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Dear Editor,

Recently, Aoki *et al.* have been published an interesting paper in Biochemical and Biophysical Research Communications [445 (2014) 357-362.] in which they identified possible downstream genes required for the extension of peripheral axons in primary sensory neurons of zebrafish. *Tppp* was claimed as one of the several candidate genes. However, there is confusion in the paper since the three tppp paralogs are mixed up. In the text *tppp* is mentioned and the properties of vertebrate TPPP1 (tubulin polymerization-promoting protein) are discussed. It is claimed that its expression was investigated in various tissues. However, the supplementary table showing the genes investigated lists not *tppp* but "*tubulin polymerization-promoting protein family member 3*" i.e. *tppp3*. To reach a complete confusion, the NCBI Accession number XM_682834 is given in a table, which is the mRNA of the *tubulin polymerization-promoting protein family member 3-like* gene according to the NCBI Database.

Thus it seems to be necessary to clarify this question.

As I show in the submitted paper, it is the *tppp3-like* gene, a paralog of *tppp*, which plays this role. This finding raises further the significance of Aoki and his co-workers' paper since it is the very first one which provides experimental data about a *tppp3-like* gene.

In the other part of the paper, I clarify the position of the *tppp3-like* genes, found exclusively in fishes, in the family of TPPP-like proteins showing that they are the orthologs of human *TPPP2*.

I hope that the paper is suitable for publication in your journal.

Budapest, November 14, 2014.

Sincerely yours

Ferenc Orosz, Ph.D.

Highlights

tppp3-like not *tppp* is a possible Islet2a downstream functional target

Fish-specific *tppp3-like* is a *TPPP2* ortholog

Genomic positions of *tppp1* and *tppp3* but not those of *tppp2* are stabilized in fishes

On the tubulin polymerization promoting proteins of zebrafish

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Abbreviations: TPPP, tubulin polymerization promoting protein

Abstract

Recently, Aoki *et al.* [15] have been published a paper (Biochem. Biophys. Res. Commun. 445 (2014) 357-362.) in which they identified possible downstream genes required for the extension of peripheral axons in primary sensory neurons of zebrafish. *Tppp* was claimed as one of them but, as I show, it is the *tppp3-like* gene, a paralog of *tppp*, which plays this role. There are three tppp paralogs in fishes: *tppp1 (*named also *tppp)*, *tppp3* and *tppp3-like*. *Tppp1* and *tppp3* are the orthologs of the corresponding human genes, however, the classification of the third one is ambiguous. It is known that the genomes of the early vertebrate lineage underwent two complete genome duplications, which result in the presence of several paralogs in vertebrates. A teleost fish specific third whole genome duplication also occurred. Thus the *tppp3-like* gene can be either an ortholog of human *TPPP2* or a fourth paralog (*tppp4*) absent in tetrapods but present in fishes; finally a *tppp3a* gene which can be originated from the third, fish specific, whole genome duplication. Comparing the sequences of vertebrate and recently available lamprey tppps I show that the *tppp3-like* gene is a *TPPP2* ortholog. Synteny data are in accordance with this suggestion.

Keywords: tubulin polymerization promoting proteins (TPPPs), zebrafish, lamprey, genome duplication, synteny

1. Introduction

The family of TPPP-like proteins was described recently [1]. Its first member, tubulin polymerization promoting protein (TPPP or TPPP1) was first isolated from bovine brain [2] and later found to promote tubulin polymerization and stabilization of microtubules [3, 4]. There are three TPPP paralogous genes in human, *TPPP1*, *TPPP2* and *TPPP3* (TPPP/p25, TPPP2/p18 and TPPP3/p20 at protein level) [5]. These paralogs can also be found in mammals, birds and reptiles. In fish, three paralogs exist as well; *tppp1* and *tppp3* are the orthologs of the corresponding human genes/proteins, however, the classification of the third one is ambiguous. Sometimes it is named as *tppp3-like* gene in databases. The reason of this name can be the fact that these proteins are more similar, indeed, to tetrapod TPPP3s than to TPPP2s or TPPP1s [6]. It is known that the genomes of the early vertebrate lineage underwent two complete genome duplications, which result in the presence of several paralogs in vertebrates in comparison with the single copy of their invertebrate orthologs [7-10]. A teleost fish specific third whole genome duplication also occurred [11-14]. Earlier, I have shown by synteny analysis that the probable history of the two-rounds duplication of the single invertebrate *tppp* gene was that the diversification of *tppp1* and the precursor of *tppp2*/*tppp3* occurred in the first round of whole-genome duplication which was followed by two further splits, *tppp1*/lost and *tppp3*/ *tppp2*, in the second round [6]. However, it remained an open question the position of the fish-specific group of *tppp*s. It can be considered either as *TPPP2* ortholog or as the fourth paralog (*tppp4*) that was lost in tetrapods but remained in fish; finally as *tppp3a* gene which is originated from the third, teleost fish specific, whole genome duplication [6].

Recently, Aoki *et al.* [15] have been published a paper in Biochemical and Biophysical Research Communications, in which they identified possible downstream genes required for the extension of peripheral axons in primary sensory neurons of zebrafish. *Tppp* was claimed as one of the several candidate genes. However, there is confusion in the paper since the three tppp paralogs are mixed up. In the text *tppp* is mentioned and the properties of vertebrate TPPP1 are discussed. It is claimed that its expression was investigated in various tissues. However, the supplementary table showing the genes investigated lists not *tppp* but "*tubulin polymerization-promoting protein family* *member 3*” i.e. *tppp3*. To reach a complete confusion, the NCBI Accession number XM_682834 is given in a table, which is the mRNA of the *tubulin polymerization-promoting protein family member 3-like* gene according to the NCBI Database.

Thus it seems to be necessary to clarify this question.

