

## Cloning the *Dmrt1* and *DmrtA2* genes of ayu (*Plecoglossus altivelis*) and mapping their expression in adult, larval, and embryonic stages

Jin-Hua WANG, Liang MIAO, Ming-Yun LI<sup>\*</sup>, Xiao-Fei GUO, Na PAN, Ying-Ying CHEN, Liang ZHAO

Ningbo University, Ningbo 315211, China

**Abstract:** The *Dmrt* family of genes are involved in sex differentiation in different species of invertebrates, and some vertebrates including human. In this study, we cloned the full-length cDNA of ayu (*Plecoglossus altivelis*) *Dmrt1* and *DmrtA2*. Sequence and phylogenetic tree analyses showed ayu *Dmrt1* showed highest similarity to that of *Oncorhynchus mykiss* while ayu *DmrtA2* is most similar to that of *Oryzias latipes*. Fluorescence-based quantitative reverse transcription PCR (qRT-PCR) revealed the *Dmrt1* was predominantly expressed in the testis. At the larval stages, *Dmrt1* mRNA expression level was highest during 52–64 days post hatching (dph) and at the gastrula stage during embryonic development. *DmrtA2*, meanwhile, was specifically expressed in the ovary and was highly expressed in the female brain tissue, but not male brain tissue. During the larval stages, *DmrtA2* expression remained high before day 34, and then fluctuated while generally decreasing. During embryonic development, *DmrtA2* expression increased gradually and peaked at the hatching stage. Our data suggest that ayu *Dmrt1* might participate in the differentiation and maintenance of testis while *DmrtA2* may play a role in ovary-differentiation and mature-ovary maintenance. *DmrtA2* might also participate in brain development.

**Keywords:** Ayu; *Dmrt*; Sequence analysis; Expression

The ayu (*Plecoglossus altivelis*; sweetfish) is a popular migratory fish and one of the most economically important freshwater fish in southeastern China and Japan due to both its perceived nutrition and taste. Belonging to the class Osteichthyes, suborder Salmonoidei, and family Plecoglossidae, the ayu is also well-known for its comparatively fast reproduction cycles. Paired with the consumer preference for the fish's superior flavor and faint smell, the ayu has accordingly been called the “the King of Freshwater Fish” in Asia. The name may be more appropriately titles “the Queen of Freshwater Fish,” as the females reproduce more quickly and are more desirable for consumption, so much so that the sales price of female ayu is nearly double that of a male (Cao et al, 1981; Cao et al, 1982; Li et al, 1985; Wang et al, 1998).

Given the consumer preference for females and the rise in the fishes popularity in Asia, finding a way to improve the breeding production and develop selective breeding and female seeding techniques may prove worthwhile. Observational research on the wild and the cultured populations of ayu showed that the adult male

and female ayu display a sexual size dimorphism, but for this study we wanted to take a more cohesive view of the underlying dimorphism. Accordingly, in this study analyzed the gonad transcriptome to identify the members of the sex-related double-sex and Mab-3-related transcription factor (*Dmrt*) gene family. The *Dmrt* family of transcription factors, including the sex-determinant (*Dsx*) of *Drosophila melanogaster*, and the Mab-3 of nematode, are characterized by a DNA-binding DM-domain, an unusual zinc-finger structure (Kim et al, 2003; Ren et al, 2001). Currently, at least 8 genes in the *Dmrt* family (*Dmrt1–Dmrt8*) (de Grandi et al, 2000; Guo et al, 2005; Kettlewell et al, 2000; Kondo et al, 2002; Nanda et al, 1999; Shibata et al, 2002) have

Received: 7 August 2013; Accepted: 16 December 2013

Foundation items: Special Preliminary Study of 973 (2008CB117015), Changjiang Scholars and Innovative Research Team Projects (IRT0734), Priority Themes of Major Science and Technology in Zhejiang Province (2009C12077), and the K. C. Wong Magna Fund of Ningbo University

<sup>\*</sup> Corresponding author, E-mail: limingyun@nbu.edu.cn

been detected across many species, including mammals (de Grandi *et al.*, 2000), reptiles (Kettlewell *et al.*, 2000), birds (Nanda *et al.*, 1999), fish (Kondo *et al.*, 2002), and amphibians (Shibata *et al.*, 2002). A previous study indicated that the *Dmrt* gene family plays a major role in an organism's sex development (Capriglione *et al.*, 2010; de Grandi *et al.*, 2000; Kettlewell *et al.*, 2000; Marchand *et al.*, 2000; Yang *et al.*, 2013;), while another study pointed out that *Dmrt* participated in somite development (Seo *et al.*, 2006).

