The whale shark genome reveals how genomic and physiological properties scale with body size

Seung Gu Park[1,2]†, Victor Luria[3]†, Jessica A. Weber[4,5]†*, Sungwon Jeon[1,2], Hak-Min Kim[1,2], Yeonsu Jeon[1,2], Youngjune Bhak[1,2], Jehun Jun[6], Sang Wha Kim[7], Won Hee Hong[8], Semin Lee[1,2], Yun Sung Cho[6], Amir Karger[9], John W. Cain[10], Andrea Manica[11], Soonok Kim[12], Jae-Hoon Kim[13], Jeremy S. Edwards[14]*, Jong Bhak[1,2,6]*, George M. Church[4]*

[1]Korean Genomics Industrialization and Commercialization Center (KOGIC), Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, Republic of Korea.

[2]Department of Biomedical Engineering, School of Life Sciences, Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, Republic of Korea.

[3]Department of Systems Biology, Harvard Medical School, Boston, MA 02115, USA.

[4]Department of Genetics, Harvard Medical School, Boston, MA 02115, USA.

[5]Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA.

[6]Clinomics Inc., Ulsan 44919, Republic of Korea.

[7]Laboratory of Aquatic Biomedicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 08826, Republic of Korea.

[8]Hanwha Marine Biology Research Center, Jeju 63642, Republic of Korea.

[9]IT - Research Computing, Harvard Medical School, Boston, MA 02115, USA.

[10]Department of Mathematics, Harvard University, Cambridge, MA 02138, USA.

[11]Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

[12]National Institute of Biological Resources, Incheon 37242, Republic of Korea.

[13]College of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 63243, Korea.

[14]Department of Chemistry and Chemical Biology, UNM Comprehensive Cancer Center, University of New Mexico, Albuquerque, NM 87131, USA.

†These authors contributed equally to this work.

*These authors jointly supervised this work.

Correspondence and requests for materials should be addressed to G.M.C. (gchurch@genetics.med.harvard.edu), J.B. (jongbhak@genomics.org), J.S.E. (JSEdwards@salud.unm.edu), or J.A.W. (jessica_weber@hms.harvard.edu).

Keywords

Whale shark; lifespan; body size; metabolic rate; neural genes

Abstract

The endangered whale shark (*Rhincodon typus*) is the largest fish on Earth and is a long-lived member of the ancient Elasmobranchii clade. To characterize the relationship between genome features and biological traits, we sequenced and assembled the genome of the whale shark and compared its genomic and physiological features to those of 81 animals and yeast. We examined scaling relationships between body size, temperature, metabolic rates, and genomic features and found both general correlations across the animal kingdom and features specific to the whale shark genome. Among animals, increased lifespan is positively correlated to body size and metabolic rate. Several genomic features also significantly correlated with body size, including intron and gene length. Our large-scale comparative genomic analysis uncovered general features of metazoan genome architecture: GC content and codon adaptation index are negatively correlated, and neural connectivity genes are longer than average genes in most genomes. Focusing on the whale shark genome, we identified multiple features that significantly correlate with lifespan. Among these were very long gene length, due to large introns highly enriched in repetitive elements such as CR1-like LINEs, and considerably longer neural genes of several types, including connectivity, activity, and neurodegeneration genes. The whale shark's genome had an expansion of gene families related to fatty acid metabolism and neurogenesis, with the slowest evolutionary rate observed in vertebrates to date. Our comparative genomics approach uncovered multiple genetic features associated with body size, metabolic rate, and lifespan, and showed that the whale shark is a promising model for studies of neural architecture and lifespan.

The relationships between body mass, longevity, and basal metabolic rate (BMR) across diverse habitats and taxa have been researched extensively over the last century, and led to generalized rules and scaling relationships that explain many physiological and genetic trends observed across the tree of life. While studies of endothermic aquatic mammals have shown that selection for larger body sizes is driven by the minimization of heat loss[1], metabolic rate in ectothermic aquatic vertebrates is directly dependent on temperature, and decreased temperatures are correlated with decreased BMRs, decreased growth rates, longer generational times, and increased body sizes[2-4]. The whale shark (*Rhincodon typus*) is the largest extant fish, reaching lengths of 20 meters (m)[5] and 42 tonnes (t) in mass[6] and has a maximum lifespan estimated at 80 years[6]. Unlike the two smaller filter-feeding shark species (*Cetorhinus maximus*, *Megachasma pelagios*) that inhabit colder temperate waters with increased prey availability, whale sharks have a cosmopolitan tropical and warm subtropical distribution and have rarely been sighted in areas with surface temperatures less than 21°C[7-9]. However, recent GPS tagging studies have revealed that they routinely dive to mesopelagic (200-1,000 m) and bathypelagic (1,000-4,000 m) zones to feed, facing water temperatures of <4°C[10]. Observations of increased surface occupation following deeper dives led to the suggestion that thermoregulation is a primary driver for their occupation of the warmer surface waters[7,11]. Since larger body masses retain heat for longer periods of time, the large body mass of whale sharks may slow their cooling upon diving and maximize their dive times to cold depths, where food is abundant. Larger body mass could thus play a role in metabolic regulation.

Body size, environmental temperature, metabolic rate, and generation time are all correlated with variations in evolutionary rates[12,13]. Since many of these factors are interconnected, modeling studies have shown that observed evolutionary rate heterogeneity can be predicted by accounting for the impact of body size and temperature on metabolic rate[14], suggesting these factors together drive the rate of evolution through their effects on metabolism. Consistent with these results, the coelacanth and elephant fish have the slowest reported evolutionary rates[15,16]. Moreover, genome size and intron size have also been linked to metabolic rate in multiple clades. Intron length varies between species and plays an important role in gene regulation and splice site recognition. In an analysis of amniote genomes, intron size was reduced in species with metabolically demanding powered flight and was correlated with overall reductions in genome size[17,18]. However, since most previous studies were limited by poor taxonomic sampling and absence of genome data for the deepest branches of the vertebrate tree, comprehensive comparative genomic analyses across gnathostomes are necessary to gain a deeper understanding of the evolutionary significance of the correlations between genome size, intron size and metabolic demands.

Here we sequenced and analyzed the genome of the whale shark and compared its genome and biological traits to those of 81 eukaryotic species, with a focus on gnathostomes such as fishes, birds, and mammals. In particular, we identified scaling relationships between body size, temperature, metabolic rates, and genomic features, and found general genetic and physiological correlations that span the animal kingdom. We also examined characteristics unique to the whale shark and its slow-evolving, large genome.

The whale shark genome

The DNA of a *Rhincodon typus* individual was sequenced to a depth of 164× using a combination of Illumina short-insert, mate-pair, and TSLR libraries (Table S1 and S2), resulting in a 3.2 Gb genome with a scaffold N50 of 2.56 Mb (Tables S2, S5, and S6). A sliding window approach was used to calculate GC content and resulted in a genome-wide average of 42%, which is similar to the coelacanth and elephant fish (Fig. S2). Roughly, 50% of the whale shark genome is comprised of transposable elements (TEs), which were identified using both homology-based and *ab initio* approaches[19,20]. Of these, long interspersed nuclear elements (LINEs) made up 27% of the total TEs identified (Table S7). A combination of homology based and *ab initio* genome annotation methods[19,20] resulted in a total of 28,483 predicted protein coding genes (Table S8).

Correlation of physiological characteristics with genome features across 82 taxa

Body mass is intrinsically linked to physiological traits such as lifespan and basal metabolic rate (BMR)[21]. To better understand how genomic features correlate with physiological and ecological parameters such as body weight, lifespan, temperature, and metabolic rate, we compared the whale shark to 80 animals and yeast (Table S15-16) using physiological and genomic data (Fig.1, Fig. S3-6 and Table S16). Across the 81 animals examined, we found a strong positive correlation with significant *p*-values between the log transformed values for body weight and maximum lifespan ($\rho = 0.79$, Fig. 2A and Table S17) and BMR ($\rho = 0.958$, Fig. S9A, exponent B = 0.68, Fig. S25, and Table S17), consistent with previous reports[21]. Comparisons of physiological traits and genome characteristics across the 81 animals revealed several genetic features that also scaled with body weight. Among these, total gene length, intron length, and genome size all show a moderate statistical correlation with body mass, lifespan, and BMR (ρ = ~0.5) (Fig. 2B-E and Table S17). These results are consistent with previous findings of decreased intron size with increased metabolic rates. Furthermore, genome size and relative intron size are strongly correlated ($\rho = 0.707$) (Fig. 2B and Table S17), with the whale shark being a notable outlier. Moreover, genome size, measured as golden path length, scales with gene size, measured as summed length of exons and introns per gene (B = 1.32, Fig. S26). Additionally, we found that, unlike in bacteria[22] and crustaceans[23], genome size in Chordates scales positively with temperature (B = 0.77, Fig. S27).

Exon length is remarkably constant across animals, regardless of genome size or intron length (Fig. 1C and Fig. S4C). Early observations of this phenomenon across small numbers of taxa led to the suggestion that the splicing machinery imposes a minimum exon size while exon skipping begins to predominate when exons exceed ~500 nt in length[24]. Also of note is the tight correlation ($\rho = 0.975$) between the overall GC content and GC3, the GC content of the third codon position (Fig. S9B and Table S17), while both features are negatively correlated with the codon adaptation index (CAI) ($\rho = -0.799$ and $\rho = -0.841$, respectively; Fig. 2G-H and Table S17) in Eukaryota, and negatively correlated with the genome size in Mammalia ($\rho = -0.434$ and $\rho = -0.473$, respectively) (Table S17). These results are partially supported by previous research, which showed that GC3 is negatively correlated with body mass, genome size, and species longevity within 1,138 placental mammal orthologs[25]. However, our results using whole genome data do not support the GC3 correlation with body mass and longevity ($\rho = 0.067$ and $\rho = 0.059$; Table S17). Thus, exon and intron length may affect body mass and longevity through a strong association between GC content and coding sequence length[26]. Additionally, CAI and intron size are moderately positively correlated ($\rho = 0.463$; Fig. 2I and Table S17). Since the CAI and codon usage bias have an inverse relationship, this is consistent with the negative correlation between intron length and codon usage bias in multicellular organisms[27].

Whale shark longevity and genome characteristics

The allometric scaling relationships between longevity, mass, temperature, and metabolic rate are well established[21], and the long lifespan of the whale shark can be explained by its large mass and the extremely low mass- and temperature-adjusted BMR (Fig. 1H and 1L). There has been considerable debate in the literature over the evolutionary causes and consequences of genome size, particularly as it relates to BMR. At 3.2 Gb, the whale shark has a genome that is significantly larger than those of other Chondrichthians (elephant fish), though both exon number and size are comparable. The whale shark is, however, a notable outlier, particularly among fish, for its long introns (Fig. 1E and S4E). Interestingly, the whale shark's relative intron length (Fig. 1E and S4E) is significantly longer than any of the other 81 species (Fig. S5G and S6G). Analyses of single copy orthologous gene clusters did not reveal any large intron gains or losses in the whale shark (Fig. S10), though retrotransposon analyses revealed a significant expansion of CR1-like LINEs and Penelope-like elements in the introns (Fig. 3A and S11-15). The CR1-like LINEs are the dominant family of transposable elements (TEs) in non-avian reptiles and birds[28]. In the whale shark, the summed length of CR1-like LINE elements is 176 Mbp (Fig. S13C), which is eleven times longer than that of the anole lizard, a species known for expanded CR1-like LINEs. The total length of intronic repetitive elements is as great as in the opossum genome, known to be rich in repetitive elements[29] (Fig. S12). In the whale shark genome, 38% of the CR1-like LINE, 39% of the CR1-Zenon like LINE, and 30% of the Penelope-like elements are located in intronic regions (Fig. S14). Strikingly, most genes (more than 88%) in the whale shark genome have the CR1-like LINE elements within their introns (Fig. S15) and 56% of genes also have LINE1 elements (Fig. S15). Thus, the whale shark's relatively large genome and long introns are due to repetitive elements.

Previous research has shown that there is an association between codon usage and the evolutionary age of genes in metazoans[30]. Interestingly, two principal component analyses (PCA) of relative synonymous codon usage (RSCU) from 82 and 76 species (six species having distant codon usage patterns were excluded), respectively, revealed that the whale shark pattern of RSCU is most similar to that of the coelacanth; with well separated patterns of RSCU for each class (Fig. S16). While the whale shark genome has a relatively short exon length (smaller than that of 59 species), importantly, it has a smaller number of exons per gene than all but two species (the yeast and fruit fly have the smallest number of exons) (Fig. S3B and S4G). Thus, the whale shark CDS length is shorter than all but the yellow sea squirt genome (Fig. 1D and S4D).

Evolutionary rate and historical demography

Analyses of the whale shark genome showed it is the slowest evolving vertebrate yet characterized. A relative rate test and two cluster analyses revealed that the whale shark has a slower evolutionary rate than those of the elephant fish and all other bony vertebrates examined, including coelacanth[16] (Fig. 3B, S17 and Table S18-20). These results support the previous work which predicted a slow evolutionary rate in ectothermic, large-bodied species with relatively low body temperature (compared to similarly sized warm-blooded vertebrates)[14]. They are also consistent with previous studies of nucleotide substitution rates in elasmobranchs, which are significantly lower than those of mammals[31,32].

A phylogenetic analysis of the 255 single-copy orthologous gene clusters from the whale shark and 24 other animal genomes (Fig. 3D) showed a divergence of the Elasmobranchii (sharks) and Holocephali (chimaeras) roughly 268 MYA and of the Chondrichthyes from the bony vertebrates about 457 MYA (Fig. 3D), consistent with previous estimates. To understand how many genes appeared in each evolutionary era within the whale shark genome, we evaluated the evolutionary age of whale shark protein-coding genes based on protein sequence similarity[33]. Grouping the whale shark genes into three broad evolutionary eras, we observed that while the majority (58%) of genes are ancient (older than 684 MYA), a few (~5%) are middle age (684 - 199 MYA), and many (36%) are young (199 MYA to present) (Fig. 3C). Normalizing the number of genes by evolutionary time suggests that gene turnover is highest near the present time (Fig. S18). Examining the age of genes shows many genes are ancient (PS 1) and many genes appear very young (PS 20) (Fig. S19), though the large number of young PS 20 genes may in part reflect the paucity of closely related species with fully sequenced genomes. These results highlight both the conservation of a large part of the genome as well as the innovative potential of the whale shark genome, since many new genes appeared within the last 200 million years.

Gene family expansions and contractions in the whale shark

Gene family expansion and contraction analyses across 25 species identified 101 contracted gene families in the whale shark. Of these, nucleosome assembly (GO:0006334) and chromatin assembly (GO:0031497) were significantly decreased in the whale shark compared to the Chondrichthyes common ancestor (Table S21A). Interestingly, the whale shark genome has a smaller number of histone gene families (H1, H2A and H2Bs) than other bony fishes and mammals (Fig. S20). This small number of histone gene families, especially the H1 family which encodes the linkers important for higher order chromatin structures, may be related to the long length of whale shark introns[34]. We also identified 13 expanded gene families that are enriched for several metabolic pathways, including fatty acid metabolism, along with neurogenesis and nervous system development, and cardiac conduction system development (Table S21B).

