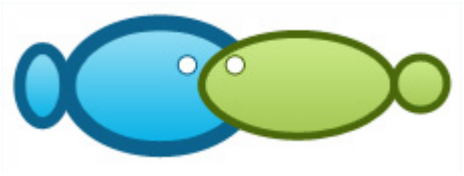

Molecular cloning and sequence analysis of insulin-like growth factors-1 cDNA of giant gourami, *Osphronemus goramy*

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Abstract. Insulin-like growth factor-1 (Igf1) plays important roles in fish growth, development and metabolism. The aims of this study were to isolate, characterize, and obtain a set of real-time PCR primers of giant gourami (*Osphronemus goramy*) Igf1-like gene. We used reverse transcription and rapid amplification of cDNA ends (RACE) to obtain the giant gourami Igf1-like gene sequences. The open reading frame (ORF) of giant gourami Igf1-like gene consisted of four characters, namely: a signal peptide, mature Igf1, E-domain, and stop codon (TAG). Signal peptide was identified in partial sequence and consists of 38 amino acids, whereas the mature Igf1 and E domain have been completely identified. The mature Igf1-like consisted of B, C, A, D, and E domains. Among all the characters of Igf1-like gene, it was found that B and A domains have the highest similarity, i.e. 93.10 to 100% compared to that of other teleost fishes. The two domains were containing six cysteine amino acid residues which are responsible to maintain the tertiary structure of Igf1. The Igf1 of giant gourami, Nile tilapia and carp were clustered in the marine fish Igf1 group, while chicken and mammals Igf1 were clustered in separate group. We have developed three pairs of specific primers that can be used to analyze the expression level of giant gourami Igf1-like gene. These findings will contribute to the understanding of the Igf1 evolution, provide basic information about the molecular basis of Igf1 metabolism, and produce recombinant Igf1 (rIgf1) to improve growth of the giant gourami and the other fishes that have a slow growth rate.

Key Words: molecular cloning, insulin-like growth factor, development, growth rate, cloning.

Introduction. Giant gourami, *Osphronemus goramy* (Lacépède, 1801) is a native freshwater fish species to Indonesia, which can reach a body length of up to 70 cm. This species is also distributed in Malaysia, India, and China (Welcomme 1988; www.fishbase.org/summary/498). Giant gourami has cultivated intensively in Indonesia because of its high market price, reaching approximately two to three times more than other freshwater farmed fish such as common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*), and stripped catfish (*Pangasionodon hypophthalmus*). However, compared to these others species, giant gourami has very slow growth rate. It takes about 1.5 years to reach marketable size and takes about 3 years to get mature. As a result the return on investment is relatively low due to the high cost production (Irmawati et al 2012).

The insulin-like growth factors-1 (Igf1) is a 7.7-kDa single-chain polypeptide of 70 amino acids that is similar in sequence to proinsulin and is greater than ninety-five percent bound to one of six insulin-like growth factor binding protein 1-6 (IGFBPs) (Humbel 1990; Mesotten & Van den Berghe 2006). This hormone is synthesized by hepatocytes in response to growth hormone. The Igf1 and insulin-like growth factor-2 (Igf2) are mitogenic peptides that regulate growth and metabolism of vertebrate. Both Igf1 and Igf2 potently activate cell proliferation and DNA synthesis (Humbel 1990;

Reinecke & Collet 1998; Pozios et al 2001). The Igf1 are members of the evolutionarily ancient insulin-like family of peptides, found throughout the metazoans. Conserved features of the insulin-like peptides include regulation by nutritional status, roles in nutrient metabolism, growth, development, reproduction, improve the immune response, and aging (Carroll 2001; Tatar et al 2003). Igf1 is thought to have arisen from insulin during the transition from chordates to primitive vertebrates (Reinecke & Collet 1998). Igf1 act through the membrane receptor, the type 1 Igf1 receptor, and the activity of Igf1 is modulated by multiple IGFBPs. Igf1 has a dual mode of action as local and as endocrine growth factors. Local Igf1 is produced throughout the body; whereas endocrine Igf1 is mainly produced by the liver.

Studies on the potential use of Igf1 in aquaculture activities have been done by identification and characterizing different types of fish Igf1, developing assays to measure blood or tissue levels of Igf1 peptide or mRNA, measuring changes in Igf1 in blood and tissue expression in response to varying nutritional conditions and season, and assessing control of Igf1 production by growth hormone and other endocrine factors, such thyroid hormone since the 1978's until now. The role of Igf1 in regulating body growth has been proved, e.g. treatment of Coho salmon (*Oncorhynchus kisutch)* with Igf1 stimulates growth (McCormick et al 1992); food restriction of rainbow trout (*Oncorhynchus mykiss*) reduces hepatic production and mRNA level of Igf1 (Duan 1998); and in many fish species, blood level of Igf1 or tissue level of its mRNA positively correlate with dietary ration, dietary protein content, and body growth rate (Perez-Sanchez et al 1995; Duan 1998; Beckman et al 2004).

