

Supplementary Materials: *Leptin* Genes in Blunt Snout Bream: Cloning, Phylogeny and Expression Correlated to Gonads Development

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Salvelinus	[MDCSMPLVSLLAHLFSMGAO—ASLSDLIVTRVKEVLGAATWVYRTOMEIKLPLSSNLVIDGLIFPPAAQDQFIEGLISIVET-MGFYQD-LIILSD]	96
Oncorhynchus mykiss	[MDCSMALLVSLLAHLFSMGAO—ASLSDLIVTRVKEVLGAATWVYRTOMEIKLPLSSNLVIDGLIFPPAAQDQFIEGLISIVET-MGFYQD-LIILFD]	96
Oncorhynchus	[MDCSMALLSLLAHLFSMGAO—ASLSLHVVTRTKVOLQATNVIR—-IKLD1SPN-L1BMDPFLPAAAATQHESLISIVET-MGFYQD-LIILVD]	91
Salmo	[MDCSMALLSLLAHLFSMGAO—ASLSLHVVTRTKVOLQATNVIR—-IKLD1SPN-L1BMDPFLPAAAATQHESLISIMET-MGFYQD-LIILVD]	91
Carassius	-MTFFALLYPCILMLSLVHPO—IPHPMLKNNWVQLAQDTTILKIDHNEAEKLVPLKLIGOFPE-LTYFVPAVDPQGLOSIMDT-LTFPQR-VLQRPF	94
Cyprinus	-MTFSALLYPCILMLSLVHPO—IPHSLSLNLVQLAQDTTILKIDHNEAEKLVPLKLIGOFPE-LTYFVPAVDPQGLOSIMDT-LTFPQR-VLQRPF	94
Labeo	-PLSLSLVHPO—IPHPMLKNNWVQLAQDTTILKIDHNEAEKLSPKLIGOFPE-LTYFVPAVDPQGLOSIVES-LSTFPH-VLQRPF	82
Schizopygopsis	-MTFAALLYPCILMLSLVHPO—IPPHPSNLNLVHQDOTTILKIDHNEAEKLSPKLIGOFPE-LTYFVPAVDPQGLOSIVDT-LTFPQR-VLQRPF	94
Ctenopharyngodon	-MNPWSVLLTTCFLSILGDIDRSPHPESLK5—VNLQADTTIIRIKERHEEKLSPKLIGOFPE-LTYFVPAVDPQGLOSIVDT-LTFPQR-VLQTLP	96
Polypterus	-MNPWSVLLTTCFLSILGDIDRSPHPESLK5—VNLQADTTIIRIKERHEEKLSPKLIGOFPE-LTYFVPAVDPQGLOSIVDT-LTFPQR-VLQTLP	96
Hypophthalmichthys	-MTFPVLLYTCFLSILGDIDRSPHPESLK5—VNLQADTTIIRIKERHEEKLSPKLIGOFPE-LTYFVPAVDPQGLOSIVDT-LTFPQR-VLQTLP	95
Cirrhinus	-VNLQADTTIIRIKERHEEKLSPKLIGOFPE-LTYFVPAVDPQGLOSIVDT-LTFPQR-VLQTLP	63
Pelteobagrus	WAVYPFLPCSCVVTIYLHAG—RALPDTSLKNSVRLQVEMTSLRQDDEPFLPFLRQDDEPFLPFLQSDPDEIQLGSSWEM-LMFNQR-VLHSPL	95
Homo	-MRNGLGFLCWMLWYLFYV—QAPV1QKQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-HP1LTLSLSMDQ7-LATYQQ-ILTSLP	90
Macaca	-MRNGLGFLCWMLWYLFYV—QAPV1QKQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-HP1LTLSLSMDQ7-LATYQQ-ILTSLP	90
Stenella	-MRGCPLCRFLWMLWYLFYV—EAPV1PKWQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-TPVLSLSLSMDQ7-LATYQQ-ILTSLP	90
