

Supplementary material

Asymmetric paralog evolution between the “cryptic” gene *Bmp16* and its well-studied sister genes *Bmp2* and *Bmp4*

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Supplementary figure

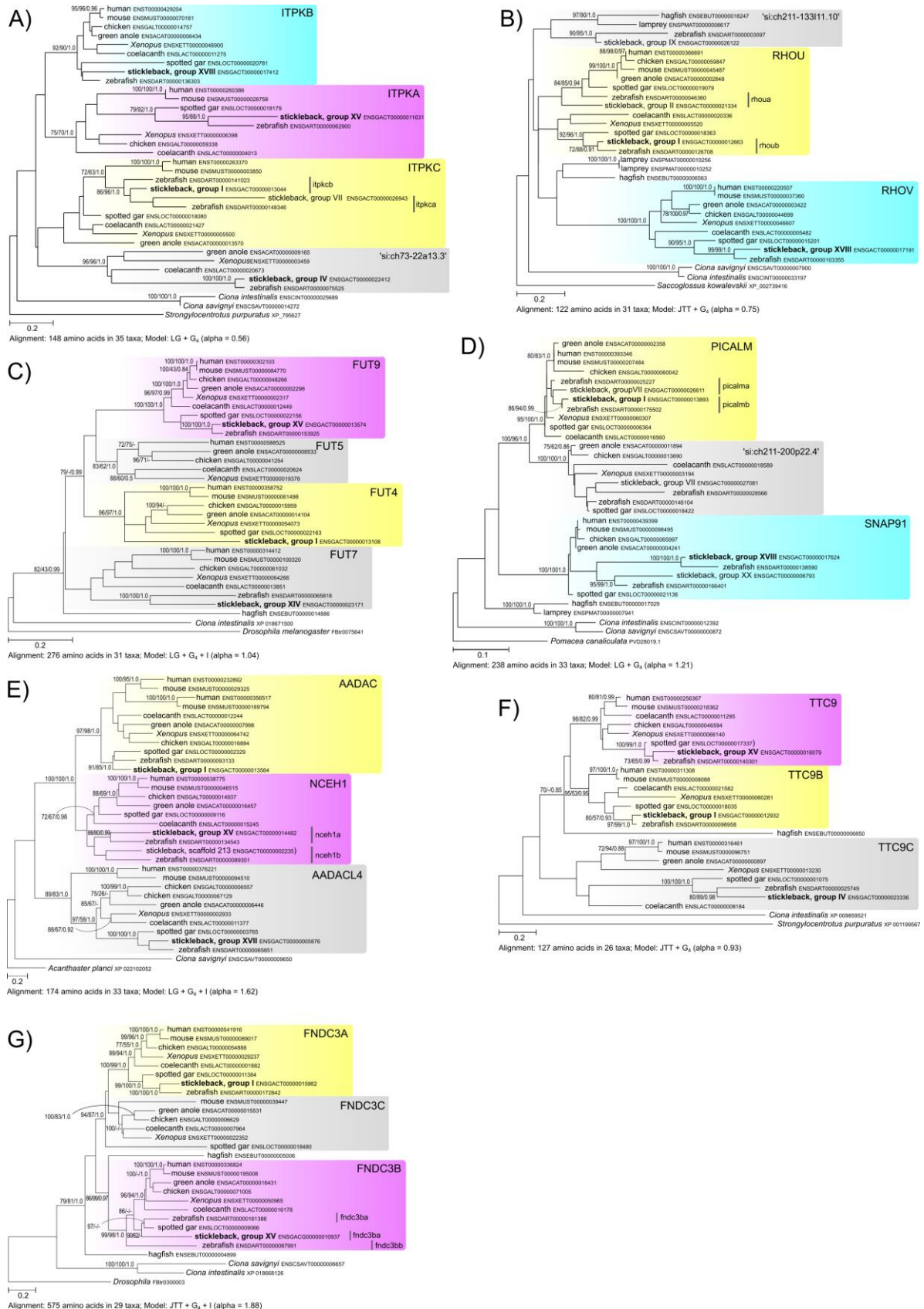


Figure S1. Phylogenetic trees of gene families whose members surround the *Bmp2*, -4 and -16 genes. Panels A-G show ML trees (inferred with MEGA7¹) of pairs or triplets of genes indicated in the syntenic blocks shown in Fig. 2. Information on tree inference parameters are given below each tree. Scale bar shows the number of substitutions per site. Colored backgrounds highlight orthology groups of gnathostome genes: yellow for stickleback genes on group I, blue for group XVIII, purple for XV, and grey for stickleback genes in other genomic regions. Names for each orthology group are based on human nomenclature. Support values at nodes are shown in order, bootstrap probabilities in the ML method and in the NJ method, and posterior probabilities in the Bayesian tree inference. Only bootstrap values no less than 70 in the ML analysis are shown. All amino acid alignments are accessible on FigShare (DOI: 10.6084/m9.figshare.6938372, 10.6084/m9.figshare.6938369, 10.6084/m9.figshare.6938366, 10.6084/m9.figshare.6938363, 10.6084/m9.figshare.6938360, 10.6084/m9.figshare.6938357 and 10.6084/m9.figshare.6938354).

Supplementary tables

Table S1. Sequences included in molecular phylogenetic analyses shown in Fig. 1 and Fig. 3A, and synteny analysis shown in Fig. 2.

Latin species name	Common name	Gene name	Accession ID	Included in Figure(s)
<i>Ambystoma mexicanum</i>	Mexican salamander	<i>Bmp2</i>	EU339232	Fig. 1
<i>Anolis carolinensis</i>	green anole	<i>Bmp2</i>	ENSACAT00000003102	Fig. 1
<i>Anolis carolinensis</i>	green anole	<i>Bmp4</i>	ENSACAT00000017970	Fig. 1
<i>Anolis carolinensis</i>	green anole	<i>Bmp16</i>	LT989953	Figs. 1, 3A
<i>Astatotilapia burtoni</i>	Burton's mouthbrooder	<i>bmp16</i>	LT989956	Fig. 3A
<i>Branchiostoma floridae</i>	Florida lancelet	<i>AmphiBMP2/4</i>	XM_002596812	Fig. 1
<i>Callorhinchus milii</i>	elephant fish	<i>Bmp2</i>	XM_007905506	Fig. 1
<i>Callorhinchus milii</i>	elephant fish	<i>Bmp4</i>	XM_007888150	Fig. 1
<i>Ciona savignyi</i>	Solitary sea squirt	<i>Bmp2/4</i>	ENSCSAVT00000004520	Fig. 1
<i>Danio rerio</i>	zebrafish	<i>bmp2a</i>	ENSDART00000019364	Fig. 1