2. Materials and methods

2.1. Database homology search

TPPP homologs were identified with an NCBI blast search using the sequences of human TPPP proteins (NP_008961; NP_776245; NP_057048) as queries. BLASTP or TBLASTN analysis [16] was performed on complete genome sequences and EST collections available at the NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Similar search was carried out on various fish databases: http://www.fugu-sg.org/; http://www.sanger.ac.uk/Projects/D_rerio/; http://www.ensembl.org/Tetraodon_nigroviridis/; http://dolphin.lab.nig.ac.jp/medaka/. The homepage of the Ensembl project [17]), http://www.ensembl.org/, was also checked for orthologs. Nucleotide sequences identified in TBLASTN searches were translated in the reading frames denoted in the TBLASTN hit, taking frame shifts or introns of genomic sequences into account.

2.2. Alignments of sequences

Multiple alignments of sequences were done by the *Clustal Omega* program [18].

2.3. Synteny Analysis

Large scale investigation of synteny among TPPP loci Genomicus (http://www.dyogen.ens.fr/genomicus-76.01/cgi-bin/search.pl) [19] was used.

3. Results and Discussion

3.1. TPPPs of the zebrafish (Danio rerio)

The zebrafish (*Danio rerio*) possesses the three TPPP-paralogs characteristic for teleost fishes (Table1). TTTP3 and TPPP3-like proteins are very similar to the corresponding fish orthologs (not shown). The chromosomal localization of the coding genes is on Dre7 and Dre5, respectively. The position of *tppp* gene has been unknown until very recently and its tentative sequence based on whole genome shotgun scaffolds; now it is localized on Dre16. It can be seen that the second and third exon of the hypothetical XP_002667767 protein fits well to the sequences of the corresponding exons of the fish orthologs (Fig. 1) but the long first exon, which is very characteristic for TPPP1 paralogs, is much shorter and absolutely different. However, there are two ESTs (5' and 3' reads) whose translated sequences are homologous to the first two exons of TPPP1s. It worth to mention that the first exon contains the characteristic KRLS sequence, a phosphorylation site [20], which is present in all vertebrate TPPP1s without exception [1, 5]. Moreover, the sequence of the second exon is completely identical with that of the second exon of XP_002667767, even at nucleotide level. (The third exon is missing which is not astonishing in the case of ESTs sequences.) Thus the TPPP1 protein of the zebrafish has very probably a similar sequence as TPPP1s of other teleost fishes and the sequence suggested here and not the hypothetical XP_002667767 corresponds to zebrafish TPPP1. This suggestion is supported by the result of TBLASTN search of the whole genome shotgun sequences of *Danio rerio* against any of the fish TPPP1s which identified the Zv8_scaffold2999. Its manual translation shows that it encodes the whole TPPP1 including the three protein coding exons with the correct sequence (Fig. 2; Supplementary figure 1). This sequence is very similar (86 % identity, 94% similarity) to that of the phylogenetically nearest relative, *Astyanax mexicanus* TPPP1 (Fig. 1).

The authors gave the sequence of the antisense oligonucleotide used to block protein translation. BLAST search and Clustal Omega alignment show unequivocally that the given sequence corresponds to a part of the mRNA of the *tppp3-like* gene exhibiting a 100% identity (data not shown). It enlightens that in Aoki and co-workers' paper identifies this gene as possible downstream gene required for the extension of peripheral axons in primary sensory neurons.

Many data are known about the properties and function of human TPPP1, much less about TPPP3 and almost nothing about TPPP2. Thus one should be very cautious if predicts the properties and function of *tppp3-like* gene/protein on the basis of mammalian TPPP1 as the authors did. E.g., it was shown that TPPP1 and TPPP3 but not TPPP2 promoted tubulin polymerization and bundling of microtubules [5]. However, it should be emphasized that Aoki and co-workers' paper is the first one which provides data about a *tppp3-like* gene.

One of their findings may have an interesting consequence which can contribute to the clarification of the nature of the third fish tppp paralog (*tppp3-like*). They studied zebrafish embryos not adult animals and found that *tppp3-like* gene is expressed in various neurons. It is known that both TPPP1 and TPPP3 can be found in adult brains of various mammals. The exact localization of TPPP3 in brain is not known but TPPP1 can be found physiologically in oligodendroglia not in neurons [21-23]. Whilst TPPP1 seems to be brain-specific, TPPP3 has been found in other tissues as well [24-26]. The developmental expression of TPPP1 in rat brain shows that it is practically absent in embryos and after birth its amount increases continuously by aging [21]. On the contrary, mammalian TPPP2 is absent in adult brain but is expressed in fetal one [27]; i.e. the novel finding by Aoki *et al.* [15] may suggest that the third fish TPPP paralog is a TPPP2 ortholog or, at least, is in accordance with this hypothesis.

3.2. Nature of the third tppp gene (tppp2, tppp3-like or tppp4)

As I mentioned, the *tppp3-like* gene/protein can be considered either as *tppp2* ortholog or as the fourth paralog (*tppp4*) absent in tetrapods or as *tppp3a* originated from the fish specific whole genome duplication. On the basis of statistical analysis of phylogenetic trees, neither these cases could be excluded since this TPPP group could be placed on the phylogenetic tree into several different positions of almost equal probabilities [6]. Since all the phylogenetic analysis showed unequivocally that *tppp3-like* genes were not sister to *tppp1* genes thus comparing the phylogenetic and synteny data (cf. Introduction), the two possibilities remained that they were fish orthologs of tetrapod TPPP2s or fish specific paralogs (*tppp3a*) resulted from the fish specific whole genome duplication.

