Turk. J. Fish.&Aquat. Sci. 19(10), 865-871
http://doi.org/10.4194/1303-2712-v19_10_06

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Similarity Analysis of the Immune-Related Genes from Chinese Sturgeon (*Acipenser sinensis*) and Dabry's Sturgeon (*Acipenser dabryanus*)

Dongdong Tang[1,2], Dan Deng[2], Shuhuan Zhang[3], Hanwen Yuan[2], Zhang Wenbing[1], Qiaoqing Xu[1,2,*], Qiwei Wei[3]

[1] Engineering Research Centre of Ecology and Agricultural Use of Wetland, Ministry of Education, Jingzhou 434020, China.
[2] Yangtze University, School of Animal Science, Jingzhou, 434020, China.
[3] Yangtze River Fisheries Research Institute, Key Lab of Freshwater Biodiversity Conservation Ministry of Agriculture Chinese Academy of Fishery Sciences, Wuhan 430223, China.

Article History
Received 17 April 2018
Accepted 18 October 2018
First Online 18 October 2018

Corresponding Author
Tel.: +86.716 8066289
E-mail: xuqiaoqing@163.com

Keywords
Chinese sturgeon
Dabry's sturgeon
Immune-related genes

Abstract

Immune-related genes are well-established positive modulators that regulate a variety of signaling pathways in cells and tissues. However, no information is available concerning the similarity of immune-related genes between Dabry's sturgeon (*Acipenser dabryanus*) and Chinese sturgeon (*Acipenser sinensis*). A previous study showed that Dabry's sturgeon is seldom affected by infectious diseases, while Chinese sturgeon frequently dies from a variety of diseases. In the present study, de novo sequencing was used to obtain abundant high-quality gene information and investigate differentially expressed genes between Dabry's sturgeon and Chinese sturgeon. Protein similarity analysis indicated that 96 immune-related genes with complete cDNA sequences were obtained from the transcriptome library of Chinese sturgeon and Dabry's sturgeon. The similarity of these immune-related genes was further classified into three groups: 100% (16 genes, 16.7%), 98–99.9% (55 genes, 57.3%), and 80–97% (25 genes, 26%). These immunity genes were further classified into five signaling pathway, including complement and coagulation cascade, the Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, JAK-STAT signaling pathway, and RIG-I-like receptor signaling pathway. Most immune-related genes studied here belong to the pathway of complement and coagulation. Collectively, the research demonstrated that similarity analysis of immune-related genes in signaling pathways might provide valuable theoretical resources to understanding the mechanism of disease susceptibility in Chinese sturgeon and Dabry's sturgeon.

Introduction

Sturgeons are one of the most ancient vertebrates, with over 200 million years of history, leading to a vital evolutionary position from invertebrates to vertebrates (Wang 2013). Chinese sturgeon (*Acipenser sinensis*) and Dabry's sturgeon (*Acipenser dabryanus*) are both first rank protected aquatic wildlife in China and are considered as precious animals because the sturgeon population has declined sharply in recent years (Gao *et al.* 2017; Li *et al.* 2016). Chinese sturgeon is a migratory fish species that matures in coastal waters and enters into rivers for spawning. This species was once widely distributed in almost all watersheds draining towards the western coasts of the Pacific Ocean; by contrast, Dabry's sturgeon is restricted to the freshwater of the Yangtze River (Li *et al.* 2016). Among the different sturgeon species, Chinese sturgeon, termed a living fossil in aquatic biology because it maintains certain

evolutionary characteristics between chondrichthyes and osteichthyes, and has an important academic value in the study of the evolutionary history of fish (Liao *et al.* 2014). A mitochondrial DNA study showed that Chinese sturgeon was genetically closest to Dabry's sturgeon (Zhang *et al.* 1999). Thus, Dabry's sturgeon was considered a land seal species of Chinese sturgeon. However, there is little research concerning the similarity of immune-related genes between Dabry's sturgeon and Chinese sturgeon.

