Single nucleotide polymorphisms (SNPs) variation in the immunoglobulin M heavy chain gene of African catfish (*Clarias gariepinus*) strains in Indonesia

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Abstract. Single nucleotide polymorphisms (SNPs) are the most common genetic variants. While the majority of SNPs are non-functional, some are functional and have significant phenotypic effects. This study aimed to evaluate SNPs in immunoglobulin M (IgM) heavy chain in five African catfish (*Clarias gariepinus*) strains in Indonesia. Strains were characterized based on morphological analysis. IgM heavy chain gene was amplified by PCR, cloned with pJET1.2, and sequenced. The results showed that Dumbo, Paiton, Masamo, and Sangkuriang strains had similar morphologies, except for Egypt. Three hundred and five nucleotide sequences were successfully sequenced from Sangkuriang, Paiton, Egypt, and Masamo that encode 101 amino acid residues, while the Dumbo strain had 229 nucleotide sequences encoding 99 amino acid residues. A total of 54, 53, 53, 53, and 52 SNPs were observed in Dumbo, Paiton, Sangkuriang, Egypt, and Masamo strains, respectively, and all were identified in the coding region. Nonsynonymous nucleotide substitutions existed in the exon, altering the amino acid product. The morphometric analysis yielded slightly different results than the molecular analysis of the IgM heavy chain, particularly regarding the genetic distance between the Masamo and Egypt strains. This study indicated a possible link between immune performance and IgM heavy chain structure gene in five different strains of African catfish. Nonetheless, more researches to investigating this correlation are required.
Key Words: catfish, cloning, DNA sequencing, genomic identification.

Introduction. The humoral immune system produces unique antibodies in response to a variety of antigens. Immunoglobulin (Ig) heavy (H) and light (L) chains make up antibodies, playing an important role in adaptive immunity (Hikima et al 2011; Yu et al 2020; Abós et al 2022; Khalid et al 2022). Ig H-chains in mammals are classified into five groups or isotypes: IgM, IgD, IgG, IgA, and IgE (Chukwuanukwu et al 2022). From the genomic sequences of various teleost fish species, three isotypes of Ig, IgM, IgD, and IgT/Z, have been determined (Mashoof & Criscitiello 2016; Teng et al 2022). The IgM and IgD have been discovered in the genome of catfish, with IgM appearing to be the major isotype that responds to pathogens (Castro et al 2013; Kordon et al 2021; Zhang et al 2022).

IgM in teleosts consists of equal proportions of heavy and light chains arranged in a basic unit containing two heavy chains and two light chains and in a tetrameric homolog (Bengtén et al 2006; Zhang et al 2010). It was discovered that the variable domain of IgM is responsible for the antibody's ability to bind to a wide range of antigens. Approximately one megabase (Mb) of the catfish germline genome encodes IgM variable and constant domains: while there are 4 exons encoding a constant domain and 2 exons for transmembrane sequences in the carboxyl terminus of IgM, there are gene segments that encode 55 variable (Vh), 6 diversity (Dh), and 12 joint gene segments, the combination of which creates a functional Vh in the amino terminus (Wilson et al 1990; Bengtén et al 2006).

African catfish (*Clarias gariepinus*) has become a species of great economic significance in Indonesian aquaculture. It has been extensively cultivated in Indonesia, primarily in Java, Sumatra, and Kalimantan. The African catfish has been introduced to Indonesia for aquaculture on several occasions, either directly from Africa or through other nations. In Indonesia, there are some African catfish strains, including Paiton, Sangkuriang, Dumbo, and Egypt strains (Iswanto et al 2015). At the outset of their introductions, each of the introduced strains displayed superior growth performance. Despite the fact that some studies have identified IgM heavy chain gene in catfish order (Galagarza et al 2019; Xu et al 2019b; Bunnoy et al 2020; Zhang et al 2022), no studies have documented the IgM heavy chain gene of African catfish, particularly in strains from Indonesia.

Intraspecific genetic variation serves substrate for hereditary evolutionary change (Clucas et al 2019; Svardal et al 2021; Dalongeville et al 2022). The quantification of variation at one or a few base pairs patterns (SNPs) has been used to observe fish genetic variation (Jaafar et al 2020). In the present work, the IgM heavy chain gene of African catfish strains was cloned to elucidate and characterize the SNP variations that thought to affect the immune performance.