The recently published sequencing data of the sea lamprey (*Petromyzon marinus*) genome [28] may help finding the answer. The analysis of the data indicated that two whole-genome duplications likely occurred before the divergence of ancestral lamprey and gnathostome lineages [28]. Since the teleost fish specific third round duplication did not take place in lampreys thus *tppp3-like*, if it is present in these species, cannot be *tppp3a* but *tppp2*. A member of the tppp family was found on the scaffold_451.1-439126, namely the PMZ_0004762 gene. The presence of some amino acid sequences characteristic only for TPPP3-like proteins suggests that this lamprey gene/protein belongs to this "fish-specific" group. E.g., the N-terminal "MAEG" sequence is the same in the vast majority of the TPPP3-like proteins [6] but does not occur in any other TPPP (cf. Fig. 3). The phenylalanine in the "FAKL" sequence of the first exon is characteristic for the fish TPPP3-like and the tetrapod TPPP2 proteins, without exception, while in TPPP1s and TPPP3s there is always tryptophan in this position. TBLASTN search of the whole genome shotgun sequences of another lamprey species, *Lethenteron camtschaticum*, a phylogenetically very near relative of *P. marinus*, revealed that its genome contains three *tppp* genes, corresponding to the three genes occurring in fishes as well: *tppp1, tppp3, tppp3-like* (Fig. 3). (The translated sequences of *tppp3-like* genes in the two lamprey species are identical in 100%.) Thus it can be concluded that the *tppp3-like* gene existed before the divergence of ancestral cyclostome (including lampreys) and gnathostome lineages which means that *tppp3-like* genes are the orthologs of tetrapod *tppp2* genes. (Detailed phylogenetic analysis will be shown elsewhere.)

Smith *et al.* [28] provides also data in their paper mentioned above (in its Supplementary Table 10) that the neighbors of this sea lamprey *tppp3-like* gene are similar to human *CEP72* (Centrosomal protein of 72 kDa) and *ZDHHC11* (Probable palmitoyltransferase ZDHHC11), the genes next to human *TPPP1*. The orthologs of these two genes (*cep72* and *zdhhc11)* are neighbor to most of the fish *tppp1*s (Fig. 3). The neighbors of human *TPPP3* are the paralogs of these two genes; a *zdhhc11* paralog, *zdhhc1*, is nearby to fish *tppp3*s. In general, the genomic positions of *tppp1* and *tppp3*, but not that of *tppp2*, are stabilized in vertebrates: there are shared synteny among mammals, birds and teleost fishes in their case [6]. It means the preserved co-localization of a group of genes on chromosomes of different species. The situation is the same among various fishes as well: the position of *tppp1* and *tppp3* are conserved (Figure 4). However, in the case of *tppp3-like (tppp2)* gene it does not hold. Comparing the chromosomal organization of *tppp1*, *tppp3* and *tppp3-like (tppp2)*, there are 22, 7 and 1 gene(s), respectively, which share their positions in a 30 gene window of the neighborhood of the *tppp* genes in the majority of the fish species (Figure 4). This instability is in accordance with the finding that the fish-specific tppp3-like gene/proteins are tppp2 orthologs.

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Legends to the Figures

Fig. 1. Multiple sequence alignment of several fish TPPP1s by ClustalOmega. The alignment was refined manually. Residues identical and similar in the majority of the species are indicated by black and grey backgrounds, respectively. The first two lines represent the first coding exon; the third and the fourth lines correspond to the second and third exons, respectively. Asterisk notes that these amino acids are coded by the last two nucleotides of the first and the first nucleotide of the second exon. Proteins and ESTs (*) are: *Homo sapiens* NP_008961; *Poecilia reticulate* XP_008430776; *Gasterosteus aculeatus* DN734108*; *Perca flavescens* GO572248*; *Oryzias latipes* XP_004078142; *Dicentrarchus labrax* FM023946*; *Tetraodon nigroviridis* CAG11971; *Takifugu rubripes* XP_003966223; *Gadus morhua* GW848004*; *Astyanax mexicanus* XP_007234837; *Danio rerio* EH550983*, *Danio rerio* XP_002667767.

Fig. 2. Suggested sequence of *Danio rerio* TPPP1. Numbers indicate the order of nucleotides in Zv8_scaffold2999 of whole genome shotgun sequences of *Danio rerio*. Gray background indicates the three exons. The corresponding amino acids are shown with bold capital letters. Small letters stands for tentative amino acids of XP_002667767 which are suggested to be erroneous.

Fig. 3. Multiple sequence alignment of several TPPPs by ClustalOmega. The alignment was refined manually. Residues identical and similar in the majority of the species are indicated by black and grey backgrounds, respectively. Some amino acids characteristic for TPPP1s, TPPP2s or TPPP3s are labeled by bold and underlined letters. Proteins and nucleotids(*) are: HsTPPP1, HsTPPP2, HsTPPP3: *Homo sapiens* NP_008961, NP_776245, NP_057048; GgTPPP1, GgTPPP2, GgTPPP3: *Gallus gallus* XP_001231864, XP_424853, CR385779*; Ac TPPP1, AcTPPP2, AcTPPP3: *Anolis carolinensis* XP_003222359, XP_003224558, XP_003225414; XtTPPP3: *Xenopus tropicalis* NP_001096466; DrTPPP1, DrTPPP2, DrTPPP,: *Danio rerio* EH550983*-XP_002667767, XP_687926, NP_958492; GaTPPP4: *Gasterosteus aculeatus* DN725593*; SsTPPP4: *Salmo salar* GE789580*; PmTPPP2: *Petromyzon marinus* AEFG01009639*; LcTPPP1, LcTPPP2, LcTPPP3:

Lethenteron camtschaticum APJL01058685-APJL01058681*, APJL01053114-APJL01053112*, APJL01048780*. Fish species are indicated by italic letters.

Fig. 4. Co-localization of *tppp* genes with other genes on chromosomes of various bony fishes. Genomicus version 76.01 was used to obtain the data for the figure. Genes conserved in the majority of the species are indicated with black background. Genes conserved in two and at least in three but not more than in the half of the species have white and gray background, respectively. Non-conserved genes are represented with empty boxes. Question marks label unknown genes.

