DExH/H-box RNA helicase genes are differentially expressed between males and females during the critical period of male sex differentiation in channel catfish

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ABSTRACT

DExH/H-box RNA helicases are motor proteins participating in nearly all aspects of cellular processes, especially in RNA metabolism. In this study, a total of 54 DExH/H-box RNA helicase genes including 37 DDX (DEAD-box) and 17 DHX (DEAH-box) genes were characterized in channel catfish (*Ictalurus punctatus*), and annotated through phylogenetic and syntenic analyses. All the catfish RNA helicases contained conserved helicase signature motifs, demonstrating that the RNA helicase gene family was highly conserved. Analysis of the relative rates of synonymous (dS) and nonsynonymous (dN) substitutions revealed that the RNA helicase genes were subjected to strong negative (purifying) selection. Meta-analysis was conducted to determine expression of the RNA helicase genes during the critical period (90–110 days post-fertilization, dpf) of male gonad differentiation. At 90 dpf, 24 RNA helicase genes were highly differentially expressed in the gonad tissues between the males and females; similarly, 24 and 18 RNA helicase genes were found highly differentially expressed in the gonad tissues between the males and females at 100 and 110 dpf, respectively ($p < 0.01$). In general, the vast majority of the RNA helicase genes (31) were expressed at higher levels in females than in males. In the male gonad, a set of 8 RNA helicases were expressed at a significantly higher level at 110 dpf than at 90 dpf. These findings suggested that RNA helicases may play important roles in sex development and differentiation in teleosts.

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1. Introduction

DExH/H-box RNA helicases are a group of motor proteins with important roles in regulating the reproductive-related genes by modulating mRNA structures (Schütz et al., 2010; Kotov et al., 2014). The majority of the RNA helicases are in the DEAD-box and DEAH-box families which are named after the single-letter designation of the amino acid sequence of motif II (Staley and Guthrie, 1998; Abdelhaleem et al., 2003). The core of these RNA helicases contains two tandem repeats of RecA-like globular domains connected by a flexible linker region. The N-terminal and C-terminal regions are designated as DEAD-domain and helicase domain, respectively (Cordin et al., 2006; Pyle, 2008). These domains participate in RNA binding activities involving ATP hydrolysis. Nine highly conserved motifs in DEAD-box proteins (Q, I, Ia, Ib, II, III, IV, V, and VI) and eight in DEAH-box proteins (I, Ia,

Ib, II, III, IV, V, and VI) are arranged sequentially and located at defined positions (Tanner et al., 2003; Tuteja and Tuteja, 2004a, 2004b). Generally, DExH/H-box RNA helicase gene families are large (Suthar et al., 2016), especially in plants. For instance, *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, and *Glycine max* have 161, 149, 136, and 213 different RNA helicase genes, respectively (Xu et al., 2013). Fewer genes were found in animal genomes. For instance, 58 RNA helicase genes were found in *Homo sapiens* (Umate et al., 2011), 54 in *Bos taurus* (Suthar et al., 2016), 32 in *Caenorhabditis elegans*, and 29 in *Drosophila melanogaster* (Boudet et al., 2001).

DExH/H-box RNA helicases function through the modulation of RNA structures, and participate in various biological processes including pre-mRNA splicing, ribosome biogenesis, nucleocytoplasmic transport, translation and RNA decay that ultimately regulate organelle gene expression for specific biological functions (Rocak and Linder, 2004). The interest in RNA helicases was further enhanced by their roles in development and reproductive processes (Owtrim, 2013). Of the RNA helicases, DDX4 (also known as *Vasa*) has been extensively studied; it is a germ cell marker in animals, reported to be involved in gametogenesis in *Drosophila* (Styhler et al., 1998; Tomancak et al., 1998) as well as

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Table 1

The channel catfish DEXD/H-box RNA helicase genes. Abbreviations for protein locations are: N, nucleus; M, mitochondria; C, cytoplasm.

Gene	Chromosome	CDS (bp)	Amino acids	Localization	Accession number
DDX1	3	2223	740	N	KX372721
DDX2A	8	1221	406	C,N	JT419373
DDX2B	8	1236	411	N	JT408181
DDX3X	12	1923	640	N	JT316448
DDX4	25	2016	671	N,C	KM874267.1
DDX5	12	1836	611	N	JT419440
DDX6	1	1437	478	N,M	JT418362
DDX10	13	2520	839	N	JT405947
DDX11	4	1875	624	N,C	KX372728
DDX13	12	3747	1248	C,N	JT407074
DDX17	12	1992	663	N,C	KX372722
DDX18	17	1977	658	N	JT417911
DDX19A	15	1440	479	C,N	JT410544
DDX19B	21	1440	479	C,N,M	JT414565
DDX20	11	2094	697	N,M	KX372723
DDX21	29	2298	765	C,N	KX372724
DDX23	15	2427	808	N	JT407143
DDX24	16	2679	892	N	JT418479
DDX27	7	2328	775	N	JT405367
DDX28	27	1698	565	M,C,N	JT408085
DDX31	26	2142	713	N	JT410639
DDX39A	12	1311	430	C,N	KX372725
DDX39B	12	1311	436	C,N	JT417326
DDX41	3	1845	614	N,C,M	JT405580
DDX42	3	2532	843	C,N	JT416942
DDX43	25	1968	655	N	KX372729
DDX46	13	2613	870	N,C	JT419149
DDX47	12	1410	469	N	KX372726
DDX48	20	1221	406	N	JT419460
DDX49	20	1434	477	N,C	JT407853
DDX51	11	1977	658	N	JT405402
DDX52	13	1842	613	C,N	JT415605
DDX54	14	2583	860	N	JT410739
DDX55	25	1809	602	N,C	JT407803
DDX56	25	1683	560	C,N	JT405896
DDX58	15	2814	937	C,M,N	JT412252
DDX59	20	1890	629	N,C	JT413136
DHX8	5	3609	1201	N	KX372718
DHX9	8	3579	1193	N,C	KX372719
DHX15	8	2313	770	N	JT405987
DHX16	22	3165	1054	N	JT413192
DHX29	25	4074	1357	C,N,M	JT415226
DHX30	8	3501	1166	N	JT414412
DHX32A	5	2232	743	N,C	JT409383
DHX32B	6	2202	733	C,N	JT415634
DHX33	14	2043	680	C,N,M	JT414119
DHX34	4	3351	1116	N	JT416192
DHX35	15	2079	692	N	JT412741
DHX36	4	2820	939	M,C	KX372730
DHX37	11	3453	1150	N	JT411419
DHX38	20	3813	1270	N	JT412543
DHX40	22	2214	737	C,N,M	KX372727
DHX57	6	3945	1314	N,C	KX372720
DHX58	3	2034	677	C,M	JT410933

