**Supplementary Materials**

**Additional File 1: Connexin sequences**

**Supplementary Figures S1 – S43**

**Mikalsen, SO; í Kongsstovu, S; Tausen, M.**

**Connexins during 500 million years – from cyclostomes to mammals**

**Common to all sequences in this document, is the following color coding:**

**Color coding: Yellow, Conserved domain as defined by Cruciani and Mikalsen (2007) Biol. Chem. 388, 253-264; Green, Conserved cysteine codons (cysteine signature); Grey, 15 nt extension at the ends of the conserved domains; Turquoise, Splice site. Other colors are explained where needed.**

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# Supplementary Figure S1. Human (*Homo sapiens*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S2. Cattle (*Bos taurus*) *GJA9*.

The sequence was obtained from GenBank.

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# Supplemenatry Figure S3. Mouse (*Mus musculus*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S4. Black flying fox (*Pteropus alecto*) *GJD5*.

The sequence was obtained from GenBank.

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# Supplementary Figure S5. Opossum (*Monodelphis domestica*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S6. Tasmanian devil (*Sarcophilus harrisii*) *GJC3.*

The sequence is collected from GenBank.

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# Supplementary Figure S7. Koala (*Phascolarctos cinereus*) *GJC3*.

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# Supplementary Figure S8. Platypus (*Ornithorhynchus anatinus*) connexins

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S9. Chicken (*Gallus gallus*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S10. Zebra finch (*Taeniopygia guttata*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry. As we initially collected the sequences from Ensembl, may the sequences contain SNPs relative to the given GenBank entry.

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# Supplementary Figure S11. Mallard (*Anas platyrhyncos*) *gjb5*, *gjb7* and *gjd3*.

The sequences were obtained from GenBank.

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# Supplementary Figure S12. Atlantic canary *(Serinus canaria*) *gjd5*.

The sequence was obtained from GenBank.

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# Supplementary Figure S13. Collared flycatcher (*Ficedula albicollis*) *gjc1*.

The sequence was obtained from GenBank.

>Fa-GJC1-XM\_005059597

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AGGGAAATCAAAGTGGCTCAGGACCGCCTGGACCTTGCCATCCAGGCTTACAACAACCAA

AACAACCCCAGCAGTTCCAGAGGGAAGAAATCCAAAGCGGGCTCCAACAAGAGCAGTGCC

# Supplementary Figure S14. Australian saltwater crocodile (*Crocodylus porosus*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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gtgattacagtggtgttgtgtgttgctgagatttttgagatcttatgtaggaggctgggt

ttcttaaacaatcagtga

# Supplementary Figure S15. Chinese alligator (*Alligator sinensis*) *gja3*.

The sequence was obtained from GenBank.

>As-gja3-XM\_025196654-GJA3

atgggtgactggagctttctggggagactattagagaatgcacaggagcactccacggtc

attggcaaagtttggctgaccgtgctgtttatcttcagaatcctggtgctgggagctgct

gctgaggaggtctggggagatgagcaatcggactttacctgcaacactcagcaacctggt

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ctgctcatagacacacggcgcttaagcagggctagtaagtccagcagcagcagagccagg

tcagatgacttggctgtgtag

# Supplementary Figure S16. American alligator (*Alligator mississippiensis*) *gjb4*.

The sequence was obtained from GenBank.

>Am-gjb4-XM\_006269885-GJB4

atgaactggtcctcactgcaggacctcctgagtggggtgaacaagtactccacagccctg

gggcgcatctggctctctgtggtctttgtcttccgcttcctggtctacgtggtagcggcg

gagcaggtgtgggctgatgaccagcgggactttgagtgcaacactctccagcccggctgc

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tccacccaggagaagaaccaggttgttaatataacctcctcaggacagactacattcagg

actgtccacttgatatag

# Supplementary Figure S17. Chinese softshell turtle (*Pelodiscus sinensis*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