Material and Method

Fish collection and sampling. The morphological characterization analyses were conducted at the Research Institute for Fish Breeding (RIFB) and genetic molecular analyses were carried out at genomic laboratory, National Research and Innovation Agency Republic of Indonesia in January to December 2022. Five strains of African catfish were collections of RIFB collected from different locations scattered in Indonesia (Iswanto et al 2015), and they were maintained separately in the concrete ponds (200 m^2). The Sangkuriang strain consisted of 34 fish specimens weighing 2.05±1.2 kg and 18 individuals of Dumbo weighing 2.6±1.1 kg were obtained from the Freshwater Aquaculture Development Center Sukabumi in 2014. Thirty-three individuals of Paiton weighing 2.9±1.1 kg were collected in 2010 from the Catfish Breeding Model Mojokerto. They were the fourth generation of the Charoen Pokphand Group's African catfish strain, introduced in Thailand in 1998. The Egypt strain comprised 31 fish weighing 2.2±0.7 kg. They were the first generation of African catfish introduced from Egypt as a donation by the Head of West Java Provincial Agency for Fisheries and Marine Affairs in 2011. Thirty-four individuals of Masamo weighing 3.1±1.1kg were transferred from the PT Matahari Sakti hatchery in Mojokerto in late 2011, and they were the first generation of African catfish introduced from Thailand in 2010. Fish were reared in flowing water and fed twice daily at 3-5% biomass using commercial pellet feed (Cargill, Indonesia) with a protein content of 30%. Fish were anesthetized with 75 mg L^{-1} MS-222, and subsequently morphological data were recorded, and the fin clips were collected from each species and kept in 70% ethanol at 4°C until further analysis.

Morphological characterization of the African catfish strains. Morphometric and meristic characteristics of living fish specimens were utilized to characterize the morphology of African catfish strains. The characterizations were conducted using standard biometric characterization techniques mostly used in morphological characterization studies of the African catfish (Teugels & Legendre 1998; Hanssens 2009). Principal component analysis (PCA) based on a covariance matrix was used to analyze morphometric data. For morphometric data analysis, log-transformed data were subjected to PCA to minimize the effect of fish sample size differences. The first principal component (PC1) considered as size factor was not used in the interpretation of the results, only the PC2 and PC3 considered as shape factors used (Sudarto 2007; Pouyaud & Paradis 2009; Iswanto et al 2015). On the covariance matrix, an independent PCA was performed on untransformed meristic data. The principal component scores of all samples were then plotted alongside 95% confidence ellipses to characterize the distribution of fish samples. The computer program SYSTAT 11 (SYSTAT Software Inc., www.systat.com) was used to assist with data analysis.

DNA extraction. DNA was extracted from the caudal fins of five African catfish strains using GeneJet Genomic DNA Purification Kit following the manufacturer's recommended protocols (Thermo Scientific). Approximately 10 mg of the tissue was used, and gDNA was run on mini horizontal gel electrophoresis. The sample was loaded into a 1.5% (w/v) agarose gel and ran for 50 min at 65-volt (6.5 V cm^{-1}). The gel was then stained with 1 g mL^{-1} peqGREEN (Vwr, UK) and viewed under ultraviolet transillumination using the gel documentation system. DNA concentration and purity were measured spectro-photometrically on a Thermo Scientific NanoDropTM One/OneC Microvolume UV-Vis Spectrophotometer and stored at -20°C for further analysis.

Polymerase chain reaction (PCR) analysis. The IgM heavy chain gene was amplified using a thermal cycling system (mycycler, Biorad). PCR standard was conducted in a final volume of 25 µL using a commercial kit master mix fast DreamTaq PCR Master Mix PCR master (Thermo Scientific, USA) and 50 ng DNA template. A 305-bp fragment on the constant region of the IgM heavy chain gene was amplified using primers F (5'-TCC CCA AGG TTT ACT TGC TCG CTC C-3') and R (5'-CGA TGG ATC TGG ATA TGT GGC GCA C-3') (Thongpan et al 1997), for 35 cycles: denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec, and extension at 72°C for 30 sec. As an internal control, the ß-actin gene was amplified using the following primers: bact-F (5'-TAT GAA GGT TAT GCT CTG CCC-3') and bact-R (5'-CAT ACC CAG GAA AGA TGG CTG-3') (Marnis et al 2016). PCR products were separated on a 1.5% (w/v) agarose gel stained with peqGREEN (Vwr, UK) 1 g mL^{-1} and visualized using ultraviolet transillumination.

DNA cloning and sequencing. The PCR products were resolved in an agarose gel, and the amplicon of the expected size was excised, gel purified, and cloned into plasmid pJET 1.2 cloning vector using CloneJETTM PCR Cloning Kit (ThermoFisher Scientific,USA). Cloned DNA fragments were Sanger sequenced by primer walking at firstbase (Singapore). Sequence reads were assembled and analyzed in Geneious v11 (Kearse et al 2012).