Gene length of neural genes and correlation with physiological features

Gene length has recently emerged as an important feature of neural genes, as long genes are preferentially expressed in neural tissues and their expression is under tight transcriptional and epigenetic control[35]. Within the 81 animal species, we compared the dimensions of average genes with those of ten categories of neural genes (neuronal connectivity, cell adhesion, olfactory receptors, ion channels, unfolded protein response associated genes, neuronal activity and memory, neuropeptides, homeobox genes, synaptic genes, and neurodegeneration) (Fig. 4A and S21). Interestingly, we found that neuronal connectivity genes are longer than average genes in most vertebrates, with the length increase being significant in whale shark and most mammals, as well as in coelacanth and platypus (Fig. 4A and S22A). Surprisingly, we found that neural genes are scaled to average genes with an exponent greater than 1 (B = 1.038, Fig. S28), with the whale shark showing an extreme lengthening of neural genes. Moreover, we found that cell adhesion, ion channels, homeobox genes, and neurodegeneration genes are increased in length in the whale shark (Fig. 4B). Thus, the organization of whale shark neural genes may reflect the need to maintain the shape, activity, identity, and resistance to neurodegeneration in a body that is both very large and long-lived. Finally, neuronal functions are enriched in long genes in more than 60 species (Fig. 4C and Additional File 1).

To determine whether physiological traits and genomic features are linked, we examined the correlation of gene size and maximum lifespan, body weight, and BMR (Fig. 2A-F). In 155 gene families, we found that gene length was significantly correlated to maximum lifespan, body weight, and BMR. Gene ontology analyses of this gene group showed statistical enrichment of biological processes such as telomere maintenance (GO:0007004: *XRCC5, MAPKAPK5*, and

NAT10) and RNA and protein export from nucleus (GO:0006405 and GO:0006611: *SDAD1, SARNP, RAE1, NUP155, ABCE1, ENY2, XPO5, CSE1L* and *STYX;* Fig. 4D, Tables S22 and S23), both of which are associated with longevity and cancer[36,37]. Of the genes in which gene length is associated with lifespan, NUP210 (nucleoporin 210) and VWF (von Willebrand factor) are both associated with longevity[38] (Fig. S24A and Table S24). Moreover, the genes correlated to BMR include *SNX14*, which is linked to protein metabolism and whose deficiency causes ataxia and intellectual disability[39] (Fig. S24B and Table S24). The only gene previously correlated with body mass (*COX5B*; the terminal enzyme of the mitochondrial respiratory chain) is a subunit of Complex IV and is essential to energy production in the cell and ultimately to aging[40] (Fig. S24C and Table S24). Taken together, these results suggest that there is an evolutionary relationship between gene size and physiological traits size such as body size, metabolic rate, and lifespan. This holds particularly among genes whose functions are essential for living long lives, such as telomere maintenance and energy production.

Conclusions

We sequenced and assembled the genome of the whale shark (*Rhincodon typus*), an endangered species that is the largest extant fish on Earth. Its relatively large 3.2 Gb genome is the slowest evolving vertebrate genome found to date, and has a striking amount of CR1-like LINE transposable elements. In most genomes, we found that major genomic traits, including intron length and gene length, correlate with body size, temperature, and lifespan, and that GC content and codon adaptation index are negatively correlated. Unexpectedly, we found that neural connectivity genes are substantially longer than average genes. In the whale shark genome, specifically, we found that introns are longer than in most other species due to the presence of repetitive elements and that neural genes of several types, including neurodegeneration genes, are much longer than average genes of species with long lifespans. These results show the power of the comparative evolutionary approach to uncover both general and specific relationships that reveal how genome architecture is shaped by size and ecology.

Methods

Sample preparation and sequencing. Genomic DNA was isolated from heart tissue acquired from an approximately seven years old, 4.5 meter deceased male whale shark from the Hanwha Aquarium, Jeju, Korea. DNA libraries were constructed using a TruSeq DNA library kit for the short-read libraries and a Nextera Mate Pair sample prep kit for the mate pair libraries. Sequencing was performed using the Illumina HiSeq2500 platform. Libraries were sequenced to a combined depth of 164× (Tables S1 and S2).

Genome assembly and annotation. Reads were quality filtered (Table S3) and the error corrected reads from the short insert size libraries (<1 Kb) and mate pair libraries (>1 Kb) were used to assemble the whale shark genome using SOAPdenovo2[41]. As the quality of assembled genome can be affected by the *K*-mer size, we used multi-K-mer value (minimum 45 to maximum 63) with the 'all' command in the SOAPdenovo2 package[41]. The gaps between the scaffolds were closed in two iterations with the short insert libraries (<1 Kb) using the GapCloser program in the SOAPdenovo2 package[41]. We then aligned the short insert size reads to the scaffolds using BWA-MEM[42] with default options. Variants were identified using SAMtools[43]. Since at least one of alleles from the mapped reads of same individual as reference should be presented in the assembly, we corrected erroneous bases where both alleles were not present in the assembly by substituting the first variant alleles. Finally, we mapped the Illumina TruSeq synthetic long reads (TSLR) to the assembly and corrected the gaps covered by the synthetic long reads to reduce erroneous gap regions in the assembly (Table S5).

The GC distribution of the whale shark genome was calculated using a sliding window approach. We employed 10 Kb sliding windows to scan the genome to calculate the GC content.

Tandem repeats were predicted using the Tandem Repeats Finder program (version 4.07)[44]. Transposable elements (TEs) were identified using both homology-based and *ab initio* approaches. The Repbase database (version 19.02)[45] and RepeatMasker (version 4.0.5)[19] were used for the homology-based approach, and RepeatModeler (version 1.0.7)[20] was used for the *ab initio* approach. All predicted repetitive elements were merged using in-house Perl scripts. Two candidate gene sets were built to predict the protein coding genes in the whale shark genome; using AUGUSTUS[46] and Evidence Modeler (EMV)[47], respectively (Supplementary Text 1.7).

Genomic context calculations. From the 82 species (Table S15), we computed the following genomic factors: GC3 (GC content at third codon position), CAI (codon adaptation index), number and length of coding exon(s), and relative intron length between first and last exon (or coding exon). CDS sequences with premature stop codons and lengths that were multiples of three were excluded. The relative intron length was calculated by dividing the total intron length between first and last exon (or coding exon) by the CDS length (or mRNA length). GC3 was computed from concatenated third codon nucleotides using the canonical method[48]. We measured relative synonymous codon usage (RSCU) using the method from Sharp *et al.*[49] and the codon adaptation index (CAI) in a CDS using Sharp and Li's method[50] for each of the 82 species. The principle component analysis (PCA) on RSCU was performed using the R packages (version 3.3.0)[51] ggplot2[52] and ggfortify[53].

Orthologous gene family clustering. To identify orthologous gene families among the whale shark and the other 82 species, we downloaded all pair-wise reciprocal BLASTP results using the 'peptide align feature' in the Ensembl comparative genomics resources[54] (release 86). To generate pair-wise orthologous that were not available in the Ensembl resources, we performed reciprocal

BLASTP[55] with the '-evalue 1e-05 -seg no -max_hsps_per_subject 1 -use_sw_tback' options. From the pair-wise reciprocal BLASTP results among the 82 species, we generated similarity matrixes by connecting possible orthologous pairs. To constrain the computational load, we did not join additional nodes when the number of node was bigger than 1500. The normalized weights for the similarity matrix were calculated using the OrthoMCL approach[56]. We identified orthologous gene families by using an in-house C++ script based on the MCL clustering algorithm[57], with inflation index 1.3. A total of 1,461,312 genes were assigned to 225,530 clusters, including 192,174 of singletons.

Gene age estimation. Phylostratigraphy uses BLASTP-scored sequence similarity to estimate the minimal age of every protein-coding gene. The NCBI non-redundant database is queried with a protein sequence to detect the most distant species in which a sufficiently similar sequence is present and posit that the gene is at least as old as the age of the common ancestor[33]. Using NCBI for every species, the timing of lineage divergence events is estimated with TimeTree[58]. To facilitate detection of protein sequence similarity, we use the e-value threshold of 10^{-3}. We evaluate the age of all proteins with length equal or greater than 40 amino acids. First, we count the number of genes in each phylostratum, from the most ancient (PS 1) to the most recent (PS 20). Most genes are ancient (PS 1-2) and a substantial number appear young (PS 20) (Fig. S19). Second, to understand broad evolutionary patterns, we aggregate the counts from several phylostrata into three broad evolutionary eras: ancient (PS 1-7, cellular organisms to Deuterostomia, 4,204 Mya - 684 Mya), middle (PS 8-14, Chordata to Selachii, 684 Mya - 199 Mya) and young (PS 15-20, Galeomorpha to *Rhincodon typus,* 199 Mya to present). To understand the gene flow per time unit, we normalized the number of genes by the age and the duration of the evolutionary era.

Correlation tests in orthologous gene families. From the 82 species, we selected 6,929 single-copy orthologous gene families which are found in at least 40 species to calculate the correlation between gene length, i.e., exon + intron length between first and last coding exon and three physiological properties (the maximum lifespan, body weight, and BMR). We identified gene families which had significant correlations between the gene length and the maximum lifespan (2,882 genes), body mass (2,193 genes), and the BMR (2,627 genes) by Spearman's rho correlation coefficient and Benjamini & Hochberg adjustment (adjust p-value ≤ 0.01). All these gene families were subject to alignment filtering criterion of containing more than 50% of conserved exon-exon boundaries (intron position) in their CDS alignments. This step reduces the effect of gene length change due to intron gain or loss and increases the accuracy of multiple sequence alignments (Fig. S23). Finally, we acquired four sets of correlated gene families between the gene length and the three properties: 1) 25 gene families with the maximum lifespan only (Table S24), 2) one gene family with the body weight only (Table S24), 3) seven gene families with the BMR only, and 4) 155 gene families with all three physiological properties (Table S23).

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Author contributions

J.B. conceived and planned the project. J.A.W., Y.S.C., S.G.P, and J.B. coordinated the project. J.A.W., V.L., S.G.P., S.J., J.S.E., and J.B. wrote the manuscript. W.H.H., S.W.K., S.K., Y.S.C., H.M.K., and S.K. prepared the samples and performed the experiments. S.G.P., J.A.W., V.L., S.J., H.M.K., Y.J., Y.B., A.K. and J.J. performed in-depth bioinformatics and evolutionary analyses. S.G.P., J.A.W., V.L., S.J., H.M.K., Y.J., Y.B., J.J., S.W.K., W.H.H., S.L., Y.S.C., A.K., A.M., S.K., J.S.E., J.B., and G.M.C. reviewed the manuscript and discussed the work.

Competing interests

Authors declare no competing interests.

Data and materials availability

The whale shark whole genome project has been deposited at DDBJ/ENA/GenBank under the accession QPMN00000000. The version described in this paper is version QPMN01000000. DNA sequencing reads have been uploaded to the NCBI Read Archive (SRP155581).

Materials & Correspondence

Correspondence and requests for materials should be addressed to G.M.C. (gchurch@genetics.med.harvard.edu), J.B. (jongbhak@genomics.org), J.S.E. (JSEdwards@salud.unm.edu), or J.A.W. (jessica_weber@hms.harvard.edu).

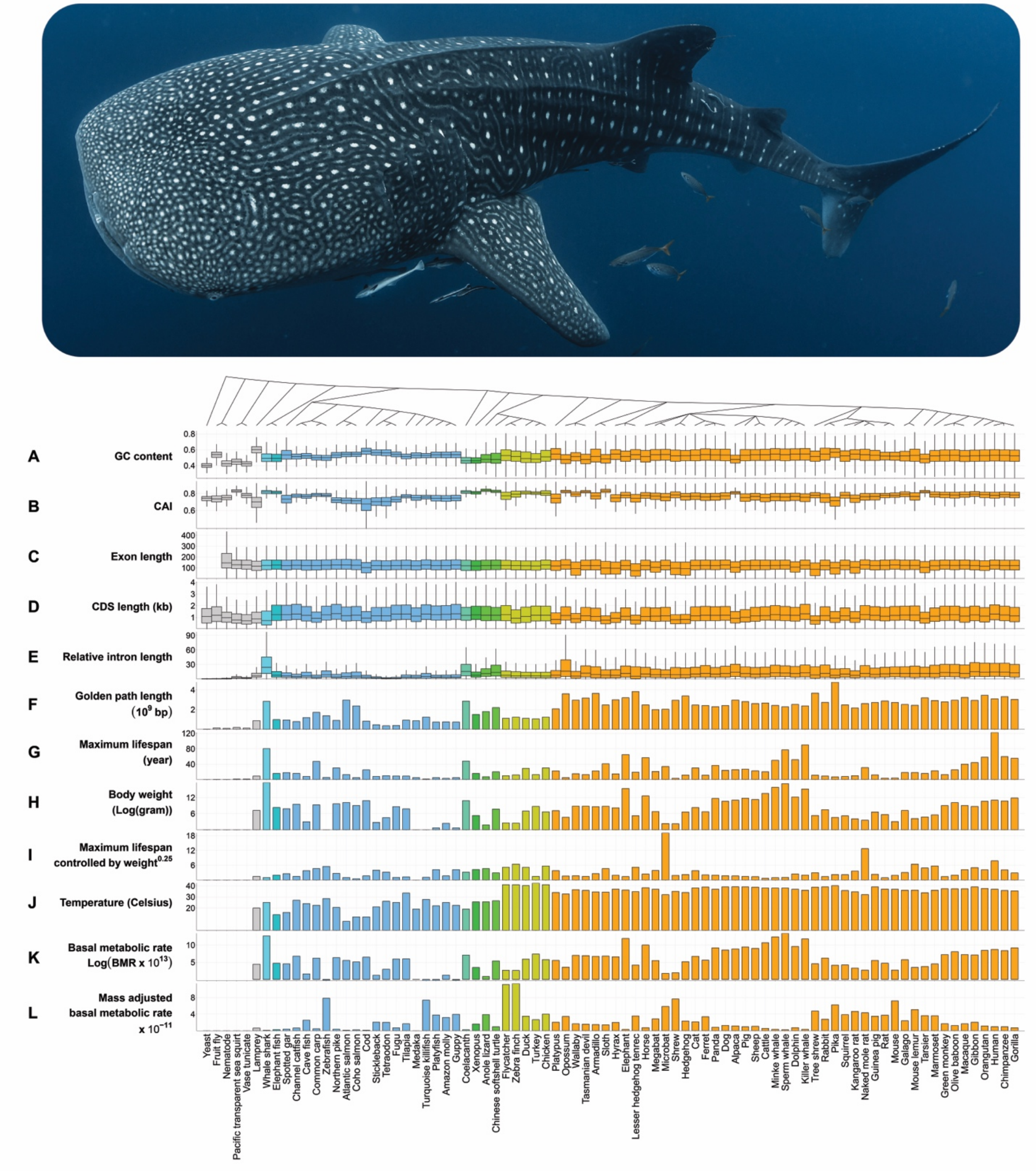


Fig. 1. Comparative genomic analysis across 82 species reveals traits linked to lifespan and bodyweight. Top panel: image of a whale shark. Bottom panel: the phylogenetic tree was constructed using the NCBI common tree