Research in sturgeons has mainly focused on sturgeon culture, including spawning and hatching (Clouthier *et al.* 2013; Du *et al.* 2016; Vicenova *et al.* 2011; Wang *et al.* 2017). With the rapid expansion of sturgeon farming, emerging infectious diseases represent a main limiting factor in sturgeon farming. For both Chinese sturgeon and Dabry's sturgeon, there is an urgent need to understand their ability to mount a specific immune response against pathogens. Meanwhile, to date, around 10 specific viruses have been found in sturgeons, including adenoviruses, papovaviruses, iridoviruses, and herpesviruses (Shchelkunov *et al.* 2009). Iridoviruses can impair growth and cause serious losses in sturgeons (Drennan *et al.* 2007). Herpesviruses are complex DNA viruses that have evolved intimately with their hosts; capturing, duplicating, and modifying host genes to aid their evasion of the host immune response (Tomofumi *et al.* 2011). A recent phylogenetic study showed that the viruses WSIV, MRSIV, and SNSV are only distantly related to the Iridoviridae, and are included in a group referred to as sturgeon nucleo-cytoplasmic large DNA viruses (NCLDVs) in the order Megavirales, a new term that has not been formally adopted by the International Committee of the Taxonomy of Viruses (Doszpoly *et al.* 2011). The pathogen *Pseudomonas alcaligenes* and several kinds of Mycobacterium have been identified in Chinese sturgeons (Xu *et al.* 2015; Zhang *et al.* 2014; Zhang *et al.* 2017). Moreover, reovirus particles were observed in sick Chinese sturgeons, suggesting the existence of primary viral illness (Zhang & Gui 2012). Systematic studies on sturgeon diseases have not yet been carried out, and reports of diseases of Dabry's sturgeon are rare. Therefore, research into their immunity-related genes would be of benefit to prevent diseases in sturgeons. De novo transcriptome sequencing has become an important method to study immunity genes (Fan *et al.* 2015). Transcriptome sequencing facilitates functional genomic studies, including global gene expression and immunity gene function. In the present study, the spleen transcriptomes of Chinese sturgeon and Dabry's sturgeon were sequenced using high-throughput sequencing, and a comparative analysis of the transcriptome data was performed. Determining the differences in immune-related gene regulation in the defense against pathogens in Chinese sturgeon and Dabry's sturgeon might serve as a valuable reference to understanding disease mechanisms in Chinese sturgeon and Dabry's sturgeon

Materials and Methods

Animals

Dabry's sturgeons (100–150 g) and Chinese sturgeons (80–90 g) were offered from the Yangtze River Fisheries Research Institute (Jingzhou, Tai Lake, China). These fish were at eight months, and were maintained with a flow-through water system for two weeks. For the A. hydrophila challenge, three healthy Dabry's sturgeons and Chinese sturgeons were challenged with an equal volume of phosphate buffered saline (PBS). At 24 h post infection, whole Dabry's sturgeons and Chinese sturgeons were sacrificed and their spleen tissues were preserved separately in RNAlater (Invitrogen, USA) at −80 °C for total RNA extraction. The experiments were conducted according to the guidelines of the China Council for Animal Care.

Establishing cDNA Library and Obtain A Transcriptome Library

Total RNA from spleen tissue of three Dabry's sturgeons and Chinese sturgeons was extracted, respectively. using the Trizol reagent (Invitrogen, USA) according to the manufacturers instructions. Total RNA was digested using DNase I (NEB, USA) to remove genomic DNA contamination, purified by poly-dT oligo-attached magnetic beads The cDNA library was used for sequencing, which was performed on the Illumina XTen platform in the paired-end read module (Sangon Biotech Co., Ltd, Shanghai, China). The spleen transcriptome library was assembled using the assembly software Trinity.

Bioinformatics Analysis

The immunity-related gene sequences from the transcriptome library were translated into amid acid sequences using translation program in ExPASy (http://web.expasy.org/translate/). Alignment of Chinese sturgeon and Dabry's sturgeon deduced proteins was performed using the Clustalw program (http://www.ebi.Uk/clustalw/). The protein sequences were aligned using the Clustal X 1.83 program in the DNAstar software. Finally, these immune-related genes were classified into several signaling pathway.

Results and Discussion

Protein Identity Analysis

The analysis of the main similarities between immune-related proteins from Chinese sturgeon and

Dabry's sturgeon are described below. Ninety-six immune-related genes were identified from the comparative transcriptome libraries between Chinese sturgeon and Dabry's sturgeon. The similarity of these immune-related genes between the two fishes was further divided into three groups: 100%, 98–99.9%, and 80–97% (Tables 1–3).