>Ps-gja1-XM\_006131857-GJA1

ATGGGTGACTGGAGTGCCTTGGGCAAACTTCTTGACAAGGTTCAAGCTTATTCTACTGCA

GGAGGGAAGGTATGGCTCTCTGTCCTCTTTATTTTCCGCATCCTACTTTTGGGGACAGCA GTGGAATCAGCCTGGGGAGATGAGCAGTCTGCTTTCCGGTGCAACACTCAACAGCCTGGT TGTGAGAATGTCTGCTATGACAAGTCCTTCCCAATCTCTCATGTGCGCTTCTGGGTTCTG CAGATCATATTTGTGTCTGTACCTACCCTCTTGTATCTAGCACATGTGTTCTATGTGATG CGGAAAGAAGAGAAACTGAACAAGAAAGAAGAAGAGCTCAAGGTTGTCCAAAATGATGGT GTGAATGTGGAGATTCACCTCCAACATATACAAAAAAAGAAATTCAAGTATGGAATTGAA GAGCATGGTAAAGTTAAAATGCGTGGAGGACTGCTCCGTACTTACATCATCAGCATCCTC TTTAAATCTGTCTTTGAGGTAGCCTTCTTGGTGATACAATGGTACGTCTATGGGTTTAGC CTGAGTGCTATTTACACTTGTGAGCGATATCCATGCCCACACAGAGTGGACTGCTTCCTC TCCCGTCCAACTGAGAAAACCATCTTCATTATCTTTATGCTGGTCGTCTCTCTAGTATCG CTTGCCTTGAACATTATTGAGCTTTTCTATGTGTTCTTCAAGGGTGTCAGAGATCGTGTG AAAGGAAAACCAGACCACTACTCTCCCACCGGTACAGTGAGTCCCTCCAAGGATTGTGGA TCCCCCAAATATGCTTATTTCAATGGCTGTTCCTCTCCCACTGCTCCTCTATCACCCATG TCTCCACCAGGGTACAAGCTCGTTACTGGTGACAGGAACAATTCCTCCTGTCGTAACTAC AATAAACAAGCCAGTGAGCAAAACTGGGCAAATTACAGTGCGGAGCAGAACAGAATGGGA CAGGCTGGCAGCACTATCTCCAACTCTCATGCCCAGCCCTTTGATTTCCCCGATGATCAC CAGAACGCTAAAAAACTGGCATCGGGGCTTGAGCTGCAGCCCCTCACCCTTGTGGACCAA AGGCCACCTAGCCGTGCCAGCAGCCGAGCCAGTAGCAGACCTAGACCTGATGACCTGGAG

ATCTAA

>Ps-gja3-XM\_025179383-GJA3 Likely not the suggested intron in the intracellular loop indicated by XM\_014581446 (when compared with genomic sequence)