fishes like zebrafish (Bárfai and Orbán, 2003), medaka (Shinomiya et al., 2000), and tilapia (Kobayashi et al., 2000). *DDX20* was essential for ovarian morphology in mice (Mouillet et al., 2008) and *DDX23* was required for the sperm-oocyte switch and differentiation of various cell types in *C. elegans* (Konishi et al., 2008). *DDX5* was highly expressed during spermatogenesis in *Cherax quadricarinatus* (Fang et al., 2011). A study about *DHX9* deletion mutant suggested its important functions on germline transcriptional control and proliferation in *C. elegans* (Walstrom et al., 2005). In addition, *DDX43*, *DDX51*, and *DDX55* were reported as male fertility and spermatogenesis related genes (He et al., 2012; Yousefi et al., 2015). In spite of the significant roles of the DEXD/H-box RNA helicases, they have not been systematically studied among teleost species, and their expression profiles during gonadogenesis in teleosts are unknown.

Channel catfish (*Ictalurus punctatus*) is a gonochoristic teleost with a XY sex-determining system (Davis et al., 1990), but the environment, especially temperature, can affect the sex ratios. It is a good model for studying the mechanisms of sex determination and differentiation because of the plasticity in sex determination (Zeng et al., 2016). The recent generation of its reference genome sequence (Liu et al., 2016), along with its gonadal transcriptome sequences generated from different developmental stages during male gonadal differentiation (Zeng et al., 2016), provided material basis for the analysis of the DEXD/H-box RNA helicase genes and their involvement in sex differentiation. In this study, we characterized a set of 54 DEXD/H-box RNA helicase genes from channel catfish and analyzed their expression patterns during the critical stages of male sex differentiation.

2. Materials and methods

2.1. Identification and analysis of RNA helicase genes

DEXD/H-box RNA helicase genes were identified in channel catfish by screening channel catfish transcriptome database (Liu et al., 2012) and whole genome database of channel catfish (Liu et al., 2016). The DDX and DHX proteins from teleost fishes (zebrafish, fugu, medaka, tilapia, platyfish, and stickleback), amphibian (*Xenopus*), reptile (turtle), bird (chicken), and mammals (human and mouse) were collected and used as query sequences to search against the RNA-Seq database using TBLASTN with E-value cutoff of e^{-10} . BLASTN program was applied to verify the retrieved cDNA sequences by aligning them with the whole genome database with E-value cutoff of e^{-10} . The open reading frames of retrieved sequences were identified by ORF Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) and verified by BLASTP against NCBI non-redundant protein database. Furthermore, Simple Modular Architecture Research Tool (SMART, <http://smart.embl-heidelberg.de>) was used to determine the conserved domains based on sequence homology. FGENESH program (Solovyev et al., 2006) was utilized to predict coding sequences from the genome assembly. Protein localization was predicted using WoLF PSORT (http://www.genscript.com/psort/wolf_psort.html).

2.2. Phylogenetic and syntenic analyses

All the amino acids of DDX and DHX from channel catfish and other selected species (zebrafish, fugu, medaka, tilapia, platyfish, stickleback, *Xenopus*, turtle, chicken, human and mouse) were used for phylogenetic analysis. Multiple protein sequences alignments were conducted by MUSCLE 3.8 (Edgar, 2004). The phylogenetic trees were constructed using the maximum likelihood method in MEGA6 with bootstrap test of 1000 replicates (Tamura et al., 2013). Jones-Taylor-Thornton (JTT) and gamma distributed rate with invariant sites (G + I) model were chosen based on the alignment results (Darriba et al., 2011).

In order to support the annotation DDX and DHX genes in channel catfish, syntenic analyses were conducted by examining the conserved co-localization of neighboring genes on reference genome sequences of channel catfish (Liu et al., 2016) and genomes of other selected species. Neighboring genes of channel catfish DDX and DHX genes were predicted by FGENESH (Solovyev et al., 2006) and BLASTP. Neighbor genes of DDX and DHX in other species were identified from the Ensembl and NCBI databases.

2.3. dN/dS analysis

In order to measure the selection pressures imposed on each fish DDX and DHX genes, we conducted the natural selection analysis based on the relative rates of synonymous (dS) and non-synonymous (dN) substitutions. The dN/dS ratios at each codon site of the different RNA helicase family members were calculated using Datamonkey