Immunity Gene Pathway Analysis

The numbers of full-length immune-related genes in the three groups were 16 (16.7%), 55 (57.3%), and 25 (26%), respectively (Figure 1). Among these genes, 76 % the deduced proteins shared high similarity between Chinese sturgeon and Dabry's sturgeon. These immune-related proteins were mostly distributed in five signaling pathways. The highest number of immune-related proteins were associated with the complement and coagulation pathway, followed by the Toll-like receptor signaling pathway, the Nucleotide-binding oligomerization domain(NOD)-like receptor signaling pathway, the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) signaling pathway, and the retinoic acid-inducible gene 1 (RIG-I)-like receptor signaling pathway.

However, these results were compared with whole transcriptome reported by (Zhu *et al.* 2016), in which some immune-related genes were found, such as interleukin (IL) 3–5, C-type lectin domain containing 7A (CLEC7), caspase recruitment domain family member 8 (CARD8) and tripartite motif containing 29 (TRIM29). CARD8, IL2, and IL3 were detected in transcriptome library of Chinese sturgeon, while CARD11, IL1, IL2, IL3, and IL5 were detected in the transcriptome library of Dabry's sturgeon. Interleukin-1-beta is an important pro-inflammatory cytokine that can mediate immune regulation in both the innate and adaptive immune defense mechanisms (Modanloo *et al.* 2017). Thus, IL1 genes in Dabry's sturgeon would directly enhance the immune response compared with Chinese sturgeon. Compared to the immune genes of the *Acipenser sinensis*, superfluous immunity immune genes had an important impact on increasing immunological responses to the invasion of pathogens for Dabry's sturgeon.

The present study revealed that 25 immune genes that had low similarity and had a remarkable effect between the two fishes. Sturgeons display innate immunity and adaptive immunity, with the innate immunity system responding before adaptive immunity (Rauta *et al.* 2012). The innate immunity system comprises signaling as the first line of defense against invasive pathogens. We performed signal pathway analysis to compare the immune-related gene difference in response to infection. Many signaling pathways associated with the immune response have been reported in numerous species, including the Toll-like receptor (TLR), chemokines, NOD, and antigen processing and presentation signaling (Zhang *et al.* 2018). Three signaling pathway are associated with immune-related gens that showed remarkable differences in similarity between Chinese sturgeon and Dabry's sturgeon, JAK-STAT signaling pathway (suppressor of cytokine signalling (SOCS)5, SOCS3), the TLR signaling pathway (nuclear factor kappa B subunit 2 (NF-k Bp100) and mitogen activated protein kinase 1 (MAPK1)) and RIG-I-like receptor signaling pathway (CARD14, CARD10) have been extensively analyzed.

The JAK-STAT signaling pathway plays a central role in transducing stress and growth signals in the

Table 1. Immune-related genes between *Acipenser sinensis* and *A. dabryanus* showing 100% similarity at the protein level

Gene name	Description	Gene name	Description
Complement and coagulation cascade		JAK-STAT signaling pathway	
C3	Complement C3 like	PIAS2	E3 SUMO-protein ligase PIAS
F8	Coagulation factor	PIAS4	E3 SUMO-protein ligase PIAS
MASP1	Mannan-binding lectin serine protease	SOCS4	Suppressor of cytokine signaling
Toll-like receptor signaling pathway		RIG-I-like receptor signaling pathway	
TLR13	Toll-like receptor 13	ATG5	Ubiquitin-like modifier activating enzyme
TLR3	Toll-like receptor 3	Chemokine	
TOLLIP	Toll-interacting protein FAS-associated	CXCL14	C-X-C motif chemokine 14
Other immune molecules		EDAR	Tumor necrosis factor receptor superfamily member EDAR
Lysozyme C	Lysozyme	TNFRSF6	Tumor necrosis factor receptor superfamily member
HSP90	Molecular chaperone HtpG		
RFX1	MHC class II regulatory factor		

Table 2. Immune-related genes between *Acipenser sinensis* and *A. dabryanus* showing 98–98.9% similarity at the protein level

