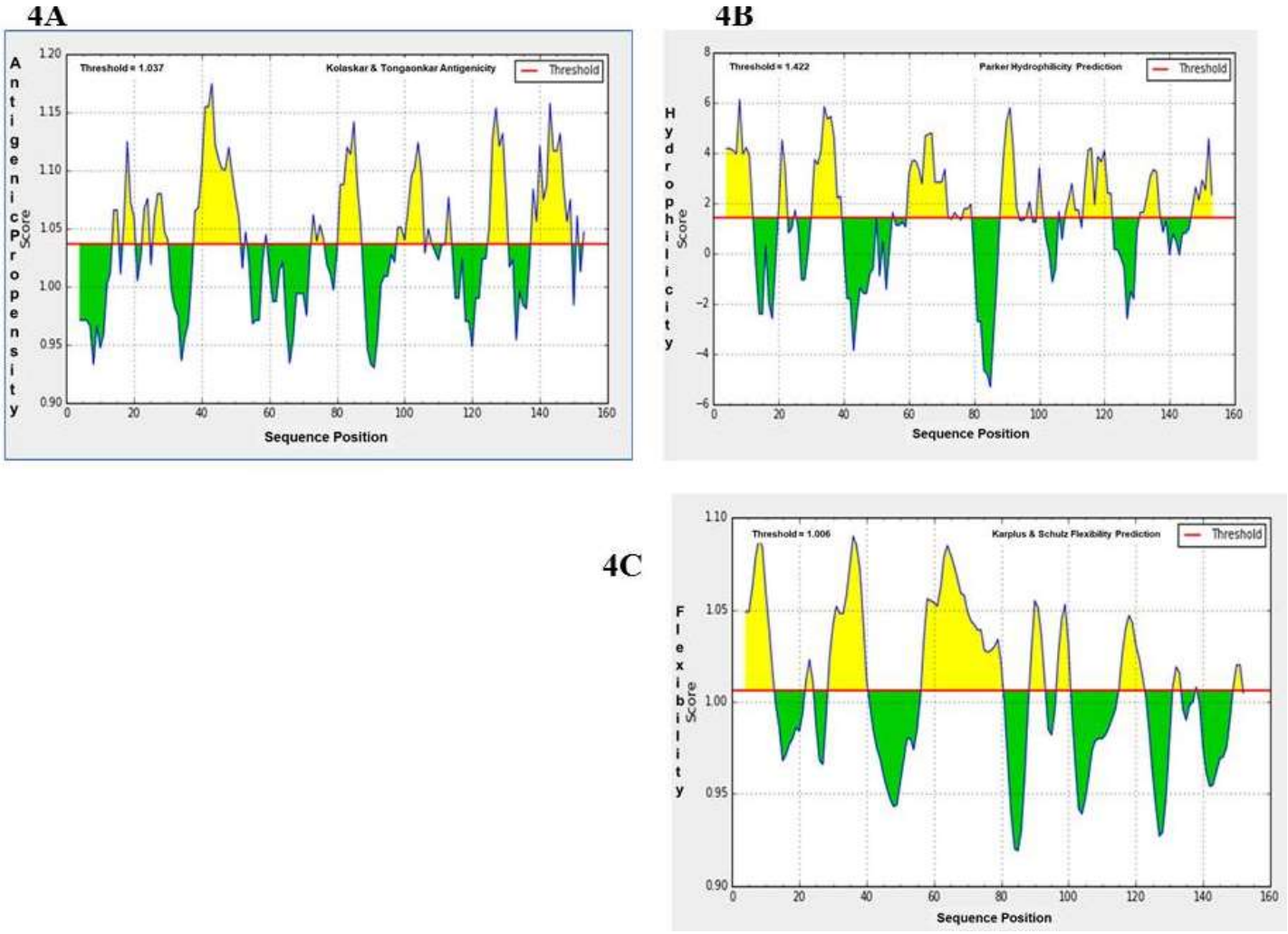


Figure 4. A) an antigenic property of GH was predicted using the Kolaskar and Tongaonkar method. Five "potential antigenic peptides have more than 1.0 antigenic propensity, and six or more amino acids in length were predicted". B) the hydrophobic regions of hybrid grouper GH and C) The flexibility regions of hybrid grouper GH.

