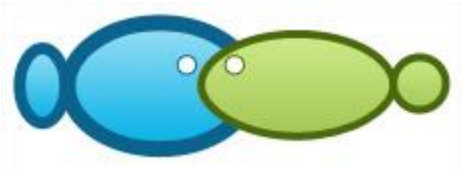

Polymorphism in exon 4 of snakehead fish (*Channa striata*) growth hormone gene from Sumatra (Indonesia) and its association with growth traits

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Abstract. Growth hormone (GH) gene can be used for selection in some aquaculture species for the best phenotypic performance during farming. This research investigated the polymorphism of the GH gene and its association with growth traits of snakehead fish. 253 snakehead fish were collected from 9 locations in Sumatra (Indonesia), i.e. rivers, lake, and floodplains. 27 were used for DNA isolation. The morphology data set included body weight (W), standard length (SL), body height (BH), length of the dorsal fin (pinna dorsalis - PD), length of the anal fin (pinna analis - PA), head length (HL), head width (HW), SL/BH, SL/PD, SL/AD, SL/HL, and SL/HW for all specimens. Exon 4 of the GH gene was amplified with PCR and sequenced to determine the possible occurrence of polymorphisms on the exon from each specimen. This exon was amplified at approximately 116-128 bp in size. Moreover, the gene alignment showed the polymorphism existence in the form of insertion, deletion (indel) and Single Nucleotide Polymorphism (SNP). Insertion-deletion were recorded in almost all specimens. However, only a few numbers of specimens showed SNP occurrences. Growth traits (SL, W, PD, PA, HL, and HW) of snakehead fish were high in Kampar River (SK), Lubuk Lampam River (LL), and Merang River, and significantly different ($p<0.05$). The polymorphism affected the SL, W and HW, with a coefficient correlation between 0.4-0.421. There was no change in the amino acids of SNP, while changes occurred at the indel. Polymorphisms significantly affected ($p<0.05$) SL (25.22±3.14) and HW (3.42±0.48). The high polymorphism scores (between 0-3) were observed in SK and SM. Exon 4 GH has a potential to be utilized as marker of growth traits for snakehead by augmenting the specimen and using other regions of the GH gene for further study.
Key Words: inland waters, insert-deletion, single nucleotide polymorphism.

Introduction. *Channa striata* is one of the favorite inland water fish for consumption with high economic value in Southeast Asia (Song et al 2013), especially Indonesia. In Indonesia, the catch of snakehead is high, 34017 tons per year in 2010 (Ministry of Maritime and Fisheries 2011). The species is also utilized by the medical field to help healing post-operation lesions (Khasani & Astuti 2019). The capturing effort of snakehead has not been followed by gene selection based-cultivation effort yet. Therefore, studies on genetic selection of snakehead for the benefit of aquaculture are required.

Aquaculture is generally performed with the selection of an individual or group of fish based on size in order to have fish with optimum growth (Dunham 2011). The growth of vertebrates is influenced by various expressed genes, exhibiting diverse morphological characteristics in similar species and populations (Canestro et al 2013). The screening of target genes for cultivation and selection is related to the phenotypic performance. Aquaculture industries need to select fish with rapid growth, as in other livestock productions. Nowadays, the marker gene of growth is implemented in aquaculture, but also in cattle, chicken and goat farming, as the indicator or marker for growth performance (Biswas et al 2003; Pal et al 2004; De Faria et al 2006).

The performance of the somatic axis as a growth regulator of fish is affected by several genes, such as GHRH (growth hormone-releasing hormone), GHIH (growth hormone-inhibitor hormone), GH (growth hormone), GHR (growth hormone receptor), myostatin, and IGF (insulin-like growth factor) (De-Santis & Jerry 2007; Gencheva & Stoyanova 2018), such as the IGF-1 gene in *Osphronemus goramy* (Irmawati et al 2016).

Gene-based selection uses common methods of Single Nucleotide Polymorphysms (SNP), Marker Assisted Selection (MAS), and Quantitative Trait Loci (QTL). These methods have been implemented in fish studies, such as MAS selection to increase the fish growth up to 20-50% (Thanh et al 2010), SNP selection on *Clupea harengus* based on different environment (Limborg et al 2012) and QTL for detecting the growth of *Cyprinus carpio* (Wang et al 2018). Such selection methods impact profit in aquaculture.

The growth hormone gene has a role as a somatic regulator for the growth of the skeleton and protein synthesis. It also affects the ribosomal translation and inhibits glucose transportation (Ni et al 2012). The gene analysis is also correlated with phenotypic indicators like the increment of muscle, length, weight, heat tolerance, ovulation, disease resistance, and muscle quality (De-Santis & Jerry 2007).

