

Isolation of growth hormone gene from the Malaysian mahseer, *Tor tambroides*

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Abstract. A study was done to clone a cDNA of the Malaysian mahseer (*Tor tambroides*) growth hormone (GH) gene. In this study, an RT-PCR technique was used to isolate the GH gene using RNA extracted from brain tissue of *T. tambroides*. The fragment obtained was purified and cloned into pGEM-T easy cloning vector. The GH cDNA of *T. tambroides* consists of 1,189 nucleotides (nt) excluding the poly (A) tail, with a 36 nt untranslated region (UTR) at the 5' region and a 520 nt UTR at the 3' region. The GH cDNA contains an ORF of 633 nt and encodes for a polypeptide of 210 amino acids (aa), including a signal peptide of 22 aa. Comparison of the *T. tambroides* GH gene with other teleost GH genes indicated that it has the highest similarity with *C. carpio* GH type 1 (GHI) with which it share 99% and 98% homology in terms of amino acid and nucleotide sequences, respectively. Molecular characterization of the *T. tambroides* GH gene showed it exhibits typical GH features similar with other teleost GH genes. Therefore, the GH sequence of *T. tambroides* was successfully isolated and the sequence added to the GenBank database (JF428142). This is the first report of the isolation of a GH gene from this Malaysian indigenous species, and will assist in understanding the molecular characteristics of the *T. tambroides* GH gene. This could lead to the use of molecular approaches to better understanding the growth of *T. tambroides* and give an insight into the molecular aspects of its growth performance in aquaculture.

Keywords: cDNA, Growth hormone, Indigenous species, *Tor tambroides*

INTRODUCTION

Growth hormone (GH) is one of the most important hormones involved in growth processes especially for promotion and maintenance of somatic growth in vertebrates (Yowe and Epping, 1999). Besides regulating growth, it also regulates other physiological functions in fish including metabolism, immune response, reproduction and osmoregulation (Cavari *et al.*, 1993; Filby and Tyler, 2007; Sakamoto *et al.*, 1997; Yada, 2007). Due to this, GH cDNA has been isolated and characterized from different orders and families of many fish species including Cypriniformes (Agellon *et al.*, 1988; Anathy *et al.*, 2001; Chang *et al.*, 1992; Filby and Tyler, 2007; Inoue *et al.*, 2003; Saito *et al.*, 1988; Venkatesh and Brenner, 1997). GH, which is a single chain polypeptide secreted in the anterior pituitary portion of the fish brain, has been studied extensively in understanding the structure-function relationships, evolution and regulation of gene expression. However, understanding of the GH gene in indigenous Malaysian fish species is still limited and has not been extensively explored.

Malaysian mahseer, also known as kelah, is one Malaysian indigenous species, a freshwater fish which is very valuable for consumption and commands a good price in the market (Nguyen *et al.*, 2006). This fish has been successfully

propagated and efforts are underway to commercialize production of this species, also reared for conservation purposes. Nevertheless, study of the GH gene which affects growth of this fish is still limited. Moreover, previous studies have shown that the coding region of GH gene sequences are less similar among teleosts species than between terrestrial species. The size of the GH gene varies among species (Law *et al.*, 1996; Marins *et al.*, 2003). Although the GH gene has been isolated from many different fish species, there is no information on GH gene sequence in *T. tambroides* and whether the sequence is similar or not with other GH genes is still unknown. The study of growth hormone in this fish is very important in providing additional information in terms of molecular development, and so the goal of this study was to isolate and characterize the GH gene fragment from *T. tambroides*.

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MATERIALS AND METHODS

In this study, five sense and five antisense primers based on the cypriniformes and *Cyprinus carpio* GH gene sequence (JF428142) were designed using Primer Premier 5 software (Table 1). In order to isolate the partial and full-length coding sequences of the *Tor tambroides* GH gene sequences, these primers were used to amplify 5 different target regions: from start codon to highly conserved region (CFKKDMHKVETYL); coding sequence from start to stop codon; 5' to 3' untranslated region (UTR); highly conserved

Table 1. The sequences of forward and reverse primers used in isolating the GH gene.