ATGGGTGACTGGAGCTTTCTGGGGAGACTATTAGAGAATGCACAAGAGCACTCCACGGTT

ATTGGCAAAGTTTGGCTGACGGTACTATTTATCTTCAGGATCCTGGTGCTGGGGGCTGCT GCTGAGGAGGTCTGGGGAGATGAACAATCGGACTTTACATGCAATACACAGCAACCTGGT TGTGAAAATGTCTGCTATGACCAAGCCTTCCCCATTTCTCACATCCGCTTCTGGGTGCTG CAGATCATTTTTGTCTCCACACCAACTCTCATCTATCTGGGCCATGTGCTGCACATTGTG CGCATGGAGGAAAAGAAGAAAGAGGCTGAGCTAAAAAAGAAGGGAAGCAGCAGGANNNNN NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGTGGGAGAGGG AGTAGCAACAACAACAGCACCACTAAGGAGGAACCCCCAAAGAAGGAGAAGCCACCAATC CGTGATGAGCGGGGTAAAATCCGCATTGGGGGGGCTTTGCTCCGCACTTATGTCTTTAAC ATCATTTTCAAAACTCTGTTCGAGGTGGGCTTCATCGTGGGCCAGTATTTCCTGTATGGC TTTGAACTAAAGCCACTCTACCGTTGCAGCCGCTGGCCTTGCCCCAACACTGTGGACTGC TTCATTTCACGGCCCACTGAAAAGACCATTTTCATCATCTTCATGCTGGTGGTGGCATGT GTCTCGCTGCTGCTCAACATGCTTGAGATGTACCACCTGGGATGGAAGAAGCTCAAGCAG GGCATGACCAACCAATACAGACCTGATCCACACCCCGCTGCCATGACGCCAGCTACCACT GTGAGGGACTCCAAACCTCTGACTCTGCCACTGCCATCTCCGGCAGTGCTCCCTGCTGTC CCACCTGCTCTGTCGGATTCCCGTGCCATCACGCCACTGCTCTCGTCACAGACTGTGCCA CCTTACTATGCTGAAGCCACTGCTAGGGCACAACCAGCCACCGCAGTTTCCTTGGCTGGC TACTCCGGGGCTCTTCCATTTGCCGAGGAACTACACAAAACTGCCACTCCCATTCCAGCC TCAACCCCAATCCCAACTCCAACTCTCGTCCCAACGCCTGTGCAAGCCATCACCTGCTAC TTCAACGGCAGCAGCCAGGCCTTAGCTGCTGAGCAGAACTGGGCCAATCTGGCAGTAGAG CAGCAGAGGAAGCCACCAGCCCGCTCAGCAACCTCCTCTTCCCCCAGCAGCACCCGACAA CAATCTCCAAAGCAAGAGGAGGCCAGTGAGCTGCCGCTGCCATTGCCACCTCTTTTGCCA CCTGTAGCAGCTGTCAGTAGTAGCAGCAGCACCAGCCTGAGCAGGGGAAGCAGCAGCAAG TGGGATGCAGAAGGTGAAGAGGAGGCCACAAAGGCATGGCCATTGTCAGTTGCCTGCACC ACAGTAGAAATGCATGAGCCGCCGCAGCTCATAGACACTCGGCGTTTAAGCAGGGCTAGT AAGTCAAGCAGCAGTAGAGCCAGGTCAGATGACTTAGCTGTGTAG

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GTGATTACAGTAATTTTGTGTGTTGCTGAGATTTTTGAGATACTATGTAGGAGGCTCGGT

TTTTTAAATAA

# Supplementary Figure S18. Green sea turtle (*Chelonia mydas*) *gja2*.

The sequence was collected from GenBank.

.

>Cmy-gja2-XM\_007056182-gja3 Chelonia mydas green sea turtle

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ctgagacccagtgacttggctgtttaa

# Supplementary Figure S19. Western painted turtle (*Chrysemus picta belli*) *gja2,* *gja3* and *gjd3*.

The sequences were collected from GenBank.

>Cpb-gja2-XM\_005286070-gja3like Highly deviating in the first 130 nt of the cds (grey font). This part of the sequence is not included in the analyses.

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# Supplementary Figure S20. Green anole (*Anolis carolinensis*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S21. Some sand lizard (*Lacerta agilis*) connexins.

These sequences were obtained from GenBank.

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# Supplementary Figure S22. Japanese gecko (*Gekko japonicus*) *gja2*, *gja9* and *gjb7*.

The sequences were collected from GenBank.

>Gj-gja2-XM\_015409011-gja3like

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ccacatgaaaaataa

# Supplementary Figure S23. Central bearded dragon (*Pogona vitticeps*) *gja4* and *gjb2*.

The sequences were collected from GenBank.

>Pv-gja4-XM\_020800133-GJA4

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gtgaaatcactgcctagtcattaa

# Supplementary Figure S24. Tropical clawed frog (*Xenopus tropicalis*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S25. Tibetan frog (*Nanorana parkeri*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S26. Coelacanth (*Latimeria chalumnae*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S27. Reedfish (*Erpetoichthys calabricus*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S28. Sterlet sturgeon (*Acipenser ruthenus*) connexins.

The sequences were initially found by blasting GenBank wgs (organism: *Acipenser ruthenus*) with sequences from the other cartilaginous fishes. The found sequences became later supported by published predicted sequences in GenBank (XM numbers). The genomic positions for the sequences are found in Supplementary Table S5. The A/B ohnologs are selected from the following criteria: (i) All sequences on a specific chromosome are selected to be either A or B. (ii) The lowest chromosome number is selected to contain the A ohnolog, and the higher chromosome number is selected to contain the B ohnolog. The A ohnologs are used in the phylogenetic analyses, except for *gje1*, as for this ohnologous pair, it seems like the A ohnolog might be a pseudogene. To distinguish between the genome duplications that generated sturgeon ohnologs and the teleost ohnologs, the sturgeon ohnologs are indicated by upper-case "A/B" (in contrast to the lower-case "a/b" for teleosts).