Gene name	Description	Similarity (%)	Gene name	Description	Similarity (%)
Complement and coagulation Pathway			Toll-like receptor signaling pathway		
F5	Coagulation factor	99.8	TLR2	Toll-like receptor 2	99.4
F4	Coagulation factor	99.1	AP-1	Activator protein 1	99.7
F10	Coagulation factor	99.5	JAK-STAT signaling pathway		
F13	Coagulation factor	98.9	SOCS2	Suppressor of cytokine signaling	99.6
C3AR	C3a anaphylatoxin chemotactic receptor	98.5	SOCS4	Suppressor of cytokine signaling	99.5
C5AR	C5a anaphylatoxin chemotactic receptor	98.1	STAT1	Signal transducer and transcription Activator	99.7
CFD	Complement factor	98.8	JAK1	Janus kinase	99.7
C5	Complement C5-like	99.4	Chemokine		
Vwf	Von willedbrand factor	99.3	TNFAIP3	Tumor necrosis factor alpha-induced protein	99.8
C1(q,r,s)C6	Complement component	98.5	CXCR4	CXC-chemokine receptor	99.7
C1(q,r,s)C7	Complement component	99.4	CXCR1	CXC-chemokine receptor	99.2
C1(q,r,s)C8	Complement component	99.5	XCR1	Chemokine XC receptor1	99.3
Other pattern receptor signaling pathway			TNIP1	TNFAIP-interacting3 protein	99
CLEC19	C-Type lectin domain family member	99.5	TNFRSF1	Tumor necrosis factor receptor superfamily member	98.7
CLEC18	C-Type lectin domain family member	99.1	TNFRSF11	Tumor necrosis factor receptor superfamily member	98.3
CLEC4	C-Type lectin domain family member	98.4	NOD-like receptor signaling Pathway		
SCARB1	Scavenger receptor	98.8	IPAF	NLR family CARD domain-containing protein 4	99.5
SCARA5	Scavenger receptor	98.3	TRAF1	TNF receptor-associated factor	99.5
SCARF2	Scavenger receptor	99.4	TRAF2	TNF receptor-associated factor	98.4
SCARF1	Scavenger receptor	99.3	CIITA	MHC class II transactivator	98.6
SCARA3	Scavenger receptor	98.2	NOD2	Nucleotide-binding oligomerization domain containing protein	99.4
RIG-I-like receptor signaling pathway					
STING	Stimulator of interferon genes protein	98.7	cGAS	Cyclic GMP-AMP synthetase	98.3
RIP1	Receptor-interacting protein 1	98.6	LRRFIP1	Leucine-rich repeat (in Flightless I) interacting	98.9
FADD	FAS-associated death domain protein	99	CARD9	Caspase recruitment domain-containing protein	99.1
B-cell receptor signaling pathway			IRF1	Interferon regulatory factor	99.7
BLNK	B-cell linker protein	98.2	IRF3	Interferon regulatory factor	98.6
IGSF3	immunoglobulin superfamily member	99.3	Other immune molecules		
IGSF5	immunoglobulin superfamily member	98.8	P53	Tumor suppressor p53	98.3
IGSF8	immunoglobulin superfamily member	99.5	MHCII	Mannose receptor	99.5
T-cell receptor signaling pathway			TF	Transferrin	99.6
PI3K	Phosphatidylinositol 3-kinase 3	99.8	Antigen processing and presentation		
IRF5	Interferon regulatory factor	99.4	GILT	Interferon gamma-inducible protein 30	98.9
IRF2	Interferon regulatory factor	98.8	CALR	Calreticulin	99.8

hypertrophic heart (Truong *et al.* 2017). The signaling pathway formed by these ligands and their IL6-a/gp130 receptor plays an important role in biology and has long been representative of the JAK-STAT pathway itself. However, the JAK-STAT signaling paradigm is representative of only a portion of the signaling pathways that use JAK and STAT proteins to transmit extracellular signals (Wagner and Siddiqui 2012). The SOCS family can trigger inverse regulation of cytokines and mediates hormone signaling through the JAK signaling pathway (Liu *et al.* 2016). Previous research showed that SOCS5 gene is expressed in tissues of

Table 3. Immune-related genes between *Acipenser sinensis* and *A. dabryanus* showing 80–89% similarity at the protein level