This paper describes the Igf1 gene sequence derived from cloned of Igf1 cDNA of giant gourami. Igf1 sequence is then used to design specific-primer for quantitative PCR (qPCR) that can be utilized to analysis expression level of Igf1 gene. In addition, it can also be used to produce the Igf1 hormone that can be employed to accelerate growth rate of giant gourami and the others other fishes that have a slow growth rate.

Material and Method

Fish. The fertilized eggs of giant gourami were collected from the farmer in Cikupa Village, Bogor, Indonesia and reared in aquarium for 10 days at Laboratory of Fish Reproduction and Genetics, Bogor Agricultural University (IPB). After the juveniles had been anesthetized with cloves leaf oil, liver tissues were dissected and put immediately in Isogen reagent (Nippon Gene, Tokyo, Japan). These liver samples were stored in -80°C until RNA isolation.

Total RNA isolation and cDNA synthesis. Total RNA isolation and cDNA synthesis were carried out in Laboratory of Fish Reproduction and Genetics, Department of Aquaculture, IPB, Indonesia on September 2011. Total RNA was isolated using an Isogen reagent according to the manufacturer's instructions. Three micrograms of total RNA were used for first strand-cDNA synthesis. Reverse transcription (RT) was performed using Ready-to-Go You Prime First Strand cDNA synthesis beads (GE Healthcare, Piscataway, NJ, USA) with an adapter-oligo (dT) primer (Table 1) according to the manufacturer's instructions. Reactions were incubated at 37°C for 1 hour. After 1 hour, 50 mL sterile distilled water (SDW) was added into the cDNA solution, and then stored at -20°C until it was used for PCR amplification.

Cloning of giant gourami Igf1 cDNA. Cloning, designing of specific primer and sequencing of Igf1 gene were conducted in Laboratory of Physiology, Department of Marine Biosciences, Faculty of Marine Science, Tokyo University of Marine Science and Technology, Tokyo, Japan on November to December 2011. The partial Igf1 cDNA was isolated by degenerate PCR amplification of cDNA derived from liver. Degenerate primers for the Igf1 gene were designed based on highly conserved region of the *Ctenopharyngodon idella* Igf1 (GenBank accession no. EU051323), *Danio aequipinnatus* Igf1 (DQ221105), *Danio rerio* Igf1 (BC114262), *Dicentrarchus labrax* Igf1 (GQ924783), *Onchorhynchus kisutch* Igf1 (M32792), *Oreochromis niloticus* Igf1 (EU272149), *Paralichtys adspersus* Igf1 (EU017533), *Paralichtys olivaceus* Igf1 (AF016922), *Salmo*

salar Igf1 (EF432852.2), and *Sparus aurata* Igf1 (AY996779) using the CLC Sequence Viewer 6 software (CLC bio, QIAGEN).

The first amplification was performed using the following pairs of degenerated primers: Igf1 degene Fw1 and Igf1 degene Rv1, Igf1 degene Fw1 and Igf1 degene Rv2, Igf1 degene Fw2 and Igf1 degene Rv1, and Igf1 degene Fw2 and Igf1 degene Rv2 (Table 1). The PCR was done for 35 cycles; each cycle consisted of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 1 minute.

The PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining. The PCR products of predicted size were purified. Purification of DNA fragments from gels was performed using GENE MATE kit (ISC BioExpress). Purified DNA fragments were then ligated with a pGEM-T Easy vector (Promega, Madison, USA) and the ligated products were designated as pT-Igf1. The pT-Igf1 plasmid was transformed into competent cell of *Escherichia coli* DH5α by heat-shock method. Isolation of pT-Igf1 plasmid was performed by using GEN ELUTE^{TM}HP Plasmid Miniprep (Sigma Life Science) kit. Nucleotide sequencing was done in both forward and reverse direction for every clone using ABI Prism 3100-Avant Genetic Analyzer. Partial sequences of giant gourami *Igf1*-like cDNA were analyzed using CLC Sequence Viewer 6 software (CLC bio, a QIAGEN company), Sequence Multi-Cloning Site of the pGEM-T Easy vector (Promega), and BLASTn from NCBI website in order to obtain nucleotide of giant gourami putative Igf1 gene.

The 3' and 5'-rapid amplification of cDNA ends (RACE) was performed using gene-specific primers which were designed based on partial sequence of the giant gourami Igf1 cDNA derived from seven bacterial clones (Table 1). The 3' and 5'-RACE PCR conditions were done for 35 cycles; each cycle consisted of denaturation at 94°C for 30 seconds, primer annealing of 3'-RACE use gradient temperature at 58°C, 60°C and 62°C for 30 seconds, extension at 72°C for 1 minute, and final extension at 72°C for 1 minute. Gradient PCR was conducted to obtain a suitable annealing temperature for Igf1 cDNA amplification. Furthermore, PCR product of nested 3'-RACE was purified and cloned into pGEM-T easy vector and sequenced according to the procedure as described previously.

