

Cloning and characterization of wnt4a gene and evidence for positive selection in half-smooth tongue sole(*Cynoglossus semilaevis*)

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Supplementary Information :

Fig. S1 Nucleotide sequence of the half-smooth tongue sole wnt4a cDNA. The red dot represents the signal peptide cleavage site. The box represents glycosylation sites. The WNT conserved domains are highlighted and the methylated island is underlined.

Fig. S2 Sense probe detected the gonads of female and male. F: female gonad, M: male gonad

Fig. S3 Multiple comparisons of the methylation level among different gonads

Fig. S4 Alignment of the 36 amino acid sequences, The WNT conserved domains are highlighted and the blue dot represents the glycosylation sites and the red dot represents the positively selected site in tongue sole.

Table. S1 Primers and their sequences used in this study.

Table. S2 Accession numbers of Wnt4 sequences used in this study.

acatg^{ggg}gagtgctgtgagaagtgaagacacaggagcaggagcagcagtgagaggaggagt 61
 agactcg^gcgcgctccgca^gcgctcaaggtaaaatctggagagaaccgaggcgcgcagtcggacagtaag 130
 tcttgtagccgctgggtgcagcagcagcaataacaacaacaaccacaaagtataagctttaagc 199
 cgtgagtgcaacaggaggtggaggtgaaggaggaggagcccgatcggagctcctgcagccggcggtcat 268
 cggactgacactcagegcatectctagtg^gctggagtgctctggaaggaaaagcgcgtgttetgetaaaag 337
 gctcgggttgatgctgagctctgactgtccagtcacaactacaactactatttatcatttgatgtatgctc 406
 gcttccttctg^gcgcgctcggagcggcagagaggacgggacatctccgtgggtggatggaccg^gggagct 475
 gcgttctcactcctccgctaacaggatgatggattaaacgcttctcctcgcgaccggtctgtttcacttctg 544
 acttttccctcaccgctctcgggattatttgatctctctg^ggcttccactccacccccaccatctcgag 613
 ATGACCGAAGACTGCGTCTGCGCTGCGTCTGATGCTCTGCTGTGCGTCTCTCCGCCAACGCCAGT 682
 M **T** E D C V L R C V L M L C C A L L S A **N** A **S** 23
 AACTGGTTATACTTGGCCAAGCTGTCGTCAGTAGGGAGCATCAGGGATGAGGAGACATGTGAGCGGTTA 751
 N W L Y L A K L S S V G S I R D E E T C E **R** L 46
 CGAGGCCTCATCCAGAGACAGGTCCAGATCTGTAAGCGCAGCGTGGAGGTGATGGATGCAGTGCCTCGC 820
R G L I Q R Q V Q I C K R **S** V E V M D A V R R 69
 GGGGCTCAGCTGGCCATAGACGAATGCCAGTTTCAGTTCGCAACCGACGATGGA^{ACT}GTTCAACTCTG 889
G A Q L A I D E C Q F Q F R N R R W **N** C S T L 92
 GAGACAATGCCTGTGTTTGGCAAAGTGGTCACACAGGGCACCCGTGAGGCTGCCTTTGTTTGTGTCATC 958
E T M P V F G K V V **T** Q G T R E A A F V C V I 115
 TCAGCGTCCATTGTGGCGTTTGGCGTAACGAGGGCCTGCAGCAGCGGAGAGCTGGAAAAATGTGGCTGT 1027
S A S I V A F A V T R A C S S G E L E K C G C 138
 GACCACAATGTACATGGAGTCAGTCCAGAGGGGTTCCAGTGGTCCGGCTGCAGTGACAACATTGCCTAC 1096
D H N V H G V **S** P E G F Q W S G C S D N I A Y 161
 GGAGTGGCCTTTTCTCAGTCCTTTGTGGACGTTAGGGAGAGGGGTTAAAGGTCA^{GT}TTCAGCCGAGCT 1165
G V A F S Q **S** F V D V R E R G K G Q S **S** **S** R A 184
 CTCATGAACCTTCACAACAATGAGGCTGGCAGAAAGGCCATTCTGTCCCACATGCGGGTGGAGTGAAG 1234
L M **N** L H N **N** E A G R K A I L **S** H M R V E C K 207
 TGTGATGGCGTGTGAGGCTCCTGTGAAGTAAAGACCTGTTGGAAGGCCATGCCACCCTTTAGAAAGGTG 1303
C H G V S G S C E V K **T** C W K A M P P F R K V 230
 GGCAATGTCATCAAGGAGAAGTTTGATGGTGCCACAGAGGTGGAGCAACGCAAGATGGGCTCAACCAA 1372
G **N** V I K E K F D G A T E V E Q R K M G S **T** K 253
 GTCCTGGTGCCTCGCAACTCTCAGTTTAAAGCCTCACACAGATGAAGACCTGTTTACCTGGACCCAGT 1441
V L V P R N S Q F K P H T D E D L V Y L D P S 276
 CCAGATTTTGTGACTATGATCCGCGAACGCCGGGCTGCTGGGCACGGTGGGCCAGTGAACAGA 1510
P D F C D Y D P R **T** P G L L G T V G R Q C **N** R 299
 ACCTCAAAGGCCATTGACGGCTGTGAGCTAATGTGCTGTGGCCGTGGCTTTCAGACACAAGAGGTGGAG 1579
T S K A I D G C E L M C C G R G F Q T Q E V E 322
 GTGGTGGACAGGTGCAGCTGTAAGTCCACTGGTGTCTATGTCAAATGCAAACAGTGCAGGAAAATG 1648
V V D R C S C K F H W C C Y V K C K Q C R K M 345
 GTGGAAATGCACACCTGCCGGTGA^tggatgtgggtggagcactgctgatggacaaagcaatcacccat 1717
V E M H **T** C R * 352
 gttctatctcaacataaaaaacagagaaggcgacagggacttttgaagatgctgcctctcccacttgaag 1786
 gccacctggccccgccactactggttgacagacagactgtcttttaaagggtcgctctatacattctta 1855
 accaccgtgagtggaataaataagtaaatgcatggggttttccccaaaaaaaaaaaaaaaaaaaaaaaaaagt 1924