In particular, *Dmrt1*, one of the prominent genes in this family, plays an important role in sex-determination and differentiation in *Drosophila melanogaster*, *Nematodes*, *Oryzias latipes*, and mice. To date, the regulatory mechanisms underlying sex determination and differentiation in some species has been studied. For example, knocking out *Dmrt1* expression during male differentiation leads to gonad feminization in chickens (Smith *et al.*, 2009) while in mice, the *Dmrt1* gene inhibits female programming in the testis after birth (Matson *et al.*, 2011). In some fish, *Dmrt1* shows testis-specific expression (Guan *et al.*, 2000; Liu *et al.*, 2004; Kobayashi *et al.*, 2004; Shin *et al.*, 2009) while in others it shows expression in both the ovary and testis, with the expression in the testis being marginally higher than that in the ovary (Guo *et al.*, 2004; Marchand *et al.*, 2000). In male fish, *Dmrt1* is generally expressed only in the sertoli cells, but the same is not true for females (Kobayashi *et al.*, 2008). Meanwhile, *DmrtA2* (*Dmrt5*), another member of the *Dmrt* gene family, has been identified in some fish, including the zebrafish (Guo *et al.*, 2004), swordfish (Veith *et al.*, 2006), and rice field eel (Zhang *et al.*, 2006). Previous studies on *DmrtA2* function mainly found relation to the brain and gonads; though in humans, the function is associated with development of the cerebral cortex (Saulnier *et al.*, 2013) while in toads it is related to the formation of the olfactory placode nerve (Parlier *et al.*, 2013).

Although many previous reports focused on the molecular heredity of ayu (Huang *et al.*, 2004; Wang *et al.*, 2011; Dong & Nobuhiko, 2003), they were restricted to the isoenzyme, biochemical heredity, and RAPD genetic diversity. To our knowledge, there are no previous studies focusing on ayu sex differentiation and its regulation. In this study, we screened the members of *Dmrt* gene family from the transcriptome and cloned *Dmrt1* and *DmrtA2*. We examined their expression in different adult tissues, and during larval and embryonic developmental stages in order to provide a better

understanding of the relevant mechanism at play in the sex differentiation among ayu.

## MATERIALS AND METHODS

### Experimental materials and reagents

Ayu fish were obtained from Qingjiang base, Zhejiang Fisheries Research Laboratory. From August-October, different tissues were sampled were frozen, immediately dissected, and then stored in liquid nitrogen at  $-80^{\circ}\text{C}$  until further analysis. The embryos of different stages were collected at 1.5 mL DNase/RNase-free centrifuge tube immediately and then stored in liquid nitrogen.

TRIzol reagent, pMD19-T vector, PrimeScript RT reagent Kit, 3'-Full RACE, and 5'-Full RACE Kit were purchased from Takara company. Other reagents were domestic analytical reagents and were purchased from Bioequip. *Escherichia coli* DH5 $\alpha$  cells were stored in our lab.

### cDNA cloning and amino acid sequence analysis

The total RNA of different tissues was isolated using RNAiso Plus (Takara) according to the manufacturer's instructions. The RNA was digested by RNase-free DNase I and purified. The reverse transcription reaction was performed in a 10- $\mu\text{L}$  volume using the PrimeScript RT reagent Kit (Takara). The reaction was performed at  $37^{\circ}\text{C}$  for 15 min and  $85^{\circ}\text{C}$  for 5 s, and then stored at  $-20^{\circ}\text{C}$ .

Specific primers were designed for RACE and RT-PCR (Table 1) based on the partial transcriptome sequence, and  $\beta$ -actin was used as a control. All the primers were synthesized by Sangon Biotech.

**Table 1** Primers for *Dmrt* cloning and analysis in ayu (*P. altivelis*)

Primer	Sequence (5'-3')
D1-3-I	AGTCAGAGACCTTCACTGTGGATT
D1-3-O	CATCAACTCCCTTGTCACACTCG
D1-5-I	GGACAGTCTTCCCACACACTCTAAT
D1-5-O	GCTCATTCTTACCACAATCTCAG
D1-y-F	CCTCAGACCTGGTGGTGGATG
D1-y-R	GTTGGGAATCTGGTACTGCTGATAG
DA2-3-I	GCAGCCAAACTCACCTCAC
DA2-3-O	AGTCGAGGACTGGCTTTCAT
DA2-5-I	CAACGCTGACACGACACCG
DA2-5-O	CAACGCTGACACGACACCG
DA2-y-F	TGAAAGGCCACAAGCGTTATT
DA2-y-R	CGGGCTTCGTTCTCTTCCT
$\beta$ -actin-F	TCGTGCGTGACATCAAGGAG
$\beta$ -actin-R	CGCACTTCATGATGCTGTTG

All procedures were performed according to users' manuals (3'-Full RACE Core Set Ver. 2.0 and 5'-Full RACE Kit, Takara). The PCR products were separated on a 2% agarose gel, cloned into the pUC-19 vector, and sequenced.

All *Dmrt* protein sequences from different species were aligned using the NCBI blast program (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic trees were constructed using MEGA5.0 (Tamura et al, 2011) using the Neighbor-joining (NJ) method, yielding an unrooted consensus tree with a 1 000 bootstrap replicates.

### ***Dmrt* expression analysis at different stages by RT-PCR**

To quantify *Dmrt* expression, we used RT-PCR according to the manufacturer's protocols. PCR cycling conditions were adjusted to the following parameters: 95°C for 30 s, then 60°C for 30 s, and finally 72°C for 30 s for 40 cycles for *Dmrt* and  $\beta$ -*actin* in a 20-L volume containing SYBR Green (TaKaRa). The primers for *Dmrt* and  $\beta$ -*actin* are listed in Table 1. All experiments were performed in triplicate to ensure concordance and eliminate potential errors. The resulting data were analyzed via the  $2^{-\Delta\Delta Ct}$  method (Livak et al, 2001).