Table 3. The antigenic amino acid sequence and its position.

No.	Start Position	End Position	Peptide	Length
1	38	51	SSVLKLLSISYRLV	14
2	81	87	GILLLIR	7
3	98	105	DSSALQLA	8
4	125	130	ELLACF	6
5	138	149	ETYLTVAKCRLS	12

below the threshold line and are represented as L104, A105, E125-C129, Y140 and V143 whilst Flexibility residues forming a flexibility loop were above the threshold value and are represented by the residues LQTEEQRQL (4-12), PIDKHETQRSSV (29-40), PSRSLSGGSAPRNQISPKLSEKT (57-80), NQDGS (97-100), PDSS (115-123), FKKD (130-133) E138 and SPEA (149-152).

Predicted Gene Ontology (GO) TERM analysis of hybrid grouper growth hormone using COFACTOR software

Two main functions were identified, namely biological process and molecular function with growth hormone mRNAs regulating relationships in the development of hybrid grouper by the GO using COFACTOR software. It

has been found that the growth hormone regulated by hybrid grouper is involved in many biological processes, including “Single-organism process, metabolic process, glucose metabolic process, biological regulation, regulation of biological process, regulation of cellular process, positive regulation of biological process, positive regulation of metabolic process, response to stimulus, regulation of biological quality, positive regulation of cellular process, regulation of cellular metabolic process, positive regulation of cellular metabolic process, regulation of transport, positive regulation of transport, response to abiotic stimulus, response to stress”, etc (Figure 5A). In addition, it also has molecular functions, including “signaling transducer activity, protein binding, signaling receptor activity, receptor binding, cytokine receptor binding, molecular transducer activity, receptor activity, transmembrane receptor activity, transmembrane signaling receptor activity and cytokine receptor activity” (Figure 5B). Also, it has cellular component, including extracellular region, extracellular space, membrane part, integral component of membrane (Figure 5C). Additionally, it also has predicted binding sites such as R10, L17, P22, P29, S26, Q36, T36, T139, T142, N22, N153, K132, K136, V136, V143, E135, E138, to nucleotides and are believed to be participating in transcriptional regulation (Figure 5D). Also present is the Predicted Enzyme Commission of growth hormone (Figure 5E).