Gene name	Description	Similarity (%)	Gene name	Description	Similarity (%)
Toll-like receptor signaling pathway			Cytokines		
TPRF	TNF receptor-associated factor	97.9	TNFRSF13	Tumor necrosis factor receptor superfamily member	87.4
TLR2	Toll-like receptor 2	97.9	TNFRSF9	Tumor necrosis factor receptor superfamily member	96.9
TLR5	Toll-like receptor 5	97.4	JAKT-STAT signaling pathway		
MAPK1	Mitogen-activated protein kinase	93.7	SOCS3	Suppressor of cytokine	97.9
SARM	Sterile alpha and TIR motif-containing protein	97.4	SOCS5	Suppressor of cytokine	91.5
NFkBp105	Nuclear factor NF-kappa-b	97.5	Complement and coagulation pathway		
NFkBp100	Nuclear factor NF-kappa-b	89	CFB	Complement factor	96.2
TANK	TRAF family member associated NF-kappa-B activator	96.1	B-cell signaling pathway		
NOD-like receptor signaling pathway			IGSF6	immunoglobulin superfamily member	95
NOD1	Nucleotide-binding oligomerization domain containing protein	97.9	RIG-I-like receptor signaling pathway		
RIP2	Receptor-interacting serine/threonine-protein kinase	96.8	CARD10	Caspase recruitment domain-containing protein	87.1
Other immune molecules			CARD14	Caspase recruitment domain-containing protein	84.6
FcgRI	High affinity immunoglobulin gamma Fc receptor	97.4	Other pattern recognition signaling pathway		
Bax	Apoptosis regulator BAX	96.8	CLEC1	C-Type lectin domain family member	97
Cytokines			IRF2BP1	Interferon regulatory factor 2-binding Protein	97.2
TNFRSF25	Tumor necrosis factor receptor superfamily member	95.9	TFR	Transferrin receptor	96.9
TNFRSF16	Tumor necrosis factor receptor superfamily member	95.4			

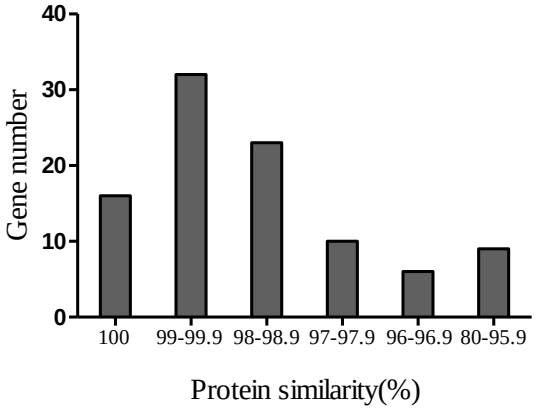

Figure 1. The number of genes with different similarities at the protein level between *Acipenser sinensis* and *A. dabryanus.*

Cynoglossus semilaevis (Hao and Sun 2016) and *Crassostrea gigas* (Li *et al.* 2015), with particularly abundant levels in the spleen and kidney, which are the major immune organs of teleosts. In this signaling pathway, SOCS5, whose gene expression varied greatly between Darby's sturgeon and Chinese sturgeon, may participate in the regulation of the immune response.

The TLR signaling pathway, a vital class of pattern recognition receptors, plays a key role in the innate immune response by recognizing antigens through inducing signaling transduction, which results in the expression of genes associated with antiviral responses, culminating in the activation of inducible transcriptional factors, such as nuclear factor kappa B (NF-κB) to increase the immune response (Wang *et al.* 2018; Zhao *et al.* 2015). NF-k Bp100 and MAPK1

showed obvious differences in protein similarity between Chinese sturgeon and Darby's sturgeon. In addition, they have different biological functions in the immune response. The differences in protein similarity observed between Darby's sturgeon and Chinese sturgeon might be in part caused differences in the fish species, life stage, and the type of immune-related genes present in each fish.