## **RESULTS**

### **Cloning and sequence analysis of ayu *Dmrt***

The cloned 1684 bp *Dmrt1* transcript (GenBank accession number: KC899210) consisted of an 882-bp open reading frame (ORF) that coded 293 amino acids, a 674-bp 3'-untranslated region (UTR), and a 116-bp 5'-UTR. NCBI conserved domain search (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) showed that the DM-domain is represented by amino acids 27–73, and the *Dmrt1* super-family conserved domain is represented by amino acids 187–246 (Figure 1).

We obtained the 2738-bp cDNA sequence of *DmrtA2* (Figure 1; GenBank accession number: KF296364), The ORF (1311 bp) coded 435 amino acids and contained the DM, DMA, and DMB conserved domains. The 3'-UTR (not including poly(A) signals) and 5'-UTR were 948 bp and 479 bp, respectively. NCBI conserved domain search (<http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) showed that the DM-domain included amino acids 51–96, and the DMA conserved domain included amino acids 187–246.

However, genomic analysis (data not shown)

indicated that this transcript includes a 297-bp intron in its coding region, suggesting that it might represent an alternative splicing form of *DmrtA2*.

### **Similarity and phylogeny of amino acids of ayu *Dmrt***

Alignment analysis showed that the identity between *Dmrt1* of ayu and other teleostean is greater than 60%, with the most similar species being the rainbow (81.5% identity) and the least similar being medaka (62% identity). The identity between the *Dmrt1* of Ayu and other vertebrates was less than 60% (Table 2). The NJ-phylogenetic tree showed that all the fish cluster into a subgroup, and the other vertebrates cluster into a separate group. In the fish subgroup, the fish from the Cypriniformes, Siluriformes, and Perciformes form a clade, and the ayu cluster with the rainbow trout (Figure 2). *DmrtA2* amino acid comparison also showed a similarity greater than 70% between ayu and other teleostean, with the highest similarity being medaka (84%). The similarity with the other vertebrates was less than 60% (Table 2). The NJ-phylogenetic tree for *DmrtA2* indicates that the ayu *DmrtA2* cluster with the other fish and stay farther away from humans and mice (Figure 2).

### ***Dmrt* gene expression in ayu adult tissue**

In different adult tissues, *Dmrt1* showed the highest expression level in the testes and the lowest level in the intestine. Using the intestinal expression level as a reference, the expression levels in testis, ovary, and female spleen were 63.1, 3.2, and 1.6 times, respectively. *DmrtA2* showed the highest expression in the female brain and the lowest expression in the male brain. Using the expression level from the male brain as a reference, the expression levels in the ovary, female brain, and female intestine were 1.3, 37.6, and 2.5 times of that in the male brain, respectively (Figure 3).

### ***Dmrt* expression in ayu larvae**

The development of ayu larvae is a slow process (Li et al, 1985), so we accordingly sampled tissue from 10 days post hatching (dph) to 70 dph. For *Dmrt1*, the stages showing highest expression levels occurred between 52 dph and 64 dph. Using the expression level at 40 dph as a baseline, the expression levels at 52 dph and 64 dph were 81.5 and 88.9 times that at 40 dph, respectively. For *DmrtA2*, the expression levels increased from 10 to 34 dph and then decreased to a minimum,



**Table 2 Comparison of amino acid sequence identities of ayu *Dmrt1* and *DmrtA2***

GenBank accession numbers	Species names	Gene names	Identity with ayu <i>Dmrt1</i> / <i>DmrtA2</i> (%)
AAG17544	<i>Oncorhynchus mykiss</i>	<i>Dmrt1</i>	81.50
ACR77511	<i>Clarias gariepinus</i>	<i>Dmrt1a</i>	78.50
HM245921	<i>Tachysurus fulvidraco</i>	<i>Dmrt1</i>	78
Q71MM5	<i>Danio rerio</i>	<i>Dmrt1</i>	73.50
ABK88911	<i>Paramisgurnus dabryanus</i>	<i>Dmrt1</i>	73
ABM54575	<i>Silurus meridionalis</i>	<i>Dmrt1b</i>	73
CAQ52797	<i>Dicentrarchus labrax</i>	<i>Dmrt1</i>	72
ABK15558	<i>Epinephelus coioides</i>	<i>Dmrt1</i>	68.50
AFA45126	<i>Gobiocypris rarus</i>	<i>Dmrt1</i>	68.50
BAM62886	<i>Parajulis poecilepterus</i>	<i>Dmrt1a</i>	62.20
ACD62373	<i>Epinephelus merra</i>	<i>Dmrt1a</i>	62.20
AAP84972	<i>Acanthopagrus schlegelii</i>	<i>Dmrt1</i>	64
BAC65996	<i>Oryzias curvinotus</i>	<i>Dmrt1</i>	62
Q9PTQ7	<i>Gallus gallus</i>	<i>Dmrt1</i>	58
AAD40474	<i>Homo sapiens</i>	<i>Dmrt1</i>	56
AAF12826	<i>Mus musculus</i>	<i>Dmrt1</i>	55.50
Q3LH63	<i>Xenopus laevis</i>	<i>Dmrt1</i>	54.50
Q76L87	<i>Oryzias latipes</i>	<i>Dmrt5</i>	84
Q5UU75	<i>Danio rerio</i>	<i>Dmrt5</i>	83
Q2I327	<i>Xiphophorus maculatus</i>	<i>Dmrt5</i>	83
ACU30591	<i>Monopterus albus</i>	<i>Dmrt5</i>	82
AFA46804	<i>Gadus morhua</i>	<i>Dmrt5</i>	81
NP_001033039	<i>Takifugu rubripes</i>	<i>DmrtA2</i>	81
Q6YHU8	<i>Oreochromis niloticus</i>	<i>Dmrt5</i>	80
AEM44777	<i>Xenopus tropicalis</i>	<i>Dmrt5</i>	72
AAN10254	<i>Mus musculus</i>	<i>Dmrt5</i>	57
AAI43801	<i>Homo sapiens</i>	<i>DmrtA2</i>	57

