

Supplementary Figure S1. Amino acid alignment of all sea lamprey and Arctic lamprey globin sequences, including the partial sequences.

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>anole_lizard_GbY
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MTDLDRRHIREIWTAAAFENPEENGRLVIRFFSDYPASKQYF---
KTVPTDGDLDKAHPQVAFHGRRIMVAFSQVIENMENWNQACVLLERLVNNHKNIHQ-V-PSGMFQLLFQAMLCTFDDLLGRT-
FTP-EKRVSWEKFFQVIQEEVEAAAYDR-----
>Arctic_lamprey_aHb1
-----MPIVD-----
SGSVPALTAAEKATIRTAWAPVYAKYQSTGVDILIKFFTSNPAAQEFFPKFQGLTSADQLKKSM DVRWHAERI INAVNDAVVA
MDDTEKMSLKLKELSSKHAKSFQ-V-DPQYFKVLA AVIVDTVL-----PGDAGLEKLMSMICILLKSSY-----
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>Arctic_lamprey_aHb10
-----MPIVD-----
SGSVGALSASEQA AVAGSWKAVYANYEAAGKAVLIKFFTSNPGVQDFFPKFKGLDSADKLKSPAVRWHAERI INAVNDAVVA
LDDPEKQNLKLKALSQKHAYEFH-V-DSQYFKVLSATILEEVDHANG-G-LSA-DGKSGWEKLLSSICIHLSAY-----
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>Arctic_lamprey_aHb12
-----MPIVD-----
SGSVGELSAAEKSLVVS AWAPVYAKYEEAGVDILVKFFSDNPGVQDFFPKFKGLDSADQLKKSPAVRWHAERIMNAVND AVVA
LDEPAKLSLKLKALS KKHAEFN-V-DPQYFKVLAGVISDAVAK-----SG-DEKAAVDKFLSQVVILLKFAY-----
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>Arctic_lamprey_aHb13
-----MPIVD-----
SGSVGAISAAEKSLIVSAWAPVYAKYEEAGVDILVKFFAANPEAQAFFPKFKGLDSADQLKKSPAVRWHAERI INAVNDAVVA
LDDPAKQSLQLKALSQKHAQELN-V-DPNYFKVLAGVISDAVAK-----GG-DAKAAVDKFLSQVVILLKSAY-----
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>Arctic_lamprey_aHb14a
-----MPIVD-----
SGSVGAISAAEKSLIVSAWAPVYAKYEEAGVDILVKFFAANPEAQAFFPKFKGLDSADKLKSPAVRWHAERI INAVNDAVVA
LDDPAKQSLQLKALSQKHAHELN-V-DPNYFKVLAGVISDAVAK-----GG-DAKAAVDKFLSQVVILLKSAY-----
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>Arctic_lamprey_aHb15
-----MPIVD-----
SGSVAPLSAAEKT KIRSAWAPVYSNYETSGVDILVKFFTSNPAAQAFFPKFKGMTSADQLKKSA D VRWHAERI INAVNDAVAS
MDDTEKMSLKLQDLSGKHAKSFQ-V-DPQYFKVLA AVIADTV A-----AGDAGFEKLMSMICILLRSAY-----
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>Arctic_lamprey_aHb2a
-----MPIVD-----
SGSVAPLSAAEKT KIRSAWAPVYSNYETSGVDILVKFFTSTPAAQEFFPKFKGMTSADQLKKSA D VRWHAERI INAVNDAVAS
MDDTEKMSMKLRDL SGKHAKSFQ-V-DPQYFKVLA AVIADTV A-----AGDAGFEKLMSMICILLRSAY-----