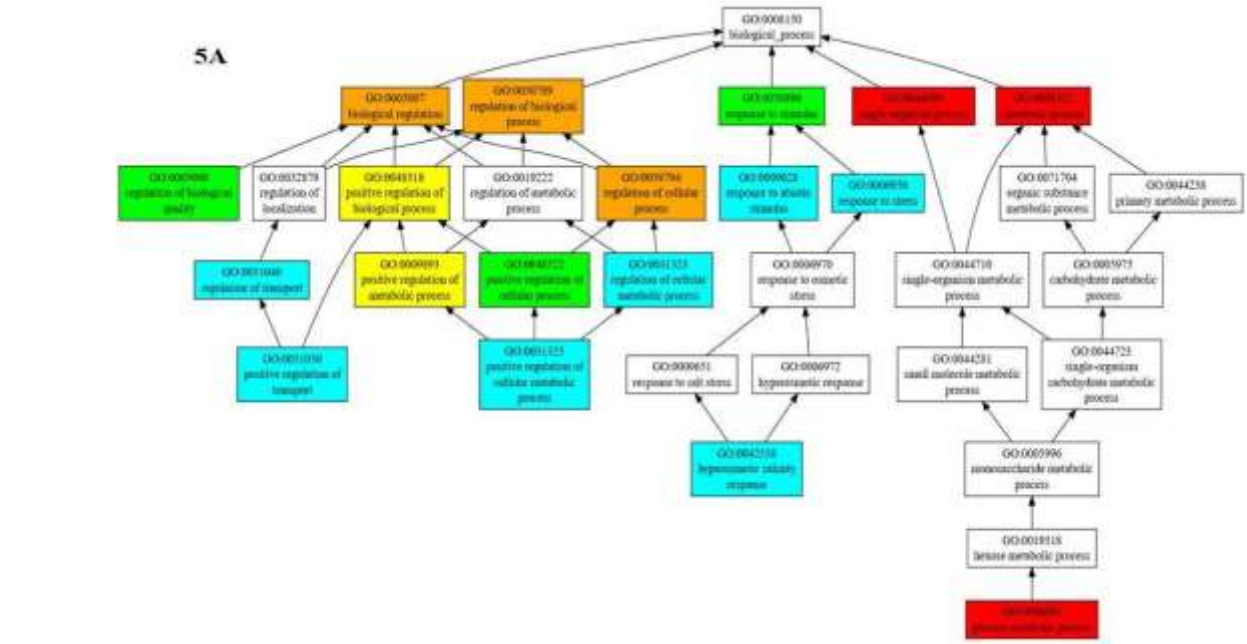
DISCUSSION

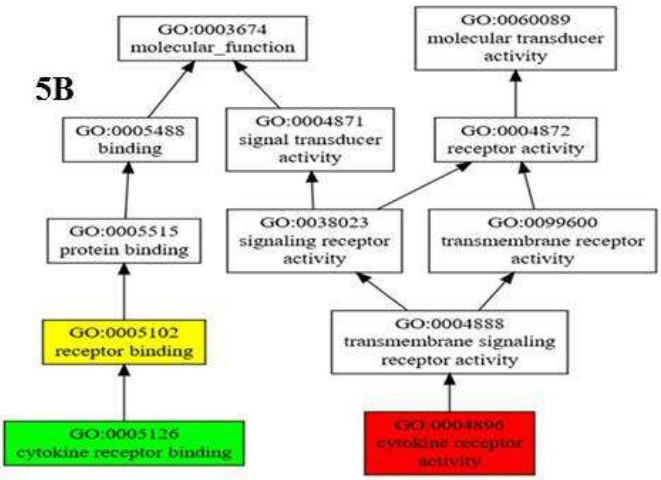
The development rate is of pivotal commercial significance in the farming of food faunas owing to the fact that the fast growth characteristically is associated with the fast-paced turnover of production. One of the potential tools for the assessment of nutritional status and growth in juvenile grouper deals with measuring the expression of a regulatory hormone, suggesting the somatic growth of the fish. The significance of growth hormone as a development-enhancing instrument has extensively acknowledged, as its possible implementation in aquaculture business (Li et al., 2005). In addition, the development of hybrid grouper fish by means of crossbreeding constitutes a practical means of yielding the grouper fish on a large scale. Nevertheless, the production of the hybrid grouper fish does not constitute a convenient strategy. The key challenge reported is slow growth. Moreover, the hybrid grouper has been effectively developed by means of the cultivation and genetic selection of hybrids of (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂). It is one of a number of groupers cultivated in China. It not only has a good taste but also huge size, making it an economically important marine water species. Growth hormone is primarily a single chain polypeptide that has two intramolecular disulphide bonds (Deng et al., 2014), known for its multifunction, for instance, regulations of several phases of growth, behaviour, immune function, metabolism, reproduction, and osmoregulation (Forsyth and Wallis, 2002; Björnsson et al., 2004; Norrelund, 2005; Norbeck et al., 2007; Møller and Jørgensen, 2009; Pérez-Sanchez, 2000; Very et al., 2005), which act through its receptor (Herrington and Carter-Su, 2001). The crucial role played by growth hormone in the promotion of growth has been reported in several vertebrates, which include fish as well (Ben-Atia et al., 1999; Acosta et al., 2008; Edens and Talamantes, 1998).