A

DDX	Q	I	Ia	Ib	II	III	IV	V	VI	Protein name
DDX1	EWLLPTDQ	AETGSGKT	PSKELAEQ	TPGR	DECD	SAT	IIF	ARGID	YVHRIGRVGRAE	RNA helicase DDX1
DDX2A	GFEKPSAIQ	AQSGTGKT	PTRELAQQ	TPGR	DEAD	SAT	VIF	ARGID	YIHRIGRGGRFG	RNA helicase eIF4A-1
DDX2B	GFEKPSAIQ	AQSGTGKT	PTRELAQQ	TPGR	DEAD	SAT	VIF	ARGID	YIHRIGRGGRFG	RNA helicase eIF4A-2
DDX3X	RYTRPTPVQ	AQSGTGKT	PTRELAQQ	TPGR	DEAD	SAT	LVF	ARGLD	YVHRIGRTGRVG	RNA helicase DDX3X
DDX4	GYVKPTPVQ	AQSGTGKT	PTRELINQ	TPGR	DEAD	SAT	MVF	ARGLD	YVHRIGRTGRVG	RNA helicase DDX4
DDX5	TWTEPTPIQ	AQSGTGKT	PTRELAQQ	TPGR	DEAD	SAT	IIF	SRGLD	YIHRIGRTARSQ	RNA helicase DDX5
DDX6	GWEKPSPIQ	AKNGTGKS	PTRELAQQ	TPGR	DEAD	SAT	IIF	TRGID	YLHRIGRSRGYQ	RNA helicase DDX6
DDX10	QYRQPTPIQ	AKTSGSGKT	PTRELAQQ	TPGR	DEAD	SAT	IVF	ARGID	YIHRVGRRTARYK	RNA helicase DDX10
DDX11	-	-	-	-	DEAH	-	-	-	MMEETGRVLSNL	RNA helicase DDX11
DDX13	-	AHTSAGKT	PIKALSNO	-	DEVH	-	-	-	YIQMAGRAGRRG	Helicase SKI2W
DDX17	NFEKPTAIQ	AQSGTGKT	PTRELAQQ	TPGR	DEAD	SAT	IIF	SRGLD	YVHRIGRTARST	RNA helicase DDX17
DDX18	GFENMTIEQ	AKTSGSGKT	PTRELAQQ	TPGR	DEAD	SAT	MVF	ARGLD	YIHRVGRRTARFI	RNA helicase DDX18
DDX19A	GFNRPSKIQ	QSQGTGKT	PTYELAIQ	TPGT	DEAD	SAT	MIF	ARGID	YLHRIGRTGRFG	RNA helicase DDX19A
DDX19B	GFNRPSKIQ	QSQGTGKT	PTYELAIQ	TPGT	DEAD	SAT	MIF	ARGID	YLHRIGRTGRFG	RNA helicase DDX19B
DDX20	GFQRPSPIQ	AKSGTGKT	PTREIAVQ	SPGR	DEAD	-	LVF	SRGID	YMHRIGRAGRFG	RNA helicase DDX20
DDX21	GVTHLFDIQ	ARTGTGKT	PTRELAQQ	TPGR	DEVQ	SAT	IVF	ARGLD	YIHRIGRTGRAG	RNA helicase DDX21
DDX23	GYKDPPTPIQ	AETGSGKT	PTRELAQQ	TPGR	DEAD	TAT	IIF	GRGID	YIHRIGRTGRAG	RNA helicase DDX23
DDX24	GFSAPTPIQ	AETGSGKT	PTRELAQQ	TPGR	DEAD	SAT	MVF	ARGLD	YVHRSGRTARAA	RNA helicase DDX24
DDX27	GFQOPTPIQ	AATGTGKT	PTRELGIQ	TPGR	DEAD	SAT	MLF	ARGLD	YVHRVGRRTARAG	RNA helicase DDX27
DDX28	NTHPTPIQ	AETGSGKT	PSRELAQQ	TPGA	DEAD	GAT	LVF	SRGLD	YIHRAGRVGRVG	RNA helicase DDX28
DDX31	NISSMTSVQ	QSQGTGKT	PTRELAQQ	TPGR	DEAD	SAT	IVF	ARGLD	YVHRVGRRTARIG	RNA helicase DDX31
DDX39A	GFEHPSEVQ	AKSGMGKT	HTRELAQQ	TPGR	DECD	SAT	VIF	GRGMD	YLHRVARAGRFG	RNA helicase DDX39A
DDX39B	GFEHPSEVQ	AKSGMGKT	HTRELAQQ	TPGR	DECD	SAT	VIF	GRGMD	YLHRVARAGRFG	RNA helicase DDX39B
DDX41	GIVHPTPIQ	AFTGSGKT	PSRELAQQ	TPGR	DEAD	SAT	LIF	SKGLD	YVHRIGRTGRSG	RNA helicase DDX41
DDX42	EYTOPTPIQ	AKTSGSGKT	PTRELCOQ	TPGR	DEAD	SAT	LIF	ARGLD	HTHRIGRTGRAG	RNA helicase DDX42
DDX43	GFQKPTPIQ	AQGTGTGKT	PTRELAQQ	TPGR	DEAD	SAT	LIF	ARGLD	YVHRVGRRTGRAG	RNA helicase DDX43
DDX46	NYEKPTPIQ	AKTSGSGKT	PTRELAQQ	TPGR	DEAD	SAT	IIF	ARGLD	YVHRAGRTGRAG	RNA helicase DDX46
DDX47	GWKSPKTIQ	AETGSGKT	PTRELAQQ	TPGR	DEAD	SAT	MVF	SRGLD	YIHRVGRRTARAG	RNA helicase DDX47
DDX48	GFEKPSAIQ	QSQGTGKT	PTRELAQQ	TPGR	DEAD	SAT	VIF	ARGLD	YIHRIGRSRGYQ	RNA helicase eIF4A-3
DDX49	GITRPTPVQ	AKTSGSGKT	PTRELAQQ	TPGR	DEAD	SAT	MVF	ARGLD	YIHRVGRRTARAG	RNA helicase DDX49
DDX51	GIQNFFPVQ	APTGSAGKT	PTRELAQQ	TPGR	DEAD	SAT	LCF	ARGID	YIHRVGRRTARAG	RNA helicase DDX51
DDX52	GFQTPPTPIQ	APTGSAGKT	PTRELAQQ	TPNR	DESD	SAT	LVF	ARGID	YIHRIGRTGRAG	RNA helicase DDX52
DDX54	GYNVPTPIQ	AKTSGSGKT	PTRELAQQ	TPGR	DEAD	SAT	VVF	ARGID	FLHRVGRVARAG	RNA helicase DDX54
DDX55	GFYMTPVQ	AVTGSAGKT	PTRELAQQ	TPGR	DEAD	SAT	LVF	ARGID	FVHRGCRGTARIG	RNA helicase DDX55
DDX56	GWAQPTLIQ	ARTGSAGKT	PTRELGGQ	TPSR	DEAD	SAT	LIF	SRGVD	YIHRVGRRTARAD	RNA helicase DDX56
DDX58	-	APTGCCKT	TKVEVYEQ	TPQI	DECH	RAL	VLF	DEGID	MQVVRGGRAGQ	RNA helicase DDX58
DDX59	GYEAPPTPIQ	ADTGSAGKT	PTRELAQQ	TPGR	DEAD	SAT	VVF	GRGLD	YVHQIGRAGRLG	RNA helicase DDX59