RIG-I-like receptors (RLRs) play crucial roles in virus recognition and antiviral innate immunity in teleosts (Shen *et al.* 2016). The RLR signaling pathway is closely associated with the regulation of the immune response (Han *et al.* 2016). CARD14 is a conserved protein that mediates TNF receptor associated factor 2 (TRAF2)-dependent activation of NF-kB signaling, which enhances the production of certain chemokines and strengthens the function of keratinocytes (Berki *et al.* 2015; Jordan *et al.* 2012). CARD10 controls signal transduction, and shows changes in its expression in response to illness, CARD10 and CARD14 in signaling pathway show significant differential function between the two fishes. It has been suggested that different immune related genes in the same signaling pathway might exert different effects on the immune functions of Darby's sturgeon and Chinese sturgeon; but the mechanisms involved remain to be clarified.

Taken together, the analysis of pathways associated with immune-related genes in Darby's sturgeon and Chinese sturgeon suggest differences between these to fishes in terms of regulation of the immune response to defend against pathogens. Further study is required to identify the functions of the immune-related proteins to better understand their involvement in defense against pathogens in Darby's sturgeon and Chinese sturgeon.

Acknowledgment

This work research was financially supported by grants from the Key Lab of Freshwater Biodiversity Conservation, Ministry of Agriculture, China (LFBC0904) and from University student innovation and entrepreneurship programme of Yangtze University (2017083).

References

Berki DM, Liu L, Choon SE, David BA, Griffiths C, Navarini AA, Tan ES, Irvine AD, Ranki A, Ogo T, Petrof G, Mahil SK, Duckworth M, Allen MH, Vito P, Trembath RC, McGrath J, Smith CH, Capon F, & Barker JN. (2015). Activating CARD14 mutations are associated with generalized pustular psoriasis but rarely account for familial recurrence in Psoriasis vulgaris. Journal of Investigative Dermatology, 135, 2964-2970. http://dx.doi.org/10.1038/jid. 2015. 288.

Clouthier SC, VanWalleghem E, Copeland S, Klassen C, Hobbs G, Nielsen O, & Anderson ED. (2013). A new species of nucleo-cytoplasmic large DNA virus (NCLDV) associated with mortalities in Manitoba lake sturgeon *Acipenser fulvescens*. Diseases of Aquatic Organisms, 102, 195-209. http://dx.doi.org/10.3354/dao02548

Doszpoly A, Benkő M, Bovo G, LaPatra SE, & Harrach B.(2011). Comparative analysis of a conserved Gene block from the genome of the members of the genus *Ictalurivirus*. Intervirology, 54, 282-289. http://dx.doi.org/10.1159/000319430

Drennan JD, LaPatra SE, Swan CM, Ireland S, & Cain KD. (2007). Characterization of serum and mucosal antibody responses in white sturgeon (*Acipenser transmontanus* Richardson) following immunization with WSIV and a protein hapten antigen. Fish & Shellfish Immunology, 23, 657-669. http://dx.doi.org/10.1016/j.fsi.2007.01.016

Du, H., Zhang, X., Leng, X., Shuhuan, Zhang J., Liu Z, Qiao X, Kynard B, & Wei Q. (2016). Gender and gonadal maturity stage identification of captive Chinese sturgeon, *Acipenser sinensis*, using ultrasound imagery and sex steroids. General and Comparative Endocrinology, 27(5), 36-43. http://dx.doi.org /10.1016/j.ygcen.2016.08.004

Fan Y, Chang M, Ma J, LaPatra S E, Hu Y, Huang L, Nie P, & Zeng L. (2015). Transcriptomic analysis of the host response to an iridovirus infection in Chinese giant salamander, *Andrias davidianus*. Veterinary Research, 46(1), 136-151. http://dx.doi.org/10.1186/s13567-015-0279 -8

Gao X, Lin P, Li M, Duan Z, & Liu H. (2016). Impact of the Three Gorges Dam on the spawning stock and natural reproduction of Chinese sturgeon in Changjiang River, China. Chinese Journal of Oceanology and Limnology, 34, 894-901. http://dx.doi.org/10.1007/s00343 -016-5027-z

Han J, Xu G, & Xu T.(2016). The miiuy croaker microRNA transcriptome and microRNA regulation of RIG-I like receptor signaling pathway after poly (I:C) stimulation. Fish & Shellfish Immunology, 54, 419-426. http://dx.doi.org/10.1016/j.fsi.2016.04.126