<i>Danio rerio</i>	zebrafish	<i>bmp2b</i>	ENSDART000000131169	Fig. 1
<i>Danio rerio</i>	zebrafish	<i>bmp4</i>	ENSDART00000075150	Fig. 1
<i>Danio rerio</i>	zebrafish	<i>bmp16</i>	NM_001171776	Figs. 1, 3A
<i>Eptatretus burgeri</i>	inshore hagfish	<i>Bmp2/4/16-A</i>	see Supplementary File 1	Fig. 1
<i>Eptatretus burgeri</i>	inshore hagfish	<i>Bmp2/4/16-B</i>	see Supplementary File 1	Fig. 1
<i>Eptatretus burgeri</i>	inshore hagfish	<i>Bmp2/4/16-C</i>	see Supplementary File 1	Fig. 1
<i>Gallus gallus</i>	chicken	<i>BMP4</i>	ENSGALT000000037192	Fig. 1
<i>Gasterosteus aculeatus</i>	stickleback	<i>bmp2</i>	ENSGACT00000016539	Fig. 2
<i>Gasterosteus aculeatus</i>	stickleback	<i>bmp4</i>	ENSGACT00000013724	Fig. 2
<i>Gasterosteus aculeatus</i>	stickleback	<i>bmp16</i>	ENSGACT00000012714	Figs. 2, 3A
<i>Gekko japonicus</i>	Japanese gecko	<i>Bmp2</i>	XM_015405598.1	Fig. 1
<i>Gekko japonicus</i>	Japanese gecko	<i>Bmp4</i>	XM_015420851.1	Fig. 1
<i>Gekko japonicus</i>	Japanese gecko	<i>Bmp16</i>	XM_015427264.1	Figs. 1, 3A
<i>Homo sapiens</i>	human	<i>BMP2</i>	ENST00000378827	Figs. 1, 3A
<i>Homo sapiens</i>	human	<i>BMP4</i>	ENST00000245451	Figs. 1, 3A
<i>Huso dauricus</i> ♀ x <i>Acipenser ruthenus</i> ♂	Bester	<i>Bmp2</i>	LT989959	Fig. 1
<i>Huso dauricus</i> ♀ x <i>Acipenser ruthenus</i> ♂	Bester	<i>Bmp4</i>	LT989960	Fig. 1
<i>Latimeria chalumnae</i>	African coelacanth	<i>Bmp2</i>	ENSLACT00000016280	Fig. 1
<i>Latimeria chalumnae</i>	African coelacanth	<i>Bmp4</i>	ENSLACT00000021864	Fig. 1
<i>Latimeria chalumnae</i>	African coelacanth	<i>Bmp16</i>	ENSLACT00000009133	Figs. 1, 3A
<i>Lepisosteus oculatus</i>	spotted gar	<i>Bmp2</i>	ENSLOCT00000020341	Fig. 1
<i>Lepisosteus oculatus</i>	spotted gar	<i>Bmp4</i>	ENSLOCT00000014951	Fig. 1
<i>Lepisosteus oculatus</i>	spotted gar	<i>Bmp16</i>	ENSLOCT00000018333	Figs. 1, 3A
<i>Oreochromis niloticus</i>	Nile tilapia	<i>bmp16</i>	ENSONIT00000007507	Fig. 3A
<i>Oryzias latipes</i>	medaka	<i>bmp2b</i>	ENSORLT00000012260	Fig. 1
<i>Oryzias latipes</i>	medaka	<i>bmp4</i>	ENSORLT00000016674	Fig. 1
<i>Paroedura picta</i>	Madagascar ground gecko	<i>Bmp16</i>	LT989964	Figs. 1, 3A
<i>Petromyzon marinus</i>	sea lamprey	<i>PmBMP24-A</i>	AY602220	Fig. 1
<i>Petromyzon marinus</i>	sea lamprey	<i>PmBMP24-B</i>	AY602221	Fig. 1
<i>Petromyzon marinus</i>	sea lamprey	<i>PmBMP24-C</i>	AY602222	Fig. 1