B

DHX	I	Ia	Ib	II	III	IV	V	VI	Protein name
DHX8	GETGSGKTT	TQPRRV	TDGMLLR	DEAH	SAT	FLTG	TNIAET	QRAGRAGR	RNA helicase DHX8
DHX9	GATGCGKTT	TQPRRI	TVGVLLR	DEIH	SAT	FLPG	TNIAET	QRKGRAGR	RNA helicase A
DHX15	GETGSGKTT	TQPRRV	TDGMLLR	DEAH	SAT	FLTG	TNIAET	QRAGRAGR	RNA helicase DHX15
DHX16	GETGSGKTT	TQPRRV	TDGMLLR	DEAH	SAT	FLTG	TNIAET	QRAGRAGR	RNA helicase DHX16
DHX29	GETGSGKST	TQPRRI	TTGILLR	DEVH	SAT	FLPG	TNIAET	QRQGRAGR	RNA helicase DHX29
DHX30	GETGCGKTT	TQPRRI	TVGVLLR	DEVH	SAS	FLPG	TNIAET	QRRGRAGR	RNA helicase DHX30
DHX32A	GTTKTGKSS	-	TDDVLLR	DQAH	-	FLAS	-	-	RNA helicase DHX32A
DHX32B	GTAKTGQST	TQIRRR	TDHMLLS	DQAA	-	FLAT	-	-	RNA helicase DHX32B
DHX33	GETGSGKTT	TQPRRV	TDGMLLR	DEAH	SAT	FMTG	TNIAET	QRAGRAGR	RNA helicase DHX33
DHX34	GDTGCGKST	TQPRRI	TEGLLLK	DEVH	SAT	FLSG	TNIAET	QRKGRAGR	RNA helicase DHX34
DHX35	GETGSGKST	TQPRRV	TDGMLVR	DEAH	SAT	FLTG	TNIAET	QRAGRGRG	RNA helicase DHX35
DHX36	GETGCGKTT	TQPRRI	TTGILLQ	DEIH	SAT	FLPG	TNIAET	QRKGRAGR	RNA helicase DHX36
DHX37	GETGSGKTT	TEPRRV	TDGVLLK	DEAH	SAT	FLTG	TNIAET	QRAGRAGR	RNA helicase DHX37
DHX38	GETGSGKTT	TQPRRV	TDGILLR	DEAH	SAT	FMPG	TNIAET	QRAGRAGR	RNA helicase DHX38
DHX40	GETGSGKTT	TQPRRV	TDGCMRLR	DEVH	SAT	FLTG	TNIAET	QRAGRAGR	RNA helicase DHX40
DHX57	GMTGCGKTT	TQPRRI	TTGVLLR	DEVH	SAT	FLPG	TNIAET	QRRGRAGR	RNA helicase DHX57
DHX58	LPTGGGKTR	THPNGK	TAQILEN	DECH	-	FLTG	TSVAEE	-	RNA helicase DHX58

Fig. 1. The amino acid sequence of conserved motifs (Q, I, Ia, Ib, II, III, IV, V and VI) constituting the RNA helicases of channel catfish DDX (A) and DHX (B) proteins.

(Delpont et al., 2010). Only family member that has sequences originated from at least two species were selected.

2.4. Expression analysis in channel catfish gonads

RNA-Seq datasets of male and female gonads of channel catfish were generated from samples collected at 90, 100, and 110 days post-fertilization (dpf) (NCBI accession no. SRP067841). Expression profiles of *DDX* and *DHX* genes were determined using these RNA-Seq datasets. Raw reads were trimmed and then mapped onto the channel catfish transcripts using CLC Genomics Workbench software (version 5.5.2; CLC bio, Aarhus, Denmark). Mapping parameters were set as $\geq 95\%$ of the reads in perfect alignment and ≤ 2 mismatches. The total mapped reads number for each transcript was determined and normalized to analyze RPKM (Reads Per Kilobase of exon model per Million mapped reads). Differentially expressed genes (DEGs) were determined based on proportions-based Kal's test with an absolute proportion fold change ≥ 2.0 and False Discovery Rate (FDR) corrected p values < 0.01 .

2.5. Validation of differentially expressed genes by RT-qPCR

We selected differentially expressed RNA helicase genes with a large fold change (> 8 fold) and FDR corrected p values < 0.01 for RT-qPCR validation. Primers of these genes were designed according to the

assembled transcripts via Primer-BLAST (NCBI primer designing tool; <http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (Supplemental Table S1). The RT-qPCR calculation of relative expression levels among three groups was conducted as described by Zeng et al. (2016): four potential endogenous reference genes—*18S*, *28S*, *gusb*, and *thp*—were tested for their expression stability. The calculated starting concentrations of these four genes were imported into NormFinder (v0.953) to rank the stability value and select the optimal endogenous reference genes. The relative expression ratio of a target gene was calculated by Pfaffl method (Pfaffl et al., 2002). The results of investigated genes were subsequently tested for significance by a randomization test. The qPCR was repeated in triplicate runs to confirm expression patterns. For comparison of male and female gonads at 90 days post-fertilization (dpf), *thp* and *18S* were selected as normalization genes; for comparison of male and female gonads at 100 and 110 dpf, *18S* and *gusb* were selected as normalization genes; for comparison of male gonads between 90 and 110 dpf, *gusb* was selected as the reference gene (Zeng et al., 2016).

3. Results

3.1. RNA helicase genes in channel catfish

A total of 54 DEXD/H-box RNA helicase genes were identified in the channel catfish genome. Among them, 37 genes belonged to DDX family

and 17 to DHX family. Characteristics of these genes, including their chromosomal locations, open reading frames and peptide sizes, predicted protein localization, and their GenBank accession numbers, are summarized in Table 1. These genes are located on a large number of chromosomes (21 out of a total of 29 chromosomes). Most of the RNA helicase genes are dispersed if they are on the same chromosome, but *DDX39A* and *DDX39B* are clustered together, so are *DDX4* and *DDX43*. The lengths of the predicted RNA helicases ranged from 406 to 1357 amino acids.

3.2. Conserved motifs and domain structures

The motifs and domain structures were well conserved in evolution as well as among the catfish DEXD/H-box RNA helicase genes. As shown in Fig. 1A, nine motifs (Q, I, Ia, Ib, II, III, IV, V, and VI) were identified in the vast majority of the 37 *DDX* RNA helicases; similarly, as shown in Fig. 1B, the eight motifs (I, Ia, Ib, II, III, IV, V, and VI) were well conserved in the vast majority of the 17 *DHX* RNA helicases. However, several motifs were missing from *DDX11* (Q, I, Ia, Ib, III, IV, and V), *DDX13* (Q, Ib, III, IV, and V), *DDX20* (III), and *DDX58* (Q). Similarly, several motifs were missing from *DHX* subfamily of the RNA helicase genes such as *DHX32A* (motifs Ia, III, V, and VI), *DHX32B* (motifs III, V, and VI), and *DHX58* (motifs III and VI).