Table 1

List of primers used for degenerate and RACE PCR

Step	*Primer name*	*Sequence*
cDNA synthesis	Adapter-oligo (dT)	5'-GTAATACGACTCACTATAGGGCACGCGTGGT-CGACGGCCCGGGCTGGTTTTTTTTTTTTTTTT-3'
Degenerate PCR	Igf1- degene Fw1	5'-TTYCWGKGGCAYKKRTGTGATGTCTT-3'
Degenerate PCR	Igf11- degene Fw2	5'-TTYCWGKGGCAYKKRTGTGATGTCTT-3'
Degenerate PCR	Igf11- degene Rv1	5'-TGTGCGACGTCAAACASACACCTCT-3'
Degenerate PCR	Igf11- degene Rv2	5'-TGTGTCTRTAVKGBTCYYGTSGRTTCT-3'
3'-RACE and RT PCR	3'-RACEIgf1GSP1	5'-TGCGACGCCTGGAGATGTACTGT-3'
3'-RACE	3'-RACE Igf1GSP2	5'-AAGCCTAGCAAGCCAGCTCGCTCTGT-3'
3'-RACE	Adaptor 1	5'-CTATAGGGCACGCGTGGT-3'
3'-RACE	Adaptor 2	5'-TAATACGACTCACTATAGGGC-3'
5'-RACE	Igf1-5Fw1	5'-GGGCATTGGTGTGATGTCTT-3'
5'-RACE	Igf1-5Fw2	5'-ATCTTCTGTAGCCACACCCTCT-3'
5'-RACE	Igf1-5Rv	5'-ACAAACTGCAGCGTGTCG-3'

Sequencing, data analysis and designing of Igf1 real-time PCR primers. Nucleotide sequence of open reading frame (ORF) was predicted by aligning of nine partial clones sequences using CLC Sequence Viewer 6 software (CLC bio, QIAGEN). Furthermore, ORF of Igf1-like cDNA was characterized using BLASTx available for the NCBI internet website (http://www.ncbi.nlm.nih.gov), GENETYX version 7, and by manual based on the typology of the Igf1 gene: a signal peptide, mature Igf1, E-domain, and the stop codon. Specific primers for giant gourami Igf1-like gene were designed using mfold software on Albani's website. Primer specificity was tested by PCR. Primers which produced a single band indicated that they are specific and can be used for further analysis.

Results and Discussion. We amplified a 1408 bp cDNA with an open reading frame (ORF) composed of 462 bp and 153 amino acids (aa). The amino acid sequence has covered all the characteristic features of Igf1, namely a signal peptide, B-C-A-D-E domains and E-domain. Moreover, six cysteine amino acid residues were found at B- and A-domain. Thus, it is most likely that the isolated sequence was giant gourami Igf1-like gene. The signal peptide was still a partial sequence that consisted of 38 aa, whereas the mature Igf1 and E-domain have been identified completely. It is assumed that there are about six amino acids sequence in the signal peptide has not been identified (Figure 1).