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>Ar-gje1A-XM\_034003916-gje1like VTUV01000005.1:49198091-4918128+49199541-49199723+49200145-49200540 We believe that exon1 is wrongly predicted in this entry. A more likely exon 1 is included here, although there are two potential errors in the sequence: Third position of initiation codon is A in assembly, and we have here exchanged it with a G, and in position 21, we have inserted a N to maintain reading frame, as this fits nicely with the gje1B ortholog. This could be a potential pseudogene.

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# Supplementary Figure S29. Spotted gar (*Lepisosteus oculatus*) connexins.

These sequences are as defined in SO Mikalsen, M Tausen, S í Kongsstovu (2020) BMC Genomics 23(1):223. doi: 10.1186/s12864-020-6620-2. The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S30. Japanese eel (*Anguilla japonica*) connexins.

GenBank was blasted both as whole-genome shotgun contigs (wgs) and as transcriptome shotgun assembly (TSA) using *Anguilla japonica* as organism. The names for the sequences are composed by the two-letter abbreviation of the Latin name - the formally correct gene name - the chromsomal scaffold accession number : position and direction (F or R). If the TSA search indicated a name for the sequence, it is given as the last part of the sequence name. The genome assembly has accession number GCA\_003597225.1 (Ajp\_01).

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>Aj-gja3a-BEWY01000008:38903519F-gja3 The genomic sequence as approx 15 N at the inicated location, but also a a repetition of a sequence 5´and 3´to the Ns. A missassembly is very likely.

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# Supplementary Figure S31. Atlantic herring (*Clupea harengus*) connexins.

These sequences are as defined in SO Mikalsen, M Tausen, S í Kongsstovu (2020) BMC Genomics 23(1):223. doi: 10.1186/s12864-020-6620-2. The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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ccatccgcagcaacagagcaagcgcaataa

>Ch-gja5a-XM\_012816449-gja5like This entry has been removed and replaced by XM\_031579948 as a result of standard genome annotation processing. However, we believe that XM\_031579948 is wrongly predicted in its 5'end. There are also differences in the low complexicity areas in the intracellular loop and the 3'tail, but these potential diffrences (we would not exclude the possibility of introns) are not importance for the present phylogenetic analyses.

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cccctcagcagcctgagtcgggcgagcagccgcgcacgctccgacgacctgaccgtatga

>Ch-gja9a-XM\_012824682-gja9like This record was removed as a result of standard genome annotation processing. However, the sequence is found in several other herring genome assemblies, and we believe this is an error in the assembly used for the updated prediction.

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>Ch-gja9b-XM\_012816385-gja9like This record was removed as a result of standard genome annotation processing, and is replaced by XM\_031585912. However, the two entries are 99.9 identical, but the newer prediction extends the 3'tail.

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taa

>Ch-gje1-XM\_012822376-gje1like Splice sites This accession number has now been replaced by XM\_031580896, that differ substantially from the present prediction for exon 1. We believe the new prediction is wrong.

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# Supplementary Figure S32. Zebrafish (*Danio rerio*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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>Dr-gjd2a-G67999-GJD2 Ensembl gene prediction ENSDARG00000067999.3

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>Dr-gjd2b-NM\_194420 Splice site.

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>Dr-gje1a-XM\_021473060-gje1like Grey font: regarded as intron, although the GenBank entry claims it to be a part of the exon. Splice site.

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>Dr-gje1b-NM\_001013546-gje1 Splice site.

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tatttaaaccagccaatgacttag

# Supplementary Figure S33. Atlantic cod (*Gadus morhua*) *gja11*, *gja12* and *gja13*.

These sequences are as defined in SO Mikalsen, M Tausen, S í Kongsstovu (2020) BMC Genomics 23(1):223. doi: 10.1186/s12864-020-6620-2. The sequences correspond to predictions in Ensembl, and the names are abbreviations for the Ensembl gene predictions in the following way: ENSGMOG00000018894 = G18894.

