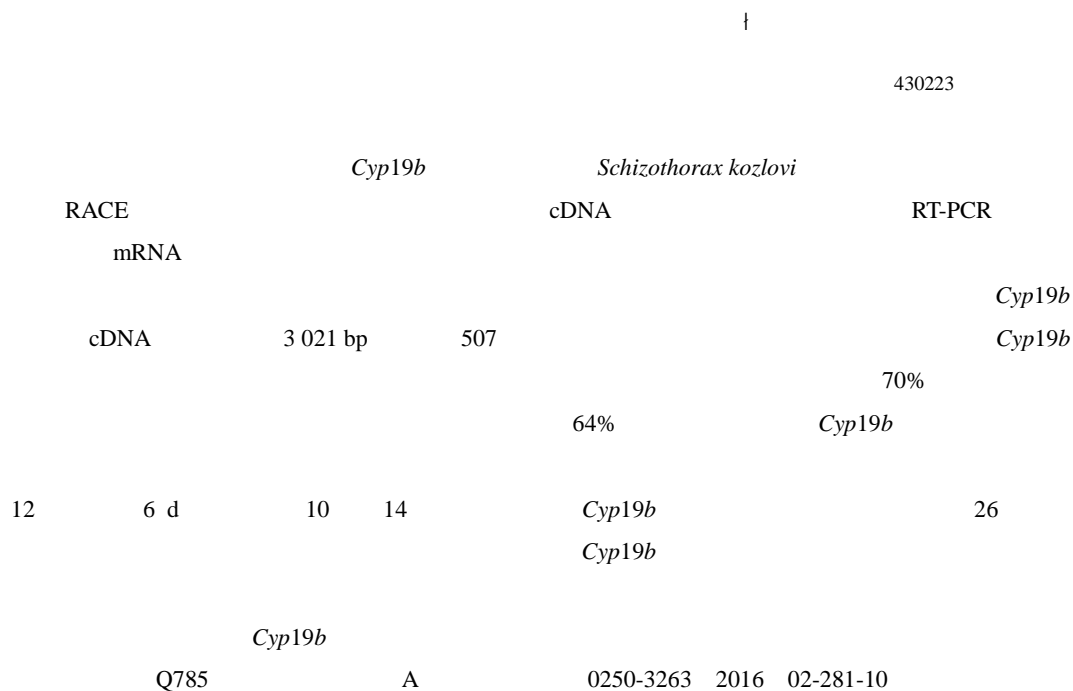


Cyp19b



Tissue Distribution of Brain Aromatase Gene *Cyp19b* in *Schizothorax kozlovi* and Temperature Effects on Its Expression

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Abstract: Previous studies by authors indicated that low temperature could result in more females of *Schizothorax kozlovi* during the early developmental stage. In order to elucidate the role of *Cyp19b* in sex differentiation of *S. kozlovi*, the full length cDNA of *Cyp19b* was cloned from the brain of *S. kozlovi* by using rapid amplification of cDNA ends (RACE) method. Relative mRNA expression levels of *Cyp19b* were determined by using quantitative real-time PCR (RT-PCR). Then the differences of *Cyp19b* mRNA

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expression levels in gills, brain, heart, liver, spleen, kidney, muscle, testis and ovary among different body-sized groups were discussed, while temperature effects on its expression during the larval stage of *S. kozlovi* were evaluated. The full length cDNA of *Cyp19b* was 3 021 bp, which encoded 507 amino acids and belonged to brain aromatase gene; the deduced amino acid sequence of *Cyp19b* of *S. kozlovi* had more than 70% identity compared with brain aromatase genes of other teleost fishes, but only around 64% when compared with gonadal aromatase genes of other teleost fishes. The mRNA expression of *Cyp19b* of *S. kozlovi* only appeared in brain but not in other tissues, presenting relatively high tissue specificity. One-Way Analysis of Variance (ANOVA) revealed that the mRNA expression levels of *Cyp19b* were not significantly different between females and males in small body-sized samples (mean standard length was 12 cm), but gradually showed significant difference between females and males in large body-sized samples (mean standard length was 26 cm) (Fig. 3). Larvae at 12 days of hatching were cultured in five different temperature groups, and ANOVA revealed that six days later the mRNA expression levels of *Cyp19b* were significantly increased by low temperature (10 °C and 14 °C), but not influenced by high temperature (26 °C) (Fig. 4). The present results suggest that brain aromatase gene *Cyp19b* may play important roles in the feminization of *S. kozlovi*, which is affected by low temperature during the early developmental stage.