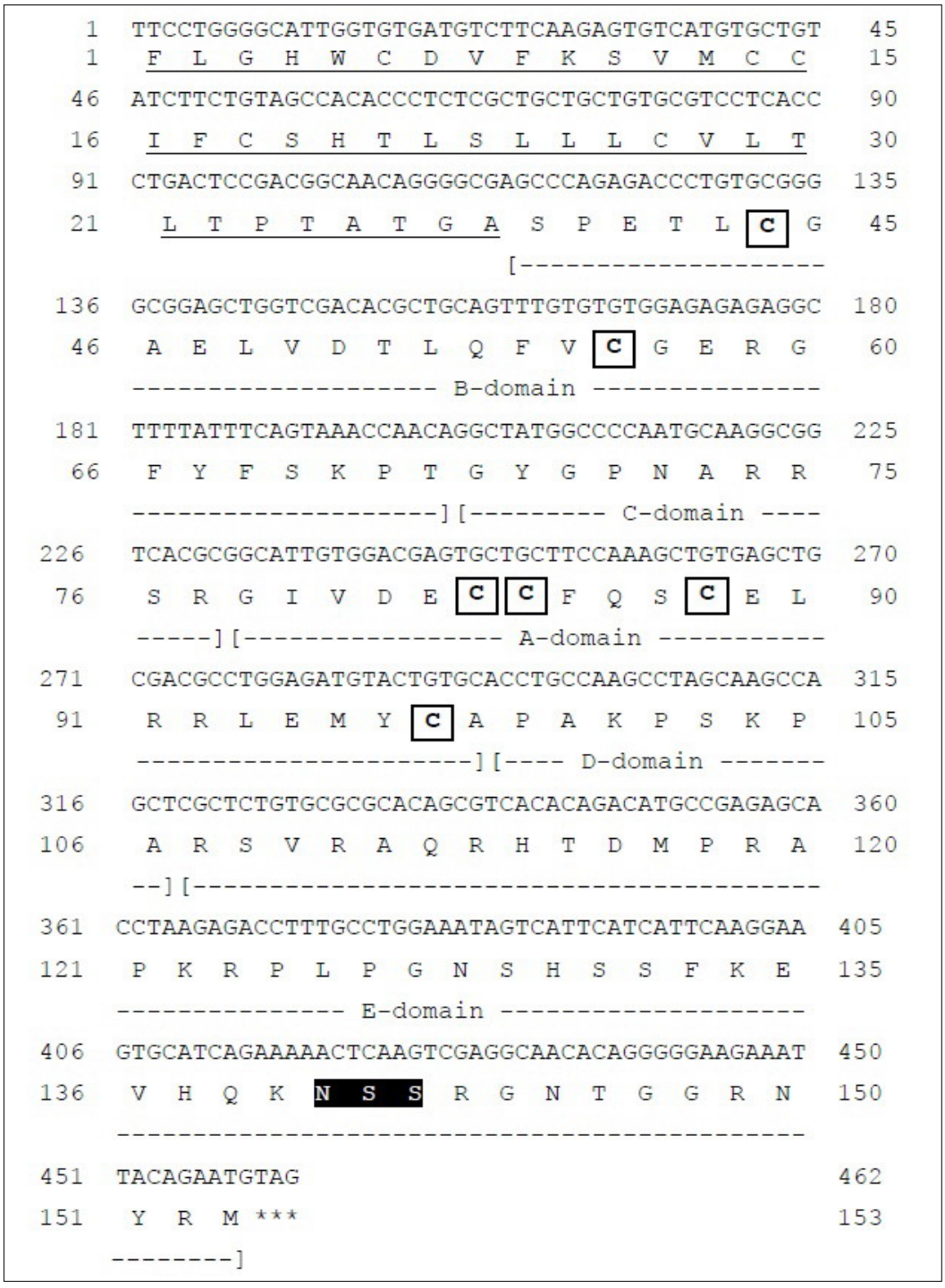

Figure 1. Partial sequences of nucleotide and amino acid residues (aa) giant gourami (*Osphronemus goramy*) insulin-like growth factor-1-like gene (Igf1-like gene). Igf1-like gene of giant gourami was consisted of 38 aa signal peptide (underlined), 68 aa mature Igf1, and 47 aa E-domain. Stop codon (TAG) is indicated by asterisks (***). Six cysteine residues are shown by letters in the box. N-glycosylation is shaded black.

The nucleotide sequences were identified and supposed as the giant gourami insulin-like growth factor-1-like gene (Igf1-like gene) has a high similarity with the amino acid residues of Igf1 tilapia and marine fishes, such as grouper (*Epinephelus coioides* and *Epinephelus lanceolatus*). The amino acids sequence of giant gourami Igf1-like gene covered 99.00% of *E. coioides* Igf1 (GenBank accession no. ADM15564.1), *E. lanceolatus* Igf1b (GenBank accession no. ABZ10841.1), and *O. niloticus* Igf1 (GenBank accession no. ABY88873.1), whereas only 95.00% covered *C. carpio* Igf1 (GenBank accession no. ABQ08938.1) and only 90.85 to 98.50% covered mammals Igf1. The amino acid residues in B and A domains were highly conserved in fishes, chicken, and mammals. The B and A domains have the highest similarity, i.e 76.19-100%, followed by the C domain (41.67-100%), D domain (37.5-75.0%), and than the E domain (34.15-100%). The high similarity of amino acid sequences in B and A domains on different species related to the functional roles of these regions in the binding of Igf1 with its receptor and Igf-binding proteins (IGFBPs) (Duval et al 2002). In human, IGFBPs act as carriers for Igf1 in the circulation, regulate the bioavailability of Igf1 to specific tissues and modulates the biological activities of Igf proteins (Yu & Rohan 2000). Although the similarity level of amino acid sequences in the E domain was low, but in giant gourami and *E. lanceolatus* 1a have a high similarity level (100%). E domain of giant gourami composed of 47 aa, the same as found in giant grouper Igf1a (Table 2 and Figure 2).

Table 2

Homology of amino acid sequences giant gourami insulin-like growth factor-1-like gene (Igf1-like gene) with Igf1 gene sequences of other vertebrates

	Query cover (%)	*Homology of CDS Igf1 (%)*	*Homology of mature IGF-1 (%)*				*Homology of E-Domain (%)*
			B	*C*	*A*	*D*	
Epinephelus coioides	99.00	94.00	100.00	100.00	100.00	75.0	62.16
E. lanceolatus 1a	99.00	-	100.00	100.00	100.00	75.0	100.00
E. lanceolatus 1b	99.00	94.00	100.00	100.00	100.00	75.0	62.16
Oreochromis niloticus	99.00	91.00	93.10	87.50	95.24	37.5	58.57
Cyprinus carpio	95.00	80.00	93.10	66.67	100.00	50.0	87.23
Gallus gallus	90.85	50.98	89.66	41.67	76.19	50.0	37.14
Bos Taurus	98.50	59.40	86.21	41.67	90.48	62.5	40.00
Mus musculus	98.50	58.65	86.21	41.67	90.48	62.5	34.15
Homo sapiens	90.85	54.90	86.21	41.67	90.48	62.5	40.00

CDS: coding sequence.

Igf1 is synthesized in the liver and then released from the liver by stimulation of growth hormone (GH). Although the liver is the primary organ where they produce Igf1, but some organs such as the pituitary, brain, ovary, spleen, and muscle, are also known to synthesize Igf1 (Dong et al 2010). The Igf1 prepropeptide has signal peptide, B-C-A-D domains and E-domain. The signal peptide and E-domain are removed by proteolytic processing of the propeptide to yield mature Igf1 which is released into the blood. The B, C, A, and D domains are a polypeptide functional linear.