Sequence and SNPs analyses. Phylogenetic analysis of the nucleotide sequence of the IgM heavy chain was carried out by the neighbor-joining method using MEGA 5.0 program, and the reliability of the estimated tree was evaluated by the bootstrap method with 1000 pseudo-replications. DNA sequences were aligned to the IgM heavy chain gene sequences of channel catfish (*Ictalurus punctatus*) (acc. number: X79482.1) (Magor et al 1994). SNPs were identified and genotyped using the EMBL-EBI search and sequence analysis tools APIs in 2019 (Madeira et al 2019). Cladogram of five strains studied and reference species was created using BLAST® (https://www.ncbi.nlm.nih.gov) based on the sequence of IgM heavy chain.

Results

Morphological characterization of the African catfish strains. The scatter plots of the remaining principal component scores revealed fewer comparable patterns. Contributions of each morphometric characters to the second (PC2) and third (PC3) principal component coefficients resulted from a PCA running on the fish samples for 16 morphometric characters (Table 1). Morphometric character scatter plot revealed that the scores of the Dumbo, Paiton, Masamo, and Sangkuriang strains overlapped, either on the PC2 or PC3 axis. While the Egypt strain's scores along the PC2 axis separated from those of the Paiton and Masamo strains, they only partially overlapped with those of the Dumbo and Sangkuriang strains (Figure 1).

Table 1
Principal component coefficients of each morphometric character in the second (PC2) and third (PC3) principal component resulted from a principal component analysis (PCA) performed on the samples of Dumbo, Sangkuriang, Paiton, Masamo, and Egypt strains of the African catfish in Indonesia

Characters	*PC1*	*PC2*	*PC3*	*PC4*	*PC5*
Head length (HL)	0.2397	-0.1013	0.0719	0.0700	0.0711
Head width (HW)	0.2654	-0.0894	-0.0080	0.0142	0.0619
Snout length (SNL)	0.2175	-0.2400	0.5633	-0.3100	-0.3319
Interorbital width (IOW)	0.2534	-0.0151	0.0476	0.0066	0.0004
Eye diameter (ED)	0.1170	-0.4593	0.3659	0.0024	0.5417
Occipital process length (OPL)	0.1943	0.0101	0.1164	0.8926	-0.1856
Occipital process width (OPW)	0.3658	-0.0824	-0.5326	-0.1437	0.3553
Occipital process to dorsal fin distance (OPDFD)	0.1644	0.7933	0.3690	-0.1506	0.2109
Predorsal length (PDL)	0.2324	0.0276	0.1052	0.0437	0.0813
Dorsal fin length (DFL)	0.3094	0.0819	-0.1251	-0.0401	-0.2081
Prepectoral length (PPEL)	0.2518	-0.1264	0.0922	-0.0076	0.0703
Prepelvic length (PPL)	0.2466	0.0492	0.0628	0.0296	0.0686
Preanal length (PAL)	0.2728	0.0918	0.0348	0.0325	0.0289
Anal fin length (AFL)	0.3064	0.0541	-0.1553	-0.0967	-0.3609
Maximum body depth at anus (MBD)	0.1808	0.1686	-0.1356	0.0944	0.2821
Caudal peduncle depth (CPD)	0.2710	-0.1056	-0.1581	-0.1870	-0.3348
Constant for centering	-3.70604	-0.37037	-0.10542	-0.02825	0.298017