Polymorphism is related to growth traits because it has a coding region affecting protein synthesis and individual growth (Thanh et al 2010). A previous research proved that SNP was found in some regions (intron and exon) of the GH gene, i.e. in the cultivation of *Oreochromis niloticus* using microsatellite markers (Dias et al 2019), intron and exon of GH gene for *Tinca tinca* (Kocour & Kohlmann 2011), intron of yellow catfish (*Pelteobagrus fulvidraco*) (Li et al 2017), intron and exon of *Cyprinis carpio* (Liu et al 2017). Based on the gene bank database, the GH gene of snakehead (*Channa striata*) with accession number EF447030.1 and 2089 bp in size, consisting of 5 introns and 6 exons is identical with *Channa punctata* (GQ214243.1) and *Channa gachua* (GQ214244.1). The polymorphism of the 3 fish species is located on exon 4, used as target gene.

Information about the polymorphism of the GH gene of snakehead (*C. striata*) and its impact on growth traits is still limited. This research was carried out to investigate the polymorphism of the GH gene on the exon 4 region of snakehead and its association with growth traits.

Material and Method

Collection of specimens. 253 snakeheads were collected from 9 inland water locations in Sumatra, Indonesia i.e. Kampar River (SK), Merang River (SM), Batanghari Sembilan River (SBS), Lubuk Lampam Floodplain (RbL), Siak Floodplain (RbS), Kumpeh Floodplain (RbK), Singkarak Lake (DS), Ranau Lake (DR), and Cala Lake (DC) as presented in Figure 1, and 27 fishes were selected for DNA isolation. The fish were collected from local fishermen and kept in a freezer at -18^{o}C for 5 min prior to the measurement of weight and morphometric characteristics. The measurements were conducted using a digital balance (OHAUS NVT 1601/3, OHAUS Instruments Co., Ltd., Shanghai, China) and a digital caliper.

The 12 morphometric characteristics determined were: body weight (W), standard length (SL), body height (BH), length of the dorsal fin (pinna dorsalis - PD), length of the anal fin (pinna analis - PA), head length (HL), head width (HW), SL/BH, SL/PD, SL/AD, SL/HL, and SL/HW. Muscle tissue was collected from 27 fish for DNA isolation using 70% ethanol. The experiment was carried out from October 2018 to March 2019, and DNA analysis was performed in the Laboratory of Fish Pest and Disease, Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Indonesia.

