

Supplementary files for the paper

Charactering the *ZFAND3* gene and mapping it in the sex-determining locus in hybrid tilapia (*Oreochromis spp.*)

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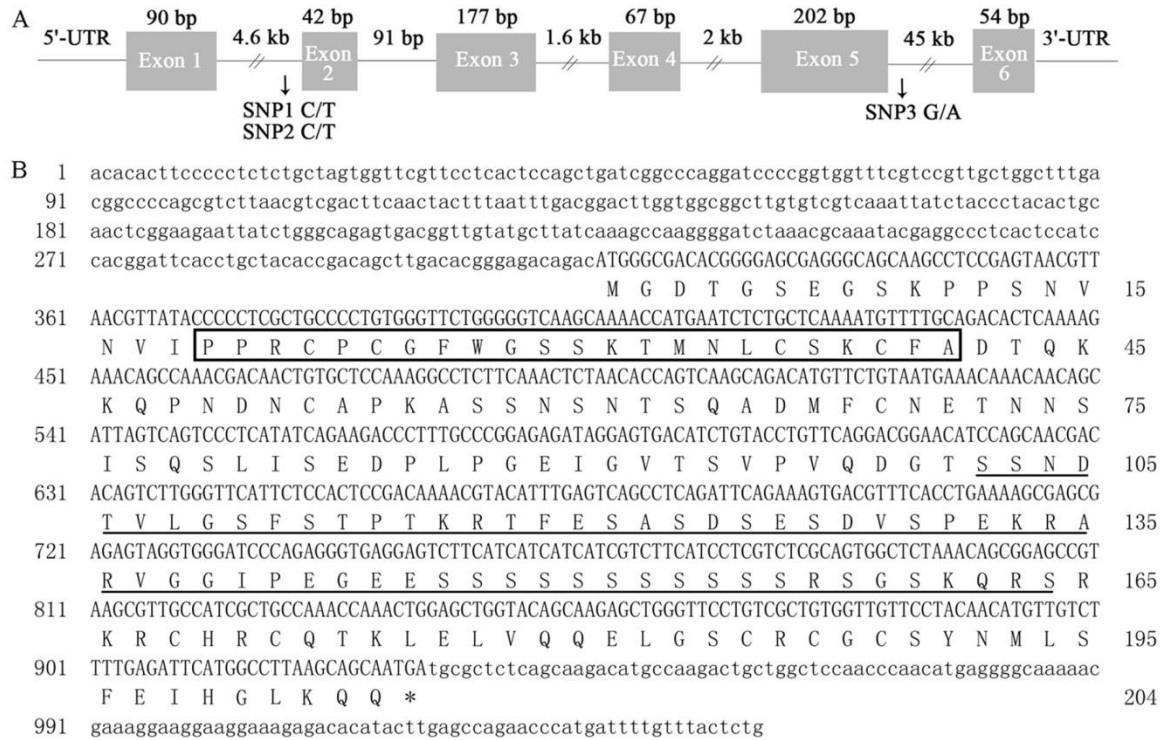
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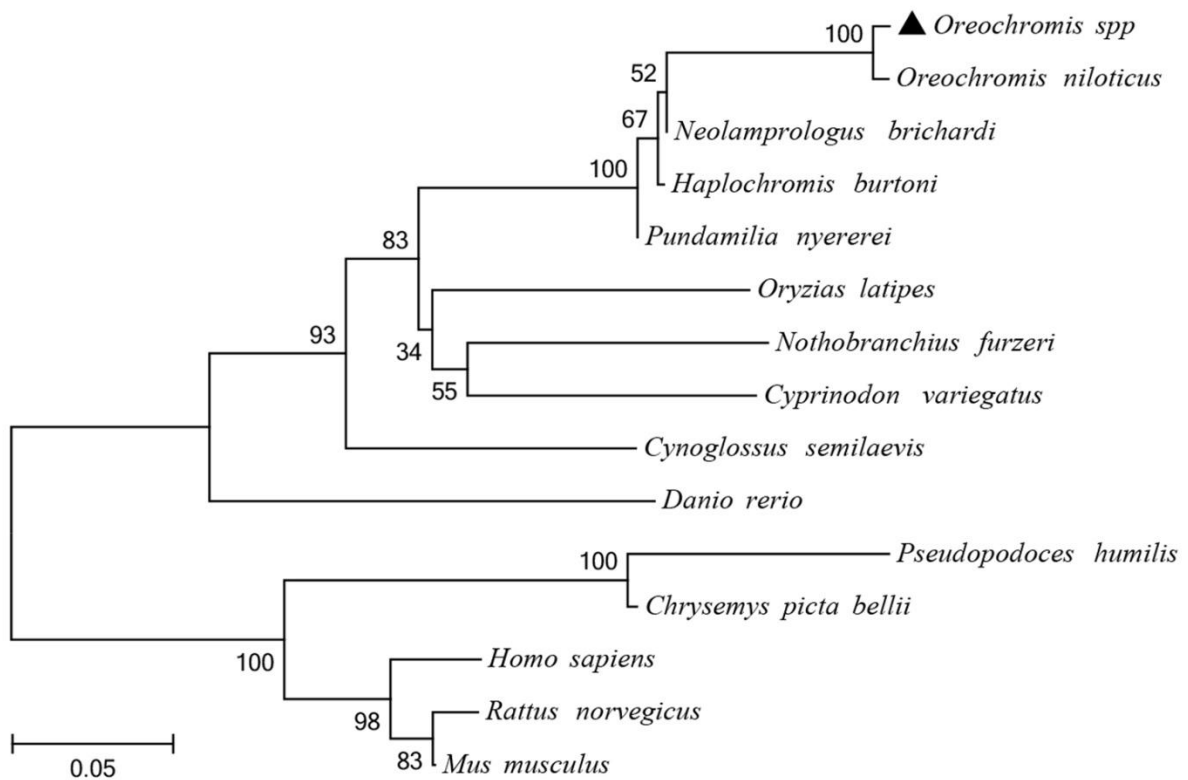
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Supplementary Figure S1. Genomic structure (A) and deduced amino acid sequence (B) of the *OsZFAND3* gene