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>Arctic_lamprey_aHb6
M-----GALQD-----
SGIVSSFKEDEKAALRESWDIFNNSHQDAGVKILARFI INNPEAKKFFPKFKGLETAEELENSAEVRVHGDKILA AVHQAVLD
LDDPDKQKNKLKDLSTSHAQQFN-V-EPAYFKTFAEVLKYVTETCGKS-FTS-EMRTSWSKLLSLIIIELQSAY-----
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>Arctic_lamprey_aHb7
-----MPIED-----
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LDEPEKNAKKLKELSEKHAVQLN-V-DAKFFKVLA EVLDKVAEKNDGS-FSD-SARSAWEKLLTYICISLKVAY-----
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>Arctic_lamprey_aHb9
-----MPIVD-----
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LDDPEKLSLKLKALS KKHAEFN-V-DPQYFKVL-----EGVSSANG-G-LGD-EAKAAWEKFLSQISILLKSQY-----
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>Arctic_lamprey_aMb1
-----MSIAD-----
SGSAPALSGDEKAAVRDTWKVVYAHAEDHGTTVLIKFLSDNADAKKFFPKFQALKTADEM KSSPVL RDHAKRIMSSINDMVVA
LDDTNAQNAQMNGLSKKHATDFK-V-DPKYFKVISNVILSVIAEGLGAQ-FND-AAKNGWSKLLTTTCIGLKSAF-----
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>Arctic_lamprey_Cygb
ME-----
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PEELIQILRAVGLSHARQATPV-DVKYYHVLGGIIMDV LLETFKDE-MSP-
TARGAWTKLLATLCTEFENAYREEGVLEQAAA-----
>Arctic_lamprey_GbX1
MGCTVSTDEHTGAQSS-----
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YQFRDCEDLQKLKMNKQLQAHGLRVMSFIEKSVARLEQECVLEQLIVEMGRKH-YKYN-A-
SPKYYSFVGIEFIATVQPFLQEK-WTN-EVEDAWQCLFRYIAAVMKRGYLEEEAASNGVNTANYDRGQGNHGATAM----

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>Atlantic_hagfish_aHb1
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HLEQDNEVKWQASRIINAVNAVVGHL DNEVAMKQYLKELSVKHSSEFQ-V-DPKMFKELSAIFVSTI-----
RGKAAYEKLFISIICTLRSTYDE-----
>Atlantic_hagfish_aHb2
-----MS-----
AHGIARTTEGERAAVRASWAVLMKDYEHAGVQILDKFFKANPAAKPFFTKMKDLHTLEDLASSADARWHVERIIQAVNFAVIN
IEDREKLSNKFVKLSQDHIEEFH-VTDPQYFMILSQTILDEVEKRN-GG-LSG-EGKSGWHKVMTIICKMLKSKY-----
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>Atlantic_hagfish_aHb3
-----MPITD-----
HGQPPTLSEGDKKAIRESWPIYKNFEQNSLAVLLEFLKKFPAQDSFPKFSAKKS--
HLEQDPAVKLQAEVIINAVNHTIGLMDKEAAMKKYLKDLSTKHSTEFQ-V-NPDMFKELSAVVFVSTM-----
GGKAAYEKLFISIATLLRSTYDA-----
>Atlantic_hagfish_aHb4
-----MPITD-----
QGPLPTLSEGDKKAIRESWPIYQNFEQTGLVVLLEFLQKNPGAQQSFPKFSATKC--