Main functional domains in 54 RNA helicase genes were found in channel catfish (Fig. 2). In *DDX* gene family, all the *DDX* genes possessed DEXDc and HELICc domain. DUF4217 domain was found in four *DDX* genes (*DDX10*, *DDX18*, *DDX31*, and *DDX55*). DBP10CT, Znf-C2HC, KH, and DSHCT domain were only found in *DDX54*, *DDX41*, *DDX43*, and *DDX13*, respectively (Fig. 2). In *DHX* gene family, all the *DHX* genes contained DEXDc and HELICc domain except *DHX32A* and *DHX32B* (Fig. 2). HA2 domain was observed in all the *DHX* genes. Two DSRM domains were found in *DHX9*, and Znf-C2HC domain was observed in

DHX34 and *DHX57*. PW1 and S1 domain were only found in *DHX16* and *DHX8*, respectively (Fig. 2).

3.3. Phylogenetic and syntenic analyses of channel catfish RNA helicase genes

Phylogenetic analysis was carried out to annotate channel catfish RNA helicases (Fig. 3A, B). In initial phylogenetic analysis, *DDX* and *DHX* genes were clustered into separate clades (data not shown). Therefore, separate phylogenetic trees were developed. In most cases, the phylogenetic analysis supported the annotation of the *DDX* and *DHX* genes based on sequence similarity (Fig. 3A, B), as they were placed in the clades compatible with phylogenetic expectations with the catfish genes being most closely related to those of zebrafish except four *DDX* genes (*DDX1*, *DDX2A*, *DDX6*, and *DDX11*) (Fig. 3A, B). For these four *DDX* genes, syntenic analyses were further conducted in order to provide additional evidence for annotation. As shown in Fig. 4, conserved syntenic blocks were identified, and the orthologies were established among *DDX1*, *DDX2A*, *DDX6*, and *DDX11* from channel catfish with those from other species, indicating proper annotations.

3.4. Gene copy number analysis of RNA helicase genes

The comparison of the copy numbers of RNA helicase genes in channel catfish and other selected vertebrates is summarized in Table 2. The total number of the DEXD/H-box RNA helicase genes were comparable among all organisms under analysis, ranging from teleost fish to amphibians, reptiles, birds, and mammals. The human DEXD/H-box RNA helicase family is comprised of 58 members including 42 *DDX* genes and 16 *DHX* genes (Umate et al., 2011). Channel catfish has almost all the orthologues of DEXD/H-box RNA helicase genes with exceptions: 1) It appeared that *DDX25* and *DDX60* were absent from the genomes of all analyzed fish species and *Xenopus*, but was present in turtle,

Fig. 2. Domain compositions of channel catfish *DDX* and *DHX* proteins. Domain analysis was conducted using Simple Modular Architecture Research Tool (SMART, <http://smart.embl-heidelberg.de>). The domain structures were download and used for figure generations.

chicken, and mammals; 2) *DDX50* was absent from the genomes of teleost fish, *Xenopus*, and turtle, but present in the genome of chicken, mouse and human; 3) *DDX53* was absent from all analyzed fish species as well as from the genomes of turtle, chicken and mouse, but was present in the genomes of *Xenopus* and human; 4) *DDX47* and *DDX58* were absent from all the analyzed fish species except in catfish and zebrafish; 5) *DDX32* was duplicated in all fish genomes but was a single-copy gene in all other major phylogenetic groups from amphibians to mammals (Table 2).

3.5. dN/dS analysis

The global and site-by-site dN/dS analyses for all of fish RNA helicase genes were conducted to delineate the evolution dynamics. The global ratios of dN/dS were well below 1.0 for all genes, a theoretical boundary for positive and negative selection (Table 3). Strong negative and rare positive selection were detected in fish DEXD/H-box RNA helicase genes (Table 3). Generally, the average dN/dS ratio of DDXs was smaller than that of DHXs. The fish *DDX20* exhibited the highest global dN/dS ratio, followed by the *DDX51*. A few positive selection sites ($p < 0.1$) were found in three *DHX* genes (*DHX36*, *DHX57*, and *DHX58*), while no positive selection sites in the *DDX* gene family (Table 3). No positive

selected amino acid sites were located at the nine motifs and main functional domains of RNA helicases.

3.6. Expression analysis of RNA helicase genes in channel catfish gonads

To determine the expression profiles of DEXD/H-box RNA helicase genes during critical period of male sex differentiation, expression of the RNA helicase genes was compared between males and females in the gonad at 90, 100, and 110 dpf. The most significant difference in expression levels of RNA helicase genes between male and female gonads was observed at 90 dpf ($p < 0.01$), followed by 100 and 110 dpf. A total of 24, 24, and 18 RNA helicase genes were highly differentially expressed in the gonad tissues between the males and females at 90 dpf, 100 dpf, and 110 dpf, respectively ($p < 0.01$; Table 4). Of these, 23 genes (17 *DDX* and 6 *DHX*) were expressed at higher levels in the female gonads than in the male gonads, while *DDX5* was expressed lower in the female gonads than in the male gonads at 90 dpf ($p < 0.01$; Table 4). At 100 dpf, 22 genes (16 *DDX* and 6 *DHX*) were expressed higher in the females than in the males, while *DDX21* and *DHX9* were expressed higher in the males than in the females ($p < 0.01$; Table 4). At 110 dpf, 17 genes (11 *DDX* and 6 *DHX*) were expressed higher in the females than in the males, while *DDX21* was expressed at a high

Fig. 3. Phylogenetic analyses of DDX (A) and DHX (B) gene families in selected fish species and mammals. DEAD and DEAH amino acid sequences were aligned with MUSLE 3.8, and phylogenetic trees were constructed using the maximum likelihood method in MEGA6 with bootstrap test of 1000 replicates. Dark dots indicated channel catfish RNA helicase genes. Number on each node represented bootstrap probability. The species names and accession numbers are provided in Supplemental Table S2.

B

Fig. 3 (continued).

level in males ($p < 0.01$; [Table 4](#)). For the most part, the vast majority of the RNA helicase genes (31) were expressed at higher levels in females than in males, with only three genes (*DDX5*, *DDX21*, and *DHX9*) being expressed higher in the male at one or more time points ([Table 4](#)).