<i>Pogona vitticeps</i>	bearded dragon	<i>Bmp2</i>	XM_020793685.1	Fig. 1
<i>Pogona vitticeps</i>	bearded dragon	<i>Bmp16</i>	XM_020810881.1	Figs. 1, 3A
<i>Polypterus senegalus</i>	Senegal bichir	<i>Bmp4</i>	LT989966	Fig. 1
<i>Protobothrops mucrosquamatus</i>	brown-spotted pit viper	<i>Bmp2</i>	XM_015813549	Fig. 1
<i>Protobothrops mucrosquamatus</i>	brown-spotted pit viper	<i>Bmp4</i>	XM_015822447	Fig. 1
<i>Protobothrops mucrosquamatus</i>	brown-spotted pit viper	<i>Bmp16</i>	XM_015829826	Fig. 3A
<i>Ptychodera flava</i>	acorn worm	<i>Pf-BMP2/4</i>	AB028219	Fig. 1
<i>Python bivittatus molurus</i>	Burmese python	<i>Bmp2</i>	XM_007427029	Fig. 1
<i>Python bivittatus molurus</i>	Burmese python	<i>Bmp4</i>	XM_007436872	Fig. 1
<i>Python bivittatus molurus</i>	Burmese python	<i>Bmp16</i>	AEQU010318313, AEQU010313080	Fig. 3A
<i>Raja clavata</i>	thornback ray	<i>Bmp2</i>	LT989967	Fig. 1
<i>Raja clavata</i>	thornback ray	<i>Bmp4</i>	LT989968	Fig. 1
<i>Rhincodon typus</i>	whale shark	<i>Bmp2</i>	XP_020370331	Fig. 1
<i>Rhincodon typus</i>	whale shark	<i>Bmp4</i>	XP_020366079	Fig. 1
<i>Rhincodon typus</i>	whale shark	<i>Bmp16</i>	XP_020370388	Figs. 1, 3A
<i>Salmo salar</i>	Atlantic salmon	<i>bmp16</i>	CK879241	Fig. 3A
<i>Scyliorhinus canicula</i>	small-spotted catshark	<i>Bmp2</i>	LT989969	Fig. 1
<i>Scyliorhinus canicula</i>	small-spotted catshark	<i>Bmp4</i>	LT989970	Fig. 1
<i>Scyliorhinus canicula</i>	small-spotted catshark	<i>Bmp16</i>	LT989971	Fig. 3A
<i>Scyliorhinus torazame</i>	cloudy catshark	<i>Bmp2</i>	see Supplementary File 1	Fig. 1
<i>Scyliorhinus torazame</i>	cloudy catshark	<i>Bmp4</i>	see Supplementary File 1	Fig. 1
<i>Scyliorhinus torazame</i>	cloudy catshark	<i>Bmp16</i>	see Supplementary File 1	Figs. 1, 3A
<i>Sparus aurata</i>	gilt-head bream	<i>bmp16</i>	FM155149	Fig. 3A
<i>Takifugu rubripes</i>	Japanese puffer	<i>bmp16</i>	ENSTRUT00000029118	Fig. 3A
<i>Tetraodon nigroviridis</i>	green spotted pufferfish	<i>bmp2b</i>	ENSTNIT00000013839	Fig. 1
<i>Tetraodon nigroviridis</i>	green spotted pufferfish	<i>bmp4</i>	ENSTNIT00000020600	Fig. 1
<i>Tetraodon nigroviridis</i>	green spotted pufferfish	<i>bmp16</i>	ENSTNIT00000010748	Figs. 1, 3A
<i>Thamnophis sirtalis</i>	garter snake	<i>Bmp2</i>	XM_014068423	Fig. 1
<i>Thamnophis sirtalis</i>	garter snake	<i>Bmp4</i>	XM_014054780	Fig. 1
<i>Thamnophis sirtalis</i>	garter snake	<i>Bmp16</i>	XM_014055083	Figs. 1, 3A
<i>Xenopus tropicalis</i>	Western clawed frog	<i>Bmp2</i>	ENSXETT00000012124	Fig. 1
<i>Xenopus tropicalis</i>	Western clawed frog	<i>Bmp4</i>	ENSXETT00000018837	Fig. 1
<i>Xiphophorus maculatus</i>	platy	<i>bmp16</i>	ENSXMAT00000004285	Fig. 3A