NLEQDNEVKWQASRIINAVNHTIGLMDKEAAMKQYLKELSAKHSSEFQ-V-DPKLFKELSAIFVSTI-----
RGKAAYEKLFISIICTLRSTYDE-----
>Australian_lamprey_aHb1
-----MPIVD-----
SGSVSPLSDAEKNKIRAAWDLVYKDYECTGVDILVKFFGTGTPAAQAFPPKFKGLTTADDLKQSSDVRWHAERIINAVNDAVKS
MDDTEKMSMKLKELSIKHAQSFY-V-DRQYFKVLAGIIADTTA-----PGDAGFEKLMISMICILLSSAY-----
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>Australian_lamprey_aHb2
-----MPIVD-----
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>Australian_lamprey_aHb3
-----MPIVD-----
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>Australian_clawed_frog_GbY
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WEVSSALTHLAQRHQDVHK-V-GVNNFQLLFLVILTIKFKEALGAD-FTP-EHCKSWEKLFISITYNFLDSCYTKSDS-----
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>Australian_clawed_frog_HbA1
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TLTDSKAAVIALWGKIAPQANAIGAEALERLFLSYPTKTYFSHF-DLSH-----GSADLANHGGKVVNALGEAAKHIND--
-LDAALSTLSDLHAYNLR-V-DPGNFKLLSHTIQVTLAIHFHKE-FDA-ATQAAWDKFLAEVATVLT SKYR-----
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>Australian_clawed_frog_HbAT5
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MTFSSAEKAAIASLWGKVS GHTDEIGAEALERLFLSYPTKTYFSHF-DLSH-----GSKDLRSHGGKVVKAI GNAATHIDD-
--IPHALSALSDLHAFKLK-V-DPGNFKLLSHAIQVTLAIHFPAE-FNA-DAQAAWDKFLAVVSAVLVSKYR-----
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>channel_catfish_GbX
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LERLDSLAVELGRSH-YRYY-A-PPKYGYVGTEFICAVRPILKEK-WTP-
ELEKAWKTLFQYVTWLMRRGYNEEEEEAKRNN TVGSSRERPRQRNTAL----
>chicken_Cygb
ME-----KVQGEMEIER----
WERSEEISDAEKKVIQETWSRVYANCEDVGVSILIRFFVNFPSAKQYFSQFKHMDDTLEMERSLQLRKHAQRVMGAIN TVVEN
LDDPEKVSSVLALVGKAHALKHK-V-EPVYFKKLTGVMLEVIAEAYGND-FTP-
EAHGAWTKMRTLIIYTHVTAAYKEAGWVSYP SATL-----
>chicken_GbE
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MSFSEAEVQSARGAWKMYVDAEDNGTAVLVRMFTEHPDTSYFTHFGMDSAEEMKQSDQVRGHGKRVFTAINDMVQHLDNT
EAFGLILNPLGQKHATQLK-I-DPKNFRIICDIILQLMEEFKFG-----DCKASFVKVTNEICTHLTNIYKEAGW-----
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>chicken_HbA
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MVLSAADKNNVKGIFTKIAGHAE EYGAETLERMFTTYPPTKTYFPHF-DLSH-----GSAQIKGHGKKVVAALIEAANHIDD-
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>chicken_HbG