Supplementary figure 1. Suggested sequence of *Danio rerio* TPPP1. Numbers indicate the order of nucleotides in Zv8_scaffold2999 of whole genome shotgun sequences of *Danio rerio*. Gray background indicates the three exons. The corresponding amino acids are shown with bold capital letters. Small letters stands for tentative amino acids of XP_002667767 which are suggested to be erroneous. Underlined nucleotide sequences correspond to Cep72 exons.

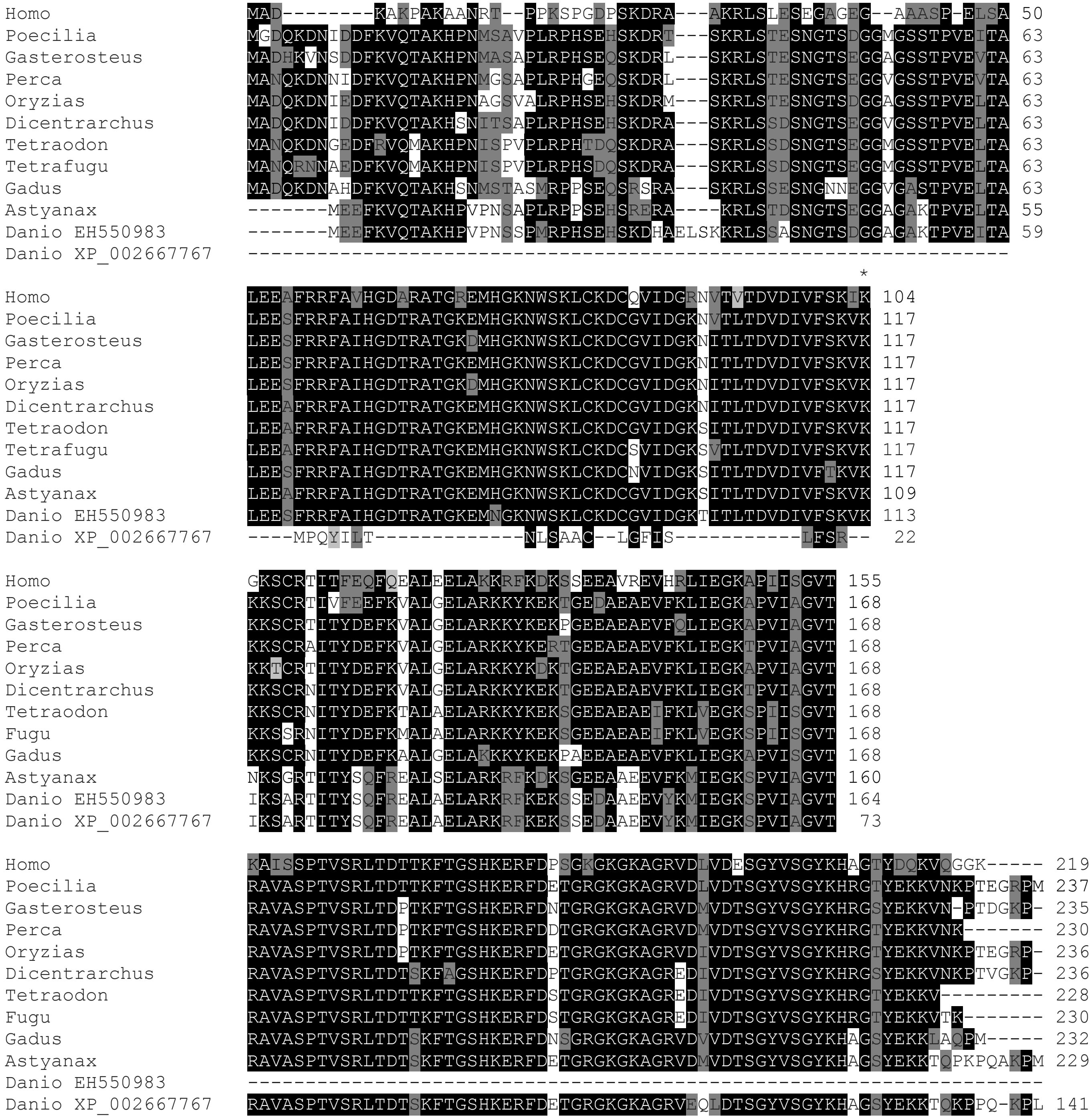
Table

Table 1. TPPP genes/proteins of *Danio rerio*

Name	Short name	Gene	GeneID	mRNA	Protein	Chromosome
tubulin polymerization-promoting protein	*tppp*	*LOC100333482*	100333482	XM_002667721	XP_002667767	Dre16
				EH550983[a] EH572114[b]		Unknown
tubulin polymerization-promoting protein family member 3-like	*tppp3-like*	*LOC559490*	559490	XM_682834	XP_687926	Dre5
tubulin polymerization-promoting protein family member 3	*tppp3*	*tppp3*	393825	NM_201335	NP_958492	Dre7