Male gonads differentiation occurs between 90 and 110 days post-fertilization. To determine the transcriptome profiles of DExD/H-box RNA helicase genes during catfish male gonadal differentiation, their expressions in the testes at 100 dpf and 110 dpf were compared with that of 90 dpf. None of DExD/H-box RNA helicase genes were expressed at significantly different levels at 100 dpf as compared with those of the 90 dpf (Table 5). However, nine RNA helicase genes were differentially expressed between 90 and 110 dpf. The vast majority (8 out of 9) of these differentially expressed genes between 90 dpf and 110 dpf were up-regulated, but *DDX3X* was expressed lower at 110 dpf than 90 dpf (Table 5). Among the upregulated genes, *DDX4*, *DDX42*, and *DDX32A* were highly differentially expressed, with their expression 15–35 times higher at 110 dpf than at 90 dpf.

3.7. Validation by RT-qPCR

To validate the differentially expressed genes identified by RNA-Seq, we selected differentially expressed RNA helicase genes with a large

fold change (>8 fold) and FDR corrected p values <0.01 for RT-qPCR validation. For comparisons of expression between males and females at 90 dpf, eight genes were analyzed including *DDX4*, *DDX19A*, *DDX20*, *DDX42*, *DDX43*, *DDX51*, *DHX32A*, and *DHX32B* (Fig. 5A). Similarly, eight genes, *DDX3X*, *DDX4*, *DDX19A*, *DDX20*, *DDX42*, *DDX43*, *DDX51*, and *DHX32A*, were analyzed at 100 dpf (Fig. 5B); five genes were analyzed for the comparison between males and females at 110 dpf including *DDX4*, *DDX19A*, *DDX43*, *DDX51*, and *DHX32A* (Fig. 5C); and three genes, *DDX4*, *DDX42*, and *DHX32A*, were analyzed to compare the expression in the male between 90 dpf and 110 dpf (Fig. 5D). The results of RT-qPCR were consistent with those of RNA-Seq analyses. However, in most cases, the fold induction or repression was larger when analyzed with RT-qPCR than with RNA-Seq, but opposite patterns were observed with several genes such as *DDX19A*, *DDX20*, *DDX42*, *DDX51*, *DDX32A* (Fig. 5A), and *DDX42* (Fig. 5B, D).

4. Discussion

RNA helicases are motor proteins involved in almost all the nucleic acid transactions (Abdelhaleem et al., 2003; Pyle, 2008). Although analyses of RNA helicases have been performed in various organisms including fish (Boudet et al., 2001; Abdelhaleem et al., 2003; Umate et al.,

Fig. 4. Syntenic analysis of partial *DDX* genes using genomic contexts of human, chicken, zebrafish, cave fish and channel catfish. A) *DDX1*, B) *DDX2A*, C) *DDX6*, D) *DDX11*.

2011; Xu et al., 2013), systematic analysis of RNA helicases among teleost fish species has been lacking. In this study, 54 DExD/H-box RNA helicase genes were identified in channel catfish, of which 37 genes belonged to *DDX* family and 17 genes to *DHX* family (Table 1). We further analyzed the structural characteristics of these genes including the conserved motifs and domains, conducted phylogenetic and syntenic analyses, delineated the evolution dynamics, determined gene copy numbers and expression profiles of the RNA helicases in the gonads of males and females.

Combining phylogenetic and syntenic analyses, all 54 DExD/H family genes were annotated. For the most part, the motifs were well conserved, spanning a broad evolutionary spectrum of species ranging from bacteria, plants, fish, to humans (Owtrim, 2013; Xu et al., 2013), suggesting RNA helicase motifs were highly conserved throughout evolution. In catfish, nine highly conserved motifs in DEAD-box proteins (Q, I, Ia, Ib, II, III, IV, V, and VI) and eight in DEAH-box proteins (I, Ia, Ib, II, III, IV, V, and VI) were also identified for the vast majority of RNA helicase genes. Although some motifs were missing from five RNA helicase genes (*DDX11*, *DDX13*, *DHX32A*, *DHX32B*, and *DHX32C*), several motifs were also missing from the *DDX49*, *DHX32*, *DHX38*, and *DHX58* genes in cattle (Suthar et al., 2016) (Fig. 1). Overall, the catfish RNA helicase

genes' motifs were high conserved and may contribute to the high RNA structural similarity, but their functions remained to be defined by biochemical analysis.

In this study, most of DExD/H-box RNA helicase genes showed a single-copy orthology in all the selected vertebrate species, while multiple copies were found in ten genes (*DDX2*, *DDX3*, *DDX5*, *DDX6*, *DDX17*, *DDX19*, *DDX39*, *DHX8*, *DHX15*, and *DHX32*). Almost all the genes do not have duplicated gene copies in teleosts, but just a single copy as observed with higher vertebrates. The only exception is *DHX32* gene that has two copies in most of the teleost species analyzed to date (Table 2). This is quite notable as teleost fish were believed to have gone through an additional round of whole genome duplication, and therefore teleosts should harbor double copies for single copy genes in higher vertebrates. With DExD/H-box RNA helicase genes, the vast majority of the 54 catfish genes are single copy genes, suggesting the loss of gene copies after whole genome duplication was not random. Rather, it appears that most of these genes with exception of few lost the duplicated copies. Perhaps the gene copy dosage of these genes is crucially important in terms of accurate level of expression, but future research is warranted to address why almost all the RNA helicase genes are single copied in fish. In a couple of

Table 2

Comparison of copy numbers of RNA helicase genes in selected vertebrate genomes. Species names were abbreviated: Cha: Channel Catfish; Zeb: Zebrafish; Fug: Fugu; Med: Medaka; Til: Tilapia; Sti: Stickleback; Pla: Platyfish; Xen: Xenopus; Tur: Turtle; Chi: Chicken; Mou: Mouse; Hum: Human. Yellow shading indicated duplicated genes, and gray shading indicates the absence of the specific RNA helicase genes.