Primer name	Primer sequence (5'-3')
TGHS1	ATGGCTAGAGCATTAGTGCTATTGT
TGHS2	ATGGCTAGAGCATTAGTGCTATTG
TGHA1	GTAAGTTTCGACCTTGTGCATGT
TGHA2	CTGAGGTAAGTTTCGACCTTGTG
TGHA3	CTACAGGGTGCAGTTGGAATCC
CGHS1	AAGCCTGCAAGAGTTTGTCTACC
CGHS2	AACTAAGCCTGCAAGAGTTTGTCTAC
CGHS3	GGCTTGCTTTAAGAAGGACAT
CGHA1	TGGATGCAATTAAAACCTTAATAGAG
CGHA2	TGTTACAGGCATTGACTAACAATACA

S=sense; A=antisense

region to stop codon; and 3' UTR (Figure 1). The primer pairs TGHS1 and TGHA1 (A) and THGS2 and TGHA2 (B) which are composed of 23 to 25-mer oligos were used in RT-PCR to generate 582 and 587 bp partial fragments, respectively, from ATG to highly conserved region, 100 bp from the stop codon. The combinations of TGHS1, TGHS2 and TGHA3 (C) (D) and CGHS1, CGHS2 with

CGHA2 (E) (F) which are composed of 22 to 26-mer oligos, generated 633, 701 and 705 bp full-length GH sequences, respectively. Meanwhile, the combination of specific CGHS3 with TGHA3 (G) and CGHSA1 (H) primers which are composed of 21-26-mer oligos, generated 127 and 586 bp partial GH fragments from the target fish, respectively (Table 2). The GH gene fragment of *T. tambroides* was

Table 2: Primer combinations and resulting product sizes.

Primer combination	Primer code	Target size (bp)
TGHS1 + TGHA1	(A)	582
TGHS2 + TGHA2	(B)	587
TGHS1 + TGHA3	(C)	633
TGHS2 + TGHA3	(D)	633
CGHS1 + CGHA2	(E)	701
CGHS2 + CGHA2	(F)	705
CGHS3 + TGHA3	(G)	127
CGHA2 + CGHA1	(H)	586

isolated with the One-Step PCR method using a commercially BioScript™ One-Step RT-PCR Kit (Bioline, USA) following the manufacturer's protocol. A 25µl reaction, consisting of 1x one-step RT-PCR buffer, 1µl one-step enzyme mix, 50 pmol of upstream primer, 50 pmol of downstream primer, 3µg of total RNA, 10µl RNase inhibitor, 5% Dimethyl sulfoxide (DMSO), was prepared and mixed into 0.2 ml PCR tube. DEPC-treated water was added to make up to 25µl. The mixtures were mixed by tapping and spun briefly for 5 seconds. The PCR samples were then put into the thermal cycler (MJ PTC200, USA) and reverse transcribed at 42°C for the initial reverse-transcription step and 95°C to denature the RT enzyme and activate DNA polymerase. After that, 35 cycles of amplification was performed which

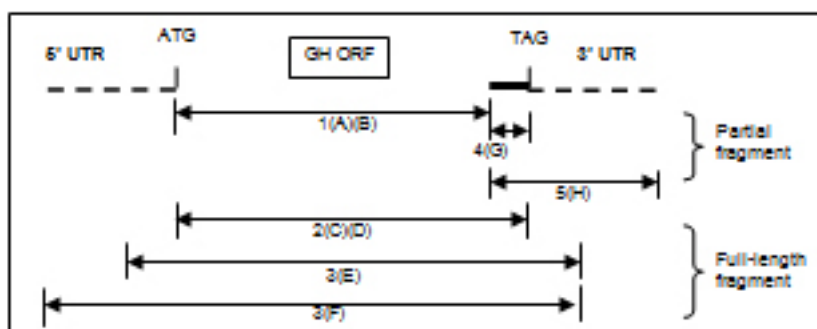


Figure 1. The amplification strategy of the *Tor tambroides* growth hormone (GH) gene. Arrows indicate the amplified sequences. The stippled line indicates a short region of highly conserved sequence

consisted of denaturation at °C for 30 seconds, annealing at 58 to 62°C for 30 seconds and extension at 72°C for 30 seconds, followed by final extension at 72°C 10 minutes.