Delphinapterus	-PYLSTY—EAPV1PKWQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-TPVLSLSLSMDQ7-LATYQQ-ILTSLP	77
Balaenoptera	-MRGCPLCRFLWMLWYLFYV—EAPV1PKWQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-LPVLSLSLSMDQ7-LATYQQ-ILTSLP	90
Neophocaena	-MRGCPLCRFLWMLWYLFYV—EAPV1PKWQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-TPVLSLSLSMDQ7-LATYQQ-ILTSLP	90
Lagenorhynchus	-VQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-TPVLSLSLSMDQ7-LATYQQ-ILTSLP	84
Gallus	-MCWRPLCR—LWSLSTVY—QAPCQ1PQDQITKTLKTYTRVTRND1SHTQSVSQQNQVTLGD-FIPO1—-HP1LSLSLSMDQ7-LATYQQ-ILTSLP	86
Xenopus	MQYHHLSEW61FWMLLPYSGQ—RAIKARVYNDNARLAKSTLITRIGEPFQGLPFSQD—QLELSLHMEDOT-LEVFQK-LISSLSP	92
Takifugu	-MDY-TLALVIALPL—LSLCVALWMSKRYTVWAAQQLVARY—DHFPT—DNLGEFTD-DVXEGTSVWAS-LESTNN-L1SDR	83
Tetradon	-MDY-TLALVIALSLQ—LSCTPVP—HMDGDRGKETKAKWVWQQLYVRLDNWVHPHMDFPITPSA-DDLDSGASAVAR-LENFSN-L1SDN	83
Morone saxatilis	-MDY-TLALVIALSLQ—LSCTPVP—LPEVYKMKRSKVWMAEVLVRLRNDFEVPEAGLTSPPA-DDLDGLSSTVTL-LEGNS-L1SDS	84
Morone	-MDY-TLALVIALSLQ—LSCTPVP—LPEVYKMKRSKVWMAEVLVRLRNDFEVPEAGLTSPPA-DDLDGLSSTVTL-LEGNS-L1SDS	84
Siniperca	-PVEVEMCSKSVWMAEVLVRLRNDFEVPEAGLTSPPA-DDLDGPSSTVWV-LEGNS-L1SDP	81
Epinephelus	-MDY-TLALLFLSLHYSWVGTAAF—LPEVYKMKRSKVWMAEVLVRLRNDFEVPEAGLTSPPA-DDLDGPSSTVWV-LEGNS-L1SDP	84
Oreochromis	-MSY-TNALSLLSLHYSWVGTAAF—LPEFVYKMKRSKVWMAEVLVRLRNDFEVPEAGLTSPPA-DMLDGPSSTVWV-LEGNS-L1SDP	87
Larimichthys	-MDY-TLALLFLSLHYSWVGTAAF—LSAEVWVOMCSKSVWMAEVLVRLRNDFEVPEAGLTSPPA-DMLDGPSSTVWV-LEGNS-L1SDP	84
Thunnus	-HDYSVIALSFHLYLWVGTAAF—LVEVEKAH—VERMABOLYKLNDQDFPQRTLTPSA-DMLDGSSVWV-LEGNSN-LISEN	83
Oryzias	-MD5-ALVLFALFRLWVATAAF—VNFELQHMGSNV1DIAKESL1TS1GKFSPPS—DELNGLSSVWV-LEGNSN-QISON	84
Megalobrama	-MYSLLISLMSLALVAYSISRKPTATEDKIRIMATTI1S1X1X1DEHNPMSFEIDPGPD1DTIDGLTSIFAH—LSTLQQLRLRVPP	168
Salvelinus	MADLQLVEDASTMQRQALEVNMSH—GRQHQNTQBOGLEEALKDVRQFLPSCVVALNRKULQDRL1LNLNDLQKU—	175