(https://www.ncbi.nlm.nih.gov/Taxonomy/CommonTree/wwwcmt.cgi) without divergence times. The second to the last rows show the following values in 82 species: five genomic contexts (**A-E**), golden path length (**F**), the maximum lifespan (**G**), body weight (**H**), maximum lifespan controlled by weight$^{0.25}$ (**I**), body temperature (optimal temperature for cold-blooded animal) (**J**), basal metabolic rate (**K**), and basal metabolic rate adjusted by weight (**L**). The exon length (**C**) shows length of exons in coding region. Yeast and fruit fly exon length were removed due to their extremely long length (median exon lengths for yeast and fruit fly are 1,032 bp and 217 bp respectively). The relative intron length (**E**) was calculated by dividing the total intron length between first coding exon and last coding exon by the CDS length. The nine colors of boxes and bars indicate biological classification (gray: Hyperoartia, Ascidiacea, Chromadorea, Insecta and Saccharomycetes, turquoise: Chondrichthyes (the cyan color indicates whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

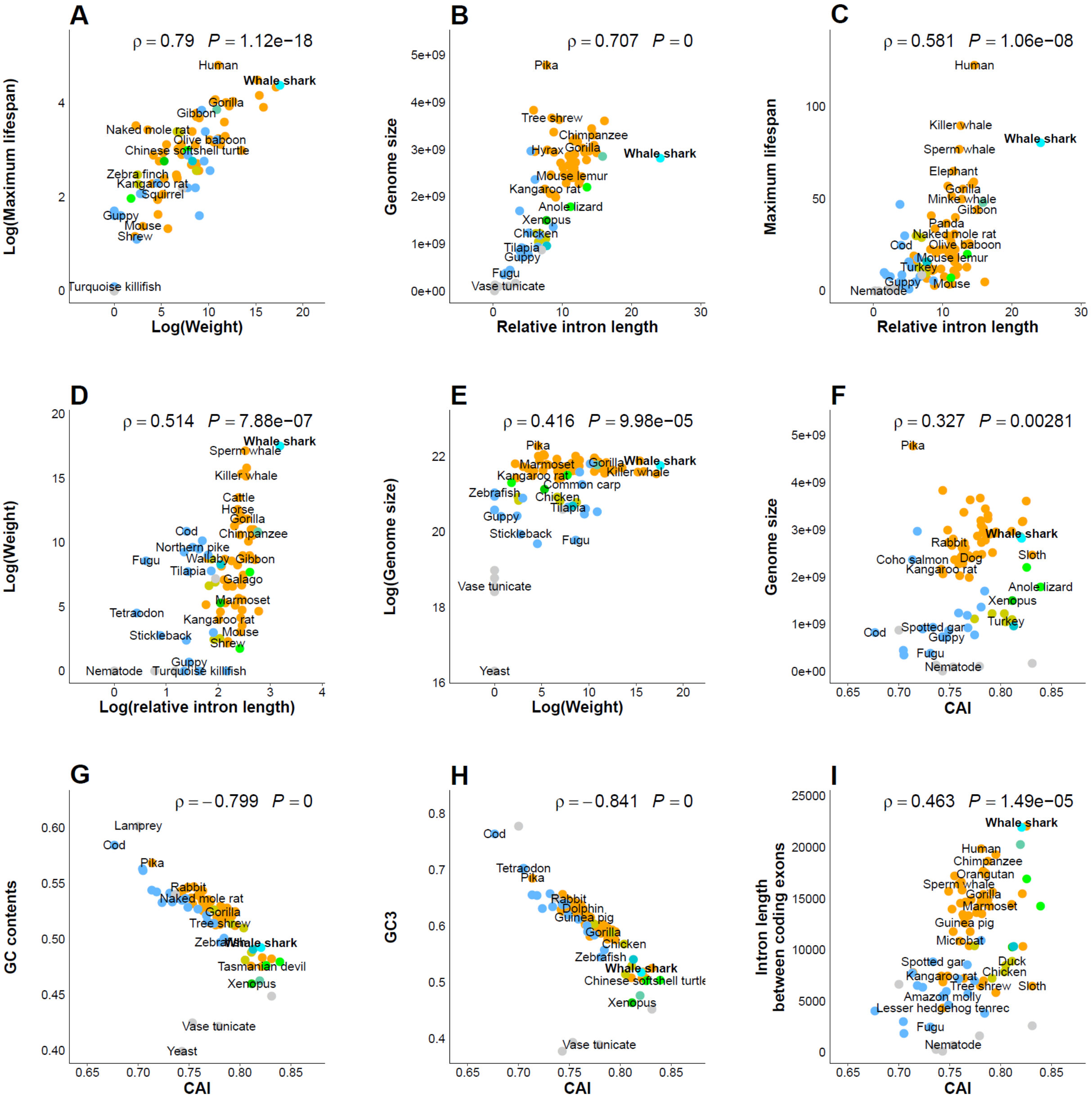


Fig. 2. Scaling relationships between genomic and physiologic properties across 82 species. The properties on the x-axis and y-axis were used to calculate Spearman's rank correlation coefficient for each plot. All *p*-values and rho values are shown at the top of each plot. Overlapping species names in the same layer were not plotted. The nine dot colors indicate biological classification (gray: Hyperoartia, Ascidiacea, Chromadorea, Insecta and Saccharomycetes,

turquoise: Chondrichthyes (cyan is whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

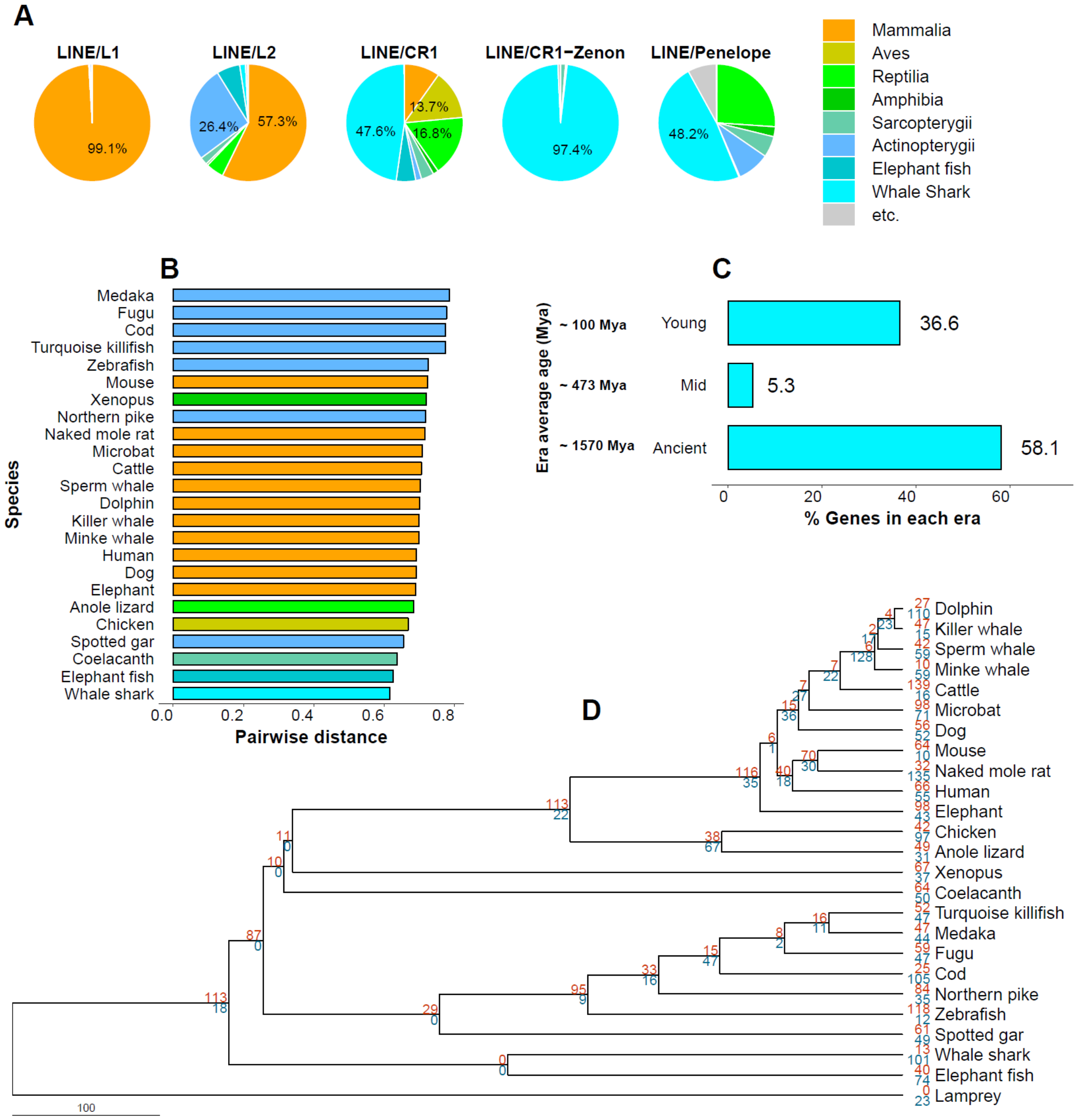


Fig. 3. Repetitive elements, evolutionary rate model, and flow of genes in the whale shark genome. (**A**) Each pie chart summarizes the length of predicted intronic repetitive elements (labeled in the top of pie). Values from the 81 species (yeast excluded) were averaged across six Classes (Mammalia, Aves, Reptilia, Amphibia, Sarcopterygii, Actinopterygii), and the whale shark and elephant fish are listed separately (yeast was excluded from these analyses). (**B**) All pairwise distances from sea lamprey were calculated using the R-package 'ape' [59]. The species were ordered by the pairwise distances. The eight bar colors indicate biological classification

(turquoise: Chondrichthyes (the cyan color indicates whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia). (**C**) While most genes (~58%) in the whale shark genome are ancient, a few (~5%) are of intermediate age and a significant fraction (~37%) are relatively young. (**D**) Maximum likelihood phylogenetic tree. Red and blue numbers refer to the number of expanded and contracted gene families at each node compared to the common ancestor, respectively.

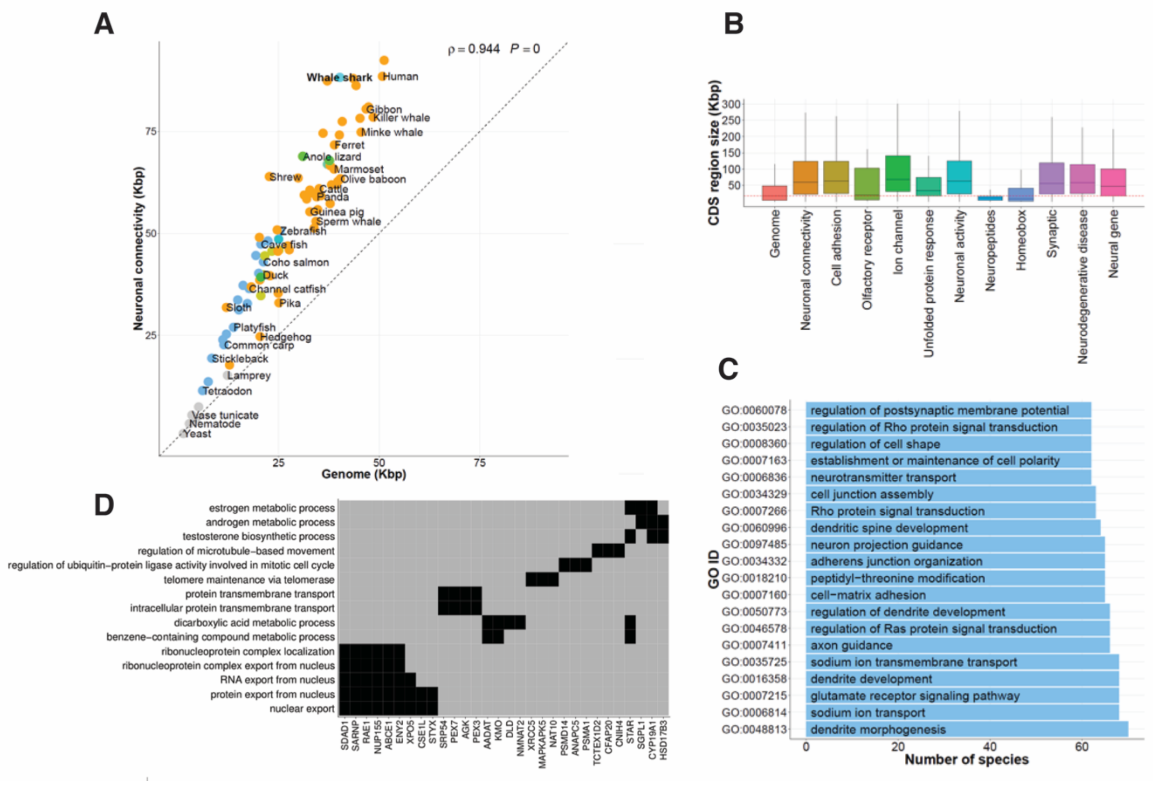


Fig. 4. The relationship between gene length and neural genes, and single-copy orthologous gene families with correlations between gene length and maximum lifespan, weight, and BMR. (**A**) Neuronal connectivity genes are longer in 81 species (yeast excluded). The x- and y-axes show the average gene length and the gene length of neuronal connectivity-related genes, respectively. The dashed diagonal line represents 'y = x'. Spearman's rho correlation coefficient and *p*-value are shown in top right corner of the plot. (**B**) Of the 12 categories of neural genes we analyzed in the whale shark genome, several are longer than average genes. (**C**) Most common GO terms are relevant to neural function. GO terms are shown based on the number of species they were found in, and were computed with Gene Set Enrichment Analysis (GSEA). (**D**) Enriched GO functions in single-copy orthologous gene families in which relative intron length positively

correlates with maximum lifespan. For each GO term, black boxes indicate representative human gene symbols representative of the family.

The whale shark genome reveals how genomic and physiological properties scale with body size

Seung Gu Park[1,2]†, Victor Luria[3]†, Jessica A. Weber[4,5]†*, Sungwon Jeon[1,2], Hak-Min Kim[1,2], Yeonsu Jeon[1,2], Youngjune Bhak[1,2], Jehun Jun[6], Sang Wha Kim[7], Won Hee Hong[8], Semin Lee[1,2], Yun Sung Cho[6], Amir Karger[9], John W. Cain[10], Andrea Manica[11], Soonok Kim[12], Jae-Hoon Kim[13], Jeremy S. Edwards[14]*, Jong Bhak[1,2,6]*, George M. Church[4]*

†These authors contributed equally to this work.

*These authors jointly supervised this work. Correspondence and requests for materials should be addressed to G.M.C. (gchurch@genetics.med.harvard.edu), J.B. (jongbhak@genomics.org), J.S.E. (JSEdwards@salud.unm.edu), or J.A.W. (jessica_weber@hms.harvard.edu).