The resulting PCR fragment was purified using a commercially available Wizard® SV gel and PCR clean-up system by Promega, and later cloned into pGEM-T Easy cloning vector (Promega, USA). The positive clones were sequenced and analyzed. The sequence was imported into BLAST (NCBI) in order to identify the similarity of the sequence, in terms of their E-value and query coverage, with other GH gene sequences in the Genbank database (<http://www.ncbi.nlm.nih.gov>). Later, the nucleotide and protein sequences obtained were compared with other teleost GH gene sequences from the Genbank database.

Multiple alignments from 36 nucleotide sequences of GH ORF were conducted using the ClustalX program and were used in phylogenetic analysis, carried out using PAUP* version 4.0 b10 (Swofford, 2000) with Maximum Likelihood and Neighbor Joining algorithms in order to estimate the phylogeny.

For Maximum Likelihood analysis, a hierarchical likelihood ratio test (hLRT) was performed using Modeltest version 3.7 (Posada and Crandall, 1998) to determine the best model of evolution with an α value of 0.01. The best fit evolutionary model selected for the sequence data set was the Kimura 2 Parameter model with Gamma distribution (K80 + G) and estimated base frequencies of A= 0.25, C= 0.25, G= 0.25, T= 0.25; Base=equal Nst=2; a Ti/tv (transition/transversion) rate of 1.8089; a G (gamma) distribution shape of 0.7729 and zero proportion of invariable sites. The K80+ G model and maximum likelihood parameters were then used to reconstruct the maximum likelihood tree using PAUP*. Neighbor-Joining trees were constructed from dissimilarity values computed using the same maximum likelihood settings. Data were resampled using 1000 bootstrap replicates ($P>0.001$) to determine the confidence limits of tree topology using a heuristic search with a tree bisection-reconnection (TBR) branch swapping algorithm. In this analysis, *Bos taurus* was used as an outgroup (Accession number: NM180996).

RESULTS AND DISCUSSION

The sequences of cDNAs were determined using different amplification products covering the entire GH cDNAs from *Tor tambroides*. Sequence results showed that the GH encoding cDNA of Malaysian mahseer, *T. tambroides* was 1,189 nucleotides long, with a 36 nt untranslated region (UTR) at the 5' terminus and a 520 nt UTR at the 3' terminus. The GH cDNA contains a 633 nt ORF starting at the first ATG codon and ending with a TAG stop codon. The analysis of the predicted amino acids encoded by *T. tambroides* GH CDNA indicated that

the sequence encodes for a protein of 210 aa. The primary structure of *T. tambroides* GH polypeptides deduced from the ORF contains a putative 22 aa signal peptide with a signal peptide cleavage site at Ser₂₃ and 188 aa of the mature peptides (Figure 2). Since the first 22 aa residues from the N-terminus are highly hydrophobic (77%) and have a high degree of homology to the signal peptide of other fish GHs, this 22 aa residues might comprise the signal peptide

The N-terminus aa residue of the mature *T. tambroides* GH is serine. This mature protein start aa can vary among species. For example, the mature GH of bluefin tuna and red seabream start with glutamine (Momoto *et al.*, 1988; Sato *et al.*, 1988). On the other hand, the first aa residue of the mature eel GH is valine (Saito *et al.*, 1988) and other species like chum salmon, coho salmon and rainbow trout GH start with isoleucine (Agellon *et al.*, 1988; Gonzalez-Villasenor *et al.*, 1988; Sekine *et al.*, 1985). The present study has shown that the signal peptide of *T. tambroides* was the same as observed in the GHs of carp and salmonid which are cleaved upon hormone secretion (Koren *et al.*, 1989; Meire *et al.*, 2006). However, this signal peptide was 4-5 amino acids shorter than the mammalian GH genes (26 aa in rat and human; 27 aa in bovine GH), and 3 to 5 residues longer than signal peptides of eel and seabream, respectively. Koren *et al.* (1989) stated that the sequence of the signal peptide is more divergent among species than that of the mature GH polypeptides.