Key words: *Schizothorax kozlovi*; *Cyp19b*; Tissue expression; Temperature

<i>Schizothorax kozlovi</i>			
Cypriniformes	Cyprinidae		
Schizothoracinae			
<i>Racoma</i>	2000		
	1981		pH
		Devlin et al. 2002	Baroiller et al.
		2009	aromatase
1989	2000	2010	
		1997	
	7 ~ 24	D Cotta et al. 2001	Ijiri et al. 2008
		Navarro-Martí et al. 2011	Shen et al. 2014
		<i>Cyp19a</i>	
		<i>Cyp19b</i>	
	2009	2013	<i>Oryctolagus</i>
	10	<i>cuniculus</i>	<i>Homo</i>
		<i>sapiens</i>	Pasmanik et al.
		100 ~ 1 000	<i>Cyp19b</i>
		1988	Piferrer et al. 2005

		ANOVA		Duncan s	
		MEGA 6.0		<i>Cyp19b</i>	
		<i>Cyp19b</i>		$P < 0.05$	
neighbor-joining NJ		2			
1 000 bootstraps					
1.4		2.1		<i>Cyp19b</i> cDNA	
Real-time PCR		<i>Cyp19b</i>		DNAStar	
				<i>Cyp19b</i> cDNA	
				GenBank	
		Dnase		KT285553	
		RNA OligodT ₁₈		open reading frame ORF	
<i>Cyp19b</i> cDNA		1 524 bp		7 7-UTR 115 bp 5	
PCR		<i>Cyp19b</i> -RT-PCR		5-UTR 1 382 bp 5-UTR	
cDNA		PCR		AATAAA Poly A	
<i>-actin</i>				507	
<i>-actin</i> -RT-PCR		1 PCR		57.8 ku	
4 2 " n 2 " n " R q y g t " U [D T " I t g g p "				P450	
PCR		2 0 4 7 " o q n 1 N -		-helix region	
7 " n PCR		95		aromatase specific substrate	
3 min 95 10 s 55 30 s 72 30 s 40		binding region		heme-binding	
65 5 s 95 15 s 2 ^{EV}		region 1			
<i>Cyp19b</i>		2.2		<i>Cyp19b</i>	
1.5				<i>Cyp19b</i>	

STATISTICA

1

*Cyp19b***Table 1 Primers used for cloning and expression of *Schizothorax kozlovi* *Cyp19b***

Primer	U g s w g p5e g+ " * 7	Usage
<i>Cyp19b</i> -P	TGGTKRYTGGGYCTNGBCC AGKATGSCYTTTCATMACCAT	Fragment PCR
<i>Cyp19b</i> - 7-P1	CAAACCTCCCAGTGTA	7 " T C E G " R E T
<i>Cyp19b</i> - 7-P2	CTTTGATCCAGACCCGAGCA	7 " T C E G " R E T
<i>Cyp19b</i> - 7-P3	TTTTCGTTGTAGTAGTTGCATG	7 " T C E G " R E T
<i>Cyp19b</i> - 5-P1	CTACCGAGTGGCGAAGGGGACGAAC	5 " T C E G " R E T
<i>Cyp19b</i> - 5-P2	ACCGTTCCCAGTCGTTACTTCCAGC	5 T C E G " R E T
<i>Cyp19b</i> -RT-PCR	TTCTGCGCTGTATCGTTGTG CCCATTTCATCCTGCAGCTC	PCR Relative real-time PCR
<i>-actin</i> -RT-PCR	TAGCCTCTCTCGGTCAGGAT ACACTGTGCCCATCTACGAG	PCR Relative real-time PCR