This PDF file includes:

Supplementary Text

Figs. S1 to S24

Tables S1 to S24

References

Other Supplementary Materials for this manuscript include the following:

Additional_file_1

SUPPLEMENTARY TEXT

SUPPLEMENTARY FIGURES

SUPPLEMENTARY TABLES

1. Whale shark genome sequencing and assembly

1.1 DNA sample preparation and sequencing.

Genomic DNA was extracted from the heart tissue of a 4.5-meter, seven year old dead male whale shark (*Rhinocodon typus,* from the Hanwha Aquarium, Jeju, Republic of Korea). DNA libraries were constructed using a TruSeq DNA library kit for the short-read libraries and a Nextera Mate Pair sample prep kit for the mate pair libraries. Libraries were sequenced using the Illumina HiSeq2500 platform. We obtained roughly 164× of paired-end short reads with varying insert sizes including mate pair (Table S1) and 848,425 TSLRs (Table S2).

Table S1. Short insert and mate pair library sequencing statistics

Insert size	Library	Read length (bp)	Number of read pairs	Total bases (bp)	Depth (genome size: 3.2Gb)	Depth sum (×)
170bp	L1	100	752,028,952	75,202,895,200	23.5	47.6
	L2	100	773,203,352	77,320,335,200	24.1	
500bp	L1	100	532,162,248	53,216,224,800	16.6	33.0
	L2	100	524,070,876	52,407,087,600	16.4	
700bp	L1	100	557,235,918	55,723,591,800	17.4	31.9
	L2	100	463,202,656	46,320,265,600	14.5	
2Kb	L1	50	329,314,538	16,465,726,900	5.1	15.0
	L2	50	360,090,428	18,004,521,400	5.6	
	L3	50	270,853,224	13,542,661,200	4.2	
5Kb	L1	50	319,466,530	15,973,326,500	5.0	17.3
	L2	50	400,800,948	20,040,047,400	6.3	
	L3	50	386,494,358	19,324,717,900	6.0	
10Kb	L1	50	257,087,152	12,854,357,600	4.0	9.0
	L2	50	321,876,522	16,093,826,100	5.0	
15Kb	L1	50	341,140,082	17,057,004,100	5.3	10.5
	L2	50	329,714,826	16,485,741,300	5.1	
Total	-	-	6,918,742,610	526,032,330,600		164.3

Table S2. Illumina TruSeq synthetic long read (TSLR) sequencing statistics

	All	**> 1,500bp only**
Number of sequences	848,425	588,325
Number of bases (bp)	3,774,313,129	3,547,450,453
N50 (bp)	8,407	8,750
The largest length (bp)	21,924	21,924
Average length (bp)	4,448	6,029

1.2 Raw data QC trimming and filtering

Low quality or contaminated reads were removed using the following filtering criteria:

1) PCR duplications (the reads were considered duplications if both paired end reads are identical).
2) Reads containing adapters.

   Left = "*GATCGGAAGAGCACACGTCTGAACTCCAGTCAC*"

   Right = "*GATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT*"
3) Reads which had more than 5% ambiguous bases (N).
4) Reads with an average base quality below 20 (<Q20).
5) Reads which had junction adapters in the mate-pair libraries.

   Left = "*CTGTCTCTTATACACATCT*"

   Right = "*AGATGTGTATAAGAGACAG*"
6) Low-quality ends were trimmed for the short-insert libraries (2bp of 5'-end and 8bp of 3'-end).

Roughly 120× depth of coverage remained after filtering (Table S3).

Table S3. Post QC short insert and mate pair library sequencing statistics

Insert size	Read length (bp)	Total Reads	Total Bases (bp)	Remained depth (X, genome size: 3.2Gb)
170bp	90	1,436,964,768	129,326,829,120	40.38963675
400bp	90	561,405,924	50,526,533,160	15.77977543
500bp	90	958,715,504	86,284,395,360	26.9471958
700bp	90	830,451,564	74,740,640,760	23.34200375
2kb	49	260,885,666	12,783,397,634	3.992340881
5kb	49	160,898,212	7,884,012,388	2.462229985
10kb	49	111,938,498	5,484,986,402	1.712998068
15kb	49	103,019,726	5,047,966,574	1.576513842
Total	-	4,424,279,862	372,078,761,398	116.2026945

1.3 Estimation of genome size using K-mer analysis

The size of the whale shark genome was estimated by *K*-mer analysis (*K*=23) using the KmerFreq_HA command of the SOAPec program in the SOAPdenovo2 package[1] (Figure S1). The genome size was calculated by dividing the total number of *K*-mers by a peak depth of *K*-mers (Table S4). The whale shark genome size was estimated to be approximately 3.14 Gb. Prior to genome assembly, the sequencing errors in the filtered reads were corrected using the *K*-mer frequency (*K*=23) information and the Corrector_HA command of the SOAPec program[1] with a three-depth criterion for low-frequency *K*-mer cutoffs.

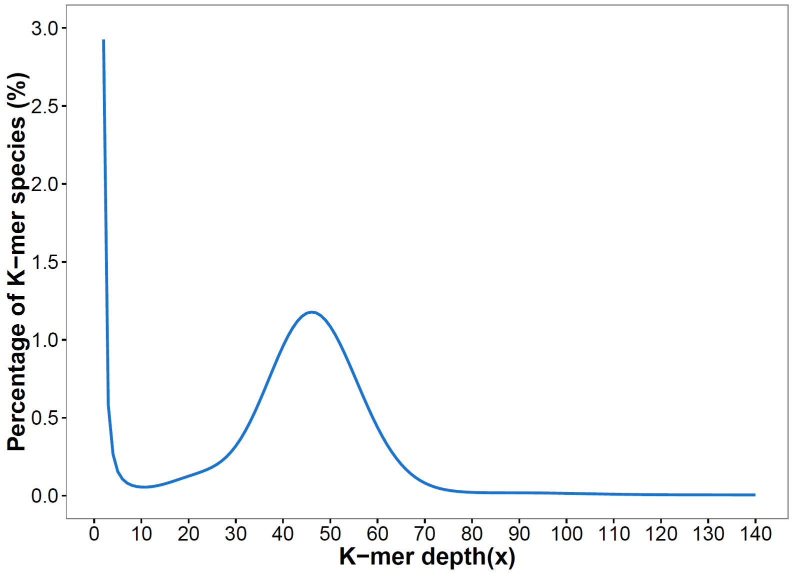


Figure S1. *K*-mer distribution frequency in the error-corrected reads, based on a 23-mer. The x-axis represents depth, and the y-axis represents proportion of *K*-mer species, as calculated by the frequency at a certain depth divided by the total frequency at all depths.

Table S4. Estimation of the whale shark genome size based on *K*-mer frequency using the error corrected reads

***K*-mer size**	**Total number of *K*-mer**	***K* depth of peak**	**Estimated genome size**
23	144,222,502,823	46	3,135,271,800

1.4 Genome assembly

The whale shark genome was assembled using the error-corrected reads from the short insert and mate pair libraries (>1 Kb) using SOAPdenovo2[1]. As the quality of assembled genome can be affected by the *K*-mer size, we used multi-*K*-mer values (minimum 45 to maximum 63) using the 'all' command in the SOAPdenovo2 package[1]. The gaps between the scaffolds were closed in two iterations with the short insert libraries (<1 Kb) using the GapCloser program in the SOAPdenovo2 package[1]. We then aligned the short insert size reads to the scaffolds using BWA-MEM[2] with default options. Variants were identified using SAMtools[3]. At least one of the alleles from the self-mapping results should be the same as the reference. Thus, the erroneous bases of the assembly which are different from both alleles of the self-mapping results were changed to one of the alleles. We mapped the Illumina TruSeq synthetic long reads (TSLR) to the assembly and corrected the gaps covered by the synthetic long reads to reduce erroneous gap regions in the assembly (Table S5). The final length of the assembly is roughly 3.2 Gb with a scaffold N50 of 2.56 Mb and a contig N50 of 36 Kb (Table S6).

Table S5. Assembly statistics after removing erroneous gap regions with the TSLRs

	Before substitution	After substitution	Difference
Number of gaps	188,018	172,567	-15,451
Number of monomer-gap	127,356	112,865	-14,491
Bases (bp)	3,202,752,364	3,201,980,496	-771,868
Sequences	3,305,708	3,305,708	0

Table S6. Final *de novo* assembly statistics

	Contig		**Scaffold**	
	All sequences	**≥200bp**	**All sequences**	**≥200bp**
N95 (bp)	115	3,298	116	46,053
N90 (bp)	127	8,207	127	293,875
N75 (bp)	12,358	20,521	582,101	1,291,341
N50 (bp)	35,692	41,993	2,564,432	3,126,012
N25 (bp)	68,429	74,123	5,777,842	6,316,425
Longest (bp)	365,232	365,232	16,092,075	16,092,075
Total Sequences	3,497,228	304,545	3,305,708	139,611
Total bases (bp)	3,159,659,671	2,780,718,445	3,201,980,496	2,826,695,639
GC content (%)	42.41%	41.74%	41.84%	41.11%

1.5 GC-content of whale shark genome

The GC distribution of the whale shark genome was calculated using a sliding window approach. We employed 10 Kb sliding windows to scan the genome to calculate the GC content. The average GC content of the whale shark is 41.6%, which is similar to that of coelacanth and elephant fish (Figure S2).

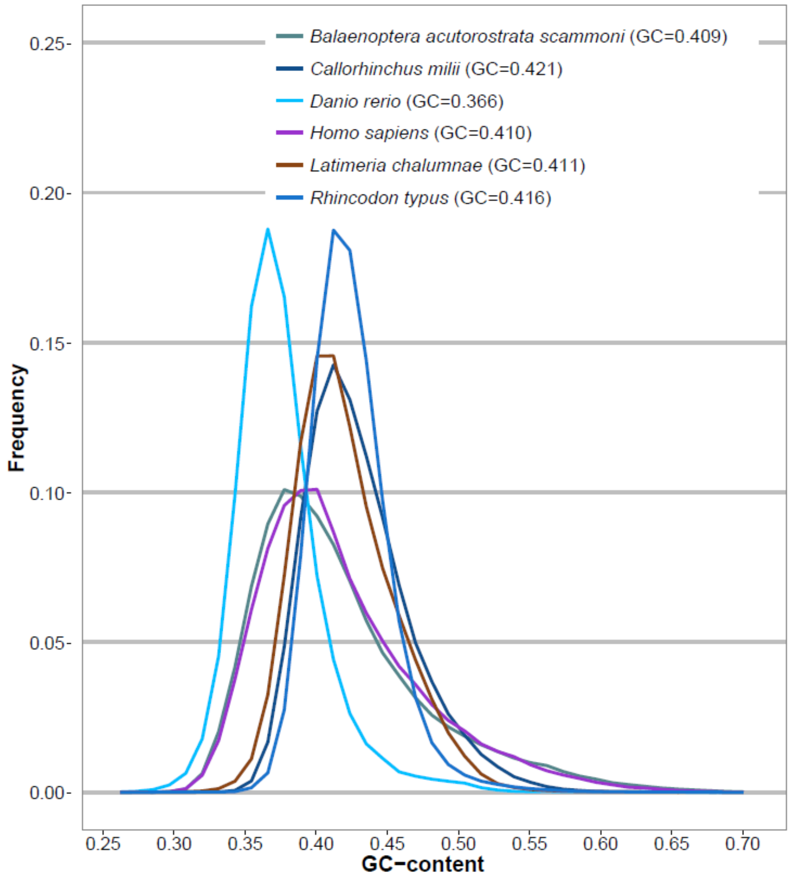


Figure S2. Genome-wide GC distribution. The x-axis represents GC content and the y-axis represents the proportion of the specified GC content. 'GC' in the legend indicates whole genome GC content of each species.

1.6 Annotation of repetitive elements

Tandem repeats were predicted using the Tandem Repeats Finder program (version 4.07)[4]. Transposable elements (TEs) were identified using both homology-based and *ab initio* approaches. The Repbase database (version 19.02)[5] and RepeatMasker (version 4.0.5)[6] were used for the homology-based approach, and RepeatModeler (version 1.0.7)[7] was used for the *ab initio* approach,. All predicted repetitive elements were merged using in-house Perl scripts. In total, 49.55% of the whale shark genome is made of TEs (Table S7).

Table S7. Repetitive element statistics for the whale shark genome

Type	*Ab initio*-based (bp)	Homology-based (bp)	Total (bp)	Percentage of genome
DNA	65,075,457	22,286,842	86,564,210	2.70%
LINE	781,235,803	260,999,963	861,138,326	26.89%
LTR	101,363,964	912,079	101,919,539	3.18%
Low complexity	415,435	0	415,435	0.01%
SINE	7,020,248	3,595,973	10,614,972	0.33%
Satellite	7,341,297	18,859	7,350,548	0.23%
Simple repeat	67,281,471		67,281,471	2.10%
Tandem repeat*			249,559,685	7.79%
Unknown	519,673,351	17,679	519,689,768	16.23%
Unspecified	6,777,305		6,777,305	0.21%
Total	1,508,223,137	287,829,289	1,586,543,783	49.55%

*Tandem Repeat was separately predicted using TRF program.

1.7 Annotation of protein coding genes

Two candidate gene sets were built to predict the protein coding genes in the whale shark genome; using AUGUSTUS[8] and Evidence Modeler (EMV)[9], respectively.

1) For the AUGUSTUS[8] prediction, we used both homology-based and *ab initio* approaches. For the homology-based gene prediction, homologous genes were identified by aligning the protein sequences from the elephant fish, zebrafish, medaka, human, mouse, and minke whale (from NCBI) to the cartilage fish protein database (from Uniprot) using GeneBlastA[10] with e-value cutoff 10^{-5}. Homologous genes with less than 40% coverage were filtered out. Homology-based gene models were constructed using Exonerate[11]. With the Homology-based gene model and three hints: cartilage fishes' EST sequences from NCBI, transcriptomic hint from elephant fish (SRP013772), and nurse shark (SRP018197), the *ab initio* prediction of the whale shark genome was performed using AUGUSTUS 3.1 with '--species=zebrafish' option[8]. We filtered out genes which contained <30 amino acids. Gene symbols were assigned by best hit to the SwissProt or Trembl databases[12] using BLASTP[13] with e-value cutoff 10^{-5}. A total of 25,409 out of 34,708 genes were assigned. Finally, we removed possible retro-transposable single exon genes. The resulting gene model contained 28,483 protein coding genes (Table S8).

2) For the EVM approach, we performed homology-based gene prediction with additional species (Table S9) and combined the prediction results with the *ab initio* prediction results [AUGUTUS[8], MAKER[14]] using EVM[9] (the weights of intermediate gene models for EVM[9] integration is noted in Table S10). We predicted 25,915 protein coding genes using the EVM[9] approach (Table S11).