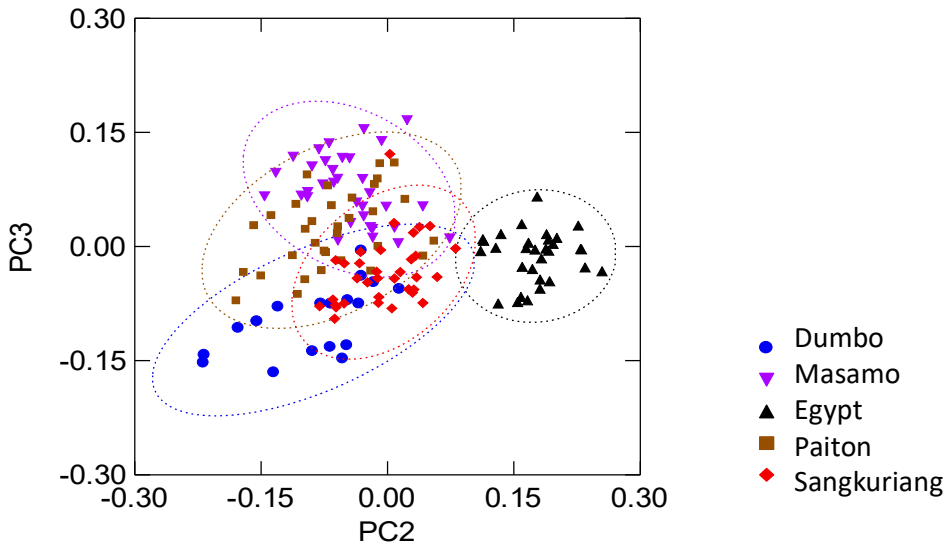

Figure 1. Scatter plot of the scores of Dumbo, Sangkuriang, Paiton, Masamo, and Egypt strains of the African catfish *Clarias gariepinus* in Indonesia along the axis of the second (PC2) and third (PC3) principal component resulted from a principal component analysis (PCA) for the morphometric characters.

Analysis of nucleotide and deduced amino acid sequence of IgM heavy chain. Nucleotide sequences of five African catfish strains (Sangkuriang, Paiton, Dumbo, Masamo, and Egypt) encoding the constant region of IgM heavy chain were deposited to the NCBI GenBank with accession numbers MZ408255, MZ408256, MZ408257, MZ408258, MZ408259, respectively. A total of 305 nucleotide sequences encoding 101 amino acid residues were obtained from each strain of Sangkuriang, Patin, Egypt, and Masamo. Only 229 bp nucleotides were successfully sequenced from Dumbo (Figure 2). Based on the number of amino acids IgM heavy chain in Sangkuriang, Paiton, Masamo, and Egypt was primary composed of valine (17%), serine (11%), tyrosine (7%). Interestingly, we observed a smaller number of valine (13%) in Dumbo with 11% and

7% for serine and tyrosine, respectively. The least abundant amino acid in all African catfish strains was histidine (1%) (Figure 3).

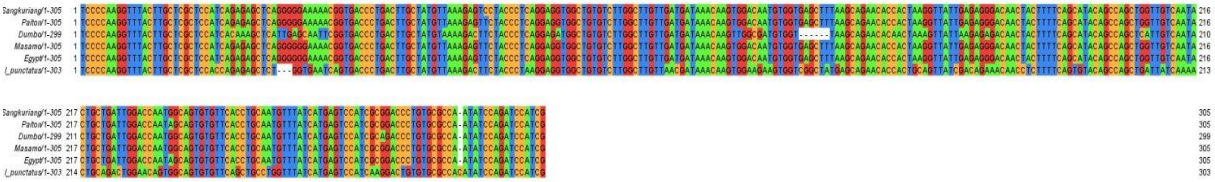

Figure 2. Alignment of nucleotide sequences from the constant region of IgM heavy chain of five African catfish strains with that of channel catfish. Hyphens between sequences represent deletions. Gaps between nucleotides show variability in the base sequence among species.

Figure 3. Alignment of deduced amino acid sequences from the constant region of IgM heavy chain of five African catfish strains. An * (asterisk) indicates positions which have a single, fully conserved residue. Symbol: (colon) indicates conservation between groups of strongly similar properties - scoring > 0.5 in the Gonnet PAM 250 matrix. Symbol. (period) indicates conservation between groups of weakly similar properties - scoring =< 0.5 in the Gonnet PAM 250 matrix.

The Paiton, Sangkuriang, Masamo, and Egypt IgM heavy chain sequences compared with the NCBI blastn revealed an 85% identity to the channel catfish (*Ictalurus punctatus*), and Dumbo had similarity 82% to channel catfish (Table S1). African catfish strains had similarity more/less than 80% compared to other catfish. The phylogenetic analysis showed that the evolutionary African catfish strains were related to bighead catfish (*Clarias macrochephalus*) (Thongpan et al 1997) and striped catfish (*Pangasianodon hypophthalmus*) (GenBank accession no. XM_034309813.1), which were in the same catfish order. As shown in the cladogram, Paiton and Egypt were one clade, while Dumbo was laid at a different clade (Figure 4).