Oncorhynchus mykiss	MADLQLVEDASTMQRQALEVNMSH—GRQHQNTQBOGLEEALKDVRQFLPSCVVALNRKULQDRL1LNLNDLQKU—	175
Oncorhynchus	WADLQLRVEDSTMGRQLEVNMSH—GRQHQNTQBOGLEEALKDVRQFLPSCVVALNRKULQDRL1LNLNDLQKU—	171
Salmo	KOHVSQLRISDLSTLGLYKERTSMH—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	171
Carassius	KOHVSQLRISDLSTLGLYKERTSMH—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	171
Cyprinus	KOHVSQLRISDLSTLGLYKERTSMH—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	171
Labeo	KOHVSQLRISDLSTLGLYKERTSMH—CTLKEPANERSALDPLNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	171
Schizopygopsis	KOHVSQLRISDLSTLGLYKERTSMH—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	141
Ctenopharyngodon	KOHVSQLHDMOSTLLEYFQDFMTPN—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	170
Polypterus	KOHVSQLHDMOSTLLEYFQDFMTPN—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	173
Hypophthalmichthys	KOHVSQLHDMOSTLLEYFQDFMTPN—CTPKEPANGSLDFTIQONQATH—	144
Cirrhinus	KOHLSQLRQIRDLSTLGLYKERTSMH—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	172
Pelteobagrus	KOHVSQLHDMOSTLLEYFQDFMTPN—CTLKEPANGSLAFLPNEATHNLVSPGVVALNRKULQDRL1LNLNDLQKU—	172
Homo	KOHVSQLHDMOSTLLEYFQDFMTPN—CTLKEPANGSLDFTIQONQATH—	172
Macaca	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Stenella	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Delphinapterus	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Balaenoptera	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Neophocaena	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Lagenorhynchus	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Gallus	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Xenopus	SRVNIQ1SISOLENLRDLHLLAHSKS—CFLPQASLTLTSLSGLQVLEASYITVWVALSLQGSLQWMLDLSPO—	167
Takifugu	FQGVQSKTEITSLAGOTLWVREGC—QEQQPK-WF—RKHFIKHTVHLAEVMEVREFLKLQDNLVLE—	152
Tetradon	LGDUV1KTAIE1S1SLGTLQDVRHNN—KEQRPKTAIVFQLPQEPOQRKQDFIQSYT1DALMSHGFMLLNLQDHLF—	160
Morone saxatilis	LIGVQSYVWD1SLSLTLQDLSVWRQHGS—SEQRPKLSPVQWLQELQRKQDFHIVSYMEALMVYEFMLLNLQDHLF—	161