Tor GH shares structural features which are also conserved in carp, chicken and human GH sequences (Figure 3). The four cysteine residues (Cys₇₁, Cys₁₈₃, Cys₂₀₀ and Cys₂₀₈) in this fish are located in similar positions in almost all GH polypeptides including fish, human, bovine and porcine GHs. Cysteine residues which are capable of forming two disulphide bonds, were assumed to contribute to the tertiary structure of the hormone molecule. This is supported by previous studies on human and bovine (Carlacci *et al.*, 1991; Somer *et al.*, 1994). Since these residues are located at nearly identical locations in all GHs, it is expected that these residues play an essential role in structural integrity and in maintaining the biologically active form of GH. Nevertheless, the extra cysteine residue (Cys₁₄₅ or Cys₁₂₃ from the mature polypeptides) in *T. tambroides* and common carp are also found in the GHs of other carps such as in goldfish and milkfish (Chang *et al.*, 1992; De Jesus *et al.*, 2002; Law *et al.*, 1996). The number of unpaired Cys residues affects the proper refolding due to the formation of oligomers (Law *et al.*, 1996). The extra Cys residue in carp was first suspected to be the cause of the low yield of properly refolded recombinant GH (Fine *et al.*, 1993). For instance, it has been found that the extra Cys is not involved in improper refolding of recombinant carp GH because mutating this Cys to Ala did not improve the yield of monomeric GH, suggesting that this extra Cys is located in the hydrophobic core of the hormone (Fine *et al.*, 1993b). Therefore, it is possible that the cysteine residue in will be similar to carp GHs.

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-36                AACTAAGCCTGCAAGAGTTTGTCTACCCTGAGCGAA        -1

1      M  A  R  A  L  V  L  L  S  V  V  L  V  S  L      15
1      ATG GCT AGA GCA TTA GTG CTG TTG TCG GTG GTG CTG GTT AGT TTG      45

                ↓
16     L  V  N  Q  G  R  A  S  D  N  Q  R  L  F  N      30
46     TTG GTG AAC CAG GGG AGA GCA TCT GAT AAC CAG CGG CTC TTC AAT      90

31     N  A  V  I  R  V  Q  H  L  H  Q  L  A  A  K      45
91     AAC GCA GTC ATT CGT GTG CAA CAC CTG CAC CAG CTG GCC GCA AAA      135

46     M  I  N  D  F  E  D  S  L  L  P  E  E  R  R      60
136    ATG ATT AAC GAC TTT GAG GAC AGC CTG TTG CCT GAG GAG CGC AGA      180

61     Q  L  S  K  I  F  P  L  S  F  (C)  N  S  D  Y      75
181    CAG CTG AGT AAA ATC TTC CCA TTG TCT TTC TGC AAT TCT GAC TAC      225

76     I  E  A  P  T  G  K  D  E  T  Q  K  S  S  M      90
226    ATT GAG GCG CCC ACT GGA AAA GAT GAA ACA CAG AAG AGC TCT ATG      270

91     L  K  L  L  R  I  S  F  R  L  I  E  S  W  E      105
271    TTG AAG CTC CTT CGC ATC TCT TTC CGC CTC ATT GAG TCT TGG GAG      315

106    F  P  S  Q  T  L  S  G  T  V  S  N  S  L  T      120
316    TTC CCC AGC CAG ACC CTG AGT GGA ACC GTC TCA AAC AGC CTG ACC      360

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121      V G I P N Q I I E K L A D L K      135
361    GTC GGG ACC CCC AAC CAG ATC ACT GAG AAG CTG GCC GAC TTG AAA      405

136      N G I S V L I K A (C) L D G Q P      150
406    ATG GGC ATC AGT GTG CTC ATC AAG GCA TGT CTC GAT GGT CAA CCA      450

151      N N D D (N D S) L P L P F E D F      165
451    AAC ATG GAT GAT AAC GAT TCC CTG CCA CTG CCT TTT GAG GAC TTC      495

166      Y L I K G E N N L R E S F R L      180
496    TAC TTG ACC ATG GGG GAG AAC AAC CTC AGA GAG AGC TTT CGT CTG      540

181      L A (C) F K K D N N K V E I Y L      195
541    CTG GCT TGC TTT AAG AAG GAC ATG CAC AAG GTC GAA ACC TAC CTG      585