-----MVHWTAEKQLITGLWGKV--
NVAECGAEALARLLIVYPWTQRFFASFGNLSSPTAILGNPMVRAHGKKVLSFGDAVKNLDN---IKNTFSQLSELHCDKLH-
V-DPENFRLLDILIIVLAHFHFKD-FTP-ECQAAWQKLVRVVAHALARKYH-----
>chicken_Mb
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-----G
>chicken_Ngb
ME-----SGM-----
LSRTQQALIRESWRRVSGSPVQHGVLFSRLFDLDPDLLPLFYQYNCKFASPQECLAPEFLDHIRKVMLVIDAAVSHLEDLPC
LEFYLCNLGKKH-QAVG-V-KVESFSTVGESLLYMLEKCLGAA-FSP-DVREAWIELYSAVVKAMQRGWEVLPEGD-----
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>cloudy_catshark_HbA
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MIFTDNDKNEIMPIIKVLEQNAEKLGAESLARMFALYPGSKVYF-HFEDFSA-----AGPKVKKHGAKVIGAITKAAHHLDD-
--LHGHLGLELAETHGKVLL-V-DPQNFPKLCQCIEVTLASHL-TE-FLP-STHCSIDKLLTVICQELSSRYR-----
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>cloudy_catshark_HbB
-----MVHWTPEERSEITSTWSAI--
NHKAVGTKALERMFSVYPWTTRYFSTIQGFTA-----EGHAIKVVGALDKAVKHLDD---VKTFKDLKSKKHAEELH-
I-DPGSFHLLTDCFIMELAHLEKCK-FTP-HTHATWAKFFKVVDATSKQYH-----
>cloudy_catshark_Mb
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MGDWDNVNVVWAKVEPNITAIGQAILLRFLQEHKETKAHFPKFKDI-AVEQLGNNEDVSKHGTIVLRALGNILKQKGS---
HGANKVELADSHINKHK-I-PPQNFTLITNIAVKVLTEMYPGD-MTG-QMQDSFSKVFKIICTDLENLYKEANFQ-----
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>coelacanth_Cygb
ME-----KVQGEMEMDR-----
WERSDQLSDTEVESIRQIWSNVYTNCENVGVLVLRFFVNFPSAKQYFSQFRHLEDPLDMERSVQLRKHARRVMGAINTVVEN
VED-QDIASVLAPVGKAHALKHK-V-EPVYFKILSGVILEILAEFYAQH-FTP-
EVQKAWTKLMSIICCHVTATYKEVWGQLSNSTM-----
>coelacanth_GbE
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MALSDAEVQTARDVWGQIYANAENGTIILVRMFTEHPDTSYFGNFKGMGSAAMEQSAQVRTHGKKIFSAIANDMIQHLDST
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>coelacanth_GbX1
MGCVFSGSGIAPSKSTPDINGSEAESRLSEE-----LKSGSGQNSD-----
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KDKLEQIAFELGRSH-CRYN-A-PPKYYQYVGQVQFISVVKPILKEA-WTP-EVEEAWQILFKYLTAVMKGYYVEEKINI-
NNALYTKIPNALSSNPVQNNL
>coelacanth_GbX2
MGCAISGLSWRAVK-----GPGE-----AKGKEGEAEA-----
LPAISQLQIHLIQESWKLIQEDIAKVGIIMFVRLFETHPECKDVFFLFRDVDDLQRLRTSKELRAHGLRVMSFVEKSVARLEH
SERLEQLALELGKSH-YRYN-A-PPKYYQYVGIEFVCAVQPILKER-WTP-EVEEAWQTLFKYVTEVMKRGYLQEEKIKRT-
METVEPKQASRRANNAI---
>coelacanth_GbY