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1  acctcgggatgatgggacatgtaaacggcatgctgagctgctgaacagcacattaaatgaagcggatgcagatctaaagaggtgcacaagggtgctcgcagatgctg
116 ATGATGGAGCAGGTCTGTCAAAAGATACGGTAAACATCGCCACAGCGGTGCAGGGAACGCTGCTGGTGTGACT
1  M M E Q V V K D T V N I A T A V Q G T L L V L T
188 GGGACATTACTGCTGATTCTGTCACAGAATATTAACAGCCAAGAATTGGAGGAACCAATCAGCTGTCCCA
25  G L L L L L L H R I L L T A K N W R N Q S A V P
260 GGTCCAGGTTGGTGGTGGTCTCGGCCAGTTATGAGCTACAGCAGGTTCTGTGGATGGGGATCGGCTCC
49  G P G W W L G L G P V M S Y S R F L W M G I G S
332 GCATGCACTACTACAACGAAAAATATGGCAGCATTGCTCGGGTCTGGATCAAAGGAGAAGAGACCTTTATA
73  A C N Y Y N E K Y G S I A R V W I K G E E T F I
404 CTTAGCAAGTCTCTGCCGTGTATCATGTTCTGAAGAGCAGTAATTACACTGGGAGGTTTGGCAGTAAAAAA
97  L S K S S A V Y H V L K S S N Y T G R F A S K K
476 GGTCTGCAGTGCATCGGCATGTTTGAACAAGGCATTATCTTCAACAGCAACATCGCATTGTGGAATAAAGTG
121 G L Q C I G M F E Q G I I F N S N I A L W N K V
548 AGAACATATTTACCAAAGCTCTTACAGGTCCGGTCTCCAGAAGTCAGTAGATGTGTGTGCAGCGCAACC
145 R T Y F T K A L T G P G L Q K S V D V C V S A T
620 AACAAACAGCTTGACATCCTGCAGGAGTTACAGACCCTCAGACATGTGGATGTACTCAATCTTCCGCGC
169 N K Q L D I L Q E F T D H S G H V D V L N L L R
692 TGATCGTGTGGATGTTTCAATAGACTCTTCTAAGAATCCCTCTAATGAGAAAAGACTTCTGATAAAG
193 C I V V D V S N R L F L R I P L N E K D L I K
764 ATCCACCGTATTTTCAGTACCTGGCAGACGGTCTCATAACAGCCAGACATTTTCTCAGACTAAACTTTGTG
217 I H R Y F S T W Q T V L I Q P D I F F R L N F V
836 TACAAAAAATACCACCTGGCAGCAAAAAGAGTGCAGGATGAAATGGGAAAGCTTGTGGAGCAAAAGCGACAG
241 Y K K Y H L A A K E L Q D E M G K L V E Q K R Q
908 GCCATCAATAACATGGAGAAGCTGGAAGAGACAGACTTCGCAACAGAGCTGATATTTGCTCAGAACCACGAT
265 A I N N M E K L E E T D F A T E L I F A Q N H D
980 GAGTGTCTGCGGATGACGTGAGGCAGTGTGTGAGATGGTGTGATCGCCGCTCCAGACACCCCTCCATC
289 E L S A D D V R Q C V L E M V I A A P D T L S I
1052 AGTCTGTTCTTCATGCTTCTCTGCTGAAGCAGAACTCCGTCGTGGAGGAGCAGATCGTACAGGAGATACAA
313 S L F F M L L L L K Q N S V V E E Q I V Q E I Q
1124 TCTCAGATAGCGAGCGGGATGTAGAGTCCGGCCGACCTGCAGAACCTGAATGTTCTAGAGCGCTTTATCAAA
337 S Q I G E R D V E S A D L Q N L N V L E R F I K