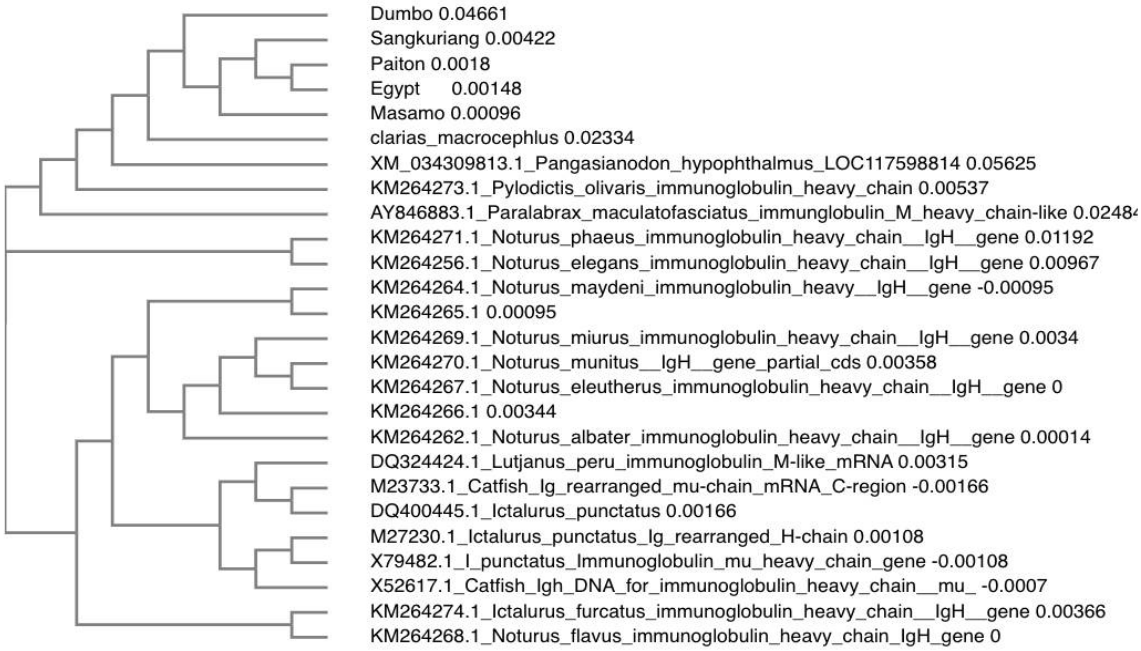

Figure 4. Neighbour-joining phylogenetic analysis of IgM heavy chain genes nucleotide sequences from Dumbo, Sangkuriang, Masamo, Paiton, and Egypt strains with the top twenty similarities in the NCBI database. The numbers next to the species names represent bootstrap support in 1000 replications.

Interspecific SNPs and their characteristics. In comparison against DNA sequence of IgM heavy chain gene from NCBI database, a total of 54 (18%), 53 (17%), 53 (17%), 53 (17%), and 52 (17%) SNPs were identified in the Dumbo, Paiton, Sangkuriang, Egypt, and Masamo strains, respectively. These interspecific SNPs were located within the coding region (CH4). One base deletion was observed in Sangkuriang, Masamo, Paiton, and Egypt when compared to Dumbo. Seven base deletions were noted in Dumbo when compared to four other strains. In addition, there were 3 base insertions observed in all African catfish strains (Table 2, Table S2).

Table 2

SNPs variation of gene encoding (exon) of the IgM heavy chain of African catfish strains against reference sequence *I. punctatus*

African catfish strains	*Total of SNPs*	*Deletion*	*Insertion*
Sangkuriang	53	A	AGG
Masamo	52	A	AGG
Paiton	53	A	AGG
Dumbo	54	CG (GA) GCT A (T) A	ATT
Egypt	53	A	AGG

Patterns of DNA and amino acid substitutions. Alignment between IgM heavy chain gene sequences of Dumbo and Singkuriang, between Dumbo and Paiton, and between Dumbo and Egypt found 20 SNPs, while alignment between Sangkuriang and Masamo discovered 5 SNPs and merely 4 SNPs were found between Sangkuriang and Egypt. Furthermore, fewer SNPs (1) were found between Masamo and Egypt. In addition, the frequency of nucleotide substitution transversion in Dumbo against other strains was significantly higher (10) than the number of nucleotide substitution transitions (3). One nucleotide substitution transversion was found in Sangkuriang when the nucleotide

sequences of Sangkuriang compared to others. Moreover, Masamo had no nucleotide substitutions, both transversion and transition (Table 3). Further analyses showed that the nucleotide substitutions occurred were nonsynonymous, meaning that mutation in DNA coding changes the resulting amino acid product (Table 4).