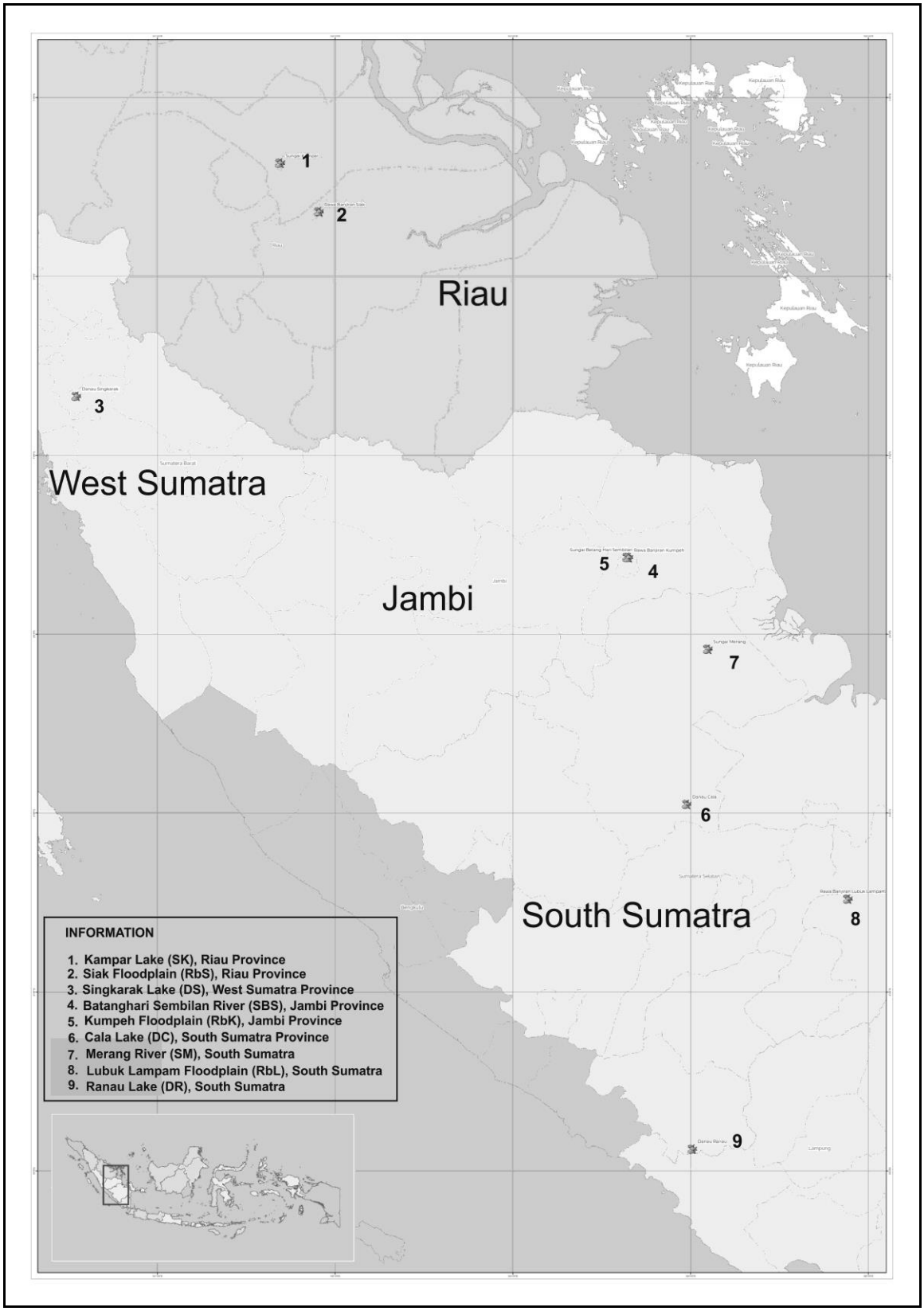

Figure 1. Location of specimen sampling in 4 provinces of Sumatra Island, Indonesia.

DNA isolation. DNA extraction was performed by the instructions of the manufacturer for sample preparation, lysis, binding, washing and elution with the Genomic DNA mini kit GB100 (Geneaid Biotech Ltd., New Taipei City, Taiwan). GH gene was expressed in muscle tissue and correlated to growth traits (Tanamati et al 2015). 10 mg of muscle

tissue of snakehead were weighed (Digital Balance, Simadzu series BX 320D, Shimadzu Corp., Kyoto, Japan), homogenized with 200 µL of Phosphate Buffer Saline (PBS) in a 1.5 mL microtube and centrifuged using Sorvall Legend Micro 17 microcentrifuge (ThermoFisher Scientific, Waltham, MA USA) at 300 rpm for 5 min. The supernatant was discarded, 800 µL of sterile distilled water was added, the mix was homogenized using a vortex (OHAUS fixed speed mini vortex mixer, OHAUS Corp., Parsippany, USA) for 20 seconds and centrifuged at 13000 rpm for 10 min. DNA extraction was continued with the addition of 250 µL TNES (10 mM of Tris-HCl pH 8, 125 mM of NaCl, 10 mM of EDTA, pH 8, and 0.5% of SDS), 50 µL of Sodium Dodecyl Sulphate (SDS) buffer, and 3 µL of proteinase K. The mixture was incubated using a hot air model oven ND0-451SD series 10422116 (Tokyo Rikakikai Co., Ltd., Japan) at 41°C for 14 h.

Polymerase Chain Reaction (PCR) and DNA sequencing. Extracted products were next amplified using T100 Thermal Cycler (Bio-Rad Laboratories Inc., Foster City California, USA). 12.5 µL of PCR mix included 6 µL of BIO-25047 MyTaq HS Red Mix (Bioline, London, UK.), 5 µL of Nuclease Free Water, 0.5 µL of each primer, and 0.5 µL of genomic DNA. The primer set for exon 4 of GH gene was: forward (F 5'CGACTGATTGACTCCTGGGA'3) and reverse (R 5'TGATCAGCAGCAGGATTCCC'3). The target gene referred to the NCBI database (https://www.ncbi.nlm.nih.gov/) with accession no. EF447030.1 and 1419-1562 bp in size.