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MAALTEADKQNIIRGIWKTVFENAEENGRTIVIRLFEEKYPETKVYFKNFKNISTMEEMQKNEQIRIHGLRVMSNLNQVIQNI
LNEVYSILTHLAKRHQYVHR-V-DVHNFKLIFGVIIKILKEALGAT-FTE-EICTSWQKMLSFTYDYLVSCHYKSSGP-----
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>coelacanth_HbA1
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MLSANDKTLISSTWNKVAANAEDIGAEALERLFLAHPQTKIYFSHM-DLSP-----GSSMLRAHGKKVMGTIEGSIKSIDK--
-LATVLSRLSDMHAYNFM-V-DPVNFKLLSQCILVALATQLMAD-FTP-EAQCAWDKFLALISEILFSKYR-----
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>coelacanth_HbA2
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MGLTAADKTLIKSIWGVKEKETEAGVEALVRLFKCFPQSKVYFDHFTDLSP-----SSQKLHAHAKVVLGALTKAVNHLDN-
--ITDTLHDISLVHAKLL-V-DPVNFELLGHGLEVALAAHFATD-FTP-EVHLAIDKFLYEVEKALFETYR-----
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>coelacanth_HbB1

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-----MVHWTETERATIETVYQKL--
HLDEVGREALTRLFIVYPWTTRYFKSFGDLSSSKAIASNPKVTEHGLKVMNKLTEAIHNLDH---IKDLFHKLSEKHFHELH-
V-DPQNFKLLSKCLIIVLATKLGKQ-LTP-DVQATWEKLLSVVVAALSREYH-----
>coelacanth_HbB2
-----MVTWTAEEKAITSVWSKV--
NPEEVGHEALIRLFIVYPWTQRYFSTFGSLSSSTVIARNFKVQQAHAHVINALTEAIRNIDN---LKASFSDLKSLHFQKLH-
V-DPENFKLLGKTLIIITLSEKLGSE-FSP-QIQAAWEKFMALVIDSLSRQYN-----
>coelacanth_Mb
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MALSEAEWGLILKVGKAEPEAASNGKSVLLRMFQEHPTDQHFQPKFKHM-TYQELQSSEELKTHGDTVLSKLGCLLKLKGN-
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>coelacanth_Ngb
ME-----KHN-----
FSVRSKELIRESWDRLGKNKLPHGTVMFTRLFELDPDLMHFLFNYNSSFPSQAGCLTSPEFIDHIHKVMMVVDAAVNSLDNLLS
LEDYLINLGKKH-QAAG-V-KMESFQVVGESLLYMLEHGLDSA-FSV-EVRHAWIALYSFVVETMARGWGANGENKLN-----
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>elephant_shark_GbY
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MTGITEADKENIHFIWEKLYENPEENGKTIVLRMFTDYPETKMYFQHFKNISTLEEMKKSPOIKRHGKIVMSALNKLIANLDN
GEELSLLAKMAERHINVHK-V-DLHNFQIIFNIIIAILEETFGNA-FTP-EIRGTWTKLFGVIYACLESYKDGAFY-----
-----P
>European_brook_lamprey_Cygb
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IEALQDIWEKVFKSAEDVGVIILVRLFTGHPASKQYFAMFKDLETANDLKASAKLRWHAGRVMGSLDKAVRSLRNPEKLIQIL
HAVGLSHARQATPV-DVKYYHILGGIIMDVLLLETFKDE-MSP-TARGAWTKLLGT-----