Hao L, & Sun L. (2016). Comparative analysis of the expression patterns of eight suppressors of cytokine signaling in tongue sole, *Cynoglossus semilaevis*. Fish & Shellfish Immunology, 55, 595-601. http://dx.doi.org /10.1016/j.fsi.2016.06.034

Jordan CT, Cao L, Roberson EDO, Duan S, Helms CA, Nair RP, Duffin KC, Stuart PE, Goldgar D, Hayashi G, Olfson EH, Feng B, Pullinger CR, Kane JP, Wise CA, Goldbach-Mansky R, Lowes MA, Peddle L, Chandran V, Liao W, Rahman P, Krueger GG, Gladman D, Elder JT, Menter A, & Bowcock AM. (2012). Rare and Common Variants in CARD14, Encoding an Epidermal Regulator of NF-kappaB, in Psoriasis. The American Journal of Human Genetics, 90, 796-808. http://dx.doi.org/10.1016/j.ajhg.2012.03.013

Kurobe T, Kelley GO, Waltzek TB, & Hedrick RP.(2011). Revised Phylogenetic Relationships among Herpesviruses Isolated from Sturgeons. Journal of Aquatic Animal Health, 20, 96-102. http: //dx.doi.org /10.1577/H07-028.1

Li J, Liu D, Ma Q, Zhang X, Dai W, Chen Y, Liu Y, & Song Z.(2015). Discriminating Dabry's sturgeon (*Acipenser dabryanus*) and Chinese sturgeon (*A. sinensis*) based on DNA barcode and six nuclear markers. Hydrobiologia, 757(1), 185-196. http://dx.doi.org/10.1007/ s10750-015-2251-z

Li J, Zhang Y, Zhang Y, Liu Y, Xiang Z, Qu F, & Yu Z. (2015). Cloning and characterization of three suppressors of cytokine signaling (SOCS) genes from the Pacific oyster, *Crassostrea gigas*. Fish & Shellfish Immunology, 44, 525-532. http://dx.doi.org/10.1016/j.fsi. 2015.03. 022

Liu C, He A, Chen L, Limbu SM, Wang Y, Zhang M, & Du Z. (2016). Molecular characterization and immune response to lipopolysaccharide (LPS) of the suppressor of cytokine signaling (SOCS)-1, 2 and 3 genes in Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 50, 160-167. http: //dx.doi.org /10.1016/j.fsi.2016.01.027

Modanloo M, Soltanian S, Akhlaghi M, & Hoseinifar SH. (2017). The effects of single or combined administration of galactooligosaccharide and Pediococcus acidilactici on cutaneous mucus immune parameters, humoral immune responses and immune related genes expression in common carp (Cyprinus carpio) fingerlings. Fish & Shellfish Immunology, 70, 391-397. http://dx.doi.org/10.1016/j.fsi.2017.09.032

Rauta PR, Nayak B, & Das S. (2012). Immune system and immune responses in fish and their role in comparative immunity study: A model for higher organisms. Immunology Letters, 148, 23-33. http: //dx.doi.org/10.1016/j.imlet.2012.08.003

Shchelkunov IS, Shchelkunova TI, Shchelkunov AI, Kolbassova YP, Didenko LV, & Bykovsky AP. (2009). First detection of a viral agent causing disease in farmed sturgeon in Russia. Diseases of Aquatic Organisms, 86,193-203. http://dx.doi.org/10.3354 /dao 02124

Shen B, Hu Y, Zhang S, Zheng J, Zeng L, Zhang J, Zhu A, & Wu C. (2016). Molecular and characterization expression analyses of three RIG-I-like receptor signaling pathway genes (MDA5, LGP2 and MAVS) in *Larimichthys crocea*. Fish & Shellfish Immunology, 55, 535-549. http://dx.doi.org/10.1016/j.fsi. 2016.06. 032

Truong AD, Rengaraj D, Hong Y, Hoang CT, Hong YH, & Lillehoj HS. (2017). Analysis of JAK-STAT signaling pathway genes and their microRNAs in the intestinal mucosa of genetically disparate chicken lines induced with necrotic enteritis. Veterinary Immunology and Immunopathology, 187, 1-9. http:// dx.doi.org /10.1016/ j.vetimm. 2017. 03.001