PCR amplification started with initial denaturation at 94ºC for 3 min, continuing with 30 cycles of denaturation at 94ºC for 3 seconds, annealing at 55ºC for 30 seconds, and extension at 72ºC for 90 seconds. The process was finalized with a final extension at 72ºC for 8 min. The size of PCR products was confirmed with 1% agarose gel (Nacalai Tesque, Inc. Kyoto, Japan) and electrophoresed in 1× Tris-borate (TBE) buffer homogenized with a portable electric stove (CRC, Chicago USA) for 3 min. Afterwards, 0.75 µL of DNA staining (1st BASE BIO-5170 fluorosafe) was added into agarose, poured into the gel formation tray and left to solidify. 2 µL of DNA ladder (Geneaid DL007) and 3 µL of PCR product were inserted into the gel and then run using Mupid-eXu (Eurogentec company, Seraing, Belgium) at 100V for 20 min. The bands of DNA were visualized under an UV transilluminator (Vilber lourmat, Eberhardzell-Germany) at a wavelength of 254 nm. DNA was purified and nucleotide bases were obtained from a sequencer device ABI 3500 Genetic Analyzer (Applied Biosystem, Foster City, CA, USA).

Statistical analysis. The sequenced products were edited using Mesquite software. The confirmation of indel polymorphism and switch was analyzed using the Molecular Evolutionary Genetics Analysis (MEGA 6) program.

The nucleotide frame was obtained from the sequencing and analyzed with BLAST using the database from the NCBI website. The sequencing product was grouped with the score based on the amount of insert/delete or indel and switch polymorphisms, namely score 1 (1-2), score 2 (3-4), score 3 (5-6). This is due to the polymorphism of one nucleotide that can affect the arrangement of amino acid (Thanh et al 2010). The DNA sequence was performed for translation to amino acid using https://web.expasy.org/, and then analyzed with BLAST-P using the NCBI database. The Pearson Bivariate Correlation was used to determine the simultaneous relationships between nucleotide polymorphism and growth characters. This analysis was continued with the variance test between correlated polymorphism-growth characters and population using ANOVA and the Duncan model. IBM SPSS 23 program was used for statistical data management.

Results and Discussion

Amplification. Amplification of sequenced DNA was 116-128 bp in size as presented in Table 1 and Figure 2. The length of nucleotide varied and was dominated by insertions and deletions. The sequenced product was confirmed with the BLAST nucleotide database on the NCBI website. The results were identical with the GH gene of *Channa striata*, accession no. EF447030.1 (Table 2) and showed indel and SNP polymorphism. Three sequences of specimen were conserved, i.e. DS3, RbK1, and RbL1. The BLAST analysis of

the 27 specimens revealed various polymorphisms; 19 were indel, 5 were SNP, 6 were indel-SNP, and 3 were not indel-SNP.

Table 1

Code of location, number of sample and length of amplified base

Location	*Code*	*N*	*Length of the DNA base*
Cala Lake	DC	3	118
Ranau Lake	DR	3	116-122
Singkarak Lake	DS	3	116-117
Kumpeh Floodplain	RbK	3	116-119
Lampam Floodplain	RbL	3	117-118
Siak Floodplain	RbS	3	116-128
Batanghari Sembilan River	SBS	3	116-118
Kampar River	SK	3	116-122
Merang River	SM	3	117-121

The insertions of new nucleotides were at 25 sites, two of them were triple bases or codon and delete. Insertion codon occurred at SK1, on site 41-43 and at SM2, on site 39-41. Deletion polymorphism was on two sites, 5 and 65. The score of indel based on the number of polymorphism sites was 1-3. The substitution of nucleotide was found in 15 sites, with a score of 1-2.