strong expression in the testis, but also a subtle expression in the ovary of zebrafish (Guo et al, 2004), rainbow trout (Marchand et al, 2000), and *Takifugu rubripes* (Shen et al, 2007). Ohmuro-Matsuyama et al (2003) found that *Dmrt1* is expressed in many different tissues of Medaka. In our study, we also detected low *Dmrt1* expression in ayu intestine and kidney, leading us to infer that *Dmrt1* not only plays an important role in testis maintenance but also may play some other yet unknown roles.

Compared to the adult tissues, *Dmrt1* expression occurred during the entire embryonic developmental stages in both *Pelteobagrus fulvidraco* (Li et al, 2012) and *Cynoglossus semilaevis* (Sun et al, 2008). Intriguingly, the expression levels different between the two fish in that the peak expression levels occurred at a different time. During this present study, we found similar results, with the expression peak at the gastrula

stage. The previous studies showed that during larval development of *Pelteobagrus fulvidraco* (Li et al, 2012), *Dmrt1* was expressed from 1 to 51 dph and reached a peak at 31 dph, while another study (Sun et al, 2008) found that in *Cynoglossus semilaevis* the expression of *Dmrt1* at 22 dph was higher than during the other developmental stages. In our study, *Dmrt1* was expressed from 10 to 70 dph, reaching a peak at 64 dph. Moreover, examination of a paraffin section of ayu gonad revealed that the period from 40–60 dph was the key time for testis formation. During this time, *Dmrt1* expression was easily detectable, implying that *Dmrt1* plays an important role in the process of testis-differentiation.

*DmrtA2* is mainly involved in the development of the brain and gonad. In mice embryos, for example, *DmrtA2* is expressed primarily in both the brain and the gonads. Moreover, expression in the female ovary is higher than that in the male testis, and very lowly