Vicenova M, Reschova S, Pokorova D, Hulova J, & Vesely T. (2011). First detection of pike fry-like rhabdovirus in barbel and spring viraemia of carp virus in sturgeon and pike in aquaculture in the Czech Republic. Diseases of Aquatic Organisms, 95, 87-95. http: //dx.doi.org /10.3354/ dao02340

Wagner MA, & Siddiqui MA. (2012). The JAK-STAT pathway in hypertrophic stress signaling and genomic stress response. JAKSTAT, 1, 131-41. http://dx.doi.org/10.4161/jkst.20702

Wang M, Wang L, Jia Z, Yi Q, & Song L. (2018). The various components implied the diversified Toll-like receptor (TLR) signaling pathway in mollusk *Chlamys farreri*. Fish & Shellfish Immunology, 74, 205-212.http://dx.doi.org//10.1016/j.fsi.2017.12.064

Wang PF, Shen YX, Wang C, Hou J, Qian J, Yu Y, & Kong N.(2017). An improved habitat model to evaluate the impact of water conservancy projects on Chinese sturgeon (*Acipenser sinensis*) spawning sites in the Yangtze River, China. Ecological Engineering, 104, 165-176. http://dx.doi.org/10.1016 /j. ecoleng. 2017.03.021

Xu J, Zeng X, Jiang N, Zhou Y, & Zeng L. (2015). *Pseudomonas alcaligenes* infection and mortality in cultured Chinese sturgeon, *Acipenser sinensis*. Aquaculture, 446, 37-41. http://dx.doi. org/10.1016/j. aquaculture.2015.04.014

Yi YJ, Zhang SH, & Wang ZY. (2013). The bedform morphology of Chinese sturgeon spawning sites in the Yangtze River. International Journal of Sediment Research, 28(3), 421-429. http://dx.doi.org/10.1016 /S1001-6279(13)60052-9

Zhang DF, Li AH, & Gong XN. (2014). Research on the mycobacteriosis and its pathogen in sturgeons. Acta Hydrobiologica Sinica, 38(3), 495-502. http://dx.doi.org/10.7541/2014.70

Zhang M, Qiao G, Li Q, Xu D, Qi Z, Wang A, Xu M, & Huang J. (2018). Transcriptome analysis and discovery of genes involved in immune pathways from coelomocytes of *Onchidium struma* after bacterial challenge. Fish & Shellfish Immunology, 72, 528-543. http://dx.doi.org/ 10.1016 /j.fsi. 2017.11.024

Zhang QY, & Gui JF. (2012). Atlas of aquatic viruses and viral disease. Beijing: Science press, 256-260. 479pp

Zhang SH, Nie P, Shu SW, Du H, Foo KL, Yang YM, Shao C, Di J, Huang Jun, Zeng LB, & Wei QW. (2017). Mycobacteriosis in cultured F2 generation Chinese sturgeon (*Acipenser sinensis*) and relevant blood physiological and biochemical indices. Journal of FisherySciences of China, 24(1), 136-145 http: //dx.doi.org/10.3724/SP.J.1118.2017.16116

Zhang SM, Zhang YP, Deng XZ, Cheng YJ, Deng H, Wang DQ, Wei QW, Zhang YW, Lie N, & Wu QJ. (1999). Twelve kinds Acipenseriformes of fish mtDNA ND4L-ND4 gene Sequence variation and molecular phylogeny. Science, 29(6), 607-614. http://dx.doi.org/10.1007/ BF02879121

Zhao BS, Zhao L, Liao H, Cheng J, Lian SS, Li X, Huang XT, & Bao ZM. (2015). Mapping toll-like receptor signaling pathway genes of Zhikong scallop (*Chlamys farreri*) with Fish. Journal of Ocean University of China, 14, 1075-1081. http://dx.doi.org//10.1007/s11802 -015-2643-8

Zhu R, Du HJ, Li SY, Li YD, Ni H, Yu XJ, Yang YY, Fan YD, Jiang N, Zeng LB, & Wang XG. (2016). De novo annotation of the immune-enriched transcriptome provides insights into immune system genes of Chinese sturgeon (*Acipenser sinensis*). Fish & Shellfish Immunology, 55, 699-716. http://dx.doi.org/10.1016/j.fsi.2016.06.051