Gene	Cha	Zeb	Fug	Med	Til	Sti	Pla	Xen	Tur	Chi	Mou	Hum
<i>DDX1</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX2</i>	2	4	2	2	2	2	2	2	2	2	2	2
<i>DDX3</i>	1	2	2	1	2	0	0	1	1	1	2	2
<i>DDX4</i>	1	1	2	1	2	1	1	1	1	1	1	1
<i>DDX5</i>	1	1	2	3	2	2	2	1	1	1	1	1
<i>DDX6</i>	1	1	2	2	3	1	1	2	1	1	1	1
<i>DDX10</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX11</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX13</i>	1	1	0	1	1	0	1	1	0	1	1	1
<i>DDX17</i>	1	1	1	2	2	3	1	1	1	1	1	1
<i>DDX18</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX19</i>	2	2	2	2	2	1	2	1	1	1	2	2
<i>DDX20</i>	1	1	1	1	1	0	1	1	1	1	1	1
<i>DDX21</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX23</i>	1	1	1	1	1	1	1	1	1	2	1	1
<i>DDX24</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX25</i>	0	0	0	0	0	0	0	0	1	1	1	1
<i>DDX27</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX28</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX31</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX39</i>	2	3	3	2	3	2	2	2	1	0	2	2
<i>DDX41</i>	1	3	2	1	1	1	1	1	1	2	1	1
<i>DDX42</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX43</i>	1	1	1	1	1	1	1	1	0	1	1	1
<i>DDX46</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX47</i>	1	1	0	0	0	0	0	1	1	1	1	1
<i>DDX48</i>	1	1	0	1	0	1	1	1	1	0	0	1
<i>DDX49</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX50</i>	0	0	0	0	0	0	0	0	0	1	1	1
<i>DDX51</i>	1	1	1	1	1	1	1	1	1	0	1	1
<i>DDX52</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX53</i>	0	0	0	0	0	0	0	1	0	0	0	1
<i>DDX54</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX55</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX56</i>	1	1	1	1	1	1	1	1	0	0	1	1
<i>DDX58</i>	1	1	0	0	0	0	0	1	0	0	1	1
<i>DDX59</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DDX60</i>	0	0	0	0	0	0	0	0	1	1	1	1
<i>DHX8</i>	1	1	3	1	1	2	1	1	1	1	1	1
<i>DHX9</i>	1	1	1	1	1	0	1	1	1	0	1	1
<i>DHX15</i>	1	1	1	1	2	2	2	1	1	1	1	1
<i>DHX16</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX29</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX30</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX32</i>	2	2	2	2	2	1	2	1	1	1	1	1
<i>DHX33</i>	1	1	1	1	1	1	1	1	1	2	1	1
<i>DHX34</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX35</i>	1	1	1	1	1	0	1	1	1	1	1	1
<i>DHX36</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX37</i>	1	1	1	1	1	1	1	2	1	1	1	1
<i>DHX38</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX40</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX57</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>DHX58</i>	1	1	1	1	1	1	1	1	1	1	1	1
Total	54	60	58	56	59	50	53	55	49	51	56	58

cases, two copies of the RNA helicase genes existed in the genomes of mammals, and these may be from lineage-specific tandem gene duplications.

Natural selection is the major force that shapes the genetic variation within populations, and selection pressure can be measured by the relative ratios of synonymous (dS) and non-synonymous (dN) substitutions. In this study, the global ratios of dN/dS were examined and they were found to be well below 1.0 for all RNA helicases (Table 3), a theoretical boundary for positive and negative selection (Kimura, 1977). It suggested that negative selection (dN/dS < 1) were working extensively

to remove nonsynonymous substitutions from the RNA helicase genes, indicating the demand for high levels of evolutionary conservation. However, few positive selection sites ($p < 0.1$) were found in three DHX genes (DHX36, DHX57, and DHX58) by site-by-site dN/dS analyses (Table 3), with no positive amino acid sites located at any motifs or main functional domains. Overall, the dN/dS ratios analysis suggested that the RNA helicase genes were highly conserved throughout the evolution.

We analyzed the expression profiles of RNA helicase genes in channel catfish gonads at 90, 100, and 110 dpf, corresponding with the period of male gonad differentiation (Patiño et al., 1996). The fact that many

Table 3

A summary description of selection pressure for each of fish DEXD/H-box RNA helicase genes (dN/dS).

Gene	Global dN/dS	No. of positive sites	No. of negative sites
DDX1	0.0716	0	245
DDX2A	0.0772	0	86
DDX2B	0.0258	0	106
DDX3X	0.1155	0	185
DDX4	0.1902	0	147
DDX5	0.1143	0	196
DDX6	0.0788	0	128
DDX10	0.1869	0	183
DDX11	0.1560	0	176
DDX13	0.1233	0	338
DDX17	0.1236	0	199
DDX18	0.1403	0	194
DDX19A	0.1510	0	135
DDX19B	0.1364	0	36
DDX20	0.2694	0	126
DDX21	0.1834	0	195
DDX23	0.0600	0	247
DDX24	0.2458	0	218
DDX27	0.1406	0	242
DDX28	0.2060	0	158
DDX31	0.2274	0	152
DDX39A	0.0297	0	141
DDX39B	0.0398	0	4
DDX41	0.0448	0	153
DDX42	0.1071	0	281
DDX43	0.2281	0	152
DDX46	0.0818	0	299
DDX47	0.1314	0	2
DDX48	0.0212	0	134
DDX49	0.1111	0	141
DDX51	0.2675	0	140
DDX52	0.2006	0	150
DDX54	0.1577	0	177
DDX55	0.1185	0	182
DDX56	0.1330	0	144
DDX59	0.1835	0	112
DHX8	0.0757	0	394
DHX9	0.1122	0	64
DHX15	0.0687	0	102
DHX16	0.0804	0	251
DHX29	0.1557	0	365
DHX30	0.2147	0	277
DHX32A	0.2260	0	135
DHX32B	0.2073	0	125
DHX33	0.0832	0	196
DHX34	0.1444	0	308
DHX35	0.0571	0	104
DHX36	0.1428	1	284
DHX37	0.1512	0	331
DHX38	0.1512	0	331
DHX40	0.1283	0	184
DHX57	0.1783	2	361
DHX58	0.2477	1	143

of these RNA helicase genes had different expression patterns between males and females and that many of them expressed at higher levels during the critical period of male gonad tissue differentiation (90–110 dpf) indicated that RNA helicases were highly relevant in catfish gonadal differentiation and development. Previous studies have shown that DEXD/H-box RNA helicase genes played important roles in gametogenesis and formation of gonads during sex differentiation (Kotov et al., 2014). Especially for *DDX4* gene, a number of studies showed that this particular gene displayed differential expression and sex-related expression differences during oogenesis and spermatogenesis in many fishes, including zebrafish (Krøvel and Olsen, 2004), medaka (Shinomiya et al., 2000), gibel carp (Xu et al., 2005), tilapia (Kobayashi et al., 2000, 2002), catfish (Raghuveer and Senthilkumaran, 2010) and European sea bass (Blázquez et al., 2011). In addition, several other RNA helicase genes, including *DDX5*, *DDX20*, *DDX23*, *DDX43*, *DDX50*, *DDX51*, and *DHX9*, are demonstrated to exert crucial functions in