Table S8. Statistics of the AUGUSTUS predicted gene set

Categories	Number or length (bp)
Number of genes	28,483
Average transcript length	39,530.27
Average number of CDSs per gene	7.45
Average CDS length per gene	1,173.7
Average CDS length per exon	157.54

Table S9. List of species used in EVM homology-based gene prediction

Common name	Scientific name	Number of protein sequences	Data Source	Assembly ID
Human	*Homo sapiens*	20,129	Ensembl 86	GRCh38.p7
Mouse	*Mus musculus*	22,294	Ensembl 86	GRCm38.p4
Anole lizard	*Anolis carolinensis*	18,520	Ensembl 86	AnoCar2.0
Xenopus	*Xenopus tropicalis*	18,000	Ensembl 86	JGI 4.2
Coelacanth	*Latimeria chalumnae*	19,198	Ensembl 86	LatCha1
Guppy	*Poecilia reticulata*	17,907	NCBI Refseq	GCF_000633615.1
Turquoise killifish	*Nothobranchius furzeri*	21,100	NCBI Refseq	GCF_001465895.1
Medaka	*Oryzias latipes*	18,937	Ensembl 86	HdrR
Tilapia	*Oreochromis niloticus*	20,467	Ensembl 86	Orenil1.0
Northern pike	*Esox lucius*	21,396	NCBI Refseq	GCF_000721915.3
Zebrafish	*Danio rerio*	24,309	Ensembl 86	GRCz10
Spotted gar	*Lepisosteus oculatus*	17,874	Ensembl 86	LepOcu1
Channel catfish	*Ictalurus punctatus*	22,463	NCBI Refseq	GCF_001660625.1
Elephant fish	*Callorhinchus milii*	15,669	NCBI Refseq	GCF_000165045.1
Lamprey	*Petromyzon marinus*	10,048	Ensembl 86	Pmarinus_7.0

Table S10. EVM weights for each gene model

Approach	Program	EVM weight
Homology based	exonerate:AnoleLizard	3
Homology based	exonerate:ChannelCatfish	3
Homology based	exonerate:Coelacanth	3
Homology based	exonerate:ElephantFish	3
Homology based	exonerate:Guppy	3
Homology based	exonerate:Human	5
Homology based	exonerate:Lamprey	3
Homology based	exonerate:Medaka	3
Homology based	exonerate:Mouse	3
Homology based	exonerate:NorthernPike	3
Homology based	exonerate:SpottedGar	3
Homology based	exonerate:Tilapia	3
Homology based	exonerate:TurquoiseKillifish	3
Homology based	exonerate:Xenopus	3
Homology based	exonerate:Zebrafish	5
ab initio	maker	5
ab initio	Augustus	15

Table S11. Statistics of the EVM predicted gene set

Categories	Number or length (bp)
Number of genes	25,915
Average transcript length	37,878.36
Average number of CDSs per gene	7.29
Average CDS length per gene	1,179.53
Average CDS length per exon	161.69

1.8 Genome assembly quality assessment

Assembly quality was assessed by mapping the paired-end DNA reads and the synthetic long read to the final scaffolds using BWA-MEM[2]. The mapping rate was 99.85% for the short reads (Table S12) and 95.14% for TSLRs (Table S13). The genome assembly and completeness of the gene annotation were also assessed using the Benchmarking Universal Single-Copy Orthologs (BUSCO) approach[15]. The two annotation methods (AUGUSTUS[8] and EVM[9]) had 88.2% and 84.3% complete BUSCO sets, respectively; which are both higher than the previously published draft genome assembly (Table S14).

Table S12. Assembly quality assessment using self-mapping of short reads

Library	Number of filtered reads	Number of mapped reads	Percentage of mapped reads
170bp	1,394,746,573	1,394,426,151	99.98%
500bp	940,285,287	940,078,838	99.98%
700bp	801,359,278	801,149,022	99.97%
2kb	236,768,852	235,357,729	99.40%
5kb	136,682,871	134,810,545	98.63%
10kb	94,350,139	93,601,841	99.21%
15kb	85,609,884	84,778,286	99.03%
Total	3,689,802,884	3,684,202,412	99.85%

Table S13. Assembly quality assessment using self-mapping of TSLRs

Read length (bp)	Number of reads	Number of ≥90% covered reads		Number of ≥50% covered reads	
0-999	178,106	142,280	79.89%	161,219	90.52%
1,000-1,999	142,565	122,562	85.97%	135,653	95.15%
2,000-2,999	88,352	78,300	88.62%	85,482	96.75%
3,000-3,999	66,397	58,688	88.39%	64,159	96.63%
4,000-4,999	51,371	45,225	88.04%	49,726	96.80%
5,000-5,999	42,530	37,009	87.02%	41,120	96.68%
6,000-6,999	38,767	33,442	86.26%	37,472	96.66%
7,000-7,999	36,124	31,037	85.92%	34,900	96.61%
8,000-8,999	39,523	33,747	85.39%	38,211	96.68%
9,000-9,999	82,517	70,444	85.37%	79,902	96.83%
10,000-10,999	56,755	48,176	84.88%	54,852	96.65%
11,000-11,999	21,202	17,840	84.14%	20,481	96.60%
12,000-12,999	3,954	3,271	82.73%	3,818	96.56%
13,000-13,999	166	118	71.08%	157	94.58%
14,000-14,999	26	9	34.62%	25	96.15%
>15,000	70	6	8.57%	53	75.71%
Total	848,425	722,154	85.12%	807,230	95.14%

Table S14. Assessment of the genome assembly and gene completeness using the BUSCO approach, compared to the initial draft whale shark assembly

	Whale shark	**Whale shark**	**Whale shark**
	(AUGUSTUS)	**(EVM)**	**(GCA_001642345.2)**
Complete (Gene)	2,279 (88.2%)	2,180 (84.3%)	1,934 (74.7%)
Duplicated (Gene)	51 (2.0%)	88 (3.4%)	84 (3.2%)
Fragmented (Gene)	136 (5.3%)	271 (10.5%)	283 (10.9%)
Missing (Gene)	171 (6.5%)	135 (5.2%)	369 (14.4%)
Number of single-copy orthologous genes	2,586	2,586	2,586

2. Comparative genomic studies

2.1 Data resources

Genome sequences and gene sets for 69 species were downloaded from Ensembl FTP (ftp://ftp.ensembl.org/pub/release-86/). An additional twelve species were added from NCBI FTP (ftp://ftp.ncbi.nlm.nih.gov/genomes/) (Table S15).

Table S15. List of 82 species and their data sources

Common name	Species name	Class	Data Source	Assembly ID
Gorilla	*Gorilla gorilla*	Mammalia	Ensembl 86	gorGor3.1
Chimpanzee	*Pan troglodytes*	Mammalia	Ensembl 86	CHIMP2.1.4
Human	*Homo sapiens*	Mammalia	Ensembl 86	GRCh38.p7
Orangutan	*Pongo abelii*	Mammalia	Ensembl 86	PPYG2
Gibbon	*Nomascus leucogenys*	Mammalia	Ensembl 86	Nleu1.0
Macaque	*Macaca mulatta*	Mammalia	Ensembl 86	Mmul_8.0.1
Olive baboon	*Papio anubis*	Mammalia	Ensembl 86	PapAnu2.0
Green monkey	*Chlorocebus sabaeus*	Mammalia	Ensembl 86	ChlSab1.1
Marmoset	*Callithrix jacchus*	Mammalia	Ensembl 86	C_jacchus3.2.1
Tarsier	*Tarsius syrichta*	Mammalia	Ensembl 86	tarSyr1
Mouse Lemur	*Microcebus murinus*	Mammalia	Ensembl 86	Mmur_2.0
Galago	*Otolemur garnettii*	Mammalia	Ensembl 86	OtoGar3
Mouse	*Mus musculus*	Mammalia	Ensembl 86	GRCm38.p4
Rat	*Rattus norvegicus*	Mammalia	Ensembl 86	Rnor_6.0
Kangaroo rat	*Dipodomys ordii*	Mammalia	Ensembl 86	dipOrd1
Guinea pig	*Cavia porcellus*	Mammalia	Ensembl 86	cavPor3
Naked mole rat	*Heterocephalus glaber*	Mammalia	NCBI	HetGla_female_1.0
Squirrel	*Ictidomys tridecemlineatus*	Mammalia	Ensembl 86	spetri2
Pika	*Ochotona princeps*	Mammalia	Ensembl 86	OchPri2.0
Rabbit	*Oryctolagus cuniculus*	Mammalia	Ensembl 86	OryCun2.0
Tree shrew	*Tupaia belangeri*	Mammalia	Ensembl 86	tupBel1
Killer whale	*Orcinus orca*	Mammalia	NCBI	GCF_000331955.2
Dolphin	*Tursiops truncatus*	Mammalia	Ensembl 86	turTru1
Sperm whale	*Physeter catodon*	Mammalia	NCBI	GCF_000472045.1
Minke whale	*Balaenoptera acutorostrata scammoni*	Mammalia	NCBI	GCF_000493695.1
Cattle	*Bos taurus*	Mammalia	Ensembl 86	UMD3.1
Sheep	*Ovis aries*	Mammalia	Ensembl 86	Oar_v3.1
Pig	*Sus scrofa*	Mammalia	Ensembl 86	Sscrofa10.2
Alpaca	*Vicugna pacos*	Mammalia	Ensembl 86	vicPac1
Dog	*Canis familiaris*	Mammalia	Ensembl 86	CanFam3.1
Panda	*Ailuropoda melanoleuca*	Mammalia	Ensembl 86	ailMel1
Ferret	*Mustela putorius furo*	Mammalia	Ensembl 86	MusPutFur1.0
Cat	*Felis catus*	Mammalia	Ensembl 86	Felis_catus_6.2
Hedgehog	*Erinaceus europaeus*	Mammalia	Ensembl 86	eriEur1
Shrew	*Sorex araneus*	Mammalia	Ensembl 86	sorAra1
Microbat	*Myotis lucifugus*	Mammalia	Ensembl 86	Myoluc2.0
Megabat	*Pteropus vampyrus*	Mammalia	Ensembl 86	pteVam1
Horse	*Equus caballus*	Mammalia	Ensembl 86	Equ Cab 2
Lesser hedgehog tenrec	*Echinops telfairi*	Mammalia	Ensembl 86	TENREC

Elephant	*Loxodonta africana*	Mammalia	Ensembl 86	Loxafr3.0
Hyrax	*Procavia capensis*	Mammalia	Ensembl 86	proCap1
Sloth	*Choloepus hoffmanni*	Mammalia	Ensembl 86	choHof1
Armadillo	*Dasypus novemcinctus*	Mammalia	Ensembl 86	Dasnov3.0
Tasmanian devil	*Sarcophilus harrisii*	Mammalia	Ensembl 86	Devil_ref v7.0
Wallaby	*Macropus eugenii*	Mammalia	Ensembl 86	Meug_1.0
Opossum	*Monodelphis domestica*	Mammalia	Ensembl 86	monDom5
Platypus	*Ornithorhynchus anatinus*	Mammalia	Ensembl 86	OANA5
Chicken	*Gallus gallus*	Aves	Ensembl 86	Gallus_gallus-5.0
Turkey	*Meleagris gallopavo*	Aves	Ensembl 86	Turkey_2.01
Duck	*Anas platyrhynchos*	Aves	Ensembl 86	BGI_duck_1.0
Zebra Finch	*Taeniopygia guttata*	Aves	Ensembl 86	taeGut3.2.4
Flycatcher	*Ficedula albicollis*	Aves	Ensembl 86	FicAlb_1.4
Chinese softshell turtle	*Pelodiscus sinensis*	Reptilia	Ensembl 86	PelSin_1.0
Anole lizard	*Anolis carolinensis*	Reptilia	Ensembl 86	AnoCar2.0
Xenopus	*Xenopus tropicalis*	Amphibia	Ensembl 86	JGI 4.2
Coelacanth	*Latimeria chalumnae*	Sarcopterygii	Ensembl 86	LatCha1
Guppy	*Poecilia reticulata*	Actinopterygii	NCBI	GCF_000633615.1
Amazon molly	*Poecilia formosa*	Actinopterygii	Ensembl 86	Poecilia_formosa-5.1.2
Platyfish	*Xiphophorus maculatus*	Actinopterygii	Ensembl 86	Xipmac4.4.2
Turquoise killifish	*Nothobranchius furzeri*	Actinopterygii	NCBI	GCF_001465895.1
Medaka	*Oryzias latipes*	Actinopterygii	Ensembl 86	HdrR
Tilapia	*Oreochromis niloticus*	Actinopterygii	Ensembl 86	Orenil1.0
Fugu	*Takifugu rubripes*	Actinopterygii	Ensembl 86	FUGU 4.0
Tetraodon	*Tetraodon nigroviridis*	Actinopterygii	Ensembl 86	TETRAODON 8.0
Stickleback	*Gasterosteus aculeatus*	Actinopterygii	Ensembl 86	BROAD S1
Cod	*Gadus morhua*	Actinopterygii	Ensembl 86	gadMor1
Coho salmon	*Oncorhynchus kisutch*	Actinopterygii	NCBI	GCF_002021735.1
Atlantic salmon	*Salmo salar*	Actinopterygii	NCBI	GCF_000233375.1
Northern pike	*Esox lucius*	Actinopterygii	NCBI	GCF_000721915.3
Zebrafish	*Danio rerio*	Actinopterygii	Ensembl 86	GRCz10
Common carp	*Cyprinus carpio*	Actinopterygii	NCBI	GCF_000951615.1
Cave fish	*Astyanax mexicanus*	Actinopterygii	Ensembl 86	AstMex102
Spotted gar	*Lepisosteus oculatus*	Actinopterygii	Ensembl 86	LepOcu1
Channel catfish	*Ictalurus punctatus*	Actinopterygii	NCBI	GCF_001660625.1
Elephant fish	*Callorhinchus milii*	Chondrichthyes	NCBI	GCF_000165045.1
Whale shark	*Rhincodon typus*	Chondrichthyes	This study	This study
Lamprey	*Petromyzon marinus*	Hyperoartia	Ensembl 86	Pmarinus_7.0
Vase tunicate	*Ciona intestinalis*	Ascidiacea	Ensembl 86	KH
Pacific transparent sea squirt	*Ciona savignyi*	Ascidiacea	Ensembl 86	CSAV 2.0
Nematode	*Caenorhabditis elegans*	Chromadorea	Ensembl 86	WBcel235
Fruit fly	*Drosophila melanogaster*	Insecta	Ensembl 86	BDGP6
Yeast	*Saccharomyces cerevisiae*	Saccharomycetes	Ensembl 86	R64-1-1

2.2 Comparison of genomic factors

Due to the absence of transcriptome data for fourteen of the comparison species (cod, sloth, hyrax, elephant, lesser tenrec, megabat, shrew, hedgehog, dolphin, tree shrew, pika, kangaroo rat, tarsier, whale shark), we focused our analyses on the genomic features in translated region (Figure 1 and Figure S3-S6).