[a] 5' read; [b] 3' read

Figure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Gasterosteus MADHKVNSDDFKVQTAKHPNMASAPLRPHSEQSKDRL---SKRLSTESNGTSEGGAGSSTPVEVTA 63
Perca MANQKDNNIDFKVQTAKHPNMGSAPLRPHGEQSKDRL---SKRLSTESNGTSEGGVGSSTPVEVTA 63
Oryzias MADQKDNIEDFKVQTAKHPNAGSVALRPHSEHSKDRM---SKRLSTESNGTSDGGAGSSTPVELTA 63
Dicentrarchus MADQKDNIDDFKVQTAKHSNITSAPLRPHSEHSKDRA---SKRLSSDSNGTSEGGVGSSTPVELTA 63
Tetraodon MANQKDNGEDFRVQMAKHPNISPVPLRPHTDQSKDRA---SKRLSSDSNGTSEGGMGSSTPVELTA 63
Tetrafugu MANQRNNAEDFKVQMAKHPNISPVPLRPHSDQSKDRA---SKRLSSDSNGTSEGGMGSSTPVELTA 63
Gadus MADQKDNAHDFKVQTAKHSNMSTASMRPPSEQSRSRA---SKRLSSESNGNNEGGVGASTPVELTA 63
Astyanax -------MEEFKVQTAKHPVPNSAPLRPPSEHSRERA----KRLSTDSNGTSEGGAGAKTPVELTA 55
Danio EH550983 -------MEEFKVQTAKHPVPNSSPMRPHSEHSKDHAELSKKRLSSASNGTSDGGAGAKTPVEITA 59
Danio XP_002667767 --
*
Homo LEEAFRRFAVHGDARATGREMHGKNWSKLCKDCQVIDGRNVTVTDVDIVFSKIK 104
Poecilia LEESFRRFAIHGDTRATGKEMHGKNWSKLCKDCGVIDGKNVTLTDVDIVFSKVK 117
Gasterosteus LEESFRRFAIHGDTRATGKDMHGKNWSKLCKDCGVIDGKNITLTDVDIVFSKVK 117
Perca LEESFRRFAIHGDTRATGKEMHGKNWSKLCKDCGVIDGKNITLTDVDIVFSKVK 117
Oryzias LEESFRRFAIHGDTRATGKDMHGKNWSKLCKDCGVIDGKNITLTDVDIVFSKVK 117
Dicentrarchus LEEAFRRFAIHGDTRATGKEMHGKNWSKLCKDCGVIDGKNITLTDVDIVFSKVK 117
Tetraodon LEEAFRRFAIHGDTRATGKEMHGKNWSKLCKDCGVIDGKSITLTDVDIVFSKVK 117
Tetrafugu LEEAFRRFAIHGDTRATGKEMHGKNWSKLCKDCSVIDGKSVTLTDVDIVFSKVK 117
Gadus LEESFRRFAIHGDTRATGKEMHGKNWSKLCKDCNVIDGKSITLTDVDIVFTKVK 117
Astyanax LEEAFRRFAIHGDTRATGKEMHGKNWSKLCKDCGVIDGKSITLTDVDIVFSKVK 109
Danio EH550983 LEESFRRFAIHGDTRATGKEMNGKNWSKLCKDCGVIDGKTITLTDVDIVFSKVK 113
Danio XP_002667767 ----MPQYILT-------------NLSAAC--LGFIS-----------LFSR-- 22
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Poecilia KKSCRTIVFEEFKVALGELARKKYKEKTGEDAEAEVFKLIEGKAPVIAGVT 168
Gasterosteus KKSCRTITYDEFKVALGELARKKYKEKPGEEAEAEVFQLIEGKAPVIAGVT 168
Perca KKSCRAITYDEFKVALGELARKKYKERTGEEAEAEVFKLIEGKTPVIAGVT 168
Oryzias KKTCRTITYDEFKVALGELARKKYKDKTGEEAEAEVFKLIEGKAPVIAGVT 168
Dicentrarchus KKSCRNITYDEFKVALGELARKKYKEKTGEEAEAEVFKLIEGKTPVIAGVT 168
Tetraodon KKSCRNITYDEFKTALAELARKKYKEKSGEEAEAEIFKLVEGKSPIISGVT 168
Fugu KKSSRNITYDEFKMALAELARKKYKEKSGEEAEAEIFKLVEGKSPIISGVT 168
Gadus KKSCRNITYDEFKAALGELAKKKYKEKPAEEAEAEVFKLIEGKAPVISGVT 168
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Danio XP_002667767 IKSARTITYSQFREALAELARKRFKEKSSEDAAEEVYKMIEGKSPVIAGVT 73
Homo KAISSPTVSRLTDTTKFTGSHKERFDPSGKGKGKAGRVDLVDESGYVSGYKHAGTYDQKVQGGK----- 219
Poecilia RAVASPTVSRLTDTTKFTGSHKERFDETGRGKGKAGRVDLVDTSGYVSGYKHRGTYEKKVNKPTEGRPM 237
Gasterosteus RAVASPTVSRLTDPTKFTGSHKERFDNTGRGKGKAGRVDMVDTSGYVSGYKHRGSYEKKVN-PTDGKP- 235
Perca RAVASPTVSRLTDPTKFTGSHKERFDDTGRGKGKAGRVDMVDTSGYVSGYKHRGTYEKKVNK------- 230
Oryzias RAVASPTVSRLTDPTKFTGSHKERFDETGRGKGKAGRVDIVDTSGYVSGYKHRGTYEKKVNKPTEGRP- 236
Dicentrarchus RAVASPTVSRLTDTSKFAGSHKERFDPTGRGKGKAGREDIVDTSGYVSGYKHRGSYEKKVNKPTVGKP- 236
Tetraodon RAVASPTVSRLTDTTKFTGSHKERFDSTGRGKGKAGREDIVDTSGYVSGYKHRGTYEKKV---------- 228
Fugu RAVASPTVSRLTDTTKFTGSHKERFDSTGRGKGKAGREDIVDTSGYVSGYKHRGTYEKKVTK------- 230
Gadus RAVASPTVSRLTDTSKFTGSHKERFDNSGRGKGKAGRVDVVDTSGYVSGYKHAGSYEKKLAQPM----- 232
Astyanax RAVASPTVSRLTDTSKFTGSHKERFDETGRGKGKAGRVDMVDTSGYVSGYKHAGSYEKKTQPKPQAKPM 229
Danio EH550983 ---
Danio XP_002667767 RAVASPTVSRLTDTSKFTGSHKERFDETGRGKGKAGRVEQLDTSGYVSGYKHAGSYEKKTQKPPQ-KPL 141