>Gm-gja11-G18894-cx34.5 Splice sites.

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ACAGAGAAGGTGTGGGGTGATGAGCAGTCCGACTTTGTGTGCAACACCAACCAGCCGGGG

TGTAAGAACGTGTGCTACGACCACGCCTTCCCCATATCACACGTCCGCTTCTGGGT**G**CTG

CAGATCATCTCGGTGGCCACGCCAACCCTGGTGTACCTGGGCCACGTCCTCCACGTCATC

CACGCTGAGCGCAAGGTGAGACTGAAGATCCAGAGGCAGGCGGAGCTGGACGAGGACGCC

CACCTGTTCCTGAAGAAGGGCTACAAGGTCCCCAAGTACAGCCACAGCAACGGCAAGATC

AACCTGCGGGGGAGCATCCTGCGCAGCTACCTGCTCAACCTGGTGGCCAGGATCCTGTTG

GAGCTGGGCTTCATCCTGGGCCAGTACTTCCTGTACGGCTTCACACTGCAGGCCCGCTAC

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>Gm-gja12-G18912-cx28.9 Splice site.

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CACAAGGAGAAAAAGCTGAGGGAGCGTATGCAGACCAGCAGCGAGCCGACCAAGAACCCA

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CAGCTCTCAGGCCCCATGTGGCCAACGGCAGAAGACGCCCGGCAGGCCAACAAGATGAAC

ATGGACTTTGAGAGCGGCCAGAGTACTGCTGGGAGCCTCAACGGGGCCAAGGAGGAGAAG

AAGCTTCTGAGTGGTCACTAG

# Supplementary Figure S34. Three-spined stickleback (*Gasterosteus aculeatus*) *gja11*, *gja12* and *gja13*.

These sequences are as defined in SO Mikalsen, M Tausen, S í Kongsstovu (2020) BMC Genomics 23(1):223. doi: 10.1186/s12864-020-6620-2. The sequences correspond to predictions in Ensembl, and the names are abbreviations for the Ensembl gene predictions in the following way: ENSGACG00000006828 = G06828.

>Ga-gja11-G06828-cx34.5 Splice sites.

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GCCGACAAGGTGTGGGGCGATGAGCAGTCCGACTTTGTCTGCAACACCCTGCAGCCCGGC

TGTGAGAACGTCTGCTACGACATGGCCTTCCCCATCTCTCACGTGCGCTTCTGGGTCCTT

CAGATCATCGCTGTGGCCACTCCAAAGTTGCTGTACCTCGGTCACGTCCTCCATGTGATC

CACCTTGAGAAGAAGATGAAGGAGAGGATGAAGAGGCATGCTGACTTGGACAACCAGATC

AGTCTGCTCCTTAGAAGGGCCTACAAAGTTCCCAAGTACACCAAGAGCACCGGCAAGATC

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>Ga-gja12-G06833-cx28.9 Splice site.

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CAGATTATCTTTGTGTCAACTCCAACGCTGATCTACCTCGGCCACGTCCTCCACATTATC

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TACATGACCTCTATTTTCTTCAGAATCCTCCTGGAGGTAGCGTTCATTGTTGGGCAGTAT

TATCTCTATGGGTTCATCATGGACCCAAGAGTGGTCTGCACCCGAGCCCCTTGTCCATTT

ACCGTGGAGTGCTACATGTCTCGGCCAACAGAGAAGACCATCTTCATTATCTTCATGCTG

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AGCCTGTCGACTCCCCGATGGCCGACGGCAGACGACGCGCTCAGGCACAACAAGGTGAAC

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CGACTACTGAGTGGTCATTAA

# Supplementary Figure S35. Japanese pufferfish (*Takifugu rubripes*) *gja11*, *gja12* and *gja13*.

These sequences are as defined in SO Mikalsen, M Tausen, S í Kongsstovu (2020) BMC Genomics 23(1):223. doi: 10.1186/s12864-020-6620-2.