1196 GAGTCTATGAGGTTCCATCCGGTGGTGGACTTCATCATGAGACAGGCTCTGGAGGACGACTCCATCGATGGC
361 E S M R F H P V V D F I M R Q A L E D D S I D K
1268 TACCGAGTGGCGAAGGGGACGAACCTCATCTGAACATCGGACGCATGCACAAGTCTGAGTTCTTCAAAAAA
385 Y R V A K G T N L I L N I G R M H K S E F F K K
1340 CCAACGAATTCAACTTGGAGAACCTTGAAGAACCCTCCAGTCTGTTACTTCCAGCCGTTCCGGCTGCGGC
409 P N E F N L E N F E N T V P S R Y F Q P F G C G
1412 CCGCGGGCCTGCGTGGGGAGCACATCTCTATGGTGTGACAAAAGCCATCCTGGTGACCTGTTGTGCGAGA
433 P R A C V G E H I S M V M T K A I L V T L L S R
1484 TTCACGGTTCATCTCTGTCACGGTGCACCGTCAAGCAGACCAACAACCTCTCCATMGACGCC
457 F T V H P R H G C T V S T I K Q T N N L S M Q P
1556 GTGGAAGAGGATCCCGACTGCTGGCCATGCGCTTATTCCACGCGCTCAGAATATCCACGGAGAACCACAC
481 V E E D P D C L A M R F I P R A Q N I H G E P H
1628 CTGAGCGCCTAA
505 L S A *
1640 aaacacaggaatgatacctttagaggtgcgcggagtaatttcctcatcaaaaatgttcagtgccctctatgtcaagactgcattgttttacttactatacacaacagttgaaact
1740 acctcaaaactgatcataccagtcgccctgcgacacagacatacttgaccaatactagacatcatgaggtgacttttggaggatggtcaccagggatgactgtctgtggtt
1840 gattactaccctcatgctcattcaaacccgtaagacctgttcgttttcggaacacaaattaaatatttttctaaagaccgggagatttctgcctcgcaggaacgaatgcaactga
1940 cttcaagcgcgacagggcgtagtaaggacactgttaaatcacactaaattttcataaaattacagttgaacctgatgacatggaatatttgacagtgcttaccgacttctgtg
2040 cctgatctgttagttgctgtcctgcgggctcagaagcctcttgatttcagaggggtgattagtgccggagttgcatgttgggtgaaactccctttaaagtgtctagc
2140 tcacctcaaaaaaagtttagtttagtttagctgacataaggcaatgacaacaaacacatcagttttgcatgactactactacataactactaaatgattattgctgtgctattttaa
2340 ataaaaftaaatgtatctttagtgcctccaactaatcaactaccaagttaacataaaaatggcattcacaacccattttaaataatgtgacgtttaaactacattaaatgagaaagaat
2440 ttatttcctggaattgactgtatttgaaaaagtgatgataggtaccagaatggagatgagtgaggggagcgtcaaaaaatgagtgatgttggatataatcaaaaaagggatga
2540 atgatgcttaagaatgaaacagtcgttattttatgaaagattagaacaaacttttggaaataatgtgacttaagtaaatgggtctgtttgttctgtttataaaaaata
2740 atctacagatgcaactgtttgaggtgactgtattacgcaactgagaaaactaaataatatttaacagaataaatactgaaactatttaatagagactgaaatatgtatattatgaaa
2840 ataaccgaaatatttgatgccgtatcgcactgttgcggaatacgaacaaactactgttttttctccaataaagtgtaccgataactactatagtaaahttacatttgaaaaa
2940 aaaaaaaaaaaaaaaaaaaaaa