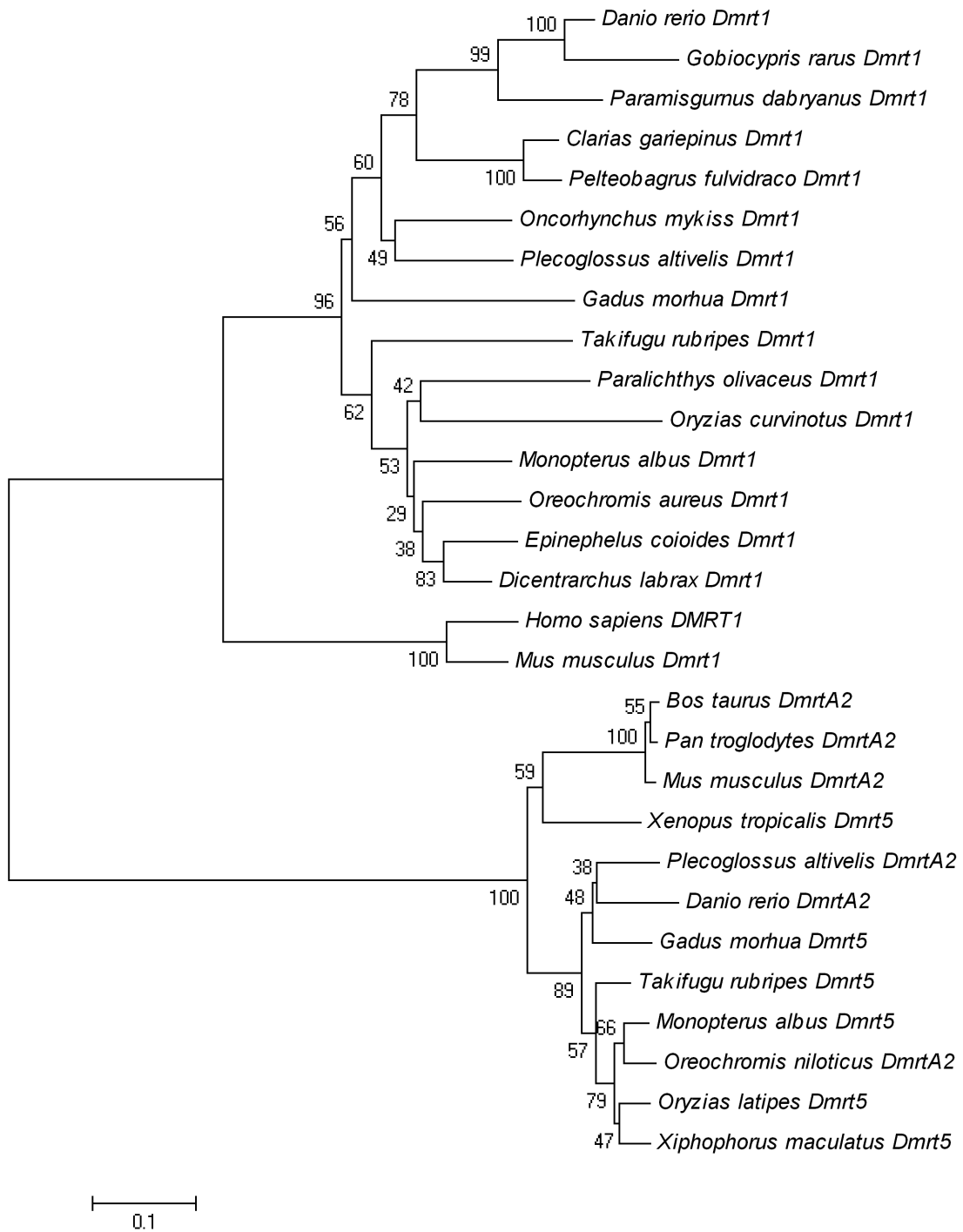


Figure 2 NJ-Phylogenetic tree based on *Dmrt1* and *DmrtA2* amino acid sequences  
 Numbers at the nodes denote the bootstrap values for 1000 replicates.

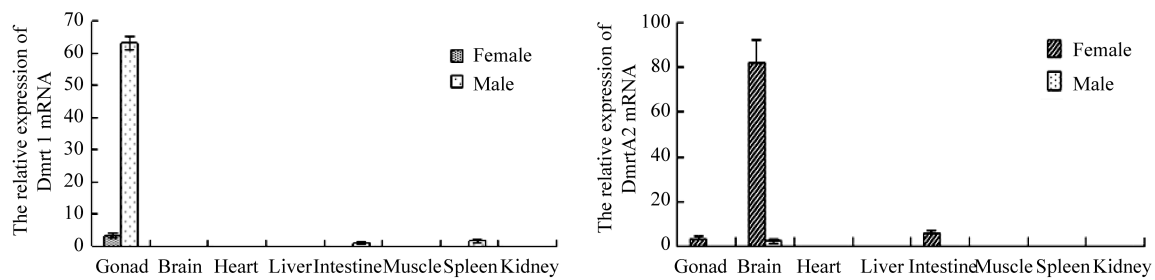


Figure 3 RT-PCR analysis of *Dmrt1* and *DmrtA2* transcripts in different ayu (*P. altivelis*) adult tissues

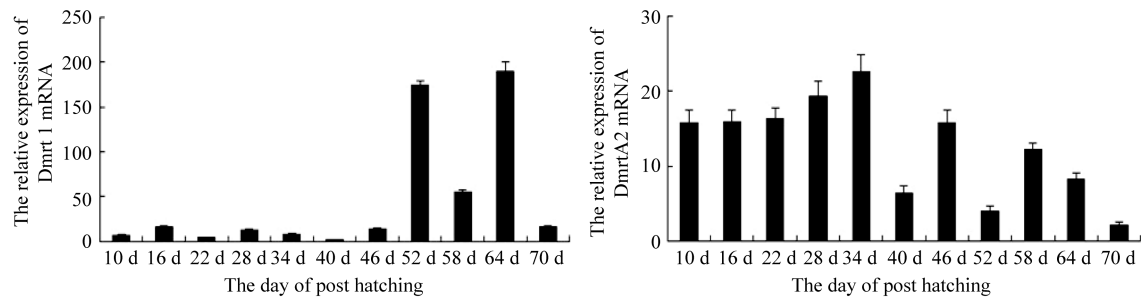


Figure 4 RT-PCR analysis of *Dmrt1* and *DmrtA2* transcripts of ayu (*P. altivelis*) at different post-hatching developmental stages