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>goldfish_GbX
MGCAISGS-----GLTAR-----APEIRAGEEET-----
PAGLTANHIRLIKESWRLTQEDIAKVGIIIMFVRLFETHPECKDVFFLFRDVEDLERLRTSRELRAHGLRVMSFIEKSVARLDQ
LERLETLALELGKSH-YRYN-A-PPKYGYVGAEFICAVRPILKDR-WTP-ELEEAWKTLFQYVTSIMREGFLEEERNKRS-
NTQTSSRERPDKRSTAI---
>green_spotted_puffer_Cygb1
ME-----RMQRDGEVDH----
VEQPGLTEKEKVMIQDSWAKVFQSCDDAGVAILVRFFVNFPSKQFFKDFKHMEPEEMQQSVQLRKHAHRVMTALNTLVES
LDNSDRVASVLKSVGRAHALKHN-V-DPKYFKILSGVILEVLGEAFTEI-ITA-
EVASAWTKLLANMCCGIAAVYKEAGWTELSSSVE-----
>green_spotted_puffer_GbX
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AAAVYPREDQIQMIKDSWKVIRDDIAKVGIIIMFVRLFETHPECKDVFFLFRDVEDLERLRSSRELRAHGLRVMSFIEKSVAR
LDQQDRLEALAVELGKSH-YHYN-A-PPKYYSYVGAEFICAVQPILKER-FTS-
ELEEAWKTLFQYVTGLMRKGHQEESRQR--HLALPPKDGPEKRTSAL---
>green_spotted_puffer_Ngb
ME-----
KLSSKDKELIRGSWDSLGNKVKPHGVILFSRLFELDPDLLNLFHYTTNCGSTQDCLSSPEFLEHVTKVMLVIDAAVSHLDDLH
SLEDFLNLGRKH-QAVG-V-KPQSFAMVGESLLYMLQCSLGQA-YTA-SLRQAWLNMYSVVVASMSRGWAKNGEDKAD----
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>gummy_shark_Mb
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VDWEKVNVSWSAVESDLTAIGQNILLRLFEQYPESQNHFPKFKNK-SLGELKDTADIKAQADTVLSALGNIVKKKGS---
HSQVPKALAATHITTHK-I-PPHYFTKITTIIVDLSEMYPSE-MNA-QVQAAFSGAFKIIICSDIEKEYKAANFQ-----
-----G
>human_CYGB
ME-----KVPGEIMEIER----
RERSEELSEAERKAVQAMWARLYANCEDVGAVAILVRFFVNFPSAKQYFSQFKHMEEDPLEMERSPQLRKHACRVMGALNTVVEN
LHDPDKVSSVLALVGKAHALKHK-V-EPVYFKILSGVILEVVAEEFASD-FPP-
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>human_HBA
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>human_HBB
-----MVHLTPEEKSAVTALWGKV--
NVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDN---LKGTFTLSELHCDKLH-
V-DPENFRLLGNVLVCVLAHHFGKE-FTP-PVQAAYQKVVAGVANALAHKYH-----
>human_HBD
-----MVHLTPEEKTAVALWGKV--
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>human_HBE
-----MVHFTAEEKAAVTSLSKSM--
NVEEAGGEALGRLLVVYPWTQRFFDSFGNLSSPSAILGNPKVKAHGKKVLTSLFGDAIKNMDN--LKPFAKLSELHCDKLH-
V-DPENFKLLGNVMVILATHFGKE-FTP-EVQAAWQKLVSAVAIALAHKYH-----
>human_HBG
-----MGHFTTEEDKATITSLWGKV--
NVEDAGGETLGRLLVVYPWTQRFFDSFGNLSSASAIMGNPKVKAHGKKVLTSLGDAIKHLDD--LKGTFACLSELHCDKLH-
V-DPENFKLLGNVLVTVLAIHFGKE-FTP-EVQASWQKMTGVSALSSRYH-----