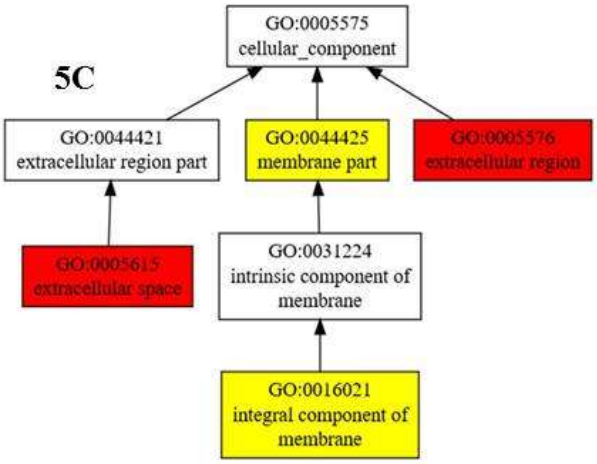
In the current research work, the juvenile grouper growth hormone was cloned for the first time, and its expression in diverse tissues was determined as well. In this research work, the sequence of deduced amino acid of growth hormone was detected as containing 156 amino acid residues as well as a putative Pfam Growth Hormone Binding Protein domain. It was discovered that the hybrid grouper growth hormone (HgGH) contained GHBP, besides being believed as prolonging the half-life of growth hormone among other functions (Chang et al., 1992). Nonetheless, it is deemed as quite essential to investigate further for the purpose of elucidating its specific role in the development of hybrid grouper.

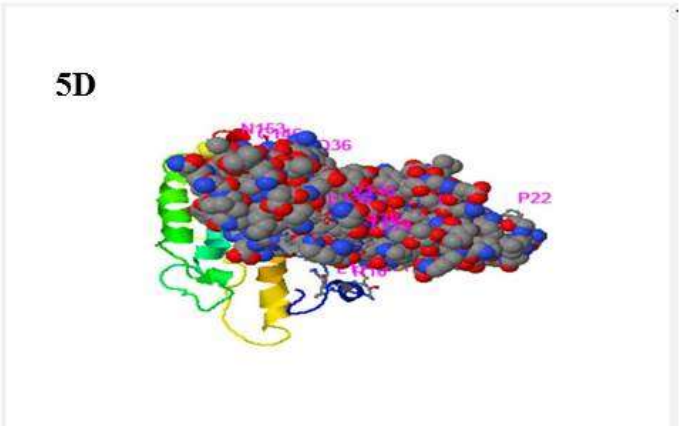
The hybrid grouper growth hormone contained the conserved cysteine residues of four and seven conserved tyrosine residues of which the four conserved cysteine residues formed two disulphide bonds. The findings of the present research work are in agreement with the findings in majority teleost growth hormones, except in goldfish and other cyprinids in which five cysteine residues were discovered (Law et al., 1996; Degani et al., 2006; Sciara et al., 2006), and might be playing a crucial role in the purpose of the hybrid grouper growth hormone (HgGH) biological activities and its stability as suggested by Deng et al. (2014) and Law et al. (1996). The four cysteine residues in hybrid grouper are found in the same locations as in virtually all of the growth hormone polypeptides, which consist of all of the studied fishes. The cysteine residues, which have the capability of establishing two disulphide bonds were anticipated to add to the tertiary structure of the hormone molecule. Owing to the fact that these residues are positioned at approximately undistinguishable positions in all of the growth hormones, “it can be predicted that these residues play an indispensable part in not only structural reliability but also sustaining the biologically energetic form of growth hormone.

Besides that, there is also a presence of double Asn-X-Thr/Ser motifs in the hybrid grouper growth hormone aa sequence and are believed to be the possible positions for N-linked glycosylation. This is in agreement with similar observation of the two comparable sequences in the tilapia, giant catfish, salmon and carp, while only one was observed in those of chicken, yellowtail, tuna, flounder, eel and sole growth hormone sequences (Pendon et al., 1994). Together with that, one tryptophan









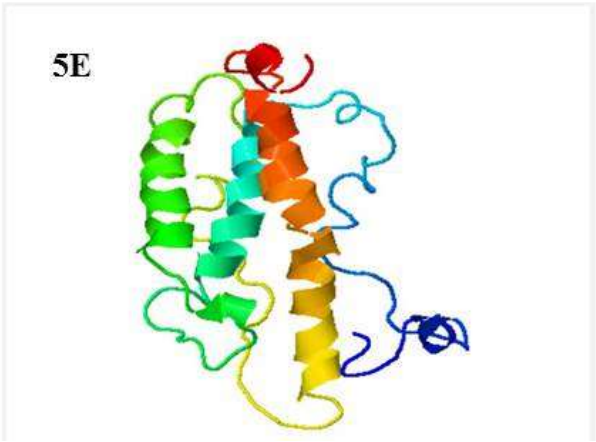


Figure 5A, B, C, D and E. Biological processes, molecular functions, cellular component, predicted ligand binding sites and predicted enzyme commission of growth hormone respectively.