The mature of giant gourami Igf1-like gene encoded 68 aa including a B domain encoded 29 aa, a C domain encoded 10 aa, an A domain encoded 21 aa, and D domain encoded 8 aa, meanwhile the E domain Igf1-like gene encoded 47 aa. The result of this study that B and A domains amino acid sequences of the teleost fishes are the most highly conserved (93.10-100.00% in similarity). Meanwhile, Dong et al (2010) reported 89 to 100% similarity in grouper with other fishes, chicken, and mamalia. Six cysteine amino acid residues were highly conserved in mature Igf1 of giant gourami, which are two in the B domain (Cys^{B6}, Cys^{B18}) and four in A domain (Cys^{A6}, Cys^{A7}, Cys^{A11}, and Cys^{A20}). In this study, position of two cysteine residues Igf1 of giant gourami in the B domain are the same as in the orange-spotted grouper (Pedroso et al 2006), but position of the other cysteine residues in the A-domain are different. The position of four cysteine residues in the orange-spotted grouper are in the Cys^{A7}, Cys^{A8}, Cys^{A12}, and Cys^{A21} whereas in this study are in the Cys^{A6}, Cys^{A7}, Cys^{A11},and Cys^{A20}. The six cysteine residues were involved in the formation of the tertiary structure of Igf (Duval et al 2002).

Futhermore, the six cysteine amino acid residues formed three disulfide bonds in mature Igf1 processing; two are between B and A domains (Cys^{B6} and Cys^{A7}; Cys^{B18} and Cys^{A20}) and one is in A domain (Cys^{A6} and Cys^{A11}) (Moriyama et al 2000). In this study, when B and A domain were compared to Igf1 of more advanced vertebrate, such as chicken, cattle, rat, and human, such domains also showed a high degree of similarity. Reinecke & Collet (1998) reported that Igf1 structure, regulation, and function appear similar in fish and mammals.