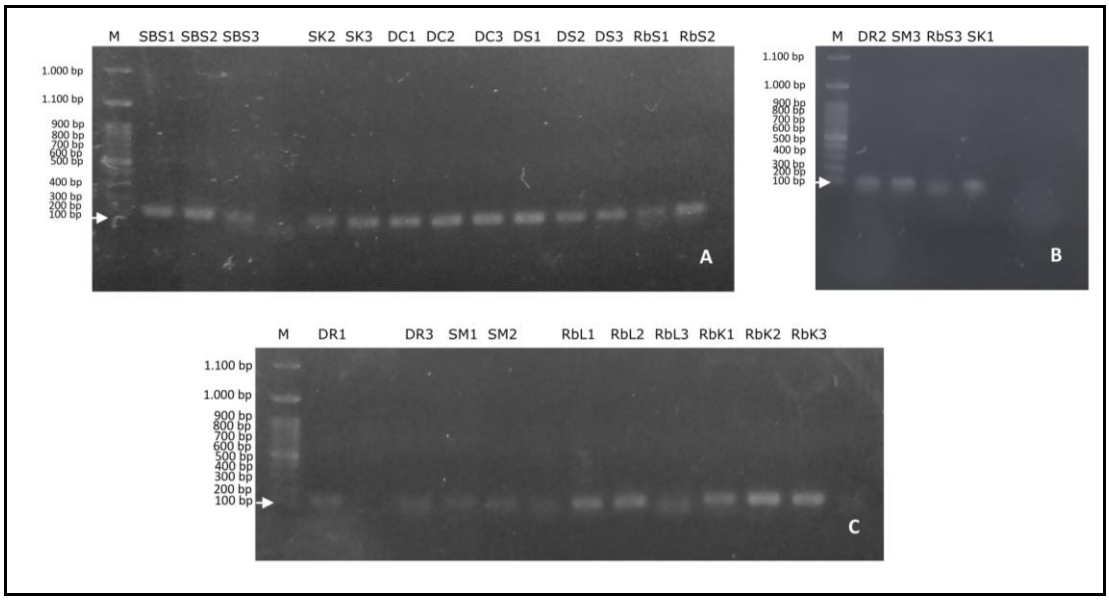

Figure 2. The performance of DNA bands from PCR products of 27 specimens at 116-128 bp in size: 13 specimens (A), 4 specimens (B) and 10 specimens C). SK - Kampar River; SM - Merang River; SBS - Batanghari Sembilan River; RbL - Lubuk Lampam Floodplain; RbS - Siak Floodplain; RbK - Kumpeh Floodplain; DS - Singkarak Lake (DS); DR - Ranau Lake; DC - Cala Lake.

The percentage of nucleotide bases of GH gene from DNA sequencing of PCR products in this research was compared with the NCBI database, accession number EF447030.1. The similarity of sequenced nucleotide was 95.08-100%, with the lowest and highest percentages in the fish group of SK (95.08-99.14%) and fish group of DS (99.15-100%), respectively.

Nucleotide changes and identity percentage of exon 4 of the GH gene in *C. striata* are presented in Table 2. Every single nucleotide change in every specimen was scored (Scr.) at column +- for indel, and S for switch. The polymorphism total score was the lowest in DS (between 0-1), and highest in SK (between 0-3).

Table 2

Summary of indel (+'/-) and SNP (S) polymorphisms in snakehead (*Channa striata*) followed by score according to +'/- and S

	Nucleotide position																																				*Scr.*		*Idn (%)*
	5	*33*	*35*	*36*	*37*	*38*	*39*	*40*	*41*	*42*	*43*	*44*	*45*	*46*	*49*	*51*	*57*	*62*	*65*	*69*	*77*	*82*	*84*	*85*	*87*	*88*	*90*	*91*	*92*	*93*	*94*	*95*	*96*	*97*	*99*	*101*	*124*	*125*	*128*	*+-*	*S*	
Ex4	A	A	G	C	C	A	G	T	T	T	C	T	G	T	G	A	T	A	G	C	C	A	A	A	A	T	A	G	A	A	C	T	G	A	A	A	G	A	G	0	0	100
DC1	*	*	*	*	*	*	*	*	*	*	*	A'	A'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	*	*	*	*	*	*	*	1	1	97.46
DC2	*	*	*	*	*	*	*	*	*	*	*	A'	G'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0	98.31
DC3	*	*	*	*	G'	A'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0	98.31
DR1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C'	*	*	*	*	*	*	*	A'	*	*	*	*	*	*	*	*	*	*	*	1	0	98.31
DR2	-	*	*	C'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0	99.15
DR3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	1	99.14
DS1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C'	*	*	*	*	*	*	*	*	*	*	1	0	99.15
DS2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C'	*	*	*	*	*	*	*	*	*	*	1	0	99.15
DS3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	100
RbK1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	100
RbK2	*	*	*	*	A'	*	*	*	*	*	*	*	*	G'	*	G'	*	*	*	*	*	*	*	C	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	2	1	95.8
RbK3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	0	100
RBL1	*	*	*	*	*	G'	*	*	*	G'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	0	98.31
RBL2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A'	*	*	*	*	*	*	*	*	*	*	*	*	1	0	99.15
RBL3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A'	*	*	*	*	*	*	*	*	*	*	*	*	1	0	99.15
RBS1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	C	*	*	*	*	*	*	*	*	*	0	1	98.28
RBS2	*	G	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	1	99.14
RBS3	*	*	*	*	*	*	*	A'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G'	*	*	*	*	*	*	*	*	A'	G	T	2	1	96
SBS1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C	*	*	*	*	*	*	*	*	*	*	*	*	0	1	99.14
SBS2	*	*	*	*	*	*	*	*	*	*	*	*	T'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C'	*	*	*	*	*	*	*	*	*	*	*	1	0	98.31
SBS3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	G	-	*	T	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	1	2	95.69
SK1	*	*	*	*	*	*	*	*	T'	A'	A'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G'	*	*	T'	C'	*	*	*	*	*	3	0	95.08
SK2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	C	*	*	*	1	1	97.44
SK3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	0	1	99.14
SM1	*	*	G'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G'	*	G'	T'	*	*	*	*	*	*	*	*	2	0	96.67
SM2	*	*	*	*	*	*	T'	T'	G'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	C'	T'	*	*	*	*	*	*	*	*	*	*	*	*	*	3	0	95.87
SM3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	G	*	*	*	*	*	*	G'	*	*	*	*	*	*	*	*	*	1	1	98.29