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1

Cyp19b cDNA

Fig. 1 cDNA and deduced amino-acid sequences of the *Schizothorax kozlovi* brain aromatase gene *Cyp19b*

Poly A

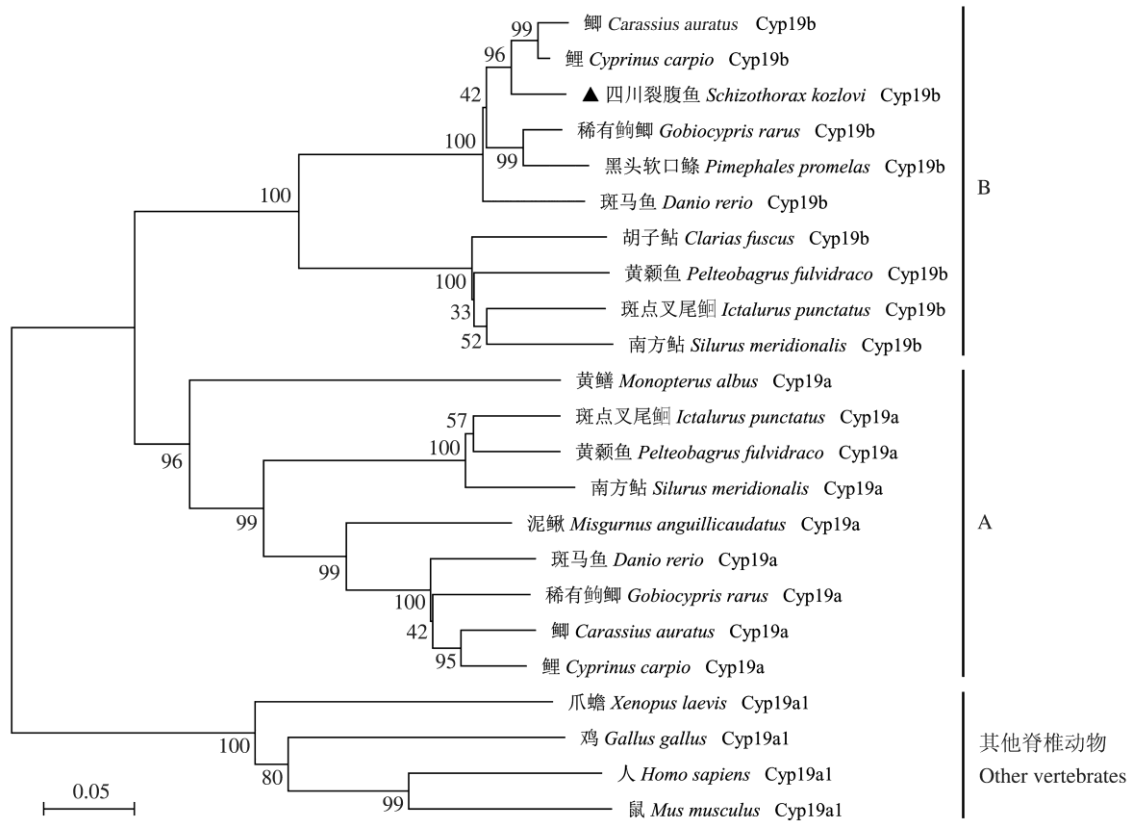
aataaa

ATG |

7- 3-

The poly-A signal is shaded in gray; the terminal aataaa signal is in dashed box; the start codon ATG is bold; the stop codon is indicated by an asterisk; the lowercase indicates 7- and 3- UTR; the putative regions of α -helix, aromatase specific substrate binding region and heme-binding region are shown in solid-line box, solid underline and dotted line, respectively.