Table 3

Frequencies of transition (Ts) and transversion (Tv) of nucleotide substitution in immunoglobulin heavy chain gene of five African catfish strains In Indonesia

Pairwise comparison	*DNA substitution (CH4, coding region)*	
	Ts	*Tv*
Dumbo	3	10
Sangkuriang	-	1
Paiton	-	1
Egypt	-	1
Masamo	-	-

Table 4

Types of nucleotide substitutions and corresponding deduced amino acids of four African catfish strains from Indonesia

Strains	*Sequence number*	*Codon (amino acid)*	*Substitutions*	*Mutations*
Dumbo	30, 32	CAA → GAG	CAA (Q) → GAG (E)	Nonsynonymous
	39, 40	TTG → GGG	TTG (L) → GGG (G)	Nonsynonymous
	42, 44	AGC → GGA	AGC (S) → GGA (G)	Nonsynonymous
	47	AAT → AAA	AAT (N) → AAA (K)	Nonsynonymous
	48	TCG → ACG	TCG (S) → ACG (T)	Nonsynonymous
	77	GAC → GAG	GAC (D) → GAG (E)	Nonsynonymous
	93	ATG → GTG	ATG (M) → GTG (V)	Nonsynonymous
	130	GGC → GAC	GGC (G) → GAC (D)	Nonsynonymous
	132	GAT → AAT	GAT (D) → AAT (N)	Nonsynonymous
	141..143	DELETION → AGC	DELETION → AGC (S)	Nonsynonymous
	144..146	DELETION → TTT	DELETION → TTT (F)	Nonsynonymous
	171	AAG → GAG	AAG (K) → GAG (E)	Nonsynonymous
	207	ATT → GTT	ATT (I) → GTT (V)	Nonsynonymous
Sangkuriang	79	TCC → TTC	TCC (S) → TTC (F)	Nonsynonymous
Paiton	304	GGC→AGC	GGC (G) → AGC (S)	Nonsynonymous
Egypt	304	GGC→AGC	GGC (G) → AGC (S)	Nonsynonymous

Discussion. The morphological characteristics of the African catfish strains were essentially identical, with the exception of the Egypt strain, which displayed a slight variation. These results corresponded to those obtained from the interstrain morphometric variation of African catfish in Africa, indicating that the Egypt strain was morphometrically distinct from the Senegal, Lake Victoria, and Swaziland strains, but intertwined with the Chad strain (Rognon et al 1998; Teugels & Legendre 1998). The morphometric and molecular analyses of the IgM heavy chain gene showed a slightly different result, particularly regarding the genetic distance between Masamo and Egypt strains. It was assumed that sequence data obtained might not cover all the genetic information since this study only sequenced a partial fragment of the IgM heavy chain gene.

Immunoglobulins (Igs) are important molecules in the adaptive immune system of jawed vertebrates since they bind antigens with high specificity (Bilal et al 2021). The capacity to produce specific antibodies in response to immunization is directly related to the repertoire of B cells, which proliferate, produce IgM, and regulate teleost systemic immunity (Yu et al 2020). Characterizing the structure of the IgM heavy chain is important for several reasons, including understanding the genetic basis of antibody functions and the evolutionary divergence of the IgM gene in fish. Previous studies have

shown that the structure of the IgM heavy chain gene has been highly conserved throughout fish evolution (Ross et al 1998; Anumukonda et al 2022). IgM heavy chain gene in many important aquaculture species including, channel catfish (Bengtén et al 2006; Zhang et al 2022), striped catfish and bighead catfish (Thongpan et al 1997), grass carp (*Ctenopharyngodon idella*), largemouth bass (*Micropterus salmoides*) (Han et al 2022), big-eye Mandarin fish (*Siniperca knerii*) (Xia et al 2021) has been characterized.

The results of the present study shed light on the genetic diversity of African catfish strains in Indonesia. The IgM heavy chain gene of African catfish strains in Indonesia had the best BLASTN hits to channel catfish (82-85% identity). This indicated that no data IgM heavy chain gene of African catfish strains in Indonesia was available in databases. Moreover, the evolutionary relationship of the African catfish strains was closely related to bighead catfish and striped catfish, both of which are classified under the same taxonomic order (Siluriformes or catfish), and IgM heavy chain of these strains shared a common ancestor with bighead catfish.

Nucleotide substitutions, both transition and transversion, occurred in an exon of IgM heavy chain gene were nonsynonymous mutations that might be affecting significant physical and chemical changes (Zou & Zhang 2021). The changes in the structure of IgM heavy chain gene could be associated with differences in immune performance among the five African catfish strains. A number of studies have shown that the IgM heavy chain gene associated to immune response against pathogens in channel catfish (Zhang et al 2022), yellow catfish (*Pelteobagrus fulvidraco*) (Xu et al 2019b), big-eye Mandarin fish (Xia et al 2021), rohu (*Labeo rohita*) (Saravanan et al 2020), and loach (*Misgurnus anguillicaudatus*) (Xu et al 2019a).