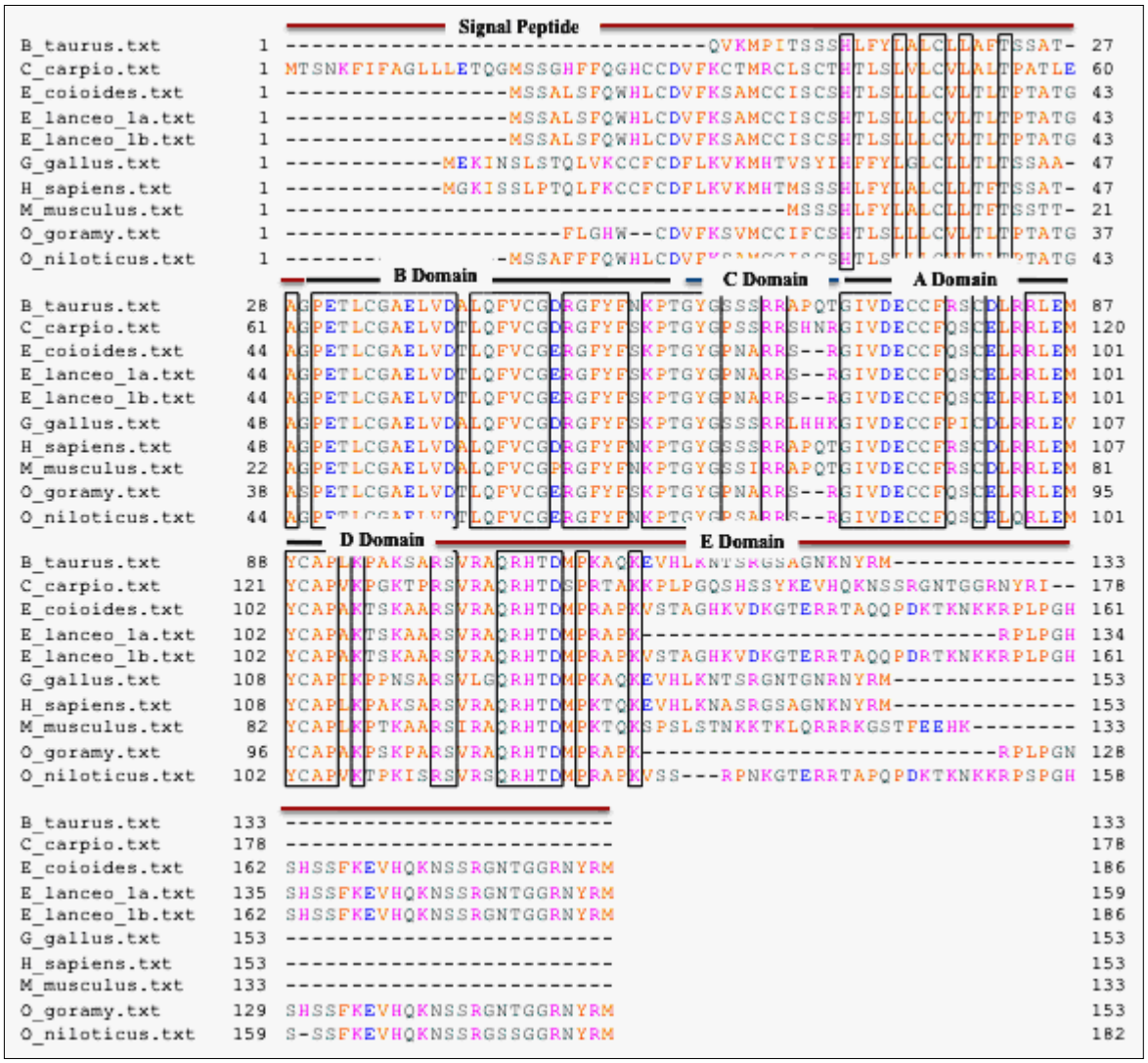

Figure 2. Alignment of amino acid sequences of giant gourami insulin-like growth factor-1-like gene (Igf1-like gene) with grouper, carp, and other vertebrate Igf1 genes. Identical residues are boxed. Motifs highly conserved among Igf1 from a wide range of organisms, the B-domain, C-domain, A-domain, and D-domain.

The similarity of amino acid sequences in C-domain of giant gourami with other teleost fishes are about 6.67 to 100%, E-domain are 62.16 to 100.00%, and then D-domain are 37.50 to 75.00%. Variety in the E-domains of Igf1 is caused by the process of alternative splicing. Salmon and trout were shown to express alternatively splicing of Igf1 mRNA transcripts coding at least four kinds of Igf1 prohormones, which encoded an identical mature Igf1 protein (Duguay et al 1994). Those transcripts are designated as Ea-1, Ea-2, Ea-3, and Ea-4 in trout (Chen et al 1994), and the predicted numbers of amino acid residues in each domain are 35, 47, 62, and 74, respectively. The E-domains of Igf1a and Igf1b of giant grouper contained 47 aa and 74 aa, respectively (Dong et al 2010). In this study, the E-domain contains 47 aa, which may be designated as Ea-2. The E-domain of giant gourami Igf1-like gene includes the N-glycosilation site at 140-143 of amino acid sequence. Ayson et al (2002) and Duguay et al (1996) reported that E-

domain may be important for intracellular transport, processing or correct folding. Tian et al (1999) demonstrates the mitogenic activity of rainbow trout proIgf1 E-peptide. In fishes, variations in the amount of peptide E-domain are supposed relate to the life of Igf1 in the blood circulation system.

Phylogenetic analysis of some vertebrates Igf1 showed that the giant gourami Igf1-like gene is more closely related with the marine fish Igf1 group compared to freshwater fishes, such as tilapia and carp Igf1 gene, whereas mammals Igf1 are clustered in separate groups (Figure 3).

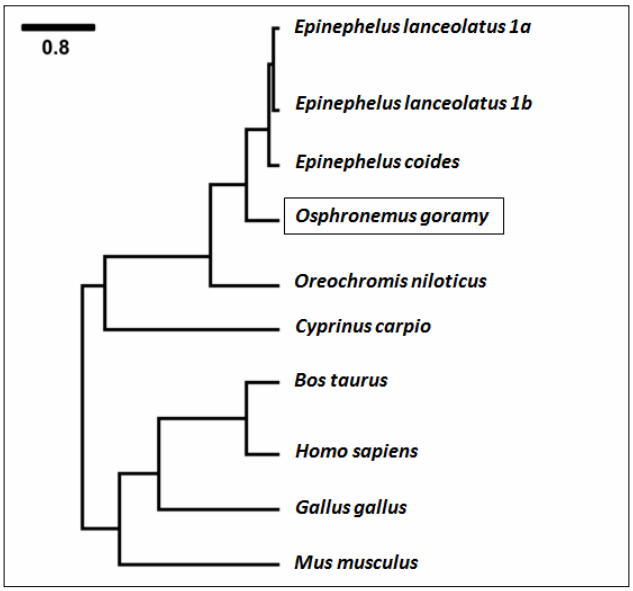

Figure 3. Phylogenetic relationship between amino acid sequences of giant gourami (*Osphronemus goramy*) insulin-like growth factor-1-like gene (igf1-like gene) and other vertebrate Igf1 genes. The GenBank accesion number of aligned amino acid sequences of Igf1 are as follows: *Cyprinus carpio* (ABQ08938.1), *Mus musculus* (AAH12409.1), *Gallus gallus* (AET50992.1), *Homo sapiens* (AAO74829.1), *Bos taurus* (AAF72409.1), *Epinephelus coioides* (ADM15564.1), *Epinephelus lanceolatus* 1a (ADM15565.1), *Epinephelus lanceolatus* 1b (ABZ10841.1), *Oreochromis niloticus* (ABY88872.1). Phylogenetyc analysis was performed by the UPGMA using the GENETYX Ver. 07 software.