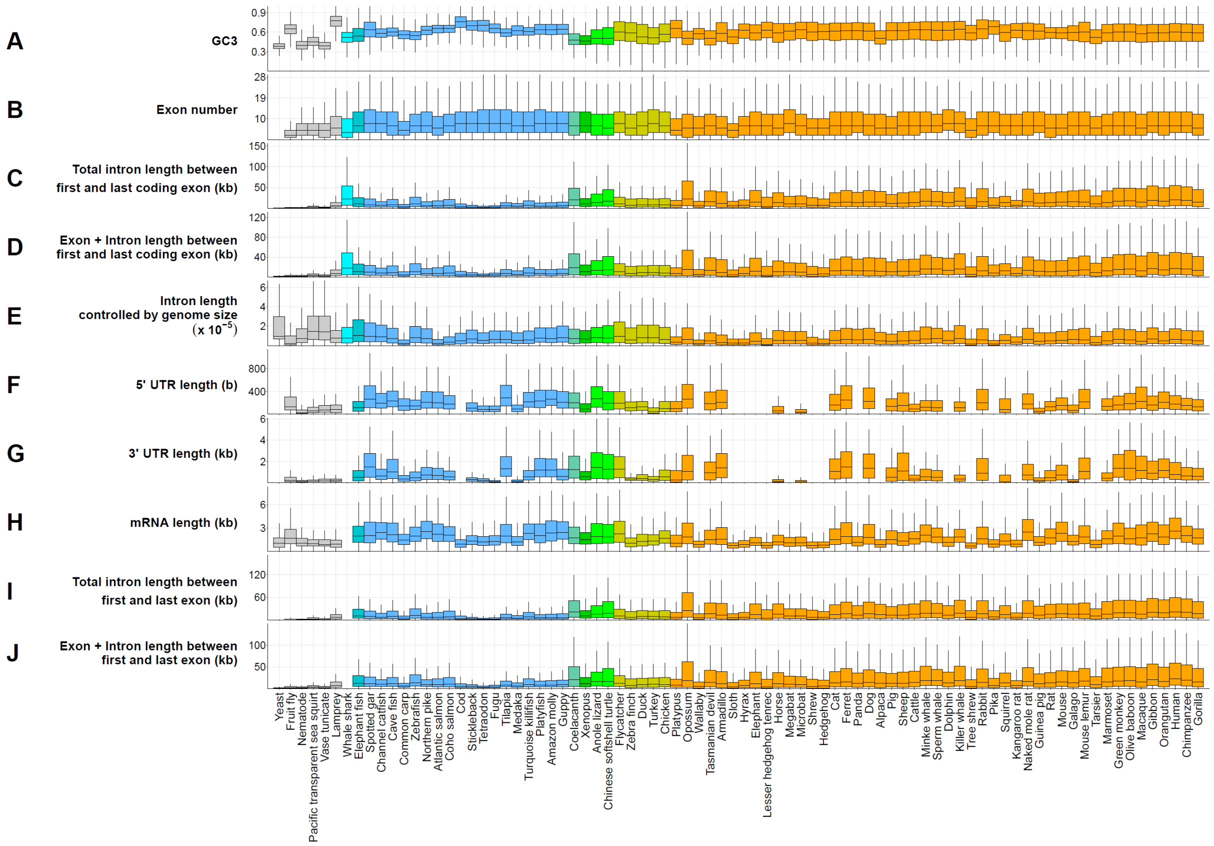


Figure S3. Comparative genomic analysis across 82 species. Extended data from Figure 1**.** The nine colors of boxes indicate biological classification (gray: Hyperoartia, Ascidiacea, Chromadorea, Insecta and Saccharomycetes, cyan: whale shark, dark turquoise: elephant fish, light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

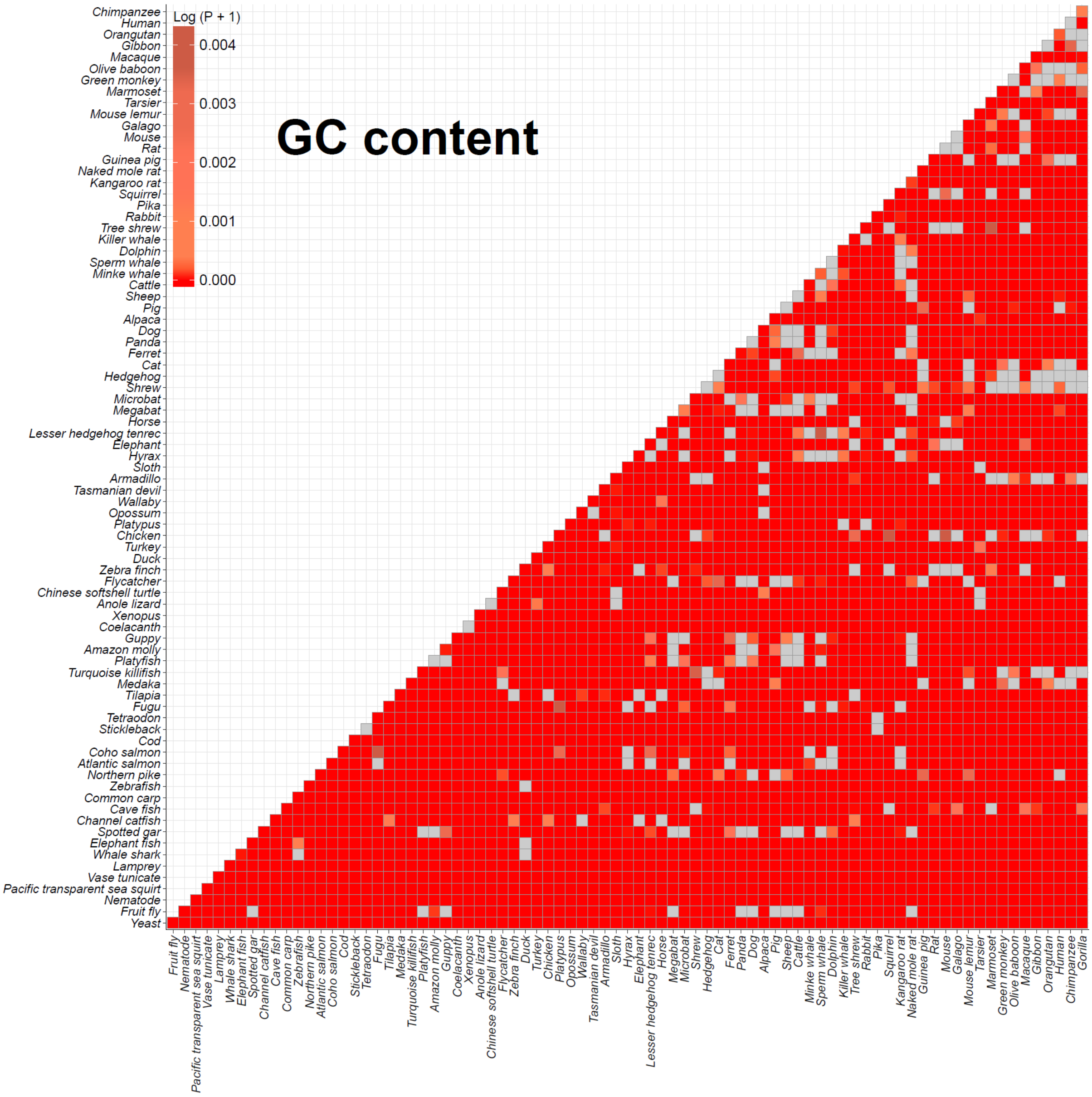


Figure S4A. Comparison of GC content in the CDS by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the GC content among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

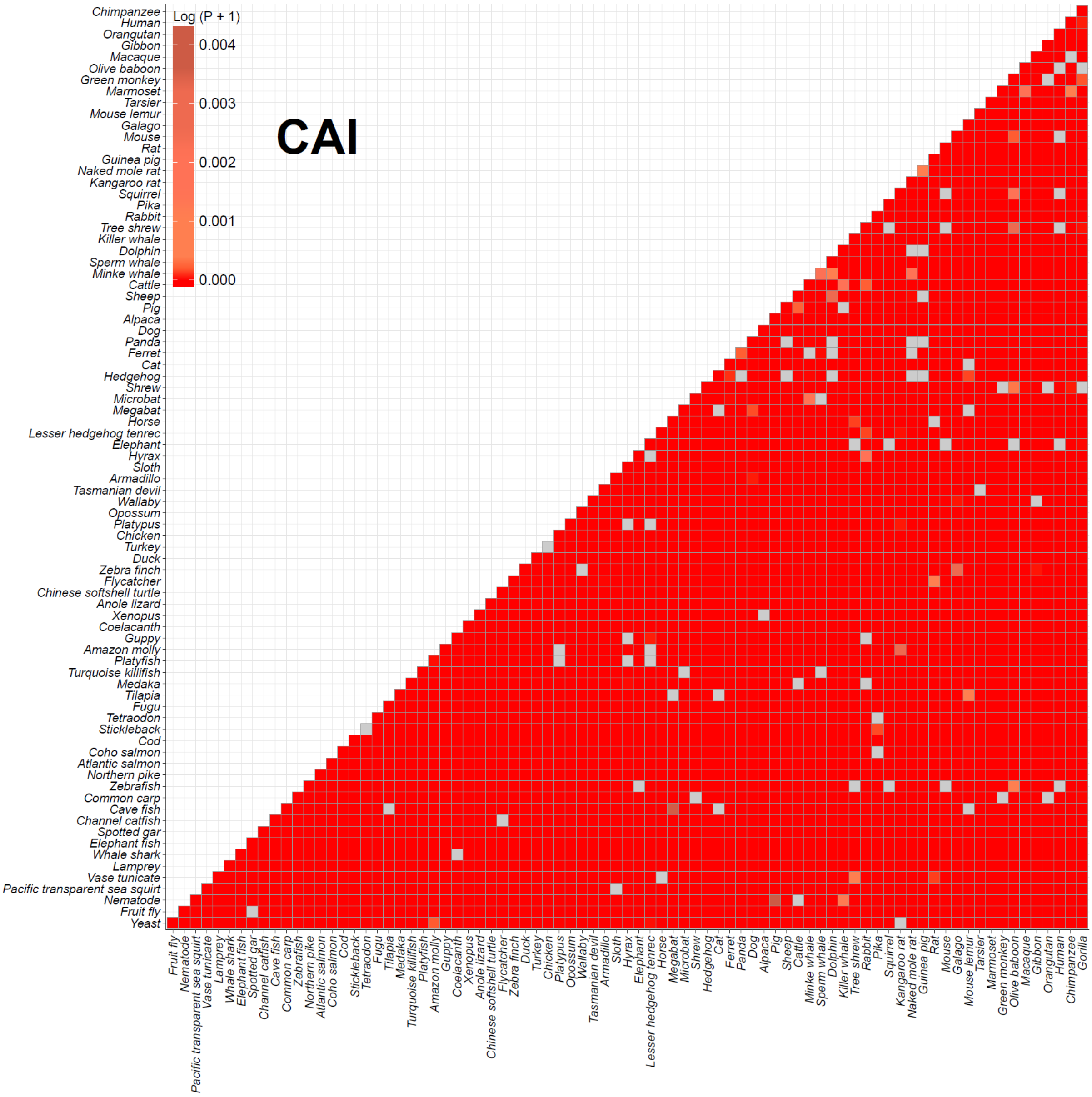


Figure S4B. Comparison of CAI by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the codon adaptation index (CAI) among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

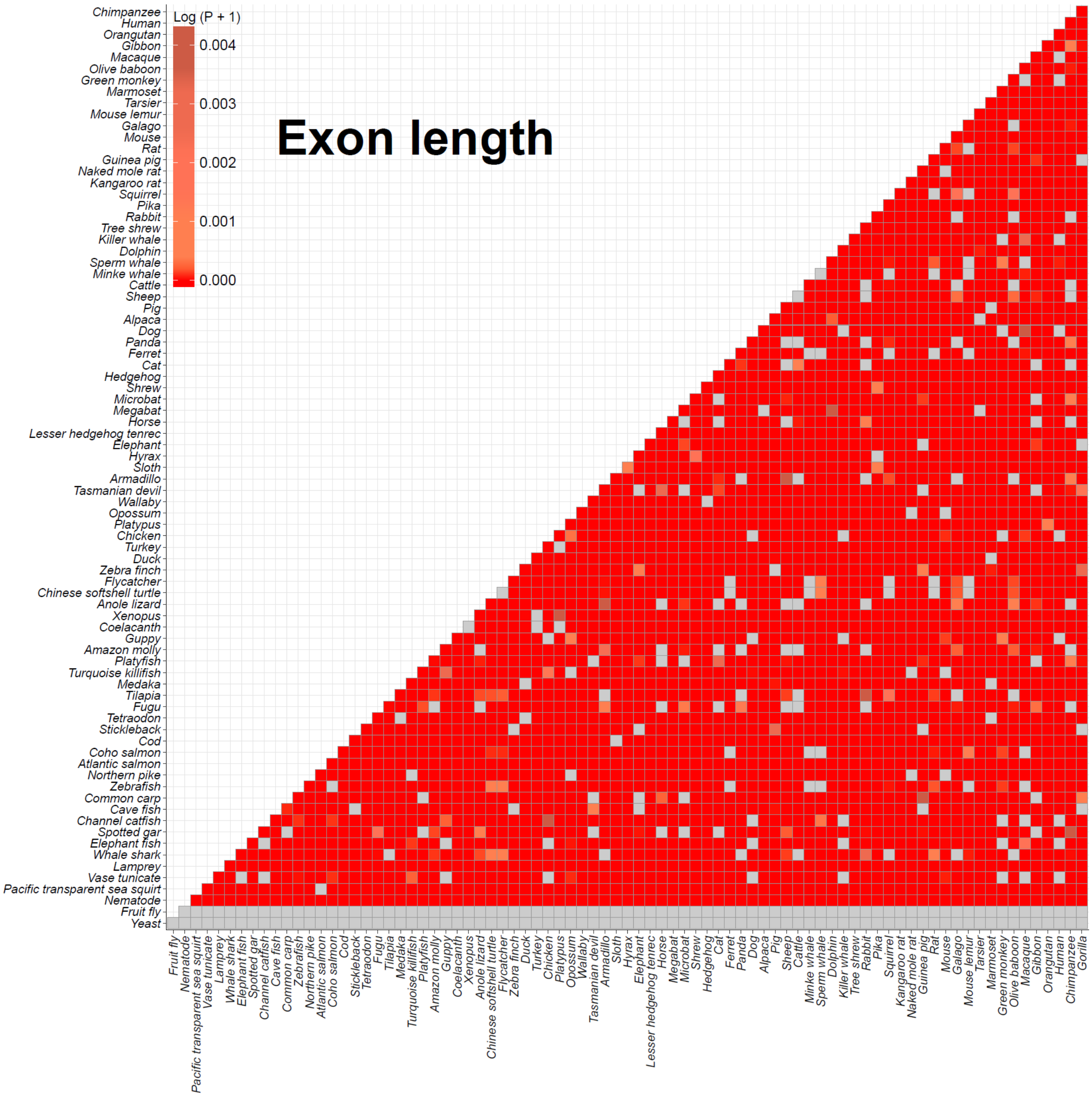


Figure S4C. Comparison of exon length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the length of exons between first and last coding exon among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01 or NA value (Yeast and Fruit fly).