	70%		<i>Cyprinus</i>						
<i>carpio</i>	GenBank	EU375456	<i>Carassius</i>	<i>Cyp19b</i>					
<i>auratus</i>	GenBank	AB009335	鲃						
<i>Gobiocypris rarus</i>	GenBank	GU220393		<i>Cyp19b</i>					
<i>Danio rerio</i>	GenBank	AF183908		9.69 ±8.89					
<i>Pimephales promelas</i>	GenBank			ANOVA				<i>Cyp19b</i>	
AJ277866									
	90%		<i>Pelteobagrus</i>						
<i>fulvidraco</i>	GenBank	AY649789	鲃					$P > 0.05$	
<i>Ictalurus punctatus</i>	GenBank	AF417239							$P <$
<i>Silurus meridionalis</i>	GenBank			0.05					
AY325907		<i>Clarias fusus</i>	GenBank		<i>Cyp19b</i>				
JN859189								$P < 0.05$	
	72%	75%	72%	71%				$P > 0.05$	3
	GenBank	EU375455	GenBank		2.4				<i>Cyp19b</i>
AF020704	鲃	GenBank	GU220394						
	GenBank	AF226620	鲃		ANOVA			12	
	GenBank	S75715	GenBank			6 d		18	
AY871802		GenBank	AY325908		<i>Cyp19b</i>	mRNA		10	14
<i>Misgurnus anguillicaudatus</i>	GenBank			22				$P < 0.05$	
AB531496	<i>Monopterus albus</i>	GenBank		18	26				$P >$
EU252487				0.05	18		<i>Cyp19b</i>	mRNA	
64% ~ 65%		<i>Cyp19b</i>			10	14	22		
								$P > 0.05$	18
		<i>Xenopus laevis</i>	GenBank					$P > 0.05$	10
AB031278	<i>Gallus gallus</i>	GenBank		22	18	26			14
NM_001001761	<i>Mus musculus</i>	GenBank			$P < 0.05$	4			
NM_007810	<i>Homo sapiens</i>	GenBank		3					
BC035959		55% ~ 57%							
A	B		2						
			B			2000			2008
		2			2009	2012			
		鲃							
						<i>Cyp19b</i>			
2.3	<i>Cyp19b</i>							<i>Cyp19b</i>	
	Real-time PCR		<i>Cyp19b</i>					<i>Cynoglossus semilaevis</i>	



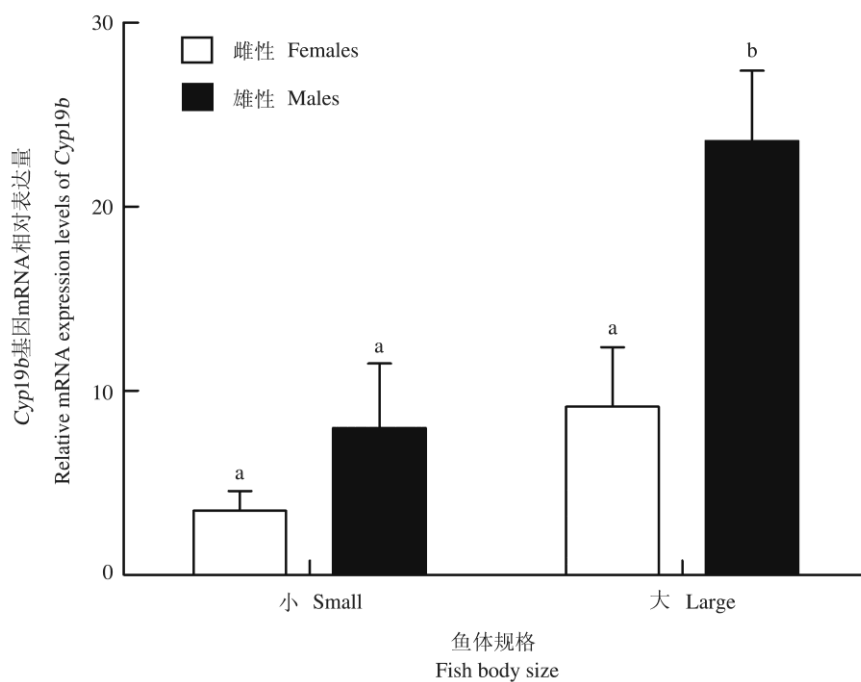
2

Fig. 2 Phylogenetic tree of amino acid sequences of *Schizothorax kozlovi* brain aromatase gene *Cyp19b* and other organisms aromatase genes based on neighbor-joining (NJ) method

1 000 bootstrap

" k p " v j g " h k i w t g " t g r t g u g p v u " v j g " v c t i g v " u v w f { " q d l g e v the frequency with r which the tree topology presented here was replicated after 1 000 bootstrap iterations, the scale represents the number of amino acid sequences changed.