Morone	LIGVQSYVWD1SLSLTLQDLSVWRQHGS—SEQRPKLSPVQWLQELQRKQDFHIVSYMEALMVYEFMLLNLQDHLF—	161
Siniperca	LNGVQSYVWD1SLSLTLQDLSGFLR—	—
Epinephelus	FNGVSYQWLTS—	—
Oreochromis	VNGVSYQWLTS—	—
Larimichthys	DGFTQVTKTE1S1SLGTLQDVRHNN—TEQRPK2SPVCPLOELOGRSEFHITVSYEALMBVZELLNLNLQDHLF—	161
Thunnus	FNGVSYQWLTS—S1SLGTLQDVRHNN—SGHLTKT1SPVQPLQJQLSRQSFHITVSYEALMBVZELLNLNLQDHLF—	160
Oryzias	FDEAKK1K1Y1S1SLMDMSNSWQHGS—GEQFQ7QAE—QTSRKF1T1S1S1QAT1LKGFLLLLQNQSDQLEIC—	155
Megalobrama	AQHLQQVQVYDLETL1S1MLECLATSQS—FLP1PFTP—KEEAFFVYT1S1NVLYLLEQ1EKL1CMMDKL1QD1D7DVAETFLF	168

Figure S1. Molecular characterization of vertebrate *leptin*. Comparison of amino acid sequences of the teleosts, amphibians, birds, mammals and human *leptin*. Multiple sequences alignment was performed by Clustal X. Conserved cysteine and aa. residues involved in the formation were shaded. The highly conserved amino acid sequences between various vertebrates were boxed. The conservative disulfide bridge were colored in blue. GenBank Accession Nos.: *Homo sapiens* (NP_000221), *Macaca mulatta* (NP_001036220), *Lagenorhynchus albirostris* (ABK88255), *Balaenoptera acutorostrata* (AER93242), *Neophocaena phocaenoides asiaeorientalis* (AER93239), *Stenella attenuata* (AER93241), *Delphinapterus leucas* (AGT57736), *Gallus gallus* (AAC60368), *Xenopus laevis* (NP_001089183), *Carassius auratus* (ACL68083), *Cyprinus carpio "jian"* (AGK24955), *Labeo rohita* (ADB22376), *Cirrhinus molitorella* (ADI70505), *Schizopygopsis pylzovi* (AFY23973), *Ctenopharyngodon idella* (ACI32423), *Polypterus senegalus* (ADIT0506), *Hypophthalmichthys molitrix* (ACI32424), *Pelteobagrus fulvidraco* (AFO67938), *Salvelinus alpinus* (BAH83535), *Oncorhynchus mykiss* (AGB34181), *Oncorhynchus keta* (AGU36310), *Salmo salar* (ACZ02412), *Takifugu rubripes* (NP_001027897), *Tetraodon nigroviridis* (BAD94451), *Oryzias latipes* (BAD94448), *Thunnus thynnus* (ADT91717), *Morone saxatilis* (AFD34357), *Morone chrysops* (AFD34356), *Epinephelus coioides* (BAI66433), *Oreochromis niloticus* (AER12722), *Siniperca chuatsi* (ACT98260), *Larimichthys crocea* (AGR51148).