A: horizontal lines and boxes indicate introns and exons in genomic structure, respectively. The locations of the three SNPs are marked with arrows. B: nucleotides (top) are numbered from 5' to 3', starting from the initiator codon (ATG), while amino acid residues (bottom) are numbered starting with the first methionine (M) residue. The asterisk (*) indicates the stop codon (TGA). A20-like zinc finger region and Serine-rich region are boxed and underline, respectively.



Supplementary Figure S2. An unrooted phylogenetic tree of the ZFAND3 gene

The phylogenetic tree was inferred by using the Neighbor-Joining method with whole deduced amino acid sequences. The values at the forks are Neighbor-Joining bootstrap supporting data. For the branch length, the distance given in the scale represents 0.05 amino acid substitution/site.

Supplementary Table S1

Summary of genotypes of parents and their F₁ progenies of tilapia at SNP1, SNP2, SNP3, Onil_1-89, Onil_19-15 and Onil_27-3 reported in this study (Please online Supplementary Dataset).

Supplementary Table S2**Amino acid identities (%) of the *ZFAND3* gene between hybrid tilapia and other representative species**

Gene	GenBank Accession No.	Amino acid	Identity	P-value
<i>Oreochromis niloticus</i>	XP_005447615.1	204	99%	2e-149
<i>Neolamprologus brichardi</i>	XP_006779955.1	238	93%	5e-123
<i>Haplochromis burtoni</i>	XP_005934639.1	238	93%	2e-122
<i>Cynoglossus semilaevis</i>	XP_008309070.1	254	69%	5e-96
<i>Homo sapiens</i>	NP_068762.1	227	52%	2e-58
<i>Rattus norvegicus</i>	NP_001012175.1	227	51%	2e-57
<i>Mus musculus</i>	NP_683728.1	205	47%	4e-50
<i>Pseudopodoces humilis</i>	XP_005533586.1	223	50%	1e-51
<i>Chrysemys picta bellii</i>	XP_008172961.1	210	45%	5e-47
<i>Pundamilia nyererei</i>	XP_005733397.1	216	82%	1e-93
<i>Oryzias latipes</i>	XP_004067319.1	244	72%	4e-86
<i>Nothobranchius furzeri</i>	XP_015808368.1	247	70%	3e-76
<i>Cyprinodon variegatus</i>	XP_015233722.1	248	72%	7e-73
<i>Danio rerio</i>	NP_001025346.1	226	58%	1e-54

Supplementary Table S3**Evolution estimates of *ZFAND3* gene by dN/dS values using maximum likelihood analysis (Please online [Supplementary Dataset](#)).**

Supplementary Table S4

Summary of primer sets used for mapping and characterizing the *OsZFAND3* gene.

Marker	Primer sequence (5'-3')	Ta (°C)	Purpose
Onil_1-89	TCTCGCGATTCTCTGCTC TTGCCATTGTCTTCCACTGTAA	55	mapping
Onil_19-15	CACCTACAATTCTAGCACATACCA GAATTTCTTCAAGGACACTGTCA	55	mapping
Onil_27-3	CCTGCAGTGGGTTTGGATT AGTCAGCGATAGAAGAGGGAATAC	55	mapping
x3 SNP1	CCCCCAGACGCACTTATG AGAGGCCTTTGGAGCACAG	50	SNP
x3 SNP2	CATCGCTGCCAAACCAACT TTCCGCCCCAATACAATCT	50	SNP
β -actin	TGACCCAGATCATGTTTCGAGAC GTGGTGGTGAAGGAGTAGCC	60	qPCR
x3	GCTGGGTTCTGTCGCTGTG TCATGGGTTCTGGCTCAAGTATG	60	qPCR, ISH
x3-pc	<u>AGCTCGAGATGGGCGACACGGGGAGC</u> <u>CCAAGCTTTTGCTGCTTAAGGCAATGAATCTCA</u>	60	Localization

Note: the letters in italics were restriction sites. The letters underlined were protective bases.