We have developed three pairs of specific primers for real-time PCR that could be used to determine the expression level of giant gourami Igf1 gene, namely: ogIgf1Fw and ogIgf1Rv1, ogIgf1Fw and ogIgf1Rv2, and ogIgf1Fw and ogIgf1Rv3. The amplicon sizes of these primers were 205, 367, and 184 bp, respectively. Furthermore, no dimers were shown in PCR amplification products (Figure 4). For internal control, we have developed β-actin primers, namely ogβactinFw and ogβactinRv (Table 3). A good primer for qPCR is primer that have high sensitivity, single-amplicon specificity, high degrees of accuracy and reliability, wide linier dinamic range, and reproducibility. Futhermore, based on thermodynamic and sequence criteria, a good real-time PCR primers should have 50-210 amplicon length, 19-23 bp primer length, 35-65% GC content, and 60-68^{o}C melting time (Tm) (Quellhorst & Rulli 2008). In this study, all primer pairs have GC content according to the criteria, and only primer pair ogIgf1Fw and ogIgf1Rv2 have 367 bp amplicon size. However, all of the primers have high specificity which they were indicated by a single band of gel electrophoretic analyses (Figure 4 and Figure 5).

Table 3

Giant gourami primers that could be used for real-time PCR

Primers name	*Sequence*	*Amplicon size (bp)*	*GC content (%)*
OgIgf1Fw	5'-TTCAAGAGTGTCATGTGCTGTA-3'	205	40.91
Og Igf1Rv1	5'-GTTTCTTGGAATGTCTGTGTGAC-3'	205	43.48
Og Igf1Fw	5'-TTCAAGAGTGTCATGTGCTGTA-3'	367	40.91
Og Igf1Rv2	5'-CATAGCCTGTTGGTTTACTGAA-3'	367	40.91
Og Igf1Fw	5'-TTCAAGAGTGTCATGTGCTGTA-3'	184	40.91
Og Igf1Rv3	5'-AATTAAAGCCTCTCTCTCCACAC-3'	184	43.48
OgβactinFw	5'-AACCATGGATGATGAAATCGCCGCA-3'	126	48.00
OgβactinRv	5'-TGATGCCTGGGGCGACCGACGATGG-3'	126	68.00

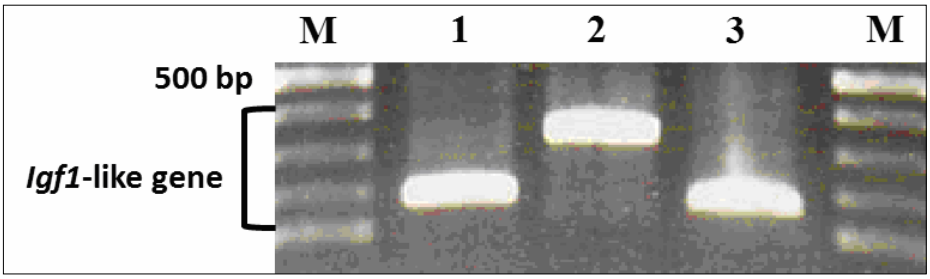

Figure 4. Real-time PCR product of giant gourami (*Osphronemus goramy*) insulin-like growth factor-1-like gene (Igf1-like gene) confirmed the primers specificity. Lane M was the molecular weight marker. Lane 1 was PCR product both of OgIgf1Fw and OgIgf1Rv1. Lane 2 was PCR product both of OgIgf1Fw and OgIgf1Rv2. Lane 3 was PCR product both of OgIgf1Fw and OgIgf1Rv3.

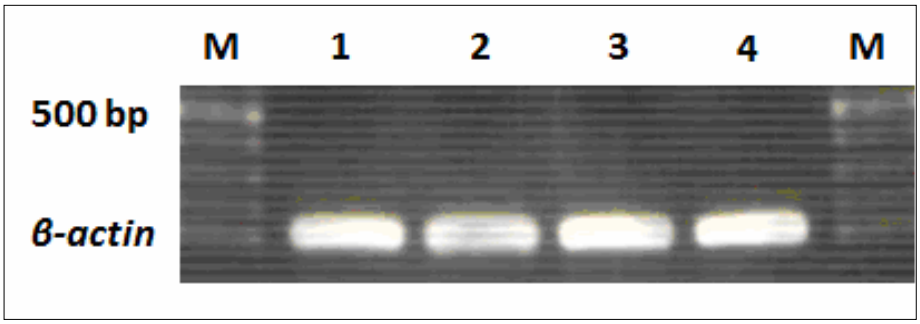

Figure 5. Real-time PCR product of giant gourami (*Osphronemus goramy*) β-actin-like gene confirmed the primers specificity. Lane M was the molecular weight marker. Lanes 1 to 4 were PCR product both of OgβactinFw and OgβactinRv.

Conclusions. The Igf1-like gene identified in this study is a giant gourami Igf1 homolog. The amino acid sequence of giant gourami-like protein contained motifs that are typically of Igf1 family, namely B, C, A, and D-domain and six conserved cysteine residues in mature peptide, E-domain; and N-glycosilation. The amino acid sequence of the giant gourami showed high similarity with giant grouper, *Epinephelus lanceolatus* Igf1a. These data could be contribute to understanding of the evolution of the Igf1 gene, understanding the expression level of Igf1 gene in king fish and provide the molecular base for improving of giant gourami growth.

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References

Ayson F. G., De Jesus E. G., Moriyama S., Hyodo S., Funkenstein B., Gertler A., Kawauchi H., 2002 Differential expression of insulin-like growth factor I and II mRNAs during embryogenesis and early larval development in rabbitfish (*Siganus guttatus*). General and Comparative Endocrinology 126:165-174.