Figure 2. *Cont.*

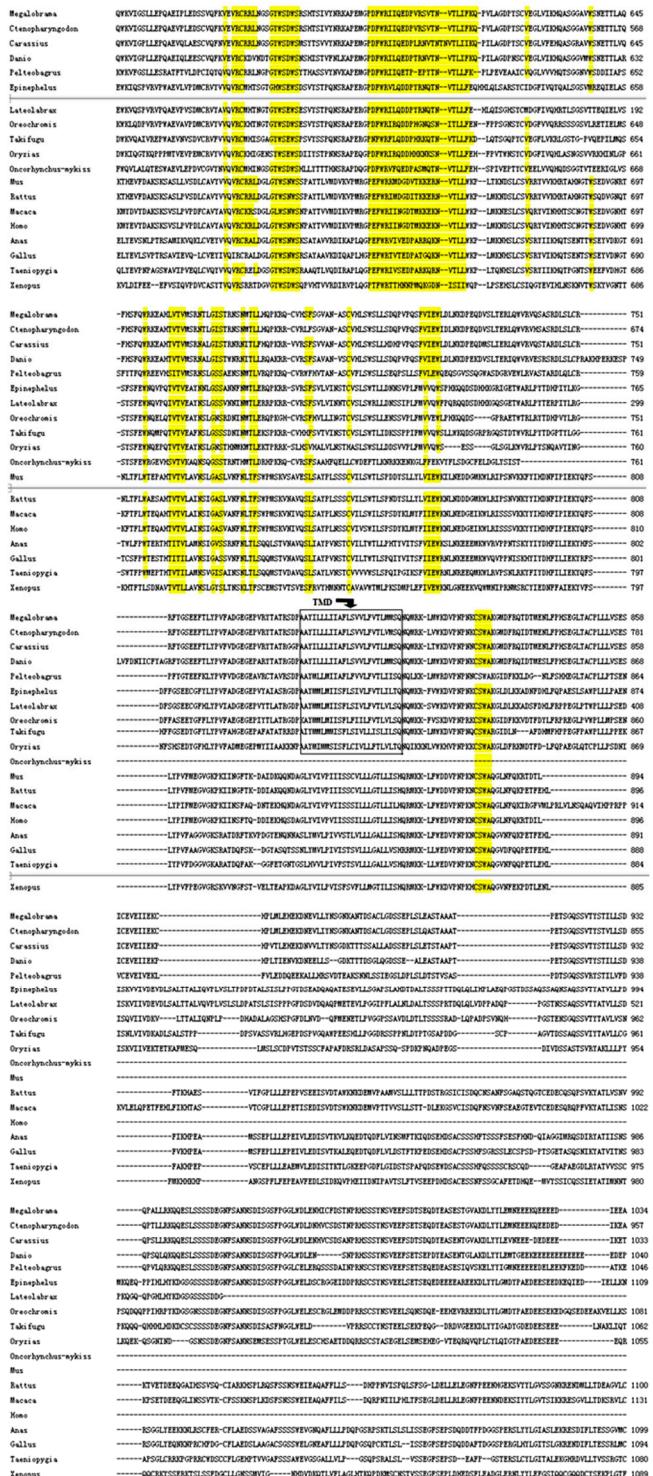


Figure S2. Molecular characterization of vertebrate *leptinR*. Comparison of amino acid sequences of the teleosts, amphibians, birds, mammalians and human *leptinR*. Multiple sequences alignment was performed by Clustal X. Conserved cysteine and aa. residues involved in the formation were shaded. The highly conserved functional domain amino acid sequences between various vertebrates were boxed and highlighted in yellow. GenBank Accession Nos.: *Carassius carassius* (ADZ75460), *Ctenopharyngodon idella* (AFU35431), *Danio rerio* (NP_001106847), *Epinephelus coioides* (AFU55262), *Lateolabrax japonicus* (AHI85769), *Oncorhynchus mykiss* (AGO59893), *Oreochromis mossambicus* (AGT28753), *Oryzias melastigma* (ABC86922), *Pelteobagrus fulvidraco* (AFO67946), *Takifugu rubripes* (NP_001124341), *Xenopus* (NP_001037866), *Mus musculus* (AAA93014), *Rattus norvegicus* (NP_036728), *Anas platyrhynchos* (ACF17729), *Gallus* (NP_989654), *Taeniopygia guttata* (AFK25169), *Macaca mulatta* (NP_001027991), *Homo sapiens* (AAC23650).

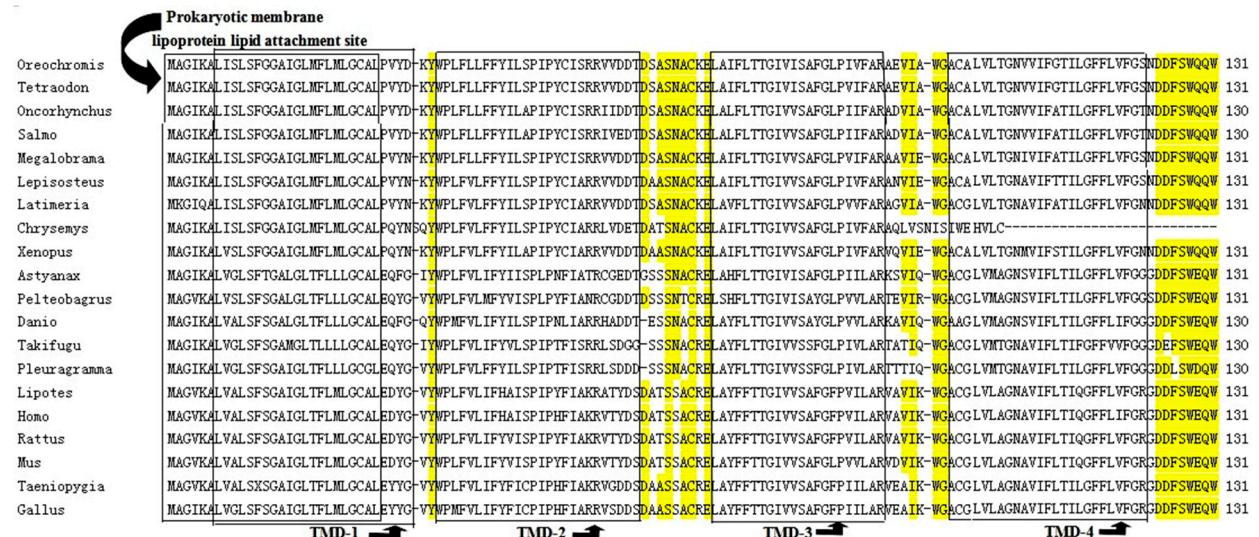


Figure S3. Molecular characterization of vertebrate *leprotl1*. Comparison of amino acid sequences of the teleosts, amphibians, birds, mammalians and human *leprotl1*. Multiple sequences alignment was performed by Clustal X. Conserved cysteine and aa.residues involved in the formation were shaded. The highly conserved functional domain amino acid sequences between various vertebrates were boxed and marked in yellow. GenBank Accession Nos.: *Homo sapiens* (AAH56250), *Lipotes vexillifer* (XP_007460146), *Rattus norvegicus* (AAH62003), *Mus