Note: Scr. - score from indel to switch; ` - nucleotide insert; Idn - identity; SK - Kampar River; SM - Merang River; SBS - Batanghari Sembilan River; RbL - Lubuk Lampam Floodplain; RbS - Siak Floodplain; RbK - Kumpeh Floodplain; DS - Singkarak Lake; DR - Ranau Lake; DC - Cala Lake.

The frame of amino acid was confirmed with BLAST Protein, accession no. ABR24501.1 (Table 3). 8 of them were synonymous, namely DC3, DR1, DS3, RbK1, RbK3, RbS2, SBS1, and SK3, while the others were non-synonymous.

Table 3

Synonymous and non-synonymous amino acid of GH in snakehead (*Channa striata*) in comparison to BLAST-P NCBI with accession no. ABR24501.1

	Amino acid position																				
	10	*11*	*12*	*13*	*14*	*15*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*27*	*26*	*28*	*29*	*30*	*31*	*32*
Ex4	S	Q	F	L	S	G	S	A	Q	R	N	P	I	S	P	L	K	S	E	L	K	T
DC1	*	*	I	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
DC2	*	*	V	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
DC3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
DR1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
DR2	A	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
DR3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	Q	*	*	*	*	*
DS1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	T	E	D
DS2	A	S	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
DS3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RbK1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RbK2	*	T	V	S	G	L'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RbK3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RBL1	*	*	V	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RBL2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	T	E	D
RBL3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	R	T	E	D
RBS1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	A	*	*	*
RBS2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
RBS3	A	R	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	R	T	E	D
SBS1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SBS2	A	S	*	C	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	E	D
SBS3	*	*	*	*	*	*	*	*	R	*	T	R	F	F	*	*	*	*	*	*	*	*
SK1	*	*	L	I'	*	*	*	*	*	*	*	*	*	*	*	*	*	*	S'	*	Q	*
SK2	*	*	*	*	*	*	R	F	C	T	E	E	*	*	*	*	*	*	*	*	*	*
SK3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
SM1	G	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	S'	*	Q	*	*
SM2	*	H	W'	*	*	*	*	*	*	*	*	*	*	*	*	N	*	*	*	*	*	*
SM3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	T	E	D

Note: * - synonymous; ` - insert; SK - Kampar River; SM - Merang River; SBS - Batanghari Sembilan River; RbL - Lubuk Lampam Floodplain; RbS - Siak Floodplain; RbK - Kumpeh Floodplain; DS - Singkarak Lake; DR - Ranau Lake; DC - Cala Lake.

Correlation of polymorphism and growth trait. There were significant differences ($p<0.05$) in W, SL, BH, PD, PA, HL, HW, SL/BH, and SL/HW characters of fish between locations (Table 4).

Indel significantly affected some growth traits of snakehead in all locations (Table 4), namely W, SL and HW. Base SNP was not correlated with growth traits. The Pearson correlation revealed that indel with those 3 characters was adequately strong with the coefficient correlation value of 0.40-0.421 and significant at $p<0.05$. Many characters were not significantly affected by the GH gene. The population from Kampar River (SK) had the highest averages for W, PD, PA, BH and HL, while Kumpeh Floodplain (RbK) exhibited the lowest average for the 5 characters.