Figure

```
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                                M  E  E  F  K  V  Q  T  A  K  H  P  V  P  N  S  S  P  M  R  P  H  S  E  H
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       S  K  D  H  A  E  L  S  K  K  R  L  S  S  A  S  N  G  T  S  D  G  G  A  G  A  K  T  P  V  E  I  T
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      A  L  E  E  S  F  R  R  F  A  I  H  G  D  T  R  A  T  G  K  E  M  N  G  K  N  W  S  K  L  C  K  D
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     C  G  V  I  D  G  K  T  I  T  L  T  D  V  D  I  V  F  S  K  V  K*

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                                                                                                  m  p  q
17101 TATATACTGACGAATTTATCAGCCGCTTGTCTTGGCTTCATCTCATTGTTTTCTAGGATCAAGTCTGCCCGCACTATTACCTACAGCCAGTTCAGAGAGG
       y  i  l  t  n  l  s  a  a  c  l  g  f  i  s  l  f  s  r  I  K  S  A  R  T  I  T  Y  S  Q  F  R  E  A
17201 CGCTGGCGGAGCTGGCCAGAAAACGCTTCAAAGAGAAGAGCAGCGAAGACGCCGCCGAGGAGGTTTACAAGATGATCGAAGGAAAATCCCCCGTCATTGC
        L  A  E  L  A  R  K  R  F  K  E  K  S  S  E  D  A  A  E  E  V  Y  K  M  I  E  G  K  S  P  V  I  A
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        G  V  T

20901 CTAAATGTGTCTGTTTGTGGACTCTCAGAGAGCCGTAGCGTCCCCGACCGTCTCCCGCCTGACGGACACCAGCAAGTTCACCGGCTCCCACAAGGAGCGT
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      F  D  E  T  G  R  G  K  G  K  A  G  R  V  E  Q  L  D  T  S  G  Y  V  S  G  Y  K  H  A  G  S  Y  E  K
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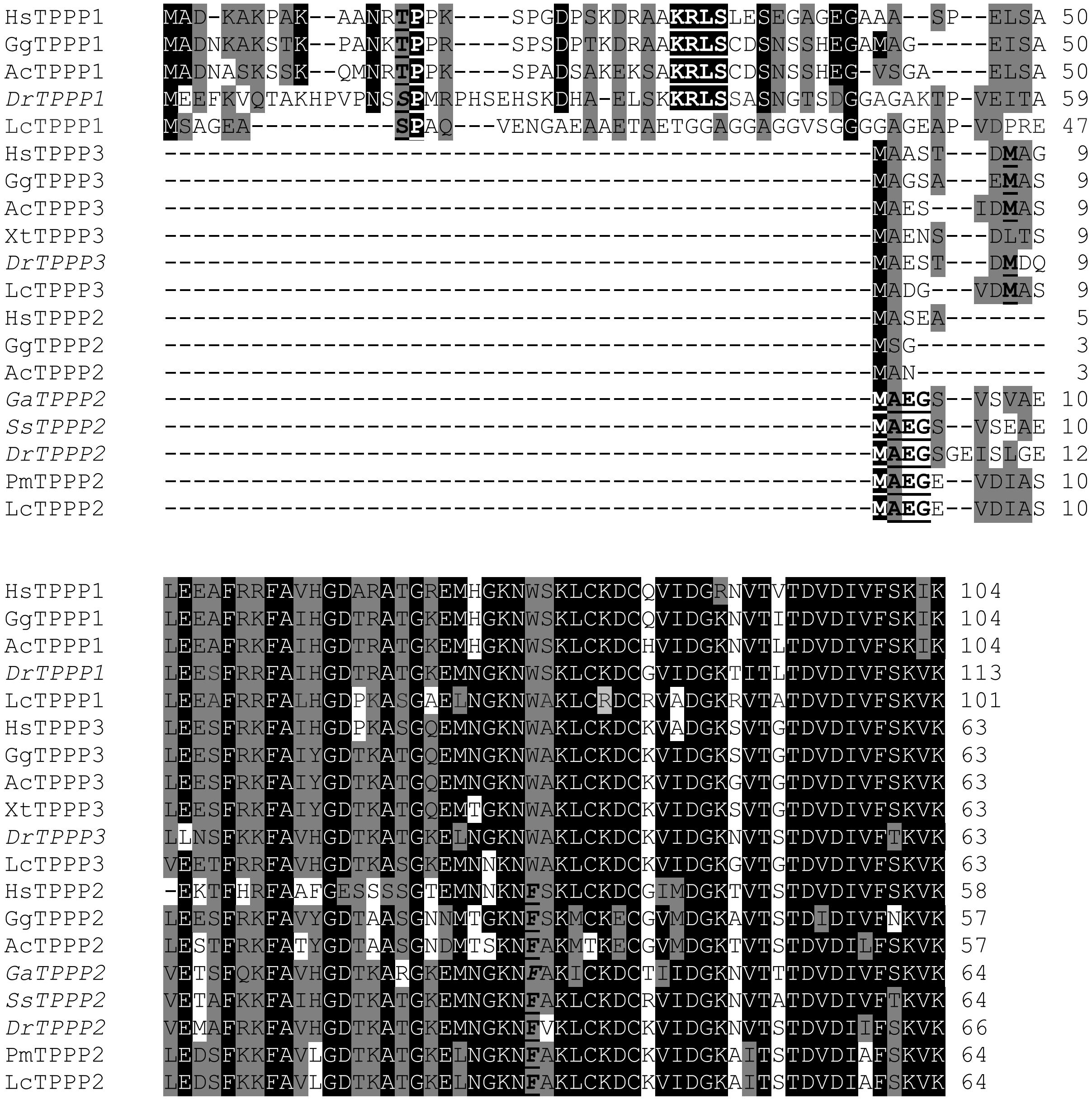
Figure



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DrTPPP1 MEEFKVQTAKHPVPNSSPMRPHSEHSKDHA-ELSKKRLSSASNGTSDGGAGAKTP-VEITA 59
LcTPPP1 MSAGEA----------SPAQ---VENGAEAAETAETGGAGGAGGVSGGGGAGEAP-VDPRE 47
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GgTPPP3 ---MAGSA---EMAS 9
AcTPPP3 ---MAES---IDMAS 9
XtTPPP3 ---MAENS---DLTS 9
DrTPPP3 ---MAEST---DMDQ 9
LcTPPP3 ---MADG---VDMAS 9
HsTPPP2 ---MASEA------- 5
GgTPPP2 ---MSG--------- 3
AcTPPP2 ---MAN--------- 3
GaTPPP2 ---MAEGS--VSVAE 10
SsTPPP2 ---MAEGS--VSEAE 10
DrTPPP2 ---MAEGSGEISLGE 12
PmTPPP2 ---MAEGE--VDIAS 10
LcTPPP2 ---MAEGE--VDIAS 10
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LcTPPP2    QKAARVITFEEFKEALQQLSCKRFKDKDEQEALEETYKLIAGKSPIIHGVT 115

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GgTPPP1    KAISSPTVSRLTDTSKFTGSHKERFDPSGKGKGRAGREDLVDASGYVSGYKHAGTYDHKVQGSK-------- 219
AcTPPP1    KAISSPTVSRLTDTTRFTGSHKERFDPSGRGKGKAGREDLVDTSGYVSGYKHAGTYDHKVQGSK-------- 218
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GgTPPP3    KAKNVGAVERLTDTSKYTGSHKERFDETGKGKGKSGRENIVDNSGYVSAYKNAGTYDAKVKK---------- 176