>Fr-gja11-XM\_003976250-32.7like Splice site. This entry has been removed in an update of the Fugu genome annotation, and has been replaced by XM\_029850045. The difference between the records is that the intron in the intracellular loop has been included as an exon in the new entry.

ATGGGCGAGTGGGATTTGTTGGGCCGCCTGTTGGATAAAGTGCAGAGTCACTCCACAGTT

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GCCGACAAGGTGTGGGGTGATGAGCAGTCTGACTTTGTCTGCAACACTCAGCAGCCGGGC

TGTGAGAACGTCTGCTACGACCTCGCGTTCCCCATCTCTCACGTGCGCTTCTGGTTTCTT

CAGATTATTGCCATAGCGACGCCGAAGCTGCTCTACCTCGGCCACGTCCTCCACGTGATC

CACATTGAGAAGAAGGAGAAGGAAAAGATGAAGAAACAGGCCGAGTTGGATGCTCAGGCG

TGTCTGTTCCTCAGGACCTACAAAGTTCCCAAGTACATCAAAAGCTCTGGCAAGATCAGC

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GGGGAGGTCCGTATCTGA

>Fr-gja12-XM\_003976251-32.2like (gja12.1) This entry has been removed in an update of the Fugu genome annotation, and has been replaced by XM\_029849996. There is a single nucleotide difference between the two records.

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aacagtgagagcaaggcctag

>Fr-gja13-XM\_011617171-32.2like (gja12.2) This entry has been removed in an update of the Fugu genome annotation, and has been replaced by XM\_029849834. There is two indel incidences approx 15 nt apart (leading to identical reading frames before and after the indels) and a single nucleotide difference between the indels.

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CACAAGGAGAACAAGCTGAGGGAGAAGCTGCTGAGCCCCGGCGGGCCCCGCCTTGCTAAG

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CAGCTGCTGCGCCATTAA

# Supplementary Figure S36. Whale shark (*Rhincodon typus*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S37. Little skate (*Leucoraja erinacea*) connexins.

GenBank was blasted as whole-genome shotgun contigs (wgs) using *Leucoraja erinacea* as organism. The names for the sequences are composed by the two-letter abbreviation of the Latin name - the formally correct gene name - the chromsomal scaffold accession number : position and direction (F or R). The genome assembly has accession number GCA\_000238235.1 (LER\_WGS\_1)). The sequences are located on very short contigs.

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gccatcacactgctcctgtgcattgctgaaatatttgagatacttttcagaaagtttggc

tatttgtctacgcaataa

# Supplementary Figure S38. Elephant shark (*Callorhinchus milii*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

>Cm-gja1-XM\_007899670-gja1-43

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GTCTAG

>Cm-gja1.2-not predicted A probable gene duplication in elephant shark, as this sequence is located on the same contig as the predicted gja1 (XM\_007899670), approx 7000 nucleotides apart. Not included in the main analyses.

ATGGCTGACTGGAGTACACTTGCAAAACTACTGGCTAAGGCACAGTCTTATTCAACTGCA

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>Cm-gjb5like-XM\_007898730 Highly divergent from all other sequences. No other chondrichthyan sequences give reasonable hits with this one, even blasting with relaxed criteria (“somewhat similar sequences”). This sequence is therefore omitted from the main analyses.

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>Cm-gjc4-XM\_007910307-gjc1like NBNB 2 nt added relative to XM\_007910307 (marked in red) as the predicted sequence (but not the present modification) gives a weird sequence in last 10-15 aa of second conserved domain.

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# Supplementary Figure S39. Small-eyed rabbitfish (*Hydrolagus affinis*) *gja5*.

The sequence was collected from GenBank.

>Ha-gja5-JAAIL010003822:14502R

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# Supplementary Figure S40. Sea lamprey (*Petromyzon marinus*) connexins.

The chronology of the sequences is according to the Greek nomenclature. Size nomenclature is indicated in parentheses. GenBank accession number is given for each entry.

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# Supplementary Figure S41. Pacific lamprey (*Entosphenus tridentatus*) connexins.