(a)	"Cscore^{GO} is the confidence score of predicted GO terms. "Cscore^{GO} values range in between [0-1]; where a higher value indicates a better confidence in predicting the function using the template".
(b)	The graph shows the predicted terms within the Gene Ontology hierachy for Molecular Function. Confidently predicted terms are color coded by CscoreGO:
	[0.4,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0]
(c)	The graph shows the predicted terms within the Gene Ontology hierachy for Biological Process. Confidently predicted terms are color coded by Cscore^{GO}:
	[0.4,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0]
(d)	The graph shows the predicted terms within the Gene Ontology hierachy for Cellular Component. Confidently predicted terms are color coded by Cscore^{GO}:
	[0.4,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0]
(a)	"Cscore^{EC} is the confidence score for the Enzyme Commission (EC) number prediction. Cscore^{EC} values range in between [0-1]; where a higher score indicates a more reliable EC number prediction".
(b)	"TM-score is a measure of global structural similarity between query and template protein".
(c)	"RMSD^{a} is the RMSD between residues that are structurally aligned by TM-align".
(d)	"IDEN^{a} is the percentage sequence identity in the structurally aligned region".
(e)	"Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein".
(a)	"CscoreLB is the confidence score of predicted binding site. Cscore LB values range in between [0-1]; where a higher score indicates a more reliable ligand-binding site prediction".
(b)	"BS-score is a measure of local similarity (sequence & structure) between template binding site and predicted binding site in the query structure". "Based on large scale benchmarking analysis, we have observed that a BS-score >1 reflects a significant local match between the predicted and template binding site".
(c)	"TM-score is a measure of global structural similarity between query and template protein".
(d)	"RMSDa the RMSD between residues that are structurally aligned by TM-align".
(e)	"IDENa is the percentage sequence identity in the structurally aligned region".
(f)	"Cov. represents the coverage of global structural alignment and is equal to the number of structurally aligned residues divided by length of the query protein".

residue (Trp) was observed in hybrid grouper as was figured out in the *Hemiramphus brasiliensis*, mullet, halfbeak, marine silverside fish, *Mugil platanus* and *Odenthesthes argentinensis*, to be one of the physiognomies of growth hormone (Marins et al., 2003; Meire et al., 2006).

The growth hormone protein sequences are termed as reflecting the molecular phylogeny of bony fishes (Bernardi et al., 1993; Schneider et al., 1992). The phylogenetic association of the designated fish species was assessed to bear in mind the growth hormone cDNA homology with the use of the neighbour-joining methodology. This methodology of examination proliferate the statistical significance of the data as well as similarity of the species. Figure 3 provides the perfect compassionate interpretation of the morphology grounded conventional taxonomy of fishes. The assemblages of fishes are linked, together with emanating beneath the same division, Teleostei. The freshly cloned hybrid grouper growth hormone gene sequence is observed as "clustered monophyletically with the solid bootstrap backing". In accordance with the expectation, it was close to the *C. altivelis* growth hormone gene sequence, besides being positioned in the identical clade and consequential from the identical family. That is why this examination illustrates the fact that the sequence cloned was the growth hormone gene with high resemblance with other teleost growth hormone genes in relations to both the structure and association.

Growth hormone is primarily produced in pituitary gland (Sciara et al., 2006); however, it is fully established that some of the other tissues express the growth hormone gene as well (Sciara et al., 2006; Yang et al., 1999). The expression of growth hormone (GH) mRNA was dominantly detected or highly expressed in the pituitary (Li et al., 2005; Sciara et al., 2006). In the present research exertion, the hybrid grouper growth hormone (HgGH) was observed as expressed in all of the tissue samples examined despite being at varying levels as well as the highest in the brain (Figure 3), which could be owing to the role it might be playing in the central nervous system. The distribution of tissue in this study is in line with the role of growth hormone in the key physiological mechanisms, for instance, metabolism and somatic growth (Reinecke et al., 2005). The expression of the hormone in various tissues proved its presence however, it is well known therefore that the malfunctioning of the growth hormone or its deficiency can results in retarded growth, therefore there is a need for further studies to elucidate the growth hormone's binding abilities to its receptors.