musculus* (AAH04744), *Gallus* (NP_001007959), *Taeniopygia guttata* (XP_004174372), *Takifugu rubripes* (NP_001129621), *Pleuragramma antarctica* (AES12469), *Danio rerio* (NP_001017787), *Astyanax mexicanus* (XP_007250701), *Pelteobagrus fulvidraco* (AFN08752), *Chrysemys picta bellii* (XP_005308635), *Lepisosteus oculatus* (XP_006629222), *Latimeria chalumnae* (XP_006010007), *Oreochromis niloticus* (XP_003443415), *Tetraodon nigroviridis* (CAG04698), *Oncorhynchus mykiss* (ACO07979), *Salmo salar* (NP_001139931), *Xenopus laevis* (NP_001079518).

Table S1. The expression levels of *M. ambycephala leptin*, *leptinR* and *leprotl1* in the different tissues during the developmental periods of gonads (mean \pm SE). The β -actin was used as an internal control to calibrate the cDNA template for all the samples. Values with one asterisk mean significantly different between females and males at $p < 0.05$ and two asterisks at $p < 0.01$.

Stage	Tissues	<i>Leptin</i>		<i>LeptinR</i>		<i>Leprotl1</i>	
		♀	♂	♀	♂	♀	♂
I	Gonad	0.00004 \pm 0.00000	0.00069 \pm 0.00011 **	0.00024 \pm 0.00000	0.00791 \pm 0.00004 **	0.29260 \pm 0.01450 **	0.01232 \pm 0.00084
	Brain	0.00002 \pm 0.00000	0.00002 \pm 0.00000	0.00256 \pm 0.00104 **	0.00146 \pm 0.00029	0.13472 \pm 0.01431	0.13513 \pm 0.00572
	Liver	0.00227 \pm 0.00220 **	0.00012 \pm 0.00001	0.01685 \pm 0.00054 **	0.00160 \pm 0.00028	0.03017 \pm 0.00806	0.03661 \pm 0.00498
II	Gonad	0.00002 \pm 0.00000 *	0.00001 \pm 0.00000	0.00007 \pm 0.00000	0.00076 \pm 0.00005 **	0.19489 \pm 0.00321 **	0.10926 \pm 0.00355
	Brain	0.00538 \pm 0.00075	0.01761 \pm 0.00097 **	0.00256 \pm 0.00104	0.01409 \pm 0.00248 **	0.15579 \pm 0.01780	0.23520 \pm 0.02510
	Liver	0.33466 \pm 0.03921 **	0.00517 \pm 0.00047	0.17830 \pm 0.02288 **	0.00806 \pm 0.00054	0.18250 \pm 0.02898 **	0.01364 \pm 0.00070
III	Gonad	0.00002 \pm 0.00000	0.00071 \pm 0.00010 **	0.00026 \pm 0.00001	0.00230 \pm 0.00009 **	0.15500 \pm 0.00076 **	0.08733 \pm 0.00719