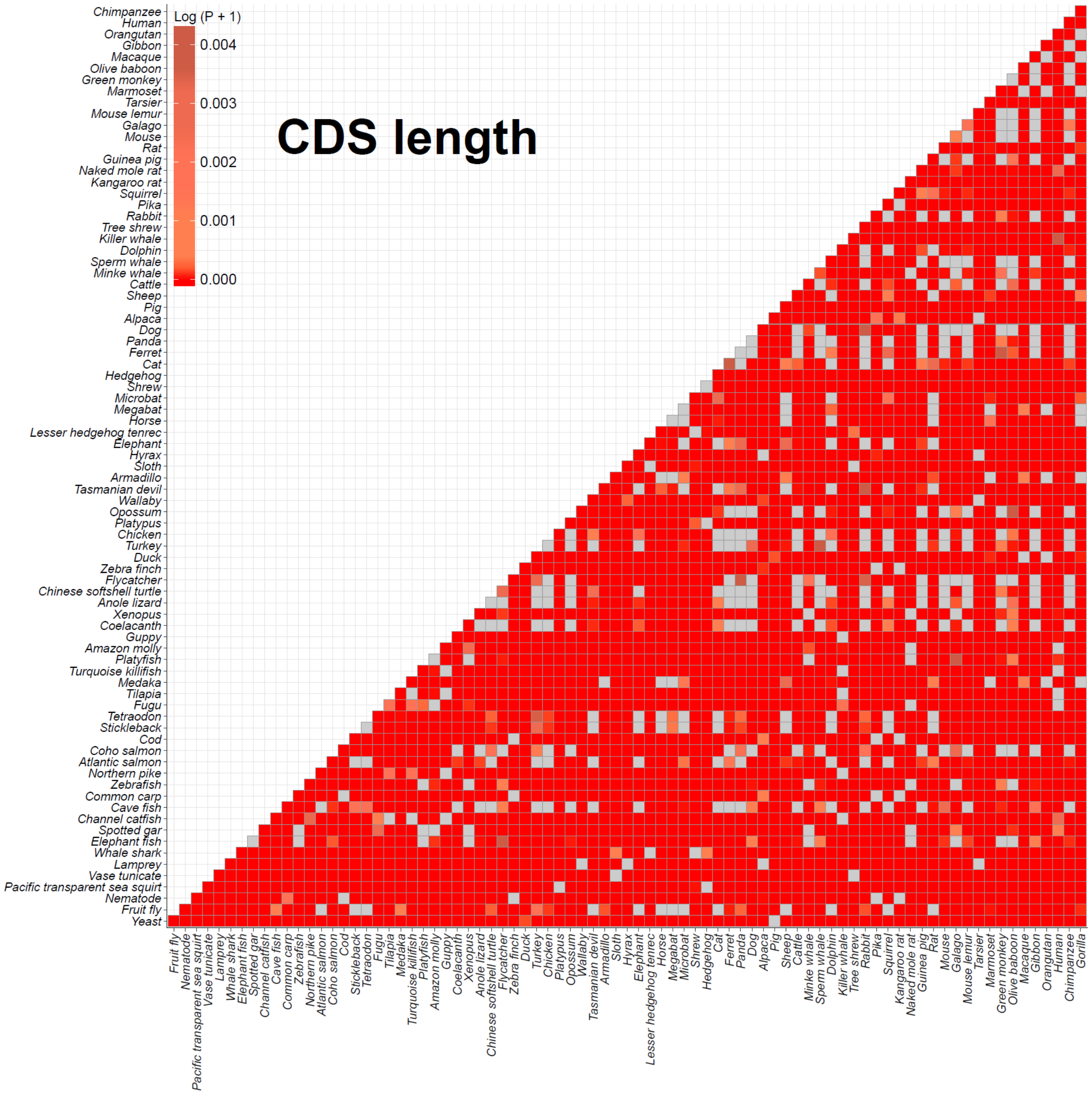


Figure S4D. Comparison of CDS length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the CDS length among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

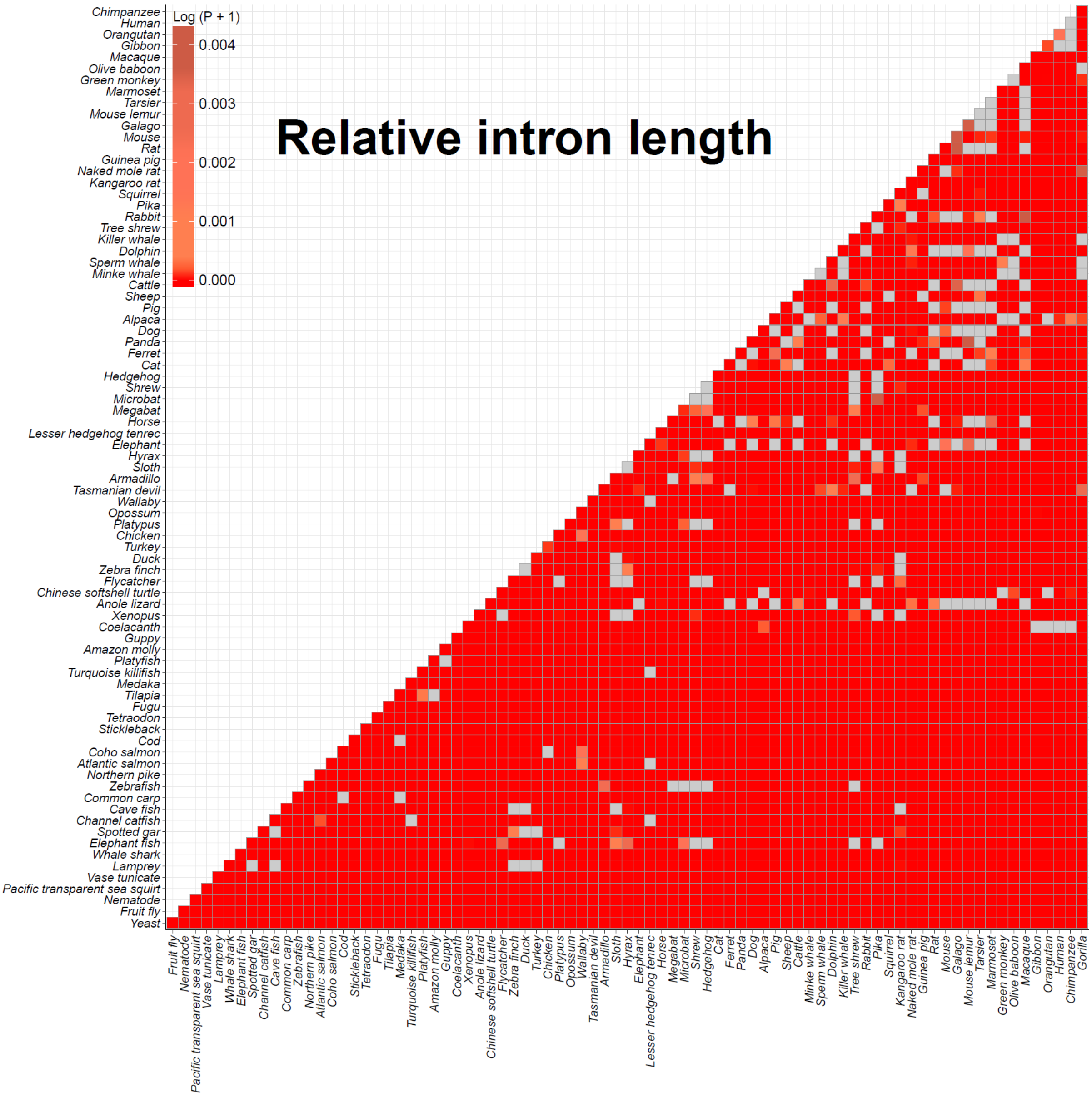


Figure S4E. Comparison of relative introns length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the relative intron length among 82 species. The relative intron length was calculated by dividing the total intron length between first and last coding exon by the CDS length. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

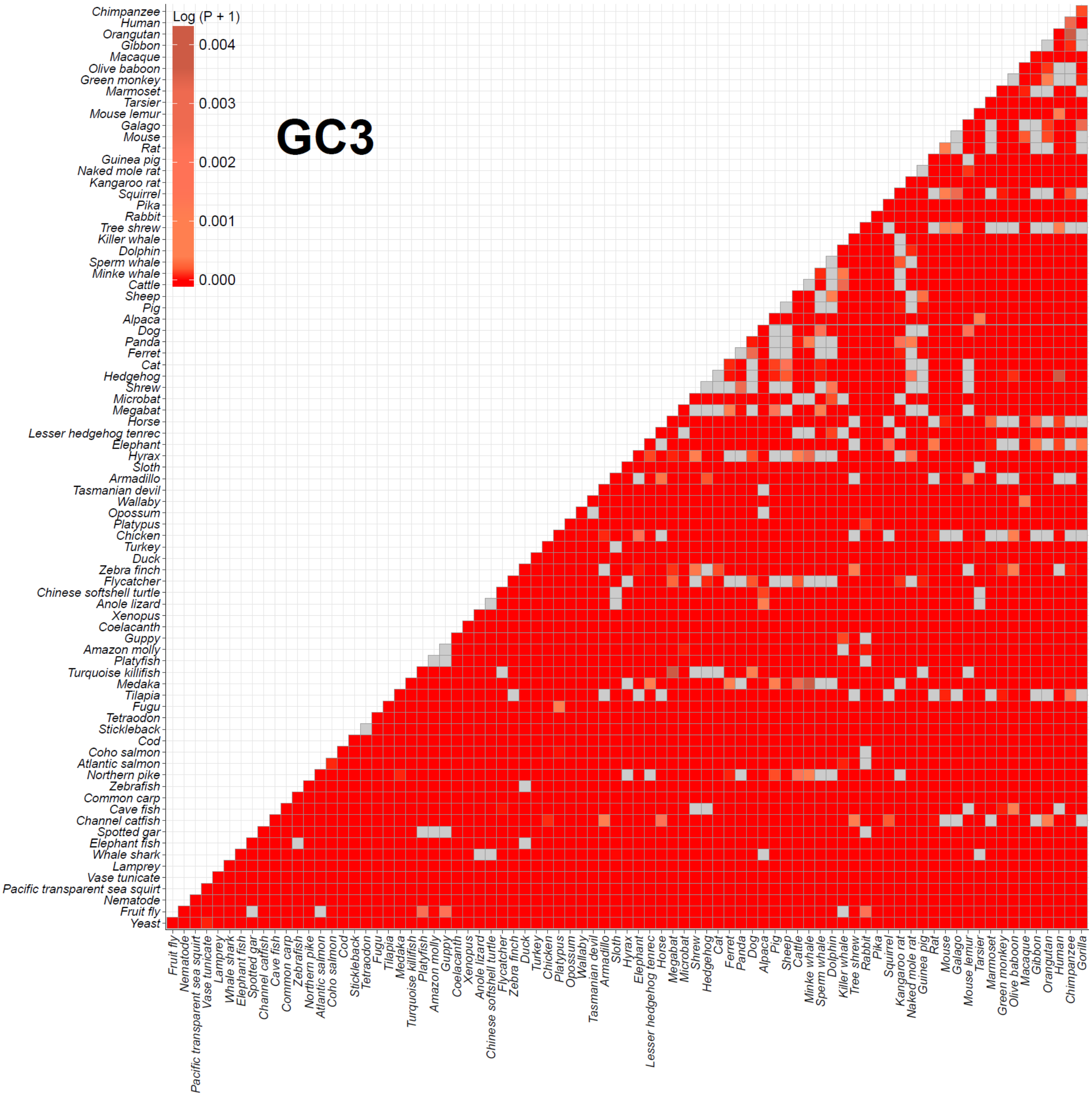


Figure S4F. Comparison of GC3 by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the GC content at the third codon position (GC3) among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

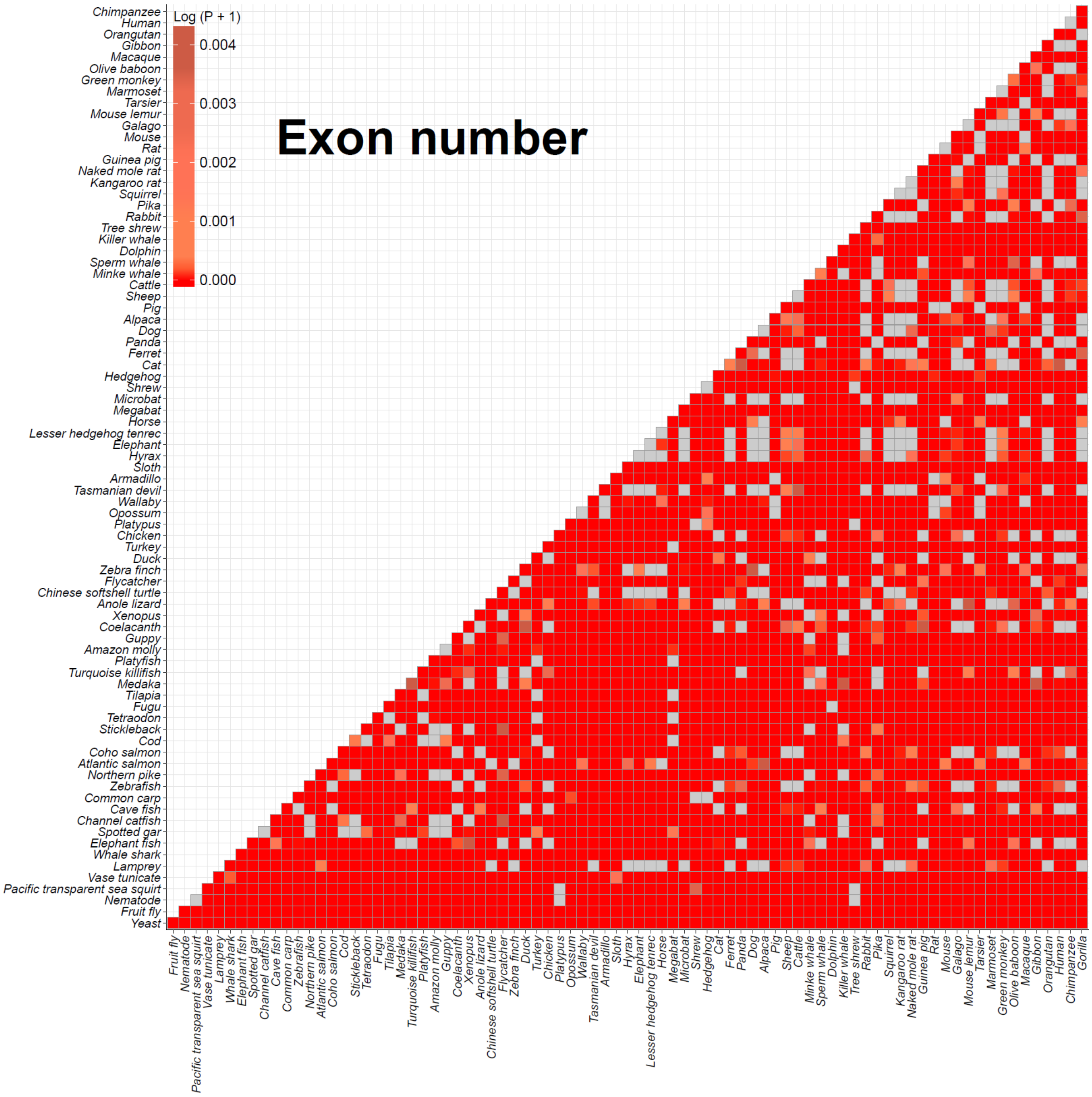


Figure S4G. Comparison of exon number by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the number of coding exons among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