Species names are shown in alphabetic order.

Table S2. Sequences identified in this study but excluded from phylogenetic analysis.

Latin species name	Common name	Gene name	Accession ID
<i>Astatotilapia burtoni</i>	Burton's mouthbrooder	<i>bmp2</i>	LT989954
<i>Astatotilapia burtoni</i>	Burton's mouthbrooder	<i>bmp4</i>	LT989955
<i>Eptatretus burgeri</i>	inshore hagfish	<i>Bmp2/4/16-A</i>	LT989957
<i>Huso dauricus</i>	Kaluga sturgeon	<i>Bmp2</i>	LT989958
<i>Lepisosteus platyrhincus</i>	Florida gar	<i>Bmp4</i>	LT989961
<i>Neoceratodus forsteri</i>	Australian lungfish	<i>Bmp4</i>	LT989965
<i>Paroedura picta</i>	Madagascar ground gecko	<i>Bmp2</i>	LT989962
<i>Paroedura picta</i>	Madagascar ground gecko	<i>Bmp4</i>	LT989963

Table S3. Information of sequences identified in this study by RT-PCR.

Species	Gene name	Source of tissue/stage	Primer set used for first identification (3'RACE)	Method used for sequence extension	Completeness of cDNA
<i>Anolis carolinensis</i>	<i>Bmp16</i>	stage 8.5	NF169/170	5'RACE	full-length
<i>Astatotilapia burtoni</i>	<i>bmp2</i>	14 / 21 dpf ^c	NF5/6	GeneRacer kit ^f	full-length
<i>Astatotilapia burtoni</i>	<i>bmp4</i>	14 / 21 dpf ^c	NF5/6	GeneRacer kit ^f	full-length
<i>Astatotilapia burtoni</i>	<i>bmp16</i>	14 / 21 dpf ^c	NF49/50	GeneRacer kit ^f	full-length
<i>Eptatretus burgeri</i>	<i>Bmp2/4-α</i>	adult brain tissue	NF5/6	5'RACE	full-length
<i>Huso dauricus</i>	<i>Bmp2</i>	^d	SK101/103 ^a	-	partial
<i>Huso dauricus</i> ♀ x <i>Acipenser ruthenus</i> ♂	<i>Bmp2</i>	stage E	NF17/18 ^a	-	partial
<i>Huso dauricus</i> ♀ x <i>Acipenser ruthenus</i> ♂	<i>Bmp4</i>	stage E	NF5/NF8	-	partial
<i>Lepisosteus platyrhincus</i>	<i>Bmp4</i>	adult muscle tissue	NF5/6	-	partial
<i>Neoceratodus forsteri</i>	<i>Bmp4</i>	stages 30-40 ^e	SK101/103 ^b	-	partial
<i>Polypterus senegalus</i>	<i>Bmp4</i>	finfold phase of larvae	SK101/103 ^b	-	partial
<i>Raja clavata</i>	<i>Bmp2</i>	stage 32	NF5/6		full-length
<i>Raja clavata</i>	<i>Bmp4</i>	stage 32	NF5/8		full-length
<i>Scyliorhinus canicula</i>	<i>Bmp2</i>	stage 33		GeneRacer kit ^f	full-length
<i>Scyliorhinus canicula</i>	<i>Bmp4</i>	stage 33		GeneRacer kit ^f	full-length
<i>Scyliorhinus canicula</i>	<i>Bmp16</i>	stage 33	NF71/72	5'RACE	partial
<i>Scyliorhinus torazame</i>	<i>Bmp2</i>	stage 23 / 25	NF5/6	-	partial
<i>Scyliorhinus torazame</i>	<i>Bmp4</i>	stage 23 / 25	NF5/6	-	partial
<i>Scyliorhinus torazame</i>	<i>Bmp16</i>	stage 23 / 25	NF71/72	5'RACE	partial
<i>Paroedura picta</i>	<i>Bmp2</i>	4 / 12 dpo	NF5/6	-	partial
<i>Paroedura picta</i>	<i>Bmp4</i>	4 / 12 dpo	NF5/6	-	partial