There are no predictions in the gene banks. The genes were found by blasting wgs in GenBank with the taxon *Entosphenus tridentatus*, and selecting the entries belonging to JAAXLI020000000 (coming from assembly ETRf\_v1 GCA\_014621495.2), which are chromosome level scaffolds. The suggested names are followed by the five last digits of the scaffold number (JAAXLIxxxxx), and the position (corresponding to the first nucleotide of the given sequence) and direction (F or R).

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CACCCATGCATCAGGCAGGTGGAGTGCTACGTGTCACGGCCCACCGAGAAGACGATCTTT

CTGCTCTTCATGTTCGGCGTCAGCGGCTTGTGCTTGGTGCTCAACCTCGCAGAGCTCAAC

CACCTGGGCTGGCGCAAGATCAAGGGGGCCGTGCAGGGTGTCCGGACACGCAAGACGTCG

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>Et-gjd6-JAAXLI01137:14425250F Chr2-CM025608.2:14425250F

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TGCCCGACCGTTTGCTACGACCGCTTTGCACCCATCTCGCATCCACGCTTCTGGATTTTC

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TCCAAAGGTGGAACTGTCGGAGATGAACGCAAAGGGATCTGTCCCATCTCAGAAGACGCC

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ACGAAATGGGGCAAGATGGGACAACCGCGGAATCGAATATGGAACTGCTATGTCCTGCAC

GTGTGCTGCAGAACTATTCTGGAAGTTGCATTTGTGTTGGGTCAGTGGTTGCTCTTTGGT

TTTAGGGTCCCCGTTCACTACAAGTGCCAGGTTTTTCCTTGTCCGCTTATAGTCGACTGC

TTTGTATCCAGGCCAACTGAAAAAAAGATCTTCTTGCTGTTCATGTTTTCTGTCAGTGTT

TTCTGCATTTGTCTCAACATCGTCGAGTTGAACCACTTGGGATGGAAGAAAATCAAGAGT

CTGATCACAAAGCGAAATAATTTGCGTGTGCAAATTGAAGAAACAAATCCCATAAGTGCA

GCTGAAGATTTCGGTTGCCAAGTCTCCCCTCATCATGATCTACAAGACAATAAATCTCAT

CTACAAACGAAGTGTTCATCTTCTTCCAGCACTTCAGCAGCTTACGATGTGTACACTCAA

GAACATCTCCACCCACACTTGAACTTAGCTCAAAAAATTACTTTGGACAAACAATTCCAA

GAACAGATACTGTACCGTATGGGTGAAGAAAGTGGAGAGATTTCACCGAAAAAGGTATCA

CGGAAAAACATTGACTTGTGGGTATAA

>Et-JAAXLI43105:359F Only first conserved domain. Not used in phylogenetic analyses. Maybe a connection to Et-JAAXLI81785:16F (below), but Et-JAAXLI81785:455F is quite deviating, so we have judged the probability of the two scaffolds to actually encode the two parts of one gene, to be too low (see below).

ATGGGCGACTGGAGCATGCTGGGAGGCGTGCTGGAGCAAGCGCACGAGCACTCGACGGCG

CTGGGCAAGGTGTGGCTCACGGTGCTCTTCGTCTTCCGCATGCTCGTGCTGGGCACGGCC

GCCGAGAGCGTTTGGGGCGACGAGCAGTCGGACTTCCACTGCAACACGCTGCAGCCGGGC

TGCGAGAACGTCTGCTACGACGCGGCCTTCCCCGTGTCCCACGTGCGCCTCTGGGTGCTC

CAGATCGTCTTCGTGTCGACGCCGTCGCTCGCCTACCTCGGACACGTGCTCCACGGAGCG

CGGGCGGCCGCGCGCAGGAGGGAGCGGCGAGCGAGGGCCGCCGCCGCCGCCGCCGAGGGA

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GGAGAGGACGGCAAGGACGAGGGGAGGGGAGAGGAGGTGG..............