Beckman B. R., Shimizu M., Gadberry B. A., Cooper K. A., 2004 Response of the somatotropic axis of juvenile coho salmon to alterations in plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41kDa IGFBP. General and Comparative Endocrinology 135:334-344.

Carroll P. V., 2001 Treatment with growth hormone and insulin-like growth factor-I in critical illness. Best Practice and Research. Clinical Endocrinology and Metabolism 15:435-451.

Chen T. T., Marsh A., Shamblott M., Chan K. M., Tang Y. L., Cheng C. M., Yang B. Y., 1994 6 structure and evolution of fish growth hormone and insulin-like growth factor genes. In: Fish physiology. Sherwood N., Hew C. (eds), Academic Press, San Diego, vol. 13, chapter 6, pp. 179-209.

Dong H., Zeng L., Duan D., Zhang H., Wang Y., Li W., Lin H., 2010 Growth hormone and two forms of insulin-like growth factors I in the giant grouper (*Epinephelus lanceolatus*): molecular cloning and characterization of tissue distribution. Fish Physiology and Biochemistry 36:201-212.

Duan C., 1998 Nutritional and developmental regulation of insulin-like growth factors in fish. Journal of Nutrition 128:306S-314S.

Duguay S. J., Swanson P., Dickhoff W. W., 1994 Differential expression and hormonal regulation of alternatively spliced IGF-I mRNA transcripts in salmon. Journal of Molecular Endocrinology 12:25-37.

Duguay S. J., Lai-Zhang J., Steiner D. F., Funkenstein B., Chan S. J., 1996 Developmental and tissue-regulated expression of IGF-I and IGF-II mRNAs in *Sparus aurata*. Journal of Molecular Endocrinology 16:123-132.

Duval H., Rousseau K., Elies G., Le Bail P. Y., Dufour S., Boeuf G., Boujard D., 2002 Cloning, characterization, and comparative activity of turbot IGF-I and IGF-II. General and Comparative Endocrinology 126:269-278.

Humbel R. E., 1990 Insulin-like growth factors I and II. European Journal of Biochemistry 190:445-462.

Irmawati, Alimuddin, Zairin M., Suprayudi M. A., Wahyudi A. T., 2012 [Growth enhancement of *Osphronemus goramy* Lac. juvenile immersed in water containing recombinant *Cyprinus carpio* growth hormone]. Indonesian Journal of Ichtiology 12(1):13-23 [in Indonesian].

Mccormick S. D., Kelley K. M., Young G., Nishioka R. S., Bern H. A., 1992 Stimulation of coho salmon growth by insulin-like growth factor I. General and Comparative Endocrinology 86:398-406.

Mesotten D., Van den Berghe G., 2006 Changes within the growth hormone/insulin-like growth factor I/IGF binding protein axis during critical illness. Endocrinology and Metabolism Clinics of North America 35:793-805.

Moriyama S., Ayson F. G., Kawauchi H., 2000 Growth regulation by insulin-like growth factor-I in fish. Bioscience, Biotechnology and Biochemistry 64:1553-1562.

Pedroso F. L., De Jesus-Ayson E. G., Cortado H. H., Hyodo S., Ayson F. G., 2006 Changes in mRNA expression of grouper (*Epinephelus coioides*) growth hormone and insulin-like growth factor I in response to nutritional status. General and Comparative Endocrinology 145:237-246.

Perez-Sanchez J., Marti-Palanca H., Kaushik S. J., 1995 Ration size and protein intake affect circulating growth hormone concentration, hepatic growth hormone binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead sea bream (*Sparus aurata*). Journal of Nutrition 125:546-552.

Pozios K. C., Ding J., Degger B., Upton Z., Duan C., 2001 IGFs stimulate zebrafish cell proliferation by activating MAP kinase and PI3-kinase-signaling pathways. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology 280:R1230-R1239.
Quellhorst G., Rulli S., 2008 A systematic guideline for developing the best real-time PCR primers. Technical Article, SABiosciences Corporation, USA, 8 pp.
Reinecke M., Collet C., 1998 The phylogeny of the insulin-like growth factors. International Review of Cytology 183:1-94.
Tatar M., Bartke A., Antebi A., 2003 The endocrine regulation of aging by insulin-like signals. Science 299:1346-1351.
Tian X., Chen M. J., Pantschenko A. G., Yang T. J., Chen T. T., 1999 Recombinant E-peptides of pro-IGF-I have mitogenic activity. Endocrinology 140:3387-3390.
Welcomme R. L., 1988 International introductions of inland aquatic species. Food and Aqriculture Organization of the United Nations, Fisheries Technical Paper Rome, Italy, 294 pp.
Yu H., Rohan T., 2000 Role of the insulin-like growth factor family in cancer development and progression. Journal of the National Cancer Institute 92:1472-1489.
*** http://www.ncbi.nlm.nih.gov.
*** FishBase [*Osphronemus goramy* Lacepède, 1801 Giant gourami]. Available at: www.fishbase.org/summary/498. Accessed: November, 2015.

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