196      R V A N (C) R R S L D S (N (C) I) L      210
586    AGG GTT GCA AAT TGC AGG AGA TCC CTG GAT TCC AAC TGC ACC CTG      630

631    TAG AGGGGGCCGATGTATTGCTAGTCAATGCCGTGTAACACTTGGCTGCAAATCTAAGAC      655
659    CAAGTTC TTA AAA CT TCC TTAAGT AAT TAT GAT CTGGTC TTATG TAT GCA GGAATTGTCA      745
749    ACCAAGCATGGCTAGGCC TGT CTT TTCATT TTC CCT CCT AT ATT TAAACC TTA TCCACCA      805
809    CTA TGT ATT TAT TC TTC TCA TTA GGG AAT GCT CAT AAA TT AAA GCT GTT CCT TAAAAAC      865
869    GTAAGGATGGAT CT GAT TTAACA TTT CAC AGT GGT GCT AAGCAATA TGGCAA TAT ATT T      925
929    TCAAAATG TGG CCAA TTGACA TTA GTC AAA GAG CTAACA AT ATG TACA AT ATT ATT GCT C      985
989    AAAAGGC TAAAGA TCAGT GTT AAG TCGAAC TTG TAA TTT AAAAG GAT GAG TTC ACT CTGG      1045
1049    C TCAAA TCT GT CTC TTT CTC TAT TAAAGT TTT AAA TT GCA TCCA      1105
1109                                     1153

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Figure 2. Nucleotide sequence of the Malaysian mahseer, *Tor tambroides* growth hormone cDNA sequence and the deduced amino acid sequence of the hormone (JF428142). The arrow indicates the possible site for signal peptide cleavage (amino acid 1-22). The mature hormone runs from amino acids 23 to 210, and the cysteine residues are circled. The tryptophan residues are underlined, and potential N-glycosylation sites (amino acids 155-157 and 207-209) are indicated with boxes.

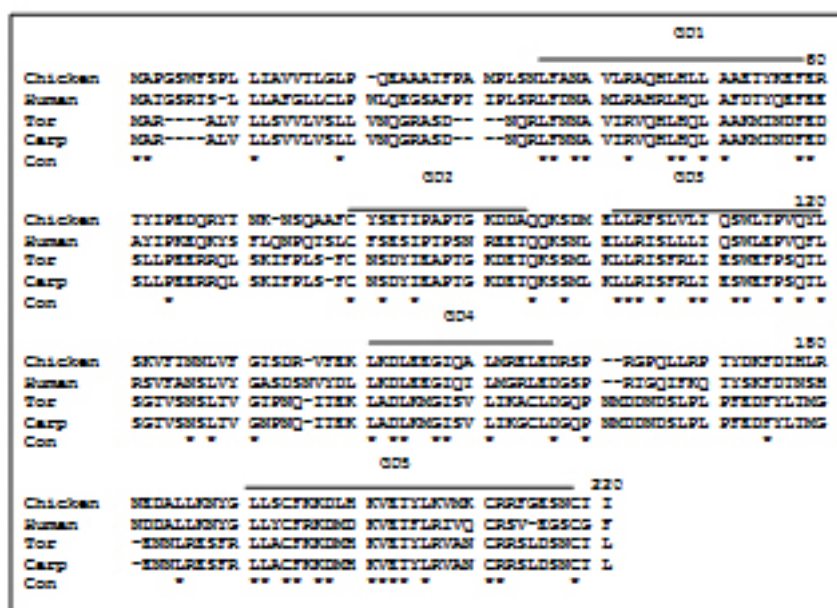


Figure 3. Comparison of deduced amino acid sequences in growth hormone (GH) genes from *Tor*, carp, chicken and human. Dashes indicate gaps which were introduced into the sequences to maximize homologies. Identical amino acids are indicated by asterisks. GD1- GD5 indicate the highly conserved domain of GH as defined by Watahiki *et al.* (1989). Consensus amino acids are shown.