2008
2012 鮡
2009 *Cyp19b*
Oreochromis niloticus Anderson et al.
1988 Vizziano-Cantonnet et al. 2011
Chang et al. 2005
Cyp19b Menuet
12 cm et al. 2005 Vizziano-Cantonnet et al. 2011
26 cm



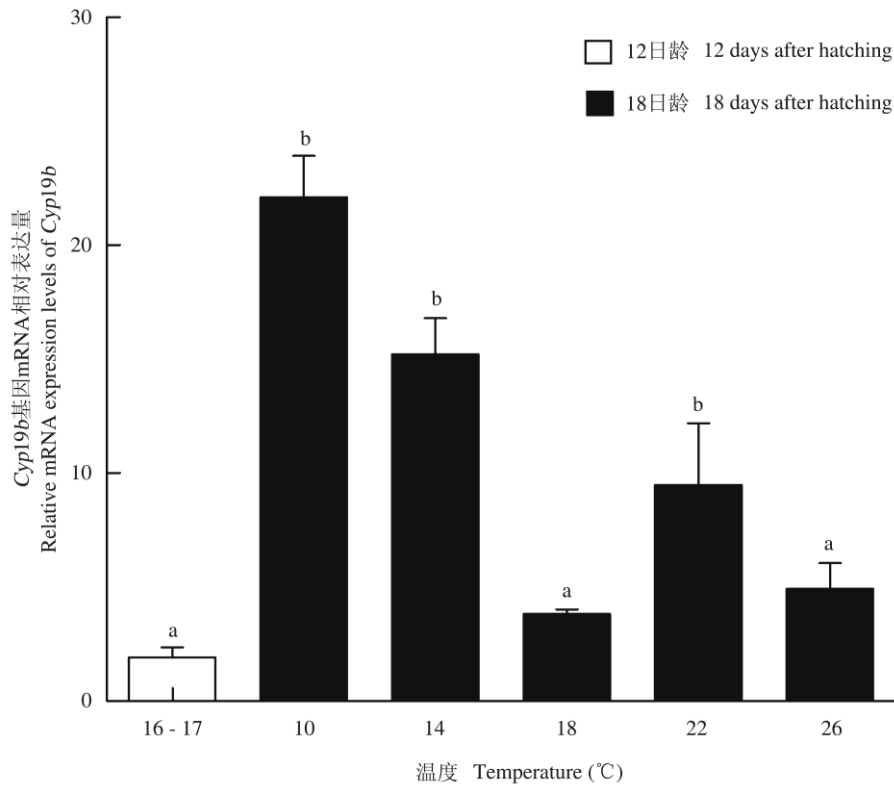
3 *Cyp19b* mRNA

Fig. 3 Relative mRNA expression levels of *Cyp19b* in the brain of *Schizothorax kozlovi* in the two different body size groups

$P < 0.05$

The same letters in the figure present no significant difference, while different letters present significant difference ($P < 0.05$).

		2005 Sawyer et al.	2006 Smith et al.	2013 Li et al.	2014	27
	2001 Menuet et al.					
2005 Piferrer et al.	2005					35
				D Cotta et al.	2001	
Nocillado et al.	2007	2009		10		<i>Cyp19b</i>
				26		
					22	<i>Cyp19b</i>
cAMP						
SF-1				10		
		<i>Cyp19a</i>				
		<i>Cyp19b</i>				<i>Cyp19a</i>
	Kitano et al.	1999	Chang et al.			



4

Cyp19b mRNA

Fig. 4 Relative mRNA expression levels of *Cyp19b* gene of *Schizothorax kozlovi* larvae before and after temperature changed

$P < 0.05$

The same letters in the figure present no significant difference, while different letters present significant difference ($P < 0.05$).

Navarro-Martí et al.

2011 Piferrer 2013 Shao et al. 2014

Cyp19b

Cyp19b

cDNA

Cyp19b

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