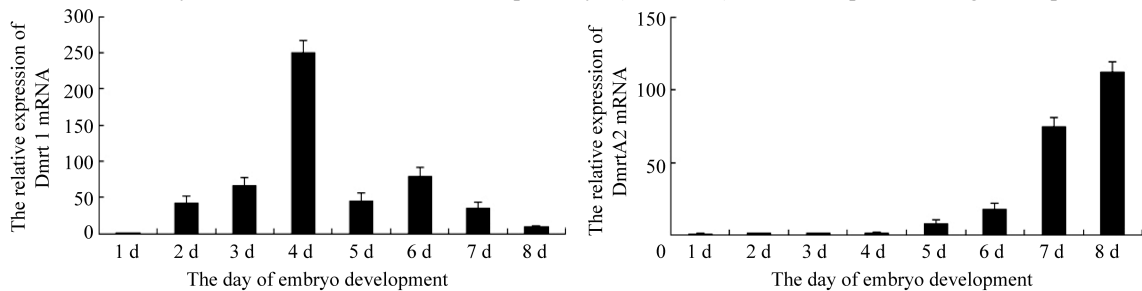


Figure 5 RT-PCR analysis of *Dmrt1* and *DmrtA2* transcripts of ayu (*P. altivelis*) at different embryonic stages

The numbers of the x-axis represent the following stages: 1 d, fertilized eggs; 2 d, morula stage; 3 d, blastula stage; 4 d, gastrula stage; 5 d, eye pouch period (neurula stage); 6 d, heartbeat period (tail-bud stage); 7 d, hatching stage; 8 d: early larva.

expressed in other tissues (Kim et al, 2003). In the puffer fish (Yamaguchi et al, 2006), *DmrtA2* was expressed in the brain and eye, with no expression in gonads, while in the swordfish (Veith et al, 2006) it was expressed in the olfactory placode and midbrain and in the zebrafish (Guo et al, 2004) it was expressed primarily in the midbrain of the embryo stage. Recently, Gennet et al (2011) also found that *DmrtA2* had an effect on the brain nerve in mice, a finding concordant with our results. It then stands to reason that *DmrtA2* may play a vital role in the development of the ayu brain. Guo et al (2004) had previously cloned the *DmrtA2* gene and found that it was expressed in the germ cells of zebrafish, mainly in the spermatogonia, spermatocytes, spermatoblasts, and oocytes, again implicating *DmrtA2* in gonad development. Zhang et al (2006) elaborated on this and found that *DmrtA2* was expressed in the gonad tissues of three types of rice field eels and that its expression in

testis was higher than that in the ovary. And again, in the Pearlescent shellfish, *DmrtA2* participates in gonad development (Yu et al, 2009). In our experiment, we found that *DmrtA2* was expressed only in the ovary and had detectable expression before 34 dph, which coincides with the critical time for ovary differentiation. Considering these results, we propose that *DmrtA2* likely participates in the regulation of gonad development, and may do so across numerous fish species.

In addition to influencing sex determination, *Dmrt* genes may be involved in the regulation of tissue development. Ayu *Dmrt1* and *DmrtA2* exhibited different expression patterns in females and males, but both were also expressed in a variety of other tissues, suggesting that in ayu, the *Dmrt* genes likely play multiple roles in organ development. Further studies may help to reveal the roles of *Dmrt1* in testis development and *DmrtA2* in brain development.