Molecular analysis and characterization of the IgM heavy chain gene in African catfish strains were essential not only due to their evolutionary history, but also to the study of their existence in harsh conditions (De Silva et al 2019), such as adapting to a high level of ammonia in the aquatic environment and occasionally inhabiting mudflats or strolls on land (Li et al 2018). Therefore, this research proposed the possibility of a novel classification of African catfish strains IgM heavy chain gene, based on the IgM C4 nucleotide variations. This report was the first record of the detailed characterization of the African catfish IgM heavy chain gene constant domain to the best of our knowledge.

Conclusions. The African catfish strains in Indonesia had similar morphologies, except for Egypt. Sequence analysis of partial gene of IgM heavy chain gave a slightly different from morphological characterization, particularly in the relation to the genetic distance between Masamo and Egypt strains. The IgM heavy chain of African catfish strains closely matched to channel catfish, and an average of 17% SNPs existed between group of African catfish and channel catfish. SNPs variation found in the exon region were nonsynonymus mutation which might be affecting the functional structure of IgM heavy chain and influencing the immune performances among the African catfish strains towards pathogens. Future studies are recommended to target the complete sequence of IgM heavy chain gene to observe comprehensively the similarity and evolutionary of African catfish strains by combining with the morphological characterizations.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Supplementary Tables

Table S1

Comparison of the African catfish strains immunoglobulin M heavy chain sequence using the NCBI blastn with published catfish species immunoglobulin M heavy chain sequences

African catfish strains	*Species name*	*NCBI accession number*	*Max*	*Total*	*Query*	*E*	*Identity*
Sangkuriang, Paiton, and Egypt	*Ictalurus punctatus*	X79482.1	335	335	100%	3,00E-87	84.64%
	Pangasianodon hypophthalmus	XM_034309813.1	331	331	100%	8,00E-82	83.89%
	Pylodictis olivaris	KM264273.1	299	299	91%	2,00E-76	83.89%
	Noturus munitus	KM264270.1	286	286	91%	1,00E-72	82.92%
	Noturus flavus	KM264268.1	286	286	91%	1,00E-72	82.92%
Dumbo	*Ictalurus punctatus*	X79482.1	300	300	100%	6,00E-77	82.35%
	Pangasianodon hypophthalmus	XM_034309813.1	286	286	97%	1,00E-72	81.54%
	Pylodictis olivaris	KM264273.1	260	260	91%	2,00E-64	81.14%
	Noturus munitus	KM264270.1	246	246	91%	1,00E-60	80.07%
	Noturus flavus	KM264268.1	251	251	91%	1,00E-61	80.43%
Masamo	*Ictalurus punctatus*	X79482.1	340	340	100%	7,00E-89	84.97%
	Pangasianodon hypophthalmus	XM_034309813.1	335	335	97%	3,00E-87	84.23%
	Pylodictis olivaris	KM264273.1	304	304	91%	5,00E-78	83.23%
	Noturus munitus	KM264270.1	290	290	91%	1,00E-73	83.27%
	Noturus flavus	KM264268.1	290	290	91%	1,00E-73	83.27%

Table S2

Interspecific SNPs of the immunoglobulin M heavy chain gene between African catfish strains and reference sequence *I. punctatus*