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LcTPPP3    KAAAVGGVDRLTDASKYTGSHKERFDADGKGKGKSGRADAAANSGYVGNYKGVGTYGDKVAK---------- 176
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GgTPPP2    KVAKVAGVDRLTDTSKYTGSHKERFDESGKGKGLAGREDLTDNSGYVGAYKGAGTYDKTH------------ 168
AcTPPP2    KAVAAGGVDRLTDTSKYTGSHKERFDESGKGKGIAGRADLAQNTGYVGNYKGSGTYDKTH------------ 168
GaTPPP2    KVAKAAAVDRLTDTTKYTGAHKERFDESGKGKGKVGREDIPDGSGYVGAYKGSGTYEEKVKEA--------- 178
SsTPPP2    KVAKAAAVDRLTDTTKFTGAHKERFDETGKGKGKAGREEIPDASGYVGAYKGKGTYEDKVKEA--------- 178
DrTPPP2    KVAKASAVDRLTDTSKYTGSHKERFDESGKGKGREGRADIPDTSGYVSAYKGQGSYDSKVKEDE-------- 181
PmTPPP2    KATNKGGVGRLTDPSKYTGTHKMRFDETGKGKGKAGREDIPDTRGYVHSYRDAGTYDQKVKGAVASPNAKKT 187
LcTPPP2    KATNKGGVGRLTDPSKYTGTHKMRFDETGKGKGKAGREDIPDTRGYVHSYRDAGTYDQKVKGAVASPNAKKT 187
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Figure

Lepis				kif13a	NHLRC1	ptdss1a			si:ch211		PLEKHG4B	nkd3	trip13	brd9	ZDHHC11	**TPPP1**	cep72									nfatc1					
Astyanax	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	**TPPP1**	cep72							trip13							?
Danio	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	**TPPP1**	cep72		?	?	?	?	?	?	?	?	?	?	?	?	?
Gadus	?	?	?	?	?	?	?	?	?	?	?	PLEKHG4B	nkd3	trip13	brd9	**TPPP1**	cep72	KIFC2	ccdc12	nbeal2	nradd	setd2	?	?	?	?	?	?	?	?	?
Tetraodon		smpd5	SPATC1	grina	RBM24	kif13a	NHLRC1	ptdss1a		PLEKHG4B	nkd3	trip13	mrpl2	brd9	ZDHHC11	**TPPP1**	KIFC2	ccdc12	nbeal2	nradd	setd2	upp1	stmn2a	hey1		fam8a1	atxn1b	itga8	fam188a	NFATC2	
Takifugu	mpp6a	14884	smpd5	SPATC1	grina	RBM24	kif13a	NHLRC1	ptdss1a	PLEKHG4B	nkd3	trip13	mrpl2	brd9	ZDHHC11	**TPPP1**	cep72	KIFC2	ccdc12	nbeal2	nradd	setd2	RBM12B	upp1	stmn2a	hey1	fabp11b	fam8a1	atxn1b	itga8	fam188a
Oreochromis	mpp6a	3003	MALSU1	smpd5	grina	RBM24	kif13a	NHLRC1	ptdss1a	si:ch211	PLEKHG4B	NKD2	trip13	brd9	ZDHHC11	**TPPP1**	cep72	KIFC2	ccdc12	nbeal2	nradd	setd2	RBM12B	gra	upp1	stmn2a	hey1	fabp11b	fam8a1	atxn1b	itga8
Gasterosteus		OSBPL3	dfna5		mpp6a	grina	RBM24	kif13a	NHLRC1	ptdss1a	PLEKHG4B	nkd3	trip13	brd9	ZDHHC11	**TPPP1**	cep72	KIFC2	ccdc12	nbeal2	nradd	setd2	RBM12B	upp1	stmn2a	hey1	fabp11b	fam8a1	atxn1b		itga8
Oryzias	OSBPL3	dfna5	mpp6a	7879	MALSU1	smpd5	grina	RBM24	kif13a	NHLRC1	ptdss1a	PLEKHG4B	nkd3	trip13	brd9	**TPPP1**	cep72	si:ch1073	KIFC2	ccdc12	nbeal2	nradd	setd2	RBM12B	upp1	stmn2a	hey1	fabp11b	atxn1b	itga8	fam188a
Xiphophorus	mpp6a	13995	MALSU1	smpd5	grina	RBM24	kif13a	NHLRC1	ptdss1a	si:ch211	PLEKHG4B	nkd3	trip13	brd9	ZDHHC11	**TPPP1**	cep72	KIFC2	ccdc12	nbeal2	nradd	setd2	gra	upp1	stmn2a	hey1	fabp11b	fam8a1	atxn1b	itga8	fam188a