## References

- Cao KJ, Li MY. 1981. The preliminary study of ayu artificial reproduction. *Fisheries Science & Technology Information*, **9**(5): 13-15.
- Cao KJ, Li MY. 1982. Studies on the reproductive biology of the ayu in Fuxi stream, Zhejiang. *Journal of Fisheries of China*, **6**(2): 107-117.
- Capriglione T, Vaccaro MC, Morescalchi MA, Tammaro S, De Iorio S. 2010. Differential DMRT1 expression in the gonads of *Podarcis sicula* (Reptilia: Lacertidae). *Sexual Development*, **4**(1-2): 104-109.
- de Grandi A, Calvan V, Bertini V, Bulfone A, Peverali G, Camerino G, Borsani G, Guioli S. 2000. The expression pattern of a mouse double sex-related gene is consistent with a role in gonadal differentiation. *Mechanisms of Development*, **90**(2): 323-326.
- Dong S, Nobuhiko T. 2003. Identification of clonal *Plecoglossus altivelis* using microsatellite DNA marker. *Journal of Fisheries of China*, **27**(4): 295-299.
- Gennet N, Gale E, Nan XS, Farley E, Takacs K, Oberwallner B, Chambers D, Li M. 2011. Doublesex and mab-3-related transcription factor 5 promotes midbrain dopaminergic identity in pluripotent stem cells by enforcing a ventral-medial progenitor fate. *Proceedings of the National Academy of Sciences of the United States of America*, **108**(22): 9131-9136.
- Guan GJ, Kobayashi T, Nagahama Y. 2000. Sexually dimorphic expression of two types of DM (Doublesex/Mab-3)-domain genes in a teleost fish, the tilapia (*Oreochromis niloticus*). *Biochemical and Biophysical Research Communications*, **272**(3): 662-666.
- Guo YQ, Cheng HH, Huang X, Gao S, Yu HS, Zhou RJ. 2005. Gene structure, multiple alternative splicing, and expression in gonads of zebrafish Dmrt1. *Biochemical and Biophysical Research Communications*, **330**(3): 950-957.
- Guo YQ, Li Q, Gao S, Zhou X, He Y, Shang X, Cheng HH, Zhou RJ. 2004. Molecular cloning, characterization, and expression in brain and gonad of Dmrt5 of zebrafish. *Biochemical and Biophysical Research Communications*, **324**(2): 569-575.
- Huang FY, Li MY. 2004. Biochemical genetic analysis of isozymes in *Plecoglossus altivelis* population in Fuxi. *Journal of Fisheries of China*, **28**(5): 579-584.
- Kettlewell JR, Raymond CS, Zarkower D. 2000. Temperature-dependent expression of turtle Dmrt1 prior to sexual differentiation. *Genesis*, **26**(3): 174-178.
- Kim S, Kettlewell JR, Anderson RC, Bardwell VJ, Zarkower D. 2003. Sexually dimorphic expression of multiple doublesex-related genes in the embryonic mouse gonad. *Gene Expression Patterns*, **3**(1): 77-82.
- Kobayashi T, Matsudua M, Kajiura-Kobayashi H, Suzuki A, Saito N, Nakamoto M, Shibata N, Nagahama Y. 2004. Two DM domain genes, *DMY* and *DMRT1*, involved in testicular differentiation and development in the medaka, *Oryzias latipes*. *Developmental Dynamics*, **231**(3): 518-526.
- Kobayashi T, Kajiura-Kobayashi H, Guan GJ, Nagahama Y. 2008. Sexual dimorphic expression of *DMRT1* and *SOX9a* during gonadal differentiation and hormone-induced sex reversal in the teleost fish Nile tilapia (*Oreochromis niloticus*). *Developmental Dynamics*, **237**(1): 297-306.
- Kondo M, Froschauer A, Kitano A, Nanda I, Hornung U, Volf JN, Asakawa S, Mitani H, Naruse K, Tanaka M, Schmid M, Shimizu N, Schartl M, Shima A. 2002. Molecular cloning and characterization of *DMRT* genes from the medaka *Oryzias latipes* and the platyfish *Xiphophorus maculatus*. *Genes*, **295**(2): 213-222.
- Li L, Liang HW, Li Z, Luo XZ, Zhang ZW, Zhu YY, Zou GW. 2012. Cloning and expression analysis of *DMRT1* gene in *Pelteobagrus fulvidraco*. *Journal of Huazhong Agricultural University*, **31**(2): 220-226.
- Li MY, Cao KJ, Xu SL. 1985. A study on the development of the egg, larva and young fish of ayu (*Plecoglossus altivelis* T.ET S.). *Journal of Zhejiang College of Fisheries*, **4**(1): 35-44.
- Liu XS, Liang B, Zhang SY. 2004. cDNA cloning, tissue distribution and mRNA transcription of *DMRT1* gene in the Protandrous Black Porgy *Acanthopagrus schlegelii*. *Zoological Research*, **25**(2): 158-161.
- Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-ΔΔCT</sup> method. *Methods*, **25**(4): 402-408.
- Matson CK, Murphy MW, Sarver AL, Griswold MD, Bardwell VJ, Zarkower D. 2011. *DMRT1* prevents female reprogramming in the postnatal mammalian testis. *Nature*, **476**(7358): 101-104.
- Marchand O, Gororoun M, D'Cotta H, McMeel O, Lareyre JJ, Bernot A, Laudet V, Guiguen Y. 2000. *DMRT1* expression during gonadal differentiation and spermatogenesis in the rainbow trout, *Oncorhynchus mykiss*. *Biochimica et Biophysica Acta*, **1493**(1-2): 180-187.
- Nanda I, Shan ZH, Schartl M, Burt DW, Koehler M, Nothwang H, Grütznert F, Paton IR, Windsor D, Dunn I, Engel W, Staeheli P, Mizuno S, Haaf T, Schmid M. 1999. 300 million years of conserved synteny between chicken Z and human chromosome 9. *Nature Genetics*, **21**(3): 258-259.
- Ohmuro-Matsuyama Y, Matsuda M, Kobayashi T, Ikeuchi T, Nagahama Y. 2003. Expression of *DMY* and *DMRT1* in various tissues of the Medaka (*Oryzias latipes*). *Zoological Science*, **20**(11): 1395-1398.
- Parlier D, Moers V, Van Campenhout C, Preillon J, Leclère L, Saulnier A, Sirakov M, Busengdal H, Kricha S, Marine JC, Rentzsch F, Bellefroid EJ. 2013. The *Xenopus* doublesex-related gene *Dmrt5* is required for olfactory placode neurogenesis. *Developmental Biology*, **373**(1): 39-52.
- Ren LL, Cheng HH, Guo YQ, Huang X, Liu L, Zhou RJ. 2001. Evolutionary conservation of *DMRT* gene family in amphibians, reptiles and birds. *Chinese Science Bulletin*, **46**(23): 1992-1996.
- Saulnier A, Keruzore M, Clercq SD, Bar I, Moers V, Magnani D, Walcher T, Filippis C, Kricha S, Parlier D, Viviani L, Matson CK, Nakagawa Y, Theil T, Gotz M, Mallamaci A, Marine JC, Zarkower D,