No	*Nucleotide position and corresponding nucleotide residue*	*I. punctatus (reference sequence)*	*Dumbo*	*Nucleotide position and corresponding nucleotide residue*	*I. punctatus (reference sequence)*	*Sangkuriang*	*Nucleotide position and correspondin g nucleotide residue*	*I. punctatus (reference sequence)*	*Masamo*	*Nucleotide position and correspondin g nucleotide residue*	*I. punctatus (reference sequence)*	*Paiton*	*Nucleotide position and corresponding nucleotide residue*	*I. punctatus (reference sequence)*	*Egypt*
1	5530	C	T	5530	C	T	5530	C	T	5530	C	T	5530	C	T
2	5533	G	C	5535	G	G	5535	G	G	5535	G	G	5535	G	G
3	5535	G	A	5544	T	G	5544	T	G	5544	T	G	5544	T	G
4	5544	T	G	5547	T	A	5547	T	G	5547	T	A	5547	T	G
5	5545	G	A	5548	G	A	5548	G	A	5548	G	A	5548	G	A
6	5547	T	C	5551	T	A	5551	T	A	5551	T	A	5551	T	A
7	5548	G	A	5553	A	G	5553	A	G	5553	A	G	5553	A	G
8	5550	A	T	5577	C	G	5577	C	G	5577	C	G	5577	C	G
9	5553	A	G	5679	T	C	5587	A	C	5587	A	C	5587	A	C
10	5574	T	A	5587	A	C	5614	A	G	5614	A	G	5614	A	G
11	5587	A	C	5614	A	G	5616	C	T	5616	C	T	5616	C	T
12	5593	G	A	5616	C	T	5631	A	C	5631	A	C	5631	A	C
13	5614	A	G	5631	A	C	5632	G	A	5632	G	A	5632	G	A
14	5616	C	T	5632	G	A	5634	A	T	5634	A	T	5634	A	T
15	5628	G	T	5634	A	T	5640	C	G	5640	C	G	5640	C	G
16	5630	A	G	5640	C	G	5641	G	A	5641	G	A	5641	G	A
17	5631	A	C	5641	G	A	5645	A	T	5645	A	T	5645	A	T
18	5634	A	T	5645	A	T	5647	G	A	5647	G	A	5647	G	A
19	5647	G	A	5647	G	A	5662	G	A	5662	G	A	5662	G	A
20	5658	C	A	5662	G	A	5663	C	A	5663	C	A	5663	C	A
21	5662	G	A	5663	C	A	5664	A	G	5664	A	G	5664	A	G
22	5663	C	A	5664	A	G	5670	C	T	5670	C	T	5670	C	T
23	5670	C	T	5670	C	T	5673	C	G	5673	C	G	5673	C	G
24	5671	G	A	5673	C	G	5676	A	G	5676	A	G	5676	A	G
25	5673	C	G	5676	A	G	5677	A	G	5677	A	G	5677	A	G
26	5677	A	G	5677	A	G	5683	C	T	5683	C	T	5683	C	T
27	5683	C	T	5683	C	T	5684	T	A	5684	T	A	5684	T	A
28	5684	T	A	5684	T	A	5693	T	C	5693	T	C	5693	T	C
29	5693	T	C	5693	T	C	5694	T	A	5694	T	A	5694	T	A
30	5694	T	A	5694	T	A	5707	A	G	5707	A	G	5707	A	G
31	5706	G	C	5707	A	G	5710	A	G	5710	A	G	5710	A	G
32	5710	A	G	5710	A	G	5715	A	T	5715	A	T	5715	A	T

33	5715	A	T	5715	A	T	5721	A	T	5721	A	T	5721	A	T
34	5721	A	T	5721	A	T	5724	C	T	5724	C	T	5724	C	T
35	5724	C	T	5724	C	T	5729	A	C	5729	A	C	5729	A	C
36	5729	A	C	5729	A	C	5732	G	A	5732	G	A	5732	G	A
37	5732	G	A	5732	G	A	5747	G	C	5734	G	A	5734	G	A
38	5747	G	C	5747	G	C	5752	C	A	5747	G	C	5747	G	C
39	5752	C	A	5752	C	A	5753	T	A	5752	C	A	5752	C	A
40	5753	T	A	5753	T	A	5754	G	T	5753	T	A	5753	T	A
41	5754	G	T	5754	G	T	5773	A	G	5754	G	T	5754	G	T
42	5773	A	G	5773	A	G	5774	A	C	5773	A	G	5773	A	G
43	5774	A	C	5774	A	C	5779	T	C	5774	A	C	5774	A	C
44	5775	G	A	5779	T	C	5780	G	C	5779	T	C	5779	T	C
45	5779	T	C	5780	G	C	5790	C	A	5780	G	C	5780	G	C
46	5780	G	C	5790	C	A	5791	A	T	5790	C	A	5790	C	A
47	5790	C	A	5791	A	T	5792	T	A	5791	A	T	5791	A	T
48	5791	A	T	5792	T	A	5793	A	T	5792	T	A	5792	T	A
49	5792	T	A	5793	A	T	5794	T	C	5793	A	T	5793	A	T
50	5793	A	T	5794	T	C	5796	C	A	5794	T	C	5794	T	C
51	5794	T	C	5796	C	A	5797	A	G	5796	C	A	5796	C	A
52	5796	C	A	5797	A	G	5798	G	A	5797	A	G	5797	A	G
53	5797	A	G	5798	G	A				5798	G	A	5798	G	A
54	5798	G	A												
	Total SNPs	54 (18%)		Total SNPs	53 (17%)		Total SNPs	52 (17%)		Total SNPs	53 (17%)		Total SNPs	53 (17%)	