Other than that, there are two Asn-Xaa-Thr/Ser motifs in the *T. tambroides* GH aa sequence (Asn₁₅₅, Asn₂₀₇) which are potential sites for N-linked glycosylation (Lemaire *et al.*, 1994; Marins *et al.*, 2003). Two similar sequences have also been observed in the GH sequences of carp, giant catfish, salmon and tilapia, while only one was found in those of chicken, yellowtail, tuna, flounder, eel and sole (Pendon *et al.*, 1994). However, none was found in mammals. Furthermore, a single tryptophan residue (Trp₁₀₄) was found in *T. tambroides* similar to that observed in carp, chicken and human GH aa sequences. This residue has been reported by Marins *et al.* (2003) in marine silverside fish, *Odentesthes argentinensis* as one of the characteristics of GH. This also has been reported in mullet, *Mugil platanus* and halfbeak, *Hemiramphus brasiliensis* (Meire *et al.*, 2006). Although this residue was not reported in most studies, it can be seen that it is conserved among the teleost GHs. Tryptophan is usually buried in hydrophobic protein cores and may involved in stacking interactions with other aromatic side chains.

In this study, the comparison with other GHs and their homologous domains has shown that the GH cDNA of *T. tambroides* shares the five main conserved domains (GD1, GD2, GD3, GD4 and GD5) located at the same location not only with common carp, human and chicken, but also with the other GHs (Watahiki *et al.*, 1989). These domains, contributing to the GH specific structural and biological features, may play an essential function in the activities of GH. Analysis of domain structure showed

that the GD1, GD2, GD3 and GD4 regions are arranged on the outside, while the GD5 region is restricted to the inside of the molecule. Thus, the GD1, GD2, GD3 and GD4 domains are considered to be involved in the specific binding of the GHs, while the GD5 domain may participate mainly in the formation and stabilization of GH-specific configurations (Watahiki *et al.*, 1989). Other than that, a stretch of 13 residues (CFKKDMHKVETYL) of the C-terminal region (183-195) of the aa sequence of *T. tambroides* is totally conserved with common carp and all known GHs except for several mammalian and high organism species (Figure 3).

It has been shown that in chicken and human GHs there are 1 and 3 different aa sequence, respectively (Saito *et al.*, 1988), and these are the only regions which have strong similarity among the various vertebrate GH sequences. Thus, these conserved regions are thought to be essential for either tertiary folding/hormone or binding/hormone receptor interactions (Venkatesh and Brenner, 1997).

The aa sequence predicted from the *T. tambroides* cDNA sequences were compared to those of GHs from other fish. In this comparison analysis, the deduced amino acid sequence of *T. tambroides* GHs showed four levels of homology with other GH molecules. High level homology, ranging from 99-75%, was found with Cypriniformes (*Cyprinus carpio*, *Carassius auratus gibelio* and *Ctenopharyngodon idella*) and Siluriformes (*Pangasius pangasius*, *Clarias gariepinus*). Salmonid fishes (*Oncorhynchus tshawytscha* and *Oncorhynchus masou*)

showed medium homology: about 63%. Fish species from Perciformes (*Trichogaster leerii* and *Channa striata*), Pleuronectiformes (*Paralichthys olivaceus*) and Scorpaeniformes (*Sebastes schlegelii* and *Cottus kazika*) displayed low homology: about 50%. Meanwhile, very low homology, about 40%, belongs to Acipenciforms (*Acipenser gueldenstaedtii* and *Acipenser sinensis*) and mammals (*Bos Taurus* and *Rattus novegius*). In this analysis, *Tor* GH had the highest similarity with *Cyprinus carpio* GH with which it shared 99% and 98% homology in terms of amino acid and nucleotide sequences, respectively (Table 3).

The phylogenetic analysis divided the studied fish into two major groups (Figure 4 and Figure 5). As discussed above, it can be seen that the fish were separated according to their intron type: while members of Ostariophysi possess only four introns, members of superorders

Protacanthopterygii and Acanthopterygii, which share a common origin, have five introns (Clements *et al.*, 2004; Venkatesh and Brenner, 1997). The Cypriniformes and Siluriformes, which are grouped together in a superorder Ostariophysi, are sister orders to Salmoniformes (*Protacanthopterygii*), Perciformes and Scorpaeniformes (superorder Acanthopterygii), probably derived from a common ancestor (Lemaire, *et al.*, 1994; Venkatesh and Brenner, 1997). Acanthopterygii, Perciformes and Scorpaeniformes have been grouped as a monophyletic lineage by Meire *et al.* (2006). Thus, the result obtained showing that the fish were clustered according to their taxonomic groups and phylogenetic grouping supported the present fish classification (Nelson, 1994).