^a Nested PCR was performed using the primer SK106 instead of an adapter primer.

^b Nested PCR was performed using the primer SK105 instead of an adapter primer.

^c see ².

^d see ³.

^e see ⁴.

^f purchased from Thermo Fisher Scientific

Table S4. Sequences of primers used in this study for identification of novel sequences.

Primers used in PCRs	Primer sequence (5' → 3')	Amino acid sequence
NF5 (F)	GACTTYWSNGAYGTNGGNTGGAA	DFSDVGWN
NF6 (F)	GGACCACCTNAAYWSNACNAAYCA	DHLNSTNH
NF8 (F)	AAGNGCNTGYTGYGTNCCNAC	P(R/K)ACCVPT
NF17 (F)	GCCAACACTGTYMGNNSTTYCA	ANTVR(S/G)FH
NF18 (F)	ACCGTYMGYKSNTTYCAYCAYGA	TVR(G/S)FHH(D/E)
NF49 (F)	ATAGCWCCNWSNGGNTAYGAYGC	IAPSGYDA
NF50 (F)	CCGASNGGMTAYGAYGCNTTYTTY	PSGYDAFF
NF71 (F)	AACTCCTCCAGCCATGCCAT	NSSSHAI
NF72 (F)	ATCGTGCAGACGCTGGTCAA	IVQTLVN
SK101 (R)	GTCGTGCCNCCNTAYATGYTNGAYYT	VVPPYMLDL
SK103 (R)	GTGCCGCCNTAYATGYTNGAYYTNTA	VPPYMLDLY
SK105	TACANCCRCANCCYTCNACNACCAT	MVVEGCGCX
SK106	TCCRCANCCYTCNACNACCATNTC	DMVVEGCG

Table S5. Number of observed synonymous and non-synonymous substitutions per group of *Bmp* genes.

Group of genes	ω	Observed number of non-synonymous substitutions	Observed number of synonymous substitutions
gnathostome <i>Bmp2</i>	0.07	18.51	76.40
gnathostome <i>Bmp4</i>	0.05	13.62	80.54
gnathostome <i>Bmp16</i>	0.12	48.8	128.14

All values are given as average for the respective group of genes as provided by the software PAML version 4.9⁵.

For more information, see Fig. 1.

Table S6. Sequences of primers used in cloning probes for *in situ* hybridization.

Gene	Species	Forward primer sequence (5' → 3')	Reverse primer sequence (5' → 3')	Length of probe
<i>Bmp2</i>	<i>A. carolinensis</i>	CCTCAACTCCACAAATCA	TTCTATTTGCTGAGCAGC	881 bp
<i>Bmp4</i>	<i>A. carolinensis</i>	TGATGGTCCTCCTATTGT	ACAATCCAATCGTTCCAG	992 bp
<i>Bmp16</i>	<i>A. carolinensis</i>	CCAAAATCAGTACCAAGTG	GATTGCTTTGATAGACTGG	760 bp
<i>Bmp2</i>	<i>S. torazame</i>	GGTTGTGGAAGGTTGTGG	AGGAGCTGCCCAGTGTTA	631 bp
<i>Bmp4</i>	<i>S. torazame</i>	ACCAGAGGAAGGGAGGAAAA	TTGTTCTTCTTGCGCCTCTG	764 bp
<i>Bmp16</i>	<i>S. torazame</i>	AGAGTACAGCACTGCAAC	CTCGTGGTTGGGAATGTT	611 bp

References

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