The flexibility residues, sequences that formed "the flexible loops" were observed as conserved in the fish species but not in other mammalians. These sites were

believed as being where the active sites are situated. The results shed light on the fact the “hydrophobic residues forming the binding interface were directed under the threshold value and represented by the residues” D24, I26, V40, E52, W54, F56, P57, S58, R59, P73, S76, E94, L95, D98, S99, L102, Q103, S113, T123, Y124, F130, T139, L141, T142, A144, K145 and C146. The transmembrane residues were directed under the threshold line, which are represented as L104, A105, E125-C129, Y140, and V143.

The predicted gene ontology analysis verifies the fact that proteins act together with other molecules for the execution of their biological functions in majority cellular mechanisms. These exchanges comprise the ligand bindings in “receptor sites, the antibodies binding to antigens, protein-DNA interactions of protein-DNA, and protein-protein interactions” (Katchalski-Katzir et al., 1992; Jones and Thornton, 1996; Berchmanski et al., 2002; Halperin et al., 2002).

Conclusion

In the present research exertion, the hybrid grouper growth hormone (GH) has been effectively sequestered and cloned. The aa sequence, which is expected from the cDNA, conveys fresh statistics regarding the growth hormone structure of a teleost, a hybrid species. The isolation of growth hormone genes is still of significance; in addition, a number of investigations merely place emphasis on the other fish species but there are just a few research works addressing the hybrid fish species. To the best of our understanding, this constituted the first to report on the isolation of the growth hormone from the hybrid grouper (*Epinephelus fuscoguttatus* ♀× *Epinephelus polyphekadion* ♂), termed as a discovery believed as helping comprehend the molecular physiognomies of the hybrid grouper growth hormone gene, besides being likely to help construct a recombinant growth hormone protein, in addition to molecular exploration, aimed at enhancing the understanding of growth enhancement by means of the growth hormone regulation. This research work provides an understanding of molecular attributes of the development concept of a species acknowledged as having a retarded growth rate. We have high hopes that these findings are going to help researchers advance the growth enactment of the cultured hybrid grouper in future.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Acosta J, Carpio Y, Besada V, Morales R, Sanchez A, Curbelo Y, Ayala J, Estrada MP (2008). Recombinant truncated tilapia growth hormone enhances growth and innate immunity in tilapia fry (Oreochromis sp.). General Comparative Endocrinology 157:49-57.

Amenyogbe E, Chen G, Wang Z (2019). Identification, Characterization, expressions and bioinformatics analysis of growth hormone receptors (GHR1 and GHR2) in hybrid grouper (*Epinephelus fuscoguttatus* ♀× *E. polyphekadion* ♂) Genomics, https://doi.org/10.1016/j.ygeno.2019.05.012

Ben-Atia I, Fine M, Tandler A, Funkenstein B, Maurice S, Cavari B, Gertler A (1999). Preparation of recombinant gilthead seabream (Sparus aurata) growth hormone and its use for stimulation of larvae growth by oral administration. General and Comparative Endocrinology 113:155-164.

Berchmanski A, Katchalski-Katzir E, Eisenstein M (2002). Electrostatics in protein-protein docking. Protein Science 11:571-587.

Bernardi G, D’Onofrio G, Caccio’ S, Bernardi G (1993). Molecular phylogeny of bony fishes based on their amino acid sequence of the growth hormone. Journal of Molecular Evolution 16:644-649.

Björnsson BT, Johansson V, Benedet S (2004). Growth hormone endocrinology of salmonids: regulatory mechanisms and mode of action. Fish Physiology and Biochemistry 27:227-242.

Brahm H, Piehl Auhlin K, Saltin B (1997). Netfluxes overworking thigh of hormones, growth factors and biomarkers of bone metabolism during short dynamic exercise. Calcified Tissue International 60(2):175-180.