Table 3. Comparison of nucleotide and amino acid sequences of *Tor tambroides* with other teleost and mammal growth hormones

Homology level	Species	Nucleotide similarity (%)	Amino acid similarity (%)
High	Cypriniformes:		
	<i>Cyprinus carpio</i>	98.0	99.7
	<i>Barbonymus gonionotus</i>	97.0	99.7
	<i>Ctenopharyngodon idella</i>	94.3	95.7
	<i>Ictiobus bubalus</i>	90.3	93.8
	<i>Catla catla</i>	93.3	87.7
Moderate	Siluriformes:		
	<i>Clarias gariepinus</i>	74.2	74.6
	<i>Pangasius pangasius</i>	76.2	75.5
	Salmoniformes:		
	<i>Oncorhynchus masou</i> <i>Oncorhynchus shawytscha</i>	73.2 72.7	63.0 63.3
Low	Perciformes:		
	<i>Trichogaster trichopterus</i>	65.8	56.2
	<i>Trichogaster leerii</i>	65.2	55.9
	Pleuronectiformes:		
	<i>Paralichthys olivaceus</i>	61.3	50.2
	<i>Platichthys stellatus</i>	62.3	51.3
	Scorpaeniformes:		
	<i>Cottus kazika</i>	64.5	55.7
	<i>Sebastes schlegelii</i>	63.8	54.5
	Acipenciformes:		
	<i>Acipenser gueldenstaedtii</i> <i>Acipenser sinensis</i>	62.0 62.4	47.6 48.0
	Mammals:		
	<i>Bos taurus</i>	60.9	44.8
	<i>Rattus novegius</i>	60.2	43.3



Figure 4. The consensus phylogenetic tree of fish growth hormone nucleotide sequences obtained using the Maximum Likelihood (ML) method. The newly cloned *Tor tambroides* growth hormone gene sequence is indicated by an arrow.

In this analysis, Cypriniformes was recovered as a monophyletic group with strong bootstrap support (Jr *et al.*, 2010). This order was divided into several groups according to family: Cobitidae, Catostomidae and Cyprinidae. The phylogenetic grouping of catostomids with cyprinids rather than cobitids in the NJ tree (Figure 5) was similar as several previous tree analyses, using both the NJ method (Clements *et al.*, 2004) and Maximum Parsimony (MP) (Jr *et al.*, 2010). This shows that Cobitidae was paraphyletic with respect to Catostomidae and Cyprinidae. The Cyprinidae was monophyletic except for *Danio rerio*. A previous study showed that Cyprinidae families were always determined as monophyletic with strong nodal support while the subfamily Rasborinae was never documented as a monophyletic and the included species were always determined to be different, unrelated clades or lineages (Myden *et al.*, 2009). The newly cloned *T. tambroides* GH gene sequence is clustered monophyletically with strong bootstrap support. As expected it was very close to the *C. carpio* GH gene sequence as they are located in the same clade and derived from the same family. Hence, this analysis proves that the sequence

obtained was GH gene has high similarity with other teleost GH genes in terms of structure and relationship.

CONCLUSION

In this study, the GH gene of *T. tambroides* has been successfully isolated and cloned. The aa sequence predicted from the cDNA brings new information about the GH structure of a teleost which a Malaysian indigenous species. In Malaysia, the isolation of GH genes is still rare and many studies only focus on fish species from temperate countries. Our data was the first to report on the isolation of the GH gene from a Malaysian indigenous species, a finding which helps to understand the molecular characteristics of the *T. tambroides* GH gene and may lead to the production of a recombinant GH gene protein, and molecular analysis in order to better understand growth determination through GH regulation. This would give an insight into the molecular aspects of the growth performance of a species which is known to have a slow growth rate. The finding is hoped to assist researchers and aquaculturists to improve the growth performance of cultured *T. tambroides* in the future.