>human_HBZ
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SLTKTERTIIIVSMWAKISTQADTIGTETLERLFLSHPQTKTYFPHF-DLHP----GSAQLRAHGSKVVAAGDAVKSIDD--
-IGGALSKLSELHAYILR-V-DPVNFKLLSHCLLVTLAARFPAD-FTA-EAHAAWDKFLSVVSSVLTEKYR-----
>human_MB
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MGLSDGEWQLVLNVWGKVEADIPGHGQEVLIIRLFKGPETLEKFDKFKHLKSEDEMKASEDLKKHGATVLTALGGILKKKGH-
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-----G
>human_NGB
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>inshore_hagfish_aHb1
-----PIID----
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DGGAEFEKLF SIIICILLRSAY-----
>mallard_GbE
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>medaka_Cygb1
ME-----RKQ--GEVDH----
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>medaka_Cygb2
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VVENLQDPEKVSSVLALVGKAHAVKHK-V-EPIYFKILSGVMSVLSEDFPEF-FTA-
EVQLVWTKLMAAVYWHVTGAYTEVGWLQVSSSAV-----
>medaka_HbA
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MSLSAKDKAAVKAFWAKVSGQADAIGSDALSRLVVYPQTKTYFAHWKDLSP----GSAPVKKHGKTVMGGIADAVGKIDD-
--ISSGLNLSELHAFTLR-V-DPTNFKILSHNIIIVMAIMFPQD-FTP-EVHVALDKFLAAVSLALSEKYR-----
>medaka_HbB
-----MVEWTEQERSIITNIFGNL--
DYEDVGSKALSRLIVYPWTQRYFASFGLNLYNAEAIKTNPNIAAHGTVLHGLDRAVKNMDN--IKATYAELSVLHSEKLH-
V-DPDNFKLLADCLTIVIAAKLGA-FSP-EIQATFQKFLAVVVSALGRQYH-----
>medaka_Mb
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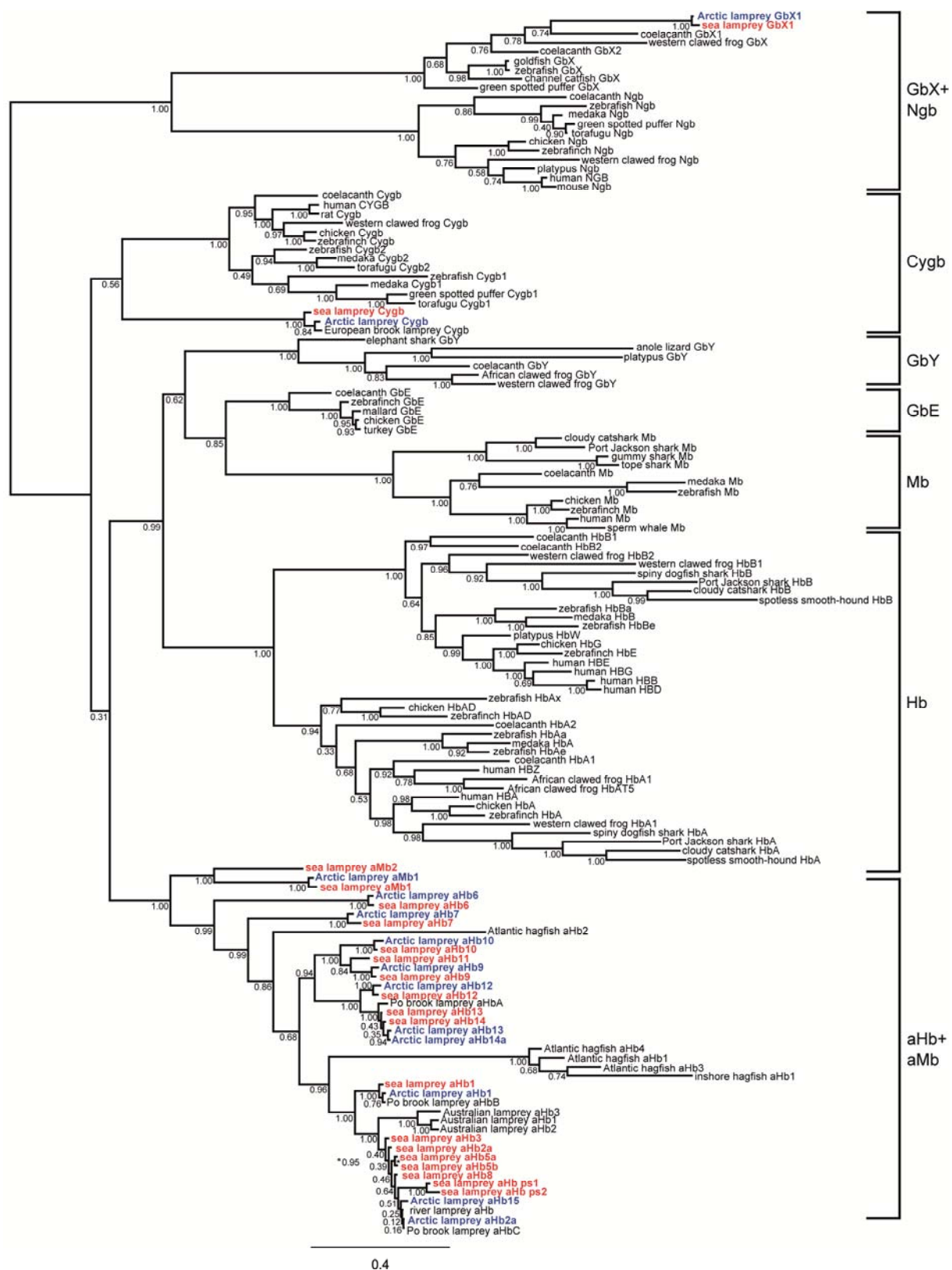
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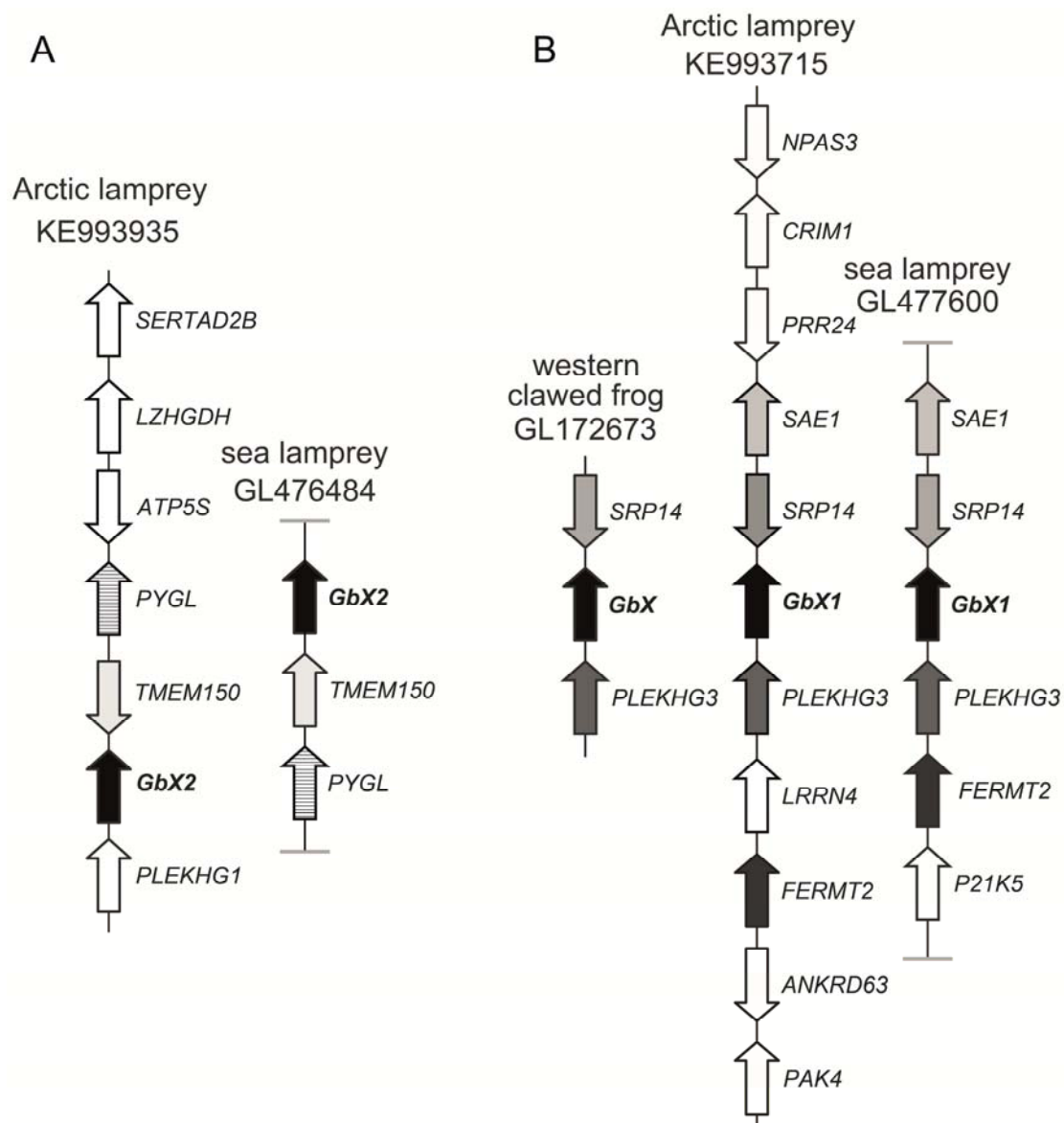
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ME-----
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TLEDFLLNLGRKH-QAVG-V-NTQSFALVGESLLYMLQSSLGPA-YTT-SLRQAWLTMYSIVVSAMTRGWAKNGEHKSN----
-----

```

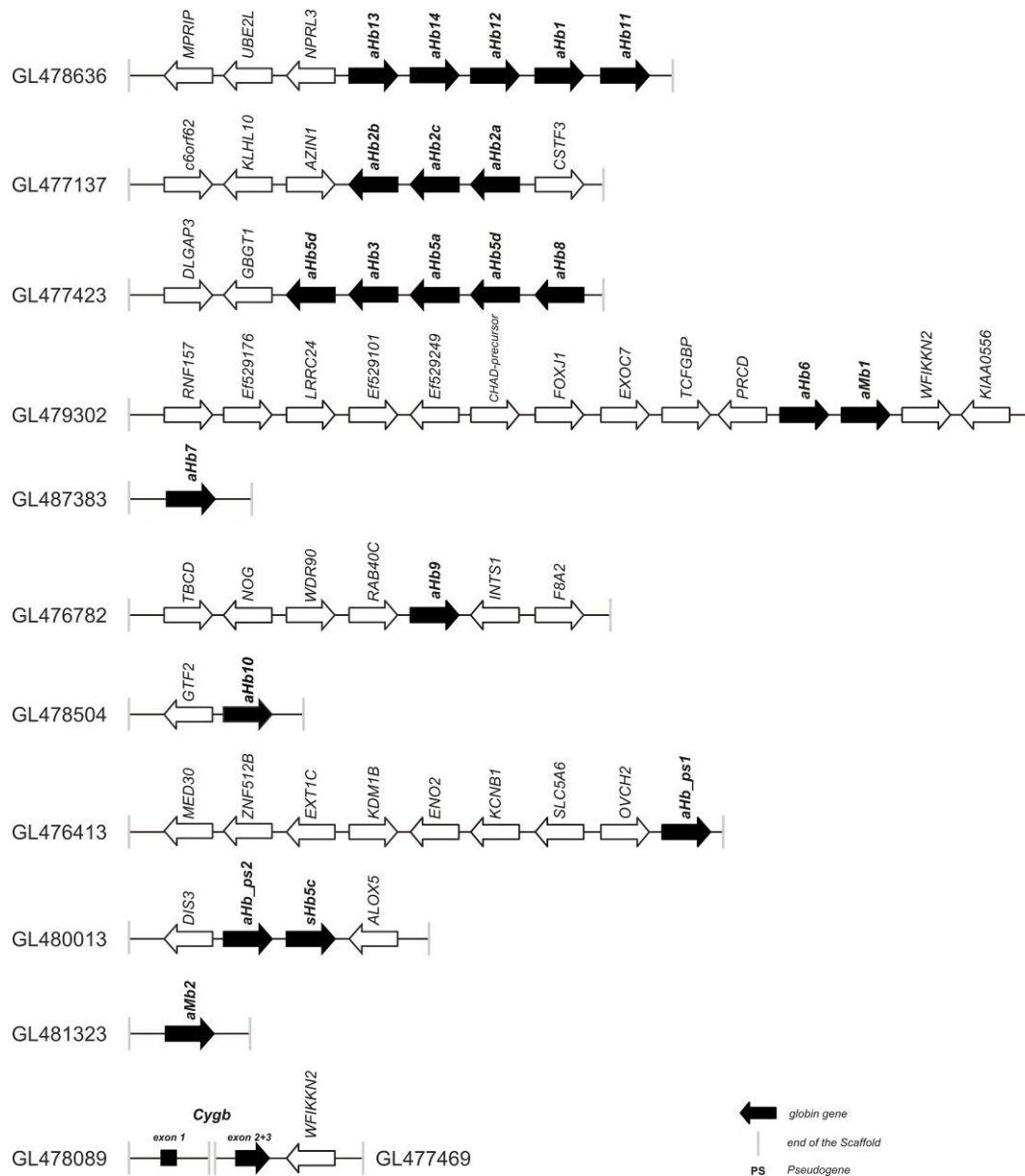
Supplementary Figure S2. Amino acid alignment of the 136 globin sequences used in phylogenetic analyses, as generated by MAFFT L-INS-i. The alignment is also available as text file (supplementary file 1, Supplementary Material online).



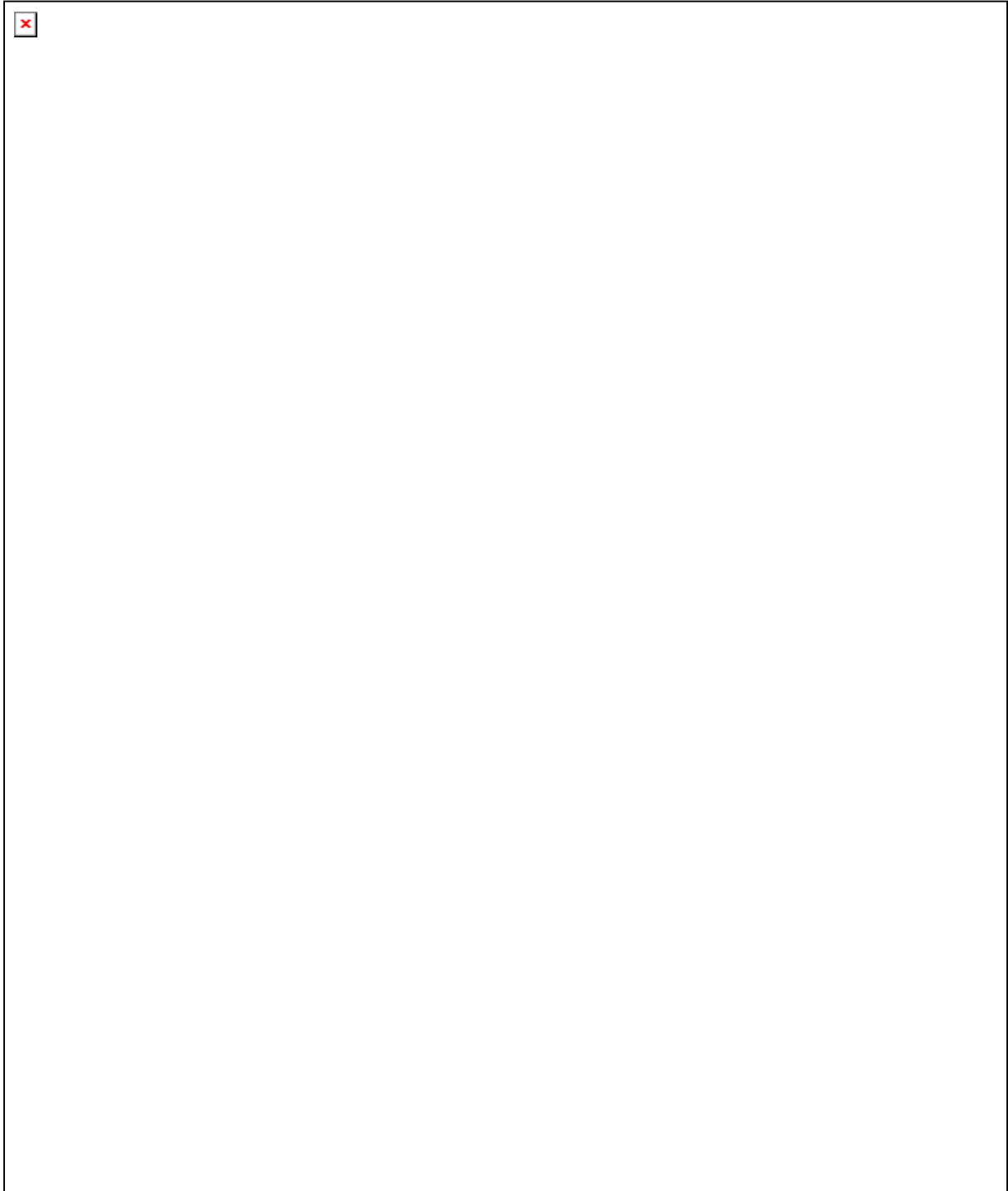
Supplementary Figure S3. Full Bayesian phylogenetic tree without collapsed branches. Posterior probabilities at each node are indicated.