- Bellefroid EJ. 2013. The doublesex homolog *DMRT5* is required for the development of the caudomedial cerebral cortex in mammals. *Cerebral Cortex*, **23**(11): 2553-2567.
- Seo KW, Wang YD, Kokubo H, Kettlewell JR, Zarkower DA, Johnson RL. 2006. Targeted disruption of the DM domain containing transcription factor *Dmrt2* reveals an essential role in somite patterning. *Developmental Biology*, **290**(1): 200-210.
- Shen XY, Cui JZ, Yang GP, Gong QL, Gu QQ. 2007. Expression Detection of DMRTs and Two Sox9 Genes in *Takifugu rubripes* (Tetraodontidae, Vertebrata). *Journal of Ocean University of China*, **6**(2): 182-186.
- Shibata K, Takase M, Nakamura M. 2002. The *Dmrt1* expression in sex-reversed gonads of amphibians. *General and Comparative Endocrinology*, **127**(3): 232-241.
- Shin HS, An KW, Park MS, Jeong MH, Choi CY. 2009. Quantitative mRNA expression of *sox3* and *DMRT1* during sex reversal, and expression profiles after GnRH $\alpha$  administration in black porgy, *Acanthopagrus schlegelii*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **154**(1): 150-156.
- Smith CA, Roeszler KN, Ohnesorg T, Cummins DM, Farlie PG, Doran TJ, Sinclair AH. 2009. The avian Z-linked gene *DMRT1* is required for male sex determination in the chicken. *Nature*, **461**(7261): 267-271.
- Sun YY, Zhang QQ, Qi J, Wang ZG, Chen YJ, Li CM, Zhong QW. 2008. Cloning and expression analysis of *DMRT1* gene in *Cynoglossus semilaevis*. *Journal of Wuhan University*, **54**(2): 221-226.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: Molecular evolutionary genetic analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**(10): 2731-2739.
- Veith AM, Schäfer M, Klüber N, Schmidt C, Schultheis C, Schartl M, Winkler C, Volff JN. 2006. Tissue-specific expression of *dmrt* genes in embryos and adults of the platyfish *Xiphophorus maculatus*. *Zebrafish*, **3**(3): 325-337.
- Wang GY, Ding WY, Chen SB, Xie QL, Zhou ZM, Ai WM, Wu JG. 2011. RAPD analysis on genetic diversity of four populations of *Plecoglossus altivelis* from Zhejiang and Fujian province. *Bulletin of Science and Technology*, **27**(6): 863-868.
- Wang HX, Li J, Xiao Y. 1998. The artificial reproduction and embryo development of ayu (*Plecoglossus altivelis*). *Fisheries Science & Technology Information*, **25**(3): 30-31, 33.
- Yamaguchi A, Lee KH, Fujimoto H, Kadomura K, Yasumoto S, Matsuyama M. 2006. Expression of the *DMRT* gene and its roles in early gonadal development of the Japanese pufferfish *Takifugu rubripes*. *Comparative Biochemistry and Physiology. Part D, Genomics & Proteomics*, **1**(1): 59-68.
- Yang Y, Gong P, Feng YP, Li SJ, Peng XL, Ran ZP, Qian YG, Gong YZ. 2013. Temporospatial expression of *Dmrt1* in chicken urogenital system (*Gallus gallus*) using whole mount in situ hybridization. *Acta Biologica Hungarica*, **64**(2): 161-168.
- Yu FF, Gui JF, Zhou L, Wang MF, Yu XY. 2009. Cloning and expression characterization of *Dmrt5* in *Pinctada Martensii*. *Acta Hydrobiologica Sinica*, 2009, **33**(5): 844-850.
- Zhang L, Chen B, Cheng HH, Zhou JR. 2006. The cloning and expression analysis of *Dmrt5* in rice fieldeel. Hubei Association for Science & Technique. Hubei: Genetic Association of Hubei Province, Genetic Association of Jiangxi, 169.

## 《动物学研究》继续接收中文稿件的启事

尊敬的老读者和作者：

《动物学研究》是昆明动物研究所和中国动物学会共同主办的国内外公开发行的动物学类学报级双月刊。在两家主办单位的共同领导下，本刊的国际影响力不断提升，已被许多国际知名数据库所收录（如 2010 年被 PubMed/Medline 收录）；继入选“2012 中国国际影响力优秀学术期刊”之后，《动物学研究》再次晋级入选“2013 中国最具国际影响力学术期刊”，其“国际他引影响因子”为 0.323。如您所知，本刊原出版语言为汉语、英语混合或前后分别编排。为进一步扩大传播和影响力，突破其语言障碍，我们拟在申报过程中，2014 年把出版语言改为全英文，刊名先继续保持原英文刊名《Zoological Research》。现就有关稿件和费用情况说明如下：

本刊除了诚邀国内外作者直接赐予英文稿件外，也继续接收中文稿件。对审理通过的高质量中文稿件，则提请作者考虑选择：第一，由作者自行改写成英文；第二，由本刊编辑部组织中译英翻译和编辑润色。本刊不收取英文稿件的版面费，但酌情收取英文润色费；对编辑部组织中译英的稿件，除了收取润色费外，还酌情收取一定的翻译费。

真诚感谢您的理解与支持！

《动物学研究》编辑部  
2014年3月18日