Poecilia	?	?	grina	RBM24	rbm24	kif13a	NHLRC1	ptdss1a	si:ch211	si:ch211	PLEKHG4B	nkd3	trip13	brd9	ZDHHC11	**TPPP1**	cep72	si:ch1073	KIFC2	ccdc12	nbeal2	nradd	setd2	RBM12B	upp1	stmn2a	hey1	fabp11b	fam8a1	atxn1b	itga8

Lepis	?	?	?				myo1c			pitpnaa			6949			**TPPP2**															
Astyanax		TRIM29	nccrp1	slc8a2a	srsf7a	hnrpl	kptn	TRIM29	crx	H3F3A	zgc	NFKBIB	TRIM29			**TPPP2**	tagln2													panx1b	epd
Danio						nccrp1	slc8a2a	srsf7a	hnrpl	kptn	crx	H3F3B	zgc	NFKBIB	TRIM29	**TPPP2**	tagln2												MYO1C	epd	panx1b
Gadus	?	?	?	?	?	?	?	?	?	?	?	?	?	?		**TPPP2**		?	?	?	?	?	?	?	?	?	?	?	?	?	?
Tetraodon																**TPPP2**															
Oreochromis	?	?	?	?	?	?	?	?	?	?	?	?	?	?	TRIM29	**TPPP2**	TUSC3	SGCZ	abca1a	zgc	tdrd7	tmod1	cplx2l	gne	clta	nans		nans	zgc	xpa	kpna7
Gasterosteus		KPNA2	bptf	nol11	APOH	APOH	prkca	cacng5a	CACNG4	cacng1	helz	psmd12	PITPNC1		TRIM29	**TPPP2**	TUSC3	SGCZ	abca1a	zgc	tdrd7	tmod1	cplx2l	gne	clta	nans	zgc	xpa	kpna7		
Oryzias	KPNA2	13783	nol11	APOH	APOH	prkca	cacng5a	cacng1	helz	helz	psmd12	PITPNC1		13962	TRIM29	**TPPP2**			TUSC3		abca1a										
Xiphophorus	nol11	APOH	APOH	prkca	cacng5a	CACNG4	cacng1	helz	psmd12		6523	6520	6517	6516	**TPPP2**	**TPPP2**		TUSC3	SGCZ	?	?	?	?	?	?	?	?	?	?	?	?
Poecilia	APOH	prkca	cacng5a	cacng1	helz	psmd12	PITPNC1	13177	13176	13171	13169	20912	13165	**TPPP2**	TRIM29	**TPPP2**		TUSC3	TUSC3	SGCZ	?	?	?	?	?	?	?	?	?	?	?

Lepis			espr2		17603	NFATC3		ddx28	8111	zgc:112160	ctrb1	agrp	atp6v0d1	hsd11b2	ZDHHC1	**TPPP3**			plekhg4	slc95a	FHOD1	?	?	?	?	?	?	?	?	?	?
Astyanax	FHOD1	slc95	plekhg4	espr2	5885	25951	NFATC3	ddx28	5876	zgc:112160	ctrb1	agrp	atp6v0d	hsd11b2	ZDHHC1	**TPPP3**	DPEP3	slc12a4	slc6a2	lpcat2	mmp2	irx6a	irx5a		fto	aktip	chd9		tox3	?	?
Danio	FHOD1	slc95	plekhg4	BX005082	espr2	NFATC3	ddx28	CR356247	zgc:112160	zgc:136141	ctrb1	agrp	atp6v0d	hsd11b2	ZDHHC1	**TPPP3**		DPEP3	slc12a4	slc6a2	lpcat2	mmp2	irx6a	irx5a	fto	aktip	chd9	tox3			
Gadus	?	?	?	?	?	?	?	?	?	?	?	?	?	?	plekhg4	**TPPP3**	slc95	FHOD1	?	?	?	?	?	?	?	?	?	?	?	?	?
Oreochromis	wdr59			gpatch1	cebpa	cebpg		FHOD1	slc95	plekhg4		idh3a	cib2	HSD11B2	ZDHHC1	**TPPP3**	trpm1a	3247	zgc:112160	ctrb1	agrp	VPS13C	emilin1b								
Gasterosteus					wdr59	gpatch1	cebpg	FHOD1	slc95	plekhg4		idh3a	cib2	hsd11b2	ZDHHC1	**TPPP3**	trpm1a	trpm1a		GABARAPL	KCNG4	rpl12	pole3	suv42oh1	ccnb2	cd82a					
Oryzias														HSD11B2	ZDHHC1	**TPPP3**	trpm1a		zgc:112160	agrp	VPS13C	emilin1b	GABARAPL2	KCNG4	rpl12	pole3	suv42oh1	ccnb2	cd82a		
Xiphophorus	?	?	?	?	?	?	?	?	?	slc95	FHOD1	cebpg	cebpa	hsd11b2	ZDHHC1	**TPPP3**	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
Poecilia	?	?	?	?	?	?	?	?	?	?	?		agrp	hsd11b2	ZDHHC1	**TPPP3**									?	?	?	?	?	?	?