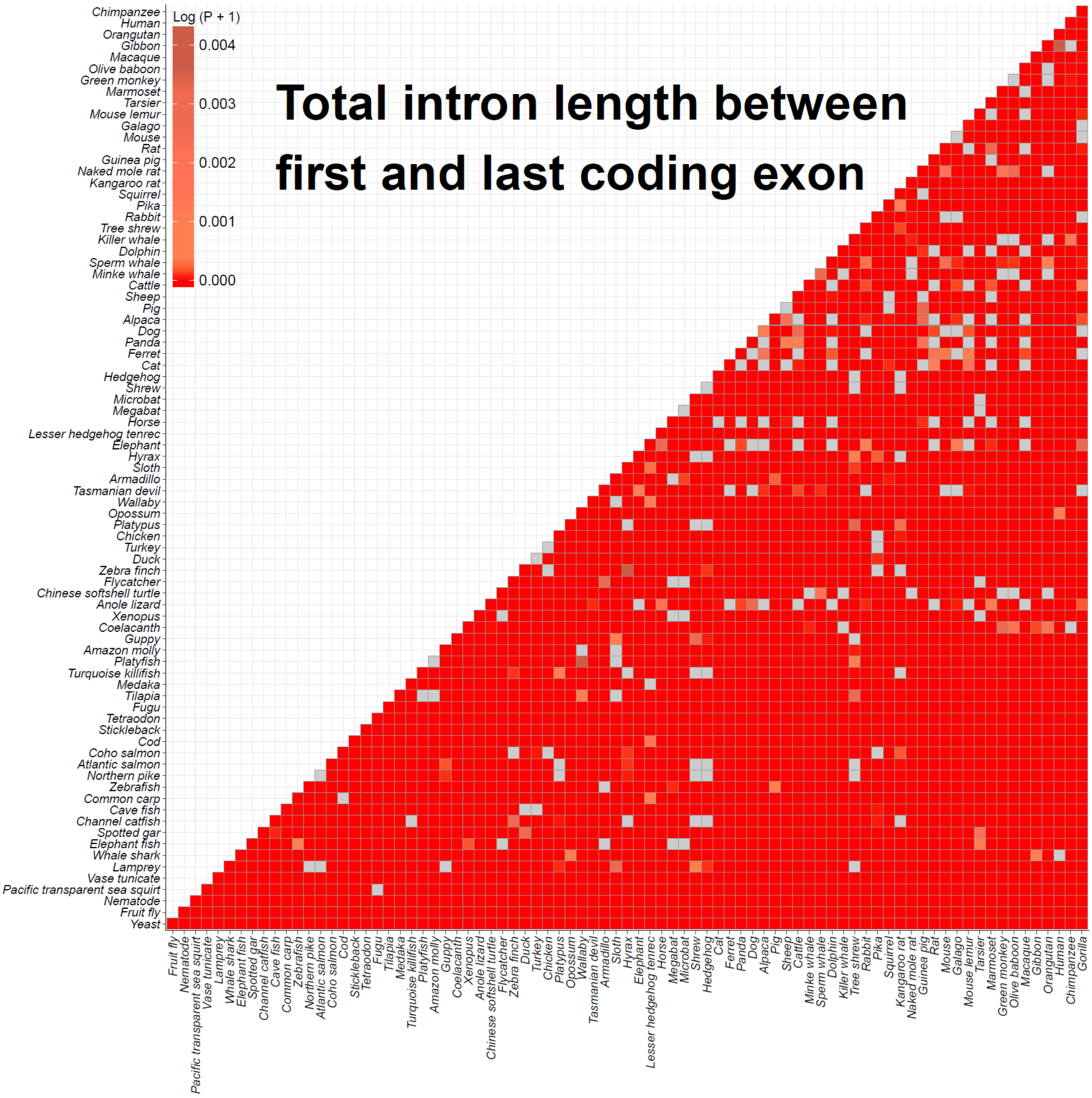


Figure S4H. Comparison of total intron length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the total length between first and last exon among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

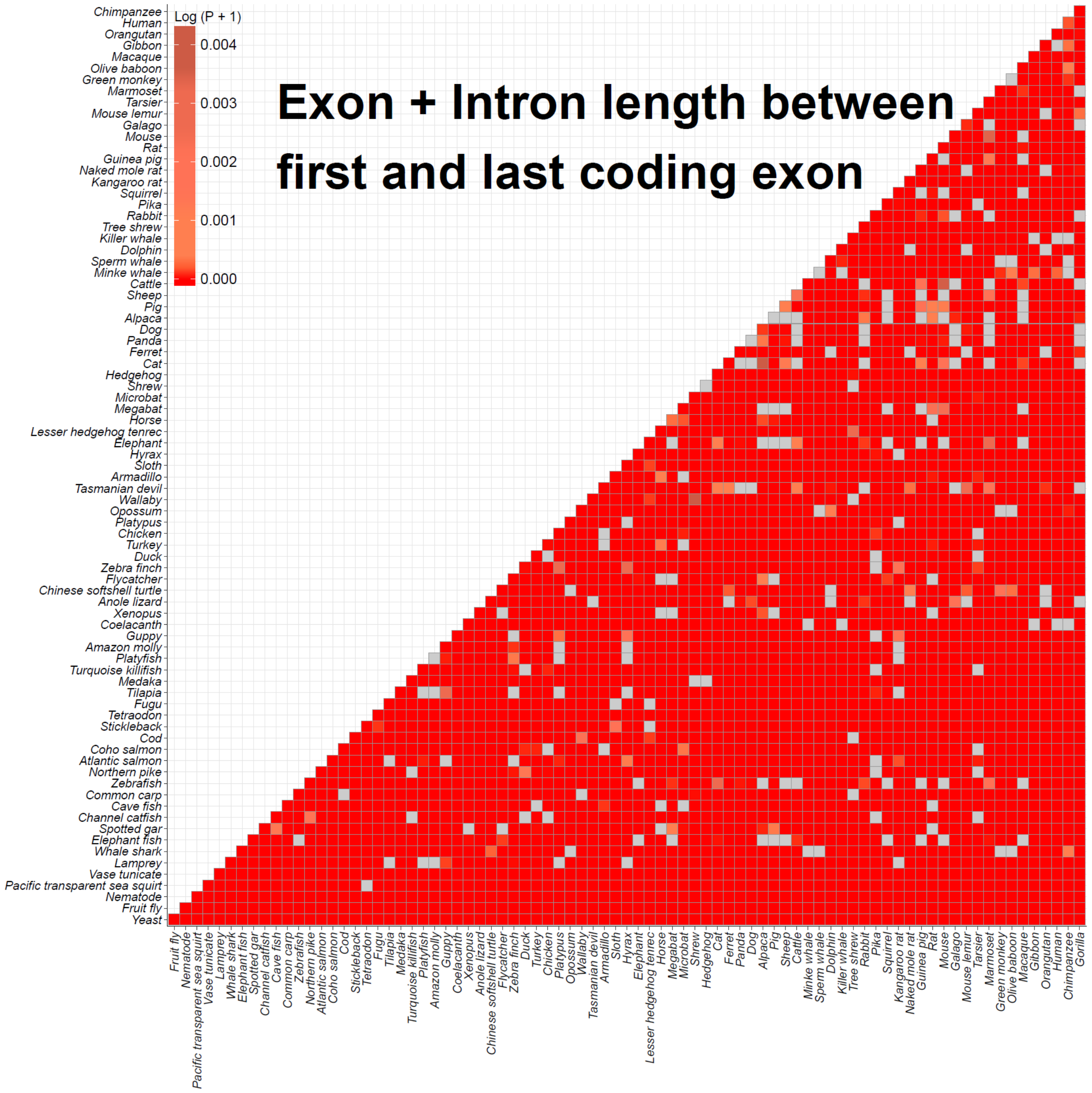


Figure S4I. Comparison of sum length of exons and introns by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the sum length of exons and introns between first and last coding exon among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

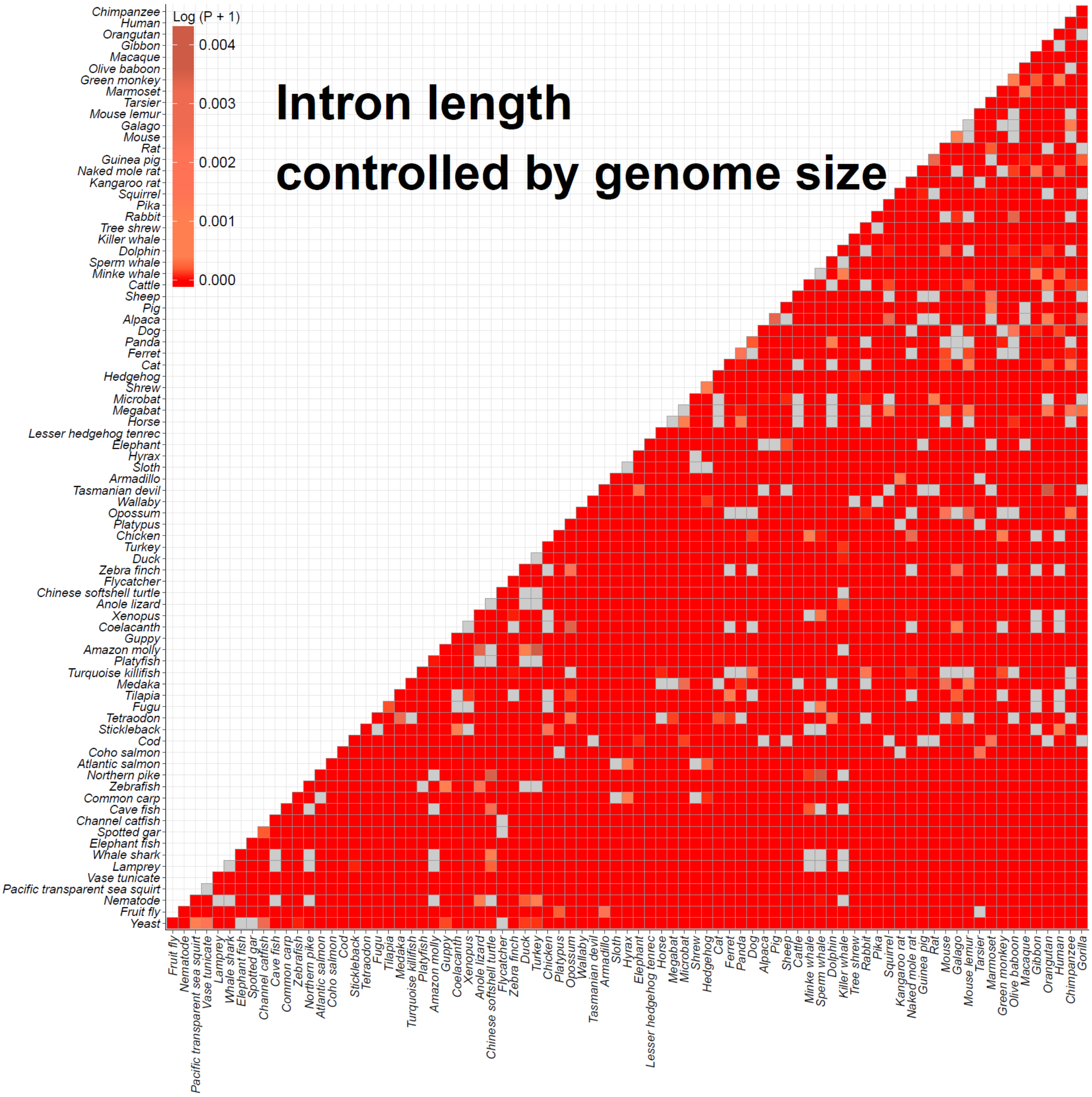


Figure S4J. Comparison of controlled introns length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the total intron length between first and last coding exon divided by genome size among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

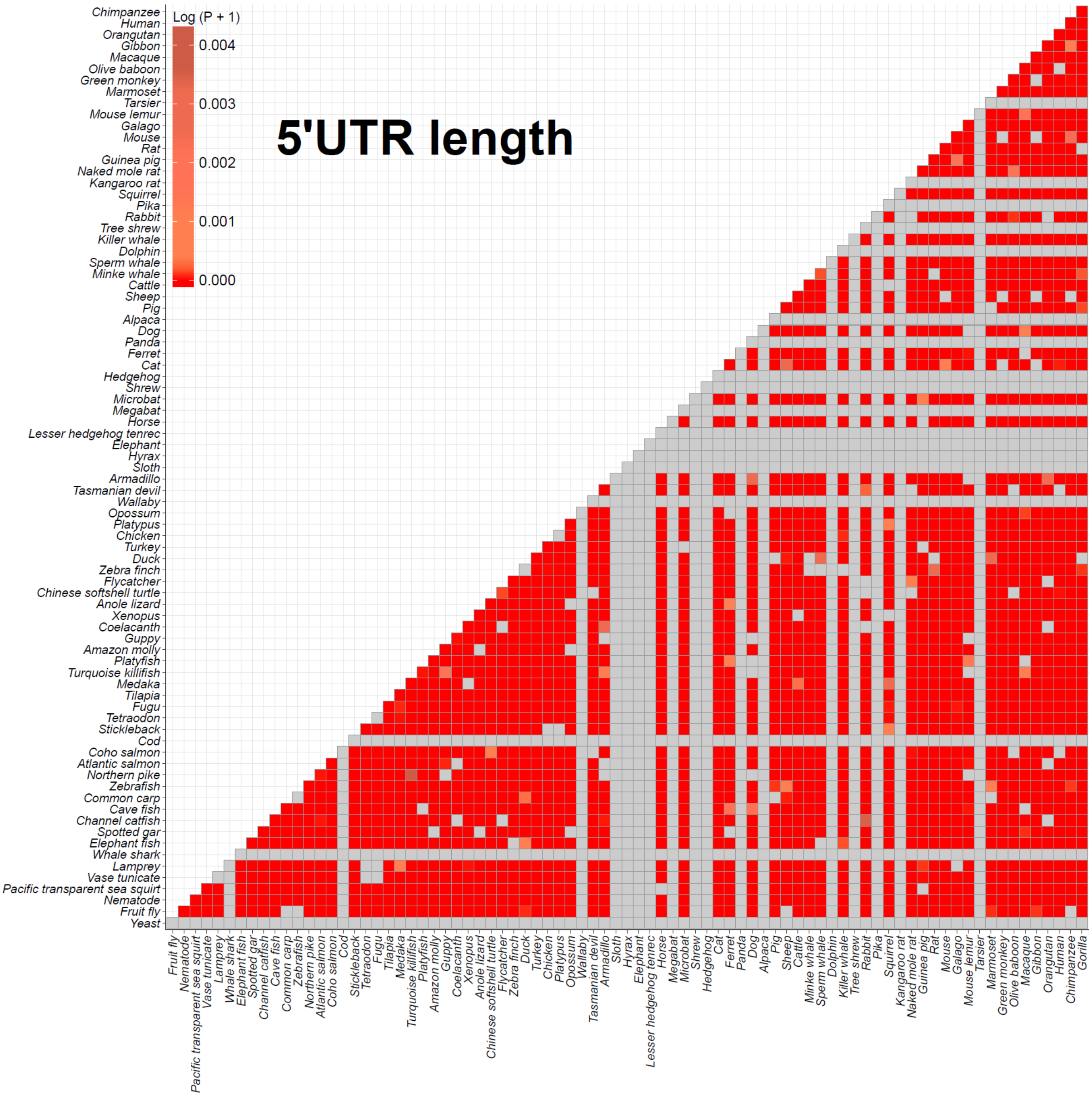


Figure S4K. Comparison of 5' UTR length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the 5' UTR length among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01 or NA values (species which have no 5' UTR information).