	Brain	0.75896 \pm 0.10136 **	0.01881 \pm 0.00056	0.66882 \pm 0.06486 **	0.01973 \pm 0.00134	9.47115 \pm 0.16127 **	0.69158 \pm 0.01815
	Liver	0.01416 \pm 0.00204 *	0.02808 \pm 0.00425	0.01651 \pm 0.00442	0.01898 \pm 0.00090	0.02690 \pm 0.00569	0.03719 \pm 0.00389
IV	Gonad	0.00000 \pm 0.00000	0.00017 \pm 0.00001 **	0.00007 \pm 0.00001	0.00353 \pm 0.00043 **	0.13534 \pm 0.00236 **	0.08337 \pm 0.00510
	Liver	0.03303 \pm 0.00398	0.07071 \pm 0.00786 **	0.02655 \pm 0.00204	0.02934 \pm 0.00869	0.02301 \pm 0.00258	0.03986 \pm 0.00793
	Pituitary	0.00005 \pm 0.00001	0.00238 \pm 0.00026 **	0.00327 \pm 0.00035	0.00644 \pm 0.00048 **	0.05043 \pm 0.01079	0.06002 \pm 0.00653
	Hypothalamus	0.00002 \pm 0.00000	0.00039 \pm 0.00008 **	0.00175 \pm 0.00011	0.00177 \pm 0.00068	0.06132 \pm 0.00132 **	0.03374 \pm 0.00575
V	Gonad	0.00002 \pm 0.00000 *	0.00001 \pm 0.00000	0.00238 \pm 0.00027 **	0.00023 \pm 0.00002	0.08383 \pm 0.00347	0.07955 \pm 0.00549
	Liver	0.05244 \pm 0.00928	0.05547 \pm 0.00677	0.04631 \pm 0.00957	0.02909 \pm 0.01057	0.04150 \pm 0.00836 **	0.01111 \pm 0.00142
	Pituitary	0.27409 \pm 0.01427	0.30982 \pm 0.02556	0.17942 \pm 0.02390 **	0.00434 \pm 0.00157	2.45742 \pm 0.44028 **	0.03102 \pm 0.00809
VI	Hypothalamus	0.00109 \pm 0.00011	0.00400 \pm 0.00068 **	0.00364 \pm 0.00047	0.32645 \pm 0.05979 **	0.02967 \pm 0.00426	0.08459 \pm 0.00440 **
	Gonad	0.00730 \pm 0.00028	0.01750 \pm 0.00134 **	0.00790 \pm 0.00093	0.01465 \pm 0.00038 **	0.04953 \pm 0.00673	0.15342 \pm 0.01387 **
	Liver	0.07953 \pm 0.00533	0.35823 \pm 0.02554 **	0.06165 \pm 0.00407	0.08643 \pm 0.01226	0.03885 \pm 0.00275	0.03308 \pm 0.01460
	Pituitary	0.26661 \pm 0.01539 *	0.17994 \pm 0.01491	0.18502 \pm 0.02369 **	0.00378 \pm 0.00053	0.10973 \pm 0.04010	0.13869 \pm 0.04312
	Hypothalamus	0.00004 \pm 0.00000	0.00733 \pm 0.00303 **	0.00356 \pm 0.00008	0.22562 \pm 0.05814 **	0.03374 \pm 0.00574	0.03374 \pm 0.00575