Brooks AJ, Dai W, Megan L, O’Mara, Abankwa D, Chhabra Y, Pelekanos RA, Gardon O, Tunny KA, Blucher KM, Morton CJ, Parker MW, Sierecki E, Gambin Y, Gomez GA, Alexandrov K, Wilson IA, Doxastakis M, Mark AE, Waters MJ (2014). Mechanism of Activation of Protein Kinase JAK2 by the Growth Hormone Receptor.Science 344; https://doi.org/10.1126/science.1249783.

Brooks AJ, Waters MJ (2010). The growth hormone receptor: Mechanism of activation and clinical implications. Nature Reviews Endocrinology 6:515-525.

Casanueva FF (1992). Physiology of growth hormone secretion and action. Endocrinology and Metabolism Clinics of North America 21(3):483–517.

Chang YS, Liu CS, Huang FL, Lo TB (1992). The primary structures of growth hormones of three cyprinid species: bighead carp, silver carp, and grass carp. General and Comparative Endocrinology 87:385-393

Degani G, Jackson K, Yom-Din S, Goldberg D (2006). cDNA cloning and mRNA expression of growth hormone in belontiidae (*anabantoidei suborder*). Bamidgeh 58(2):124-136.

Deng SP, Wu B, Zhu CH, Li GL (2014). Molecular cloning and dimorphic expression of growth hormone (GH) in female and male spotted scat *Scatophagus argus*. Fisheries Science 80:715-723.

Edens A, Talamantes F (1998). Alternative processing of growth hormone receptor transcripts. Endocrine Reviews 19:559-582.

Forsyth IA, Wallis M (2002). Growth hormone and prolactin–molecular and functional evolution. Journal of Mammary Gland Biology and Neoplasia 7:291-312.

Godfrey RJ, Madgwick Z, Whyte GP (2003). The exercise-induced growth hormone response in athletes. Sports Medicine 33(8):599-613.

Halperin I, Ma B, Wolfson H, Nussinov R (2002). Principles of docking: an overview of search algorithms and a guide to scoring functions. Proteins, 47:409-443. https://doi.org/10.1002/prot.10115.

Herrington J, Carter-Su C (2001). Signaling pathways activated by the growth hormone receptor. Trends in Endocrinology and Metabolism 12:252-257.

James CM, Al-Thobaiti SA, Rasem BM, Carlos MH (1999). Potential of Grouper Hybrid (Epinephelus fuscoguttatus x E. polyphekadion) for aquaculture. Aquaculture. Eurofish Magazine 24:35-37.

Jones S, Thornton J (1996). Pricinples of protein-protein interactions. PNAS 93:13-20. https://doi.org/10.1073/pnas.93.1.13.

Karplus PA, Schulz GE (1985). Prediction of chain flexibility in proteins. Naturwissenschaften 72:212-213.

Katchalski-Katzir E, Shariv I, Eisenstein M, Friesem A, Aflalo C, Vakser I (1992). Molecular Surface Recognition: Determination of Geometric Fit Between Proteins and Their Ligands by Correlation Techniques. PNAS 89:2195-3199.

Kohno H (1997). Why is grouper larval rearing difficult? An approach from the development of the feeding apparatus in early stage larvae of the grouper, E. coioides. Ichthyological Research 44(3):267-274.

Kolaskar AS, Tongaonkar PC (1990). A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Letters 276:172-174.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007). Clustal W and Clustal X version 2.0. Bioinformatics 23:2947-2948.

Law MS, Cheng KW, Fung TK, Chan YH, Yu KL, Chan KM (1996). Isolation and characterization of two distinct growth hormone cDNAs from the goldfish, Carassius auratus. Archives of Biochemistry and Biophysics 330:19-23.

Li H, Oehrlein SA, Wallerath T, Ihrig-Biedert I, Wohlfart P, Ulshöfer T (1998). Activation of protein kinase C alpha and/or epsilon enhances transcription of the human endothelial nitric oxide synthase gene. Molecular Pharmacology 53:630-637.

Li WS, Chen D, Wong AOL, Lin HR (2005). Molecular cloning, tissue distribution and ontogeny of mRNA expression of growth hormone in orange-spotted grouper (Epinephelus coioides). General and Comparative Endocrinology 144:78-89.

Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ Method. Methods 25:402-408.

Marins LF, Levy JA, Folch JM, Sanchez A (2003). A growth hormone-based phylogenetic analysis of euteleostean fishes including a representative species of the Atheriniformes Order, Odontesthes argentinensis. Genetics and Molecular Biology 26(3):295-300.

McArdle W, Katch FI, Katch VL (2010). Exercise Physiology: Nutrition, Energy and Human Performance. 7th ed. Baltimore: Lippincott Williams and Wilkins 1038 p.

Meire KM, Castano C, Laurino J, Levy JA, Marins LF (2006). cDNA cloning and phylogenetic analysis of growth hormones genes from the mullet Mugil platanus (Mugilomorpha, Mugulidae) and the halfbeak Hemiramphus brasiliensis (Atherinomorpha, Hemiramphidae). Rio Grandae 28(2):97-102.

Mingarro M, Vega-Rubin de Celis S, Astola A, Pendon C, Valdivia MM, Perez-Sanchez J (2002). Endocrine mediators of seasonal growth in gilthead sea bream (*Sparus aurata*): the growth hormone and somatolactin paradigm. General and Comparative Endocrinology 128:102-111.

Møller N, Jørgensen JOL (2009). Effects of growth hormone on glucose lipid and protein metabolism in human subjects. Endocrine Reviews pp. 152-177.

Norbeck LA, Kittilson JD, Sheridan MA (2007). Resolving the growth-promoting and metabolic effects of growth hormone: differential regulation of GH–IGF-I system components. General and Comparative Endocrinology 151:332-341.

Norrelund H (2005). The metabolic role of growth hormone in humans with particular reference to fasting. Growth Hormone and IGF Research 15:95-122.

Parker JM, Guo D, Hodges RS (1986). New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray derived accessible sites. Biochemistry 25:5425-5432.

Pendon C, Martinez-Barbera JP, Perez-Sanchez J, Rodriguez RB, Grenett H, Valdivia MM (1994). Cloning of the sole (Solea senegalensis) growth hormone-encoding cDNA. Gene 145:237-240.

Pérez-Sanchez J (2000). The involvement of growth hormone in growth regulation, energy homeostasis and immune function in the gilthead sea bream (*Sparus aurata*): a short review. Fish Physiology and Biochemistry 22:135-144.

Reinecke M, Björnsson BT, Dickhoff WW, McCormick SD, Navarro I, Power DM, Gutiérrez J (2005). Growth hormone and insulin-like growth factors in fish: Where we are and where to go. General and Comparative Endocrinology 142:20-24.

Schneider JF, Myster SH, Hackett PB, Guise KS, Faras AJ (1992). Molecular cloning and sequence analysis of the cDNA for northern pike Esox lucius. Molecular Marine Biology and Biotechnology 1:106-112.

Sciara AA, Rubiolo JA, Somoza GM, Arranz SE (2006). Molecular cloning, expression and immunological characterization of pejerrey (Odontesthes bonariensis) growth hormone. Comparative Biochemistry and Physiology - Part C 142:284-292.

Sekar M, Singh SD, Gupta S (2014). Cloning and Characterization of Pangasianodon hypophthalmus Growth Hormone Gene and its Heterologous Expression. Applied Biochemistry and Biotechnology 173:1446-1468.

Very NM, Kittilson JD, Norbeck LA (2005). Isolation, characterization, and distribution of two cDNAs encoding for a growth hormone receptor in rainbow trout (Oncorhynchus mykiss). Comparative Biochemistry and Physiology - Part B 140:615-628.

Wang HS (1997). The problems and situation in the artificial propagation of grouper, *Epinephelus*. Journal of Dalian Fisheries University 12(3):44-51.

Yang BY, Greene M, Chen TT (1999). Early embryonic expression of the growth hormone family protein genes in the developing rainbow trout, Oncorhynchus mykiss. Molecular Reproduction and Development 53:127-134.

Zhang C, Freddolino PL, Zhang Y (2017). COFACTOR: Improved protein function prediction by combining structure, sequence, and protein-protein interaction information. Nucleic Acids Research 45(W1):W291-W299.