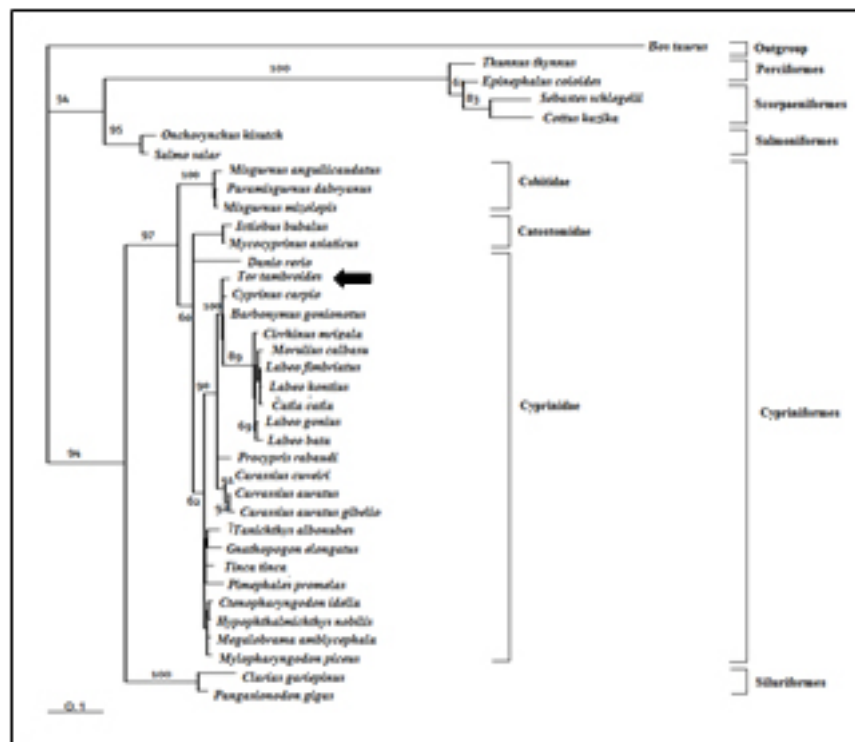


Figure 5. Consensus tree (50% majority-rule) of growth hormone nucleotide sequences using Neighbour-Joining (NJ) method in PAUP. Values at the branch points are the percentage of bootstrap replicates that support the branch out of 1,000 analyses. The newly cloned *Tor tambroides* growth hormone gene sequence is indicated by an arrow. Accession numbers are as follows: *Bos taurus* (NM180996), *Thunnus thynnus* (X06735), *Epinephelus coioides* (EU280321), *Sebastes schlegelii* (U89917), *Cottus kazika* (AB079538), *Oncorhynchus kisutch* (M19999), *Salmo salar* (X14305), *Misgurnus anguillicaudatus* (DQ350433), *Paramisgurnus dabryanus* (DQ350432), *Misgurnus mizolepis* (AF133815.1), *Ictiobus bubalus* (AY375302), *Myxocyprinus asiaticus* (DQ350434.1), *Danio rerio* (NM_001020492), *Cyprinus carpio* (X13670.1), *Barbomyx gonionotus* (FJ265053), *Cirrhinus mrigala* (AF140281.2), *Morulius calbasu* (AY691181.1), *Labeo fimbriatus* (AY691182.1), *Labeo kontius* (AY691184.1), *Catla catla* (AY053361), *Labeo gonius* (AY691183.1), *Labeo bata* (AY691182.1), *Procypris rabaudi* (EU863219), *Carassius cuvieri* (AF389237.1), *Carassius auratus* (AF069398), *Carassius auratus gibelio* (AY265352), *Tanichthys albonubes* (EU367959), *Gnathopogon elongatus* (FJ265030.1), *Tinca tinca* (DQ980027), *Pimephales promelas* (AY643399), *Ctenopharyngodon idella* (AY616661), *Hypophthalmichthys nobilis* (X60473.1), *Megalobrama amblycephala* (AY170124), *Mylopharyngodon piceus* (AF389238.1), *Clarias gariepinus* (EF411172), and *Pangasionodon gigas* (L27835).

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