Fig.S1 Hu et al

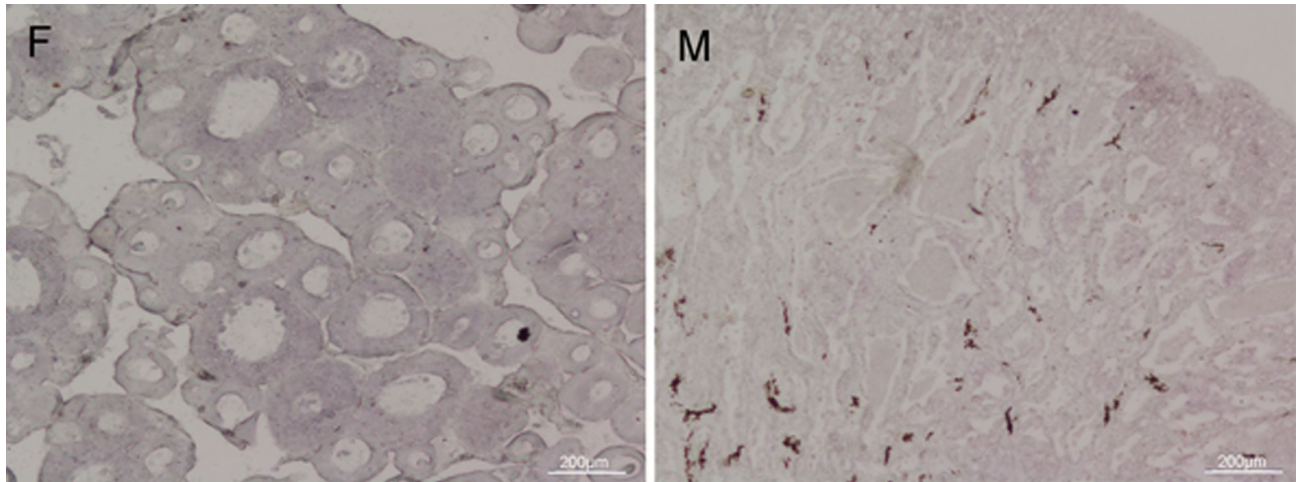


Fig.S2 Hu et al

ANOVA					
VAR00002					
	平方和	df	均方	F	显著性
组间	.380	2	.190	31.506	.000
组内	.145	24	.006		
总数	.525	26			