Table 4

Morphological characters of *Channa striata* from the 9 sampling points

SP	*N*	*W±SE (g)*	*SL±SE (cm)*	*BH±SE (cm)*	*PD±SE (cm)*	*PA±SE (cm)*	*HL±SE (cm)*	*HW±SE (cm)*	*SL/BH±SE (cm)*	*SL/PD±SE (cm)*	*SL/PA±SE (cm)*	*SL/HL±SE (cm)*	*SL/HW±SE (cm)*
DC	2	179.12±	22.83±	2.86±	14.12±	8.49±	6.79±	2.97±	7.96±	1.61±	2.69±	3.36±	7.67±
	5	4.37^{cd*}	0.18^{c*}	0.03^{b}	0.12^{d}	0.06^{b}	0.07^{c}	0.02^{b}	0.06^{f*}	0.01^{a}	0.01^{a}	0.03^{a}	0.06^{e}
DR	2	142.92±	20.59±	2.99±	12.78±	7.44±	6.20±	2.98±	6.89±	1.61±	2.76±	3.32±	6.92±
	5	8.07^{bc*}	0.42^{b*}	0.07^{bc}	0.24^{bc}	0.12^{a}	0.12^{b}	0.07^{b}	0.08^{a*}	0.01^{a}	0.02^{a}	0.04^{a}	0.10^{b}
DS	2	110.25±	19.75±	2.62±	11.95±	7.15±	6.00±	2.70±	7.60±	1.65±	2.76±	3.29±	7.35±
	6	3.36^{ab*}	0.19a^{b*}	0.06^{a}	0.12^{b}	0.09^{a}	0.06^{ab}	0.05^{a}	0.15cd^{e*}	0.01^{a}	0.02^{a}	0.02^{a}	0.11^{d}
Rb	2	97.52±	18.82±	2.44±	11.07±	7.09±	5.61±	2.60±	7.70±	2.04±	2.65±	3.35±	7.26±
K	5	4.20^{a*}	0.27^{a*}	0.04^{a}	0.43^{a}	0.11^{a}	0.09^{a}	0.05^{a}	0.09^{def*}	0.40^{a}	0.02^{a}	0.03^{a}	0.09^{cd}
Rb	3	301.32±	27.76±	3.92±	16.95±	9.70±	8.23±	4.30±	7.11±	1.63±	2.86±	3.38±	6.50±
L	4	21.06^{f*}	0.63^{e*}	0.10^{f}	0.35^{f}	0.24^{c}	0.23^{e}	0.13^{e}	0.08^{ab*}	0.01^{a}	0.03^{a}	0.03^{a}	0.09^{a}
Rb	2	167.86±	22.64±	3.10±	13.55±	8.15±	6.68±	3.17±	7.29±	1.67±	2.78±	3.38±	7.13±
S	9	14.23c^{d*}	0.69^{c*}	0.07^{cd}	0.39^{cd}	0.25^{b}	0.17^{c}	0.09^{bc}	0.13^{bc*}	0.02^{a}	0.04^{a}	0.05^{a}	0.11^{bcd}
SB	3	195.40±	22.83±	3.06±	13.97±	8.32±	6.84±	3.26±	7.48±	1.64±	3.03±	3.34±	7.01±
S	1	13.17^{d*}	0.49^{c*}	0.07^{bc}	0.34^{d}	0.28^{b}	0.17^{c}	0.08^{c}	0.09^{cd*}	0.03^{a}	0.37^{a}	0.03^{a}	0.06^{bc}
SK	2	243.33±	25.78±	3.29±	15.59±	9.43±	7.64±	3.68±	7.88±	1.65±	2.73±	3.37±	7.01±
	7	12.67^{e*}	0.47^{d*}	0.09^{de}	0.29^{e}	0.18^{c}	0.14^{d}	0.08^{d}	0.12^{ef*}	0.01^{a}	0.02^{a}	0.03^{a}	0.09^{bc}
SM	3	250.74±	25.14±	3.40±	15.77±	9.26±	7.43±	3.52±	7.45±	1.59±	2.71±	3.38±	7.14±
	1	11.82^{e*}	0.47^{d*}	0.08^{e}	0.30^{e}	0.17^{c}	0.15^{d}	0.07^{d}	0.15^{cd*}	0.01^{a}	0.02^{a}	0.03^{a}	0.07^{bcd}

Note: different superscripts show significant difference ($p<0.05$); * - significant difference in correlation; SP - sampling point; SL - standard length; W - weight; PD - pinna dorsalis; PA - pinna analis; BH - body height; HL - head length; HW - head width; SK - Kampar River; SM - Merang River; SBS - Batanghari Sembilan River; RbL - Lubuk Lampam Floodplain; RbS - Siak Floodplain; RbK - Kumpeh Floodplain; DS - Singkarak Lake; DR - Ranau Lake; DC - Cala Lake.

ANOVA test results for polymorphisms score with growth traits (SL, W, and HW) are presented in Table 5. Polymorphism score indicated significant differences on the SL and HW characters. SL was significantly different on polymorphism scores 0, 2, and 3. Moreover, HW was significantly different on polymorphism scores 0 and 3.