>Et-JAAXLI81785:16F Only part of second conserved domain. This part is quite deviating from other sequences

NAGGACGCTCCTCCGGTGCGTCTCCGCGGAGCGCTGCTCAGGACCTACGTCCTGTGCGTG

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CTGTCTCGCCCCACCGAGAAGAACATCTTCATCGTCTTCATGATGGCCGTGTCAGCGATC

TCGCTGGCGCTCAACTTGGCCGAGG

# Supplementary Figure S42. Arctic lamprey (*Lethentheron camtschaticum*) connexins.

The sequences were found by blasting directly into GenBank genome assembly LetJap1.0 GCA\_000466285.1. We have in general selected the longest scaffolds (KE numbers), but some of the found sequences are not assembled into the KE scaffolds, but are only found in shorter scaffolds/contigs (APJL numbers).

>LcAL-gja3-KE993718:2774835F Splice site

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ACGCTGTACCGCTGCAGCCGCTGGCCCTGCCCCAACGTGGTCGACTGCTTCGTGTCGCGC

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TGCTTCGTGTCGCGCGCCACCGAGAAGACCGTCTACGTGCGCCTCATGCTCGCCGCCTCG

AGCCTCGGCCTGGCCCTCAACGTCGCCGAGTTCGCCTACTTGCTGTGCCACGCCGNNNNC

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TGA

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CGGGCCAAGCGGAAGGCGCGGCGGTGCGGGCGGCCCTACGACGACGTCGCGGTGCGGAAG

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GTGGTGGCGCACGCCAAGAAGGGTGTCCACCAGGACGTGGTGGAGATCGGAGAGGCGTGA

>LcAL-gjb-gen1-KE997332.1:2950F-3338 Splice site

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GGGCGCGTCTGGCTGTCGGTGCTCTTCATCTTCCGGCTGCTCGTGCTCGTGGTGGCGGCC

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AAGAGCGTCTGCTACGACCGCTTCTTCCCCATCTCGCACATCCGCTTCTGGTCGCTGCAG

CTCATCGTGGTGACCACCCCGACGCTGCTCGTCGCCATGCACGTGGGCAGCCGCAAGATG

CGCTCGAGGCACCATCACTTCCCCGAGGGATTCGAGAACAAGATCCAGGGCGGCCTGCTC

TGGACCTACACCATCAGCGTGCTCTTTCGGATCATCTTCGAGACGGGCAGCCTCTACATC

TTCTACATCCTCTACGGGGGCTACTCGCTGCCGCGTCTCGTCAAGTGCACCGAGGAGCCG

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TCCGCCGTCAGCGGCGGGTCAGGCAACCCGGCAGG

>LcAL-gjc1/2-KE995893.1:17729F Due to stop codon in reading frame in intracellular loop, there is likely an intron here, possibly also in 3´-tail, but we have not tried to define these introns

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ACAGCACCCGGTTGGCCGTGTGGATATGA

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ACCATCATCGTCGCCGTGCCGTCCATCGTCTACCTCGGCTACGCCATGCACAGCCTGAGC

CGGGCAGAAGAAGCCCGGGGGAGCGTCGCGCCAAGGAGCAAGAGCCGACCGGCGATGGGC

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>LcAL-gjc-gen3-KE993784:162887R + 162586R + 160783-161292 + 159534-159679 + 159171-159423 Note a potential stp codon in the reading frame (red font), but as this is after the conserved domains, it does not affect the phylogenetic analyses. Splice sites

ATGAGCTGGAGCTTCCTCACACGGCTGCTGGAGGAGGTGCACCAACACTCCACCTTCCTG

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>LcAL-gjc-gen4.1-KE997621:4017R+3070R We have put a tentitative intron into the intracellular loop

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GCCGAGATGGGTTTCCTGGCCGGTCAGTATCTGCTCTACGGCCTGGAGGTGACCCCGCGC

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GAGAAAACCATCTTCCTGCTGCTCATGTACGCGGTCGCTGGTCTCAGCCTCCTGCTGGAG

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TGA

>LcAL-APJL01162196.1:421F Only possible to define first conserved domain. Sequence not included in analyses

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# Supplementary Figure S43. Inshore hagfish (*Eptatretus burgeri*) connexins.

The sequences were found by blasting directly into GenBank genome assembly Eburgeri\_3.2 GCA\_900186335.2.

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