VAR00002				
Duncan ^a				
VAR00001	N	alpha = 0.05 的子集		
		1	2	3
1.00	9	.1911		
2.00	9		.2702	
3.00	9			.4729
显著性		1.000	1.000	1.000
将显示同类子集中的组均值。				
a. 将使用调和均值样本大小 = 9.000。				

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Table S1 Hu et al

Primers	Promer sequences (5'-3')	Utilizations
UPM-long	CTAATACGACTCACTATAGGGCAAGCAGTG GTATCAACGCAGAGT	5' or 3'RACE
UPM-short	CTAATACGACTCACTATAGGGC	5' or 3'RACE
Wnt4a-5'GSP	AACTGAGAGTTGCGAGGCACCAGGAC	5'region clone
Wnt4a-3'GSP	GAACCTTCACAACAATGAGGCTGGCA	3'region clone
Wnt4ash-S	GCCGAATTCAGCACCAGTGGAACCTTACAGC	Situ hybridization
Wnt4ash-A	GCCGATATCTTTGCGGTAACGAGGGC	Situ hybridization
Wnt4a-S1	TAAAGGTCAGTCTTCCAGCCGA	qRT-PCR
Wnt4a-A1	TGGCACCATCAAACCTTCTCCTT	qRT-PCR
β -Actin-f	CCAACAGGGAAAAGATGACC	Internal control
β -Actin-r	TTCTCCTTGATGTCACGCAC	Internal control
Wnt4aMS-S	GTTGTGAGAAGTGAAGATATAGGAGTAG	DNA methylation
Wnt4aMS-A	CAAACACTCCAACCACTAAAAATAC	DNA methylation
CseF382F	5'ATTCACTGACCCCTGAGAGC3'	Genetic sex determination
CseF382R	5'TGGCACCATCATTGTAAACTA3'	Genetic sex determination

Table S2.Hu et al

Species name	Accession number
Gasterosteus aculeatus	ENSGACG00000010904
Oreochromis niloticus	XP_003442666.1
Tetraodon nigrovirdis	ENSTNIG00000007371
Epinephelus coioides	KC206549.1
Maylandia zebra	XP_004559969.1
Dicentrarchus labrax	CBN80604.1
Oncorhynchus mykiss a2	NP_001268268.1
Oncorhynchus mykiss a1	NP_001268339.1
Xiphophorus maculatus	XP_005799140.1
Oryzias latipes	NP_001153911.1
Gadus morhua	ENSGMOG00000016283
Astyanax fasciatus mexicanus	ENSAMXG00000003592
Danio rerio	NP_001035477.1
Mus musculus	NP_033549.1
Homo sapiens	NP_110388.2
Equus caballus	XP_005607536.1
Bubalus bubalis	AFH66793.1
Pan troglodytes	XP_524597.3
Rattus norvegicus	NP_445854.1
Cynoglossus semilaevis	KJ825677
Latimeria chalumnae	ENSLACG00000014986
lepisosteus oculatus	ENSLOCG00000001126
cavia porcellus	ENSCPOG00000005809
macaca mulatta	XP_001100814.1
bDanio rerio	ENSDARG00000040159
bAstyanax fasciatus mexicanus	ENSAMXG00000010599
bGadus morhua	ENSGMOG00000015702
bGasterosteus aculeatus	ENSGACG00000010089
bOreochromis niloticus	ENSONIG00000012962
bOryzias latipes	ENSORLG00000012515
bTakifugu rubripes	ENSTRUG00000007912
bTetraodon nigrovirdis	ENSTNIG00000003321
capitella teleta	ELU08380.1
Azumapecten farreri	AFU35435.1
Takifugu rubripes	XP_003973796.1
Haplochromis burtoni	XP_005933542