Table 5

Growth traits according to polymorphisms scores of exon 4 of GH gene in *Channa striata*

Polymorphism scores	*N*	*SL±SE (g)*	*W±SE (cm)*	*HW±SE (cm)*
0	3	18.10 ± 0.58^{a}	84.33 ± 6.48^{a}	2.30 ± 0.10^{a}
1	16	22.90 ± 1.07^{ab}	193.00 ± 24.67^{a}	3.23 ± 0.15^{ab}
2	3	25.66 ± 1.38^{c}	226.00 ± 30.51^{a}	3.30 ± 0.25^{ab}
3	5	25.22 ± 3.14^{c}	205.00 ± 63.51^{a}	3.42 ± 0.48^{b}

Note: different superscripts show significant differences ($p<0.05$). SL - standard length; W - weight; HW - head width.

Polymorphism and growth traits. The nucleotide at exon 4 GH gene in *C. striata* showed that indel and SNP were found in almost all locations (river, lake and floodplain), excepting 3 isolates who had a conserved sequence (DS3, RbK1, and RbK3). Former studies documented similar findings, with the possibility of indel and SNP occurring at the exon of GH gene in *Tinca tinca* (Kocour & Kohlmann 2011).

Transversion (A-C) and transition (A-G) took place on SNP exon 4 for 5 specimens, i.e. DR3, RbS1, RbS2, SBS 1, and SK3 (Table 2). This nucleotide alteration was not associated with the growth trait of snakehead. This non-synonymous SNP was only found on RbS1 (Table 3). Dissimilar results were noted for *C. carpio*, in which exon 3 of the IGF-I gene was associated with body weight and length (Feng et al 2014).

Contrarily, most specimens in this experiment underwent indel and indel-SNP (Table 2) altering codon. Consequently, BLAST protein showing translation of amino acids also differs from the NCBI protein database accession no. ABR24501.1. (Table 3). Such condition was observed on indel of IGF-I gene of *C. carpio* (Hu et al 2012) and *Tinca tinca* (Kocour & Kohlmann 2014), as well as on indel of CDC25A Shaanbei White Cashmere (SBWC) gene of goats (Cui et al 2019). This translation resulted in a protein with shorter amino acids and indicated by a stop codon or non-sense. The mutation of frameshift caused premature stop codon (Calduch-Giner et al 2003).

The polymorphism of exon 4 gene GH of *C. striata* in this study experienced the alteration of amino acid arrangement. Similar findings were reported on the exon region of hybrid *Culter alburnus x Ancherythroculter nigrocauda* (Cheng & Sun 2015), *Chlamys farreri* (Wang et al 2010), on preprosomatostatin (CcPSS3) gene of *C. carpio* (Feng et al 2015), and myostatin gene of bighead carp (*Hypophthalmichthys nobilis*) (Liu et al 2012). The exon is part of a protein coding region for DNA translation.

The non-synonymous substitution of amino acids might happen due to the genetic response to the environment. This is supported by the non-synonymous mutation on HIF-1A (hypoxia-inducible factor) gene on *Triplophysa* sp. noticed at highlands, while synonymous mutation of HIF-1B gene was recorded at lowlands (Wang et al 2015). Not all translations are finalized with non-sense mutations. Non-synonymous mutations could affect the activity and function of gene expression (Feng et al 2014).

This polymorphism variation is predicted since nature provides gene variation, so that indel or SNP can be notified on one protein-code region. This is in accordance with the investigations of Thanh et al (2010) documenting more frequent SNPs of actin and CHH genes on natural strains of giant freshwater prawn (*Macrobrachium rosenbergii*) from Dong Nai and Mekong than on cultivated ones.

Positive selection is indicated by more substitutions of nonsynonymous mutations compared to synonymous ones (Pankova et al 2017). Similarly, the wild nature of *Drosophila melanogaster* was indicated with indel polymorphism followed by non-synonymous mutations on exon 1 of Insuline-like Receptor (InR) gene (Paaby et al 2010) in different environment. This proved the availability of positive selection and gene polymorphism as adaptation components to environmental conditions.

Several fish, like *P. fulvidraco*, *C. carpio*, and *L. crocea*, can present polymorphism of the GH gene on intron associating with growth traits (Ni et al 2012; Liu et al 2017; Li et al 2017). The intron also presented a longer nucleotide base than the exon and could influence gene expression by regulating splicing mRNA on the exon (Graur & Li 2000; Kocour & Kohlmann 2011). Therefore, the identification of polymorphism on other regions relating to the growth of snakehead is required.

Conclusions. The sequence of GH gene exon 4 region of *C. striata* from Sumatera, Indonesia, presented indel and SNP polymorphisms. The Pearson correlation showed positive association (0.4-0.421) between indel and growth traits (weight, standard length and head width). Snakehead with an indel score of 2-3 that could influence length and head width were found in a floodplain and river.

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