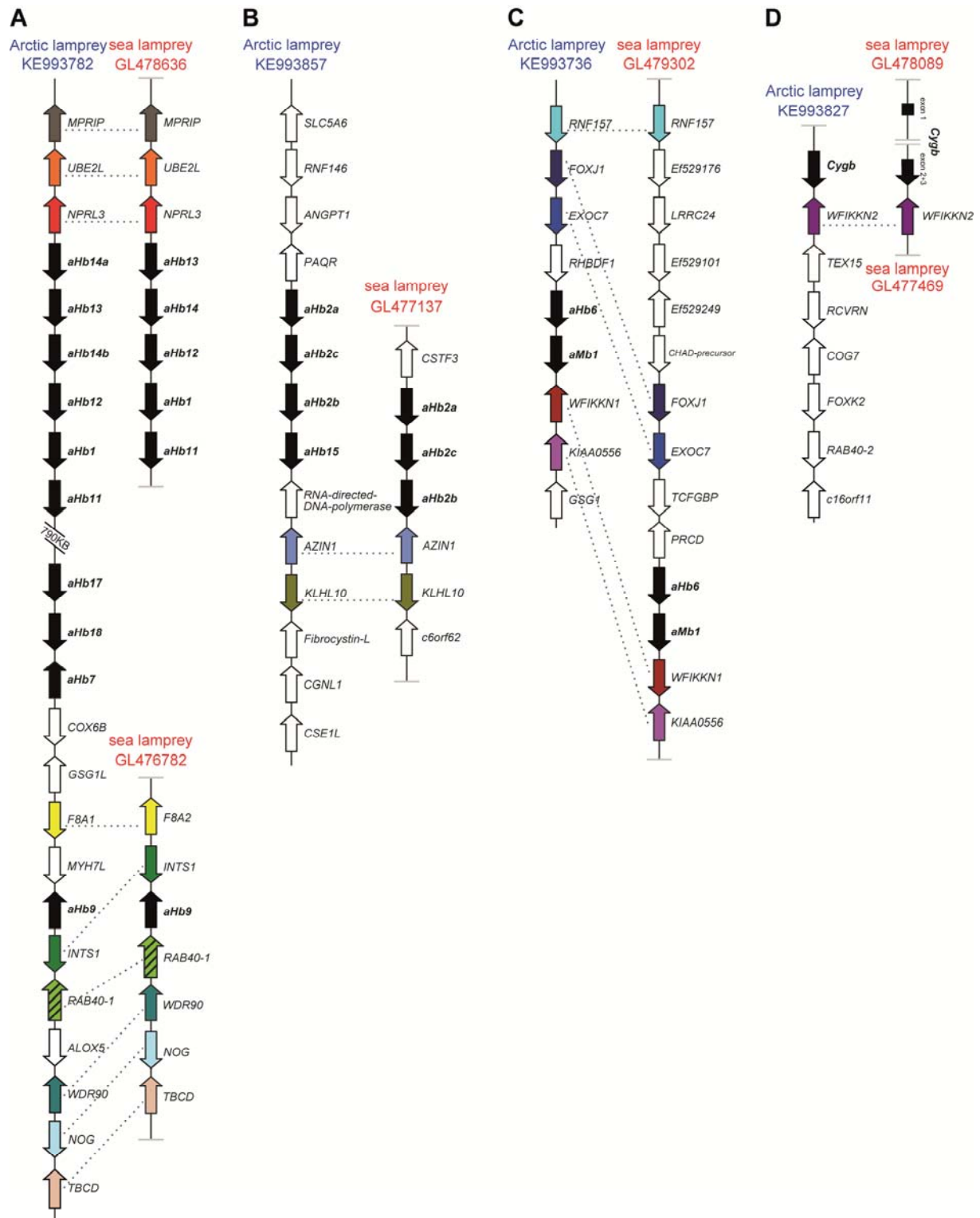
Supplementary Figure S4. Conserved synteny in the *GbX* region. The neighboring genes of the lampreys *GbX1* (B) and the *GbX2* (A) are indicated. Comparison with the western clawed frog *GbX* cluster shows the conserved synteny with the sea lamprey *GbX1*. The globin genes are colored in black, orthologous genes are indicated in the same shades of grey.



Supplementary Figure S5. The sea lamprey hemoglobin gene clusters. The neighboring genes of globin genes are indicated. The globin genes are colored in black.



Supplementary Figure S6. The Arctic lamprey hemoglobin gene clusters. The neighboring genes of globin genes are indicated. The globin genes are colored in black.



Supplementary Figure S7. Synteny analyses of the globin loci of the sea lamprey and the Arctic lamprey. Note that in (A) scaffold KE993782 of the Arctic lamprey links the sea lamprey scaffolds GL478636 and GL476782.