Table 4

Differentially expressed DEXD/H-box RNA helicase genes in the gonads between male and female at 90, 100, and 110 days post-fertilization (dpf). Only the significantly differentially expressed genes are listed (|fold change value in male / value in female| ≥ 2 with the FDR corrected *p* values < 0.01). Minus indicated genes that were expressed at lower levels in male than in female.

Gene	Fold change		
	90 dpf	100 dpf	110 dpf
DDX1	−3.3	—	—
DDX3X	—	−8.7	—
DDX4	−60.9	−106.5	−3.3
DDX5	4.6	−2.8	—
DDX10	—	−4.9	−5.0
DDX13	—	—	−4.5
DDX18	—	—	−3.2
DDX19A	−16.4	−10.0	−7.0
DDX19B	−2.2	−3.3	—
DDX20	−9.9	−7.0	—
DDX21	—	2.7	2.4
DDX23	−4.4	−4.4	−2.9
DDX24	−2.4	—	—
DDX27	−4.0	−5.9	−7.0
DDX39A	—	−2.2	—
DDX41	−5.5	−4.9	−3.3
DDX42	−94.6	−70.2	—
DDX43	−10.2	−11.8	−3.5
DDX47	—	—	−3.6
DDX49	−5.1	—	—
DDX51	−14.8	−14.5	−3.4
DDX52	−6.2	−7.2	—
DDX55	−3.0	—	—
DDX56	−2.5	—	—
DDX59	−5.6	−3.8	—
DHX9	—	3.6	—
DHX16	−3.4	−3.3	−4.3
DHX29	−3.6	−4.2	−5.3
DHX30	−7.7	−3.4	−6.7
DHX32A	−102.5	−77.4	−3.2
DHX32B	−8.1	—	—
DHX36	—	−3.5	−3.9
DHX38	−2.9	−5.1	−4.9

gametogenesis in animals (Walstrom et al., 2005; Konishi et al., 2008; Mouillet et al., 2008; Fang et al., 2011; He et al., 2012; Yousefi et al., 2015).

In this study, a total of 31 RNA helicase genes were expressed at higher levels in females, and only 3 RNA helicase genes were expressed at higher levels in males. It was shown that the gene expression of *DDX4* in female-dominant group of European sea bass were always higher than that in male-dominant group (Blázquez et al., 2011). The similar result has been found in the process of *Clarias gariepinus* gametogenesis: the *DDX4* mRNA levels gradually increased due to the increasing number of germ cells in testis and more number of primary oocytes in ovary

Table 5

Expression of DEXD/H-box RNA helicase genes in testis during channel catfish male gonadal differentiation (90, 100, and 110 dpf). Only the significantly differentially expressed genes are listed (|fold change value at 100 dpf or 110 dpf / value at 90 dpf| ≥ 2 with the FDR corrected *p* values < 0.01). Minus indicated genes that were expressed at higher levels in male at 90 dpf than at 110 dpf.

Gene	Fold change	
	100 dpf vs 90 dpf	110 dpf vs 90 dpf
DDX3X	—	−2.2
DDX4	—	14.7
DDX20	—	2.8
DDX41	—	2.8
DDX42	—	35.7
DDX51	—	4.9
DDX52	—	2.3
DDX59	—	3.2
DHX32A	—	27.8

Fig. 5. Comparison of relative fold changes between RNA-Seq and RT-qPCR results. A, B, and C represent 90 days post-fertilization (dpf), 100 dpf, and 110 dpf time points, respectively. D represents the changes between 90 dpf and 110 dpf in the male gonad.

(Raghuveer and Senthilkumaran, 2010). In channel catfish, ovarian differentiation began at 19 dpf, while testicular formation normally started between 90 and 102 dpf (Patiño et al., 1996). In that regard, sex differentiation during the studied period had already taken place in females and female gonad development was in an advanced stage. Therefore, female gonads are bigger and should contain more germ cells than male gonads. Consequently, the differential expression level of the RNA helicases in male and female could be attributed not only to sex, but also to differential gonad growth at this developmental stage (Papadaki et al., 2005; Raghuveer and Senthilkumaran, 2010; Blázquez et al., 2011). Taken together, these observations suggested that the RNA helicase genes may be regulated in a sex-specific as well as developmental stage-specific manner for germ cell development and sex differentiation in channel catfish.

In addition, nine genes were highly expressed in the male gonads at 110 dpf as compared to their levels of expression at 90 dpf in this study. In channel catfish male gonads, transcriptomic profiles at 90 dpf and 100 dpf exhibited high similarities, while drastic changes were observed during 90–110 dpf, with a group of highly up-regulated genes involved in germ cells development (Zeng et al., 2016). The gene expression patterns may suggest that the major events for male sex differentiation occur between 100 and 110 dpf, and the RNA helicase genes may be involved in catfish testicular development. Functional analysis is required to determine how the RNA helicases exert their functions for male sex differentiation.

5. Conclusions

A total of 54 DEXD/H-box RNA helicase genes of channel catfish were identified and characterized in this study. Phylogenetic and syntenic analyses were conducted to clearly establish their orthologies, supporting their annotations. Strong negative and rare positive selection were detected in fish RNA helicase genes by dN/dS analysis. The meta-analysis of DEXD/H-box RNA helicase genes in gonad

differentiation were conducted, with a fraction of genes of the most highly changed expression validated using RT-qPCR. The expression patterns, especially the significant differences between males and females, and between 90 dpf and 110 dpf, suggested significant involvement of the DEXD/H-box RNA helicases in sex development and differentiation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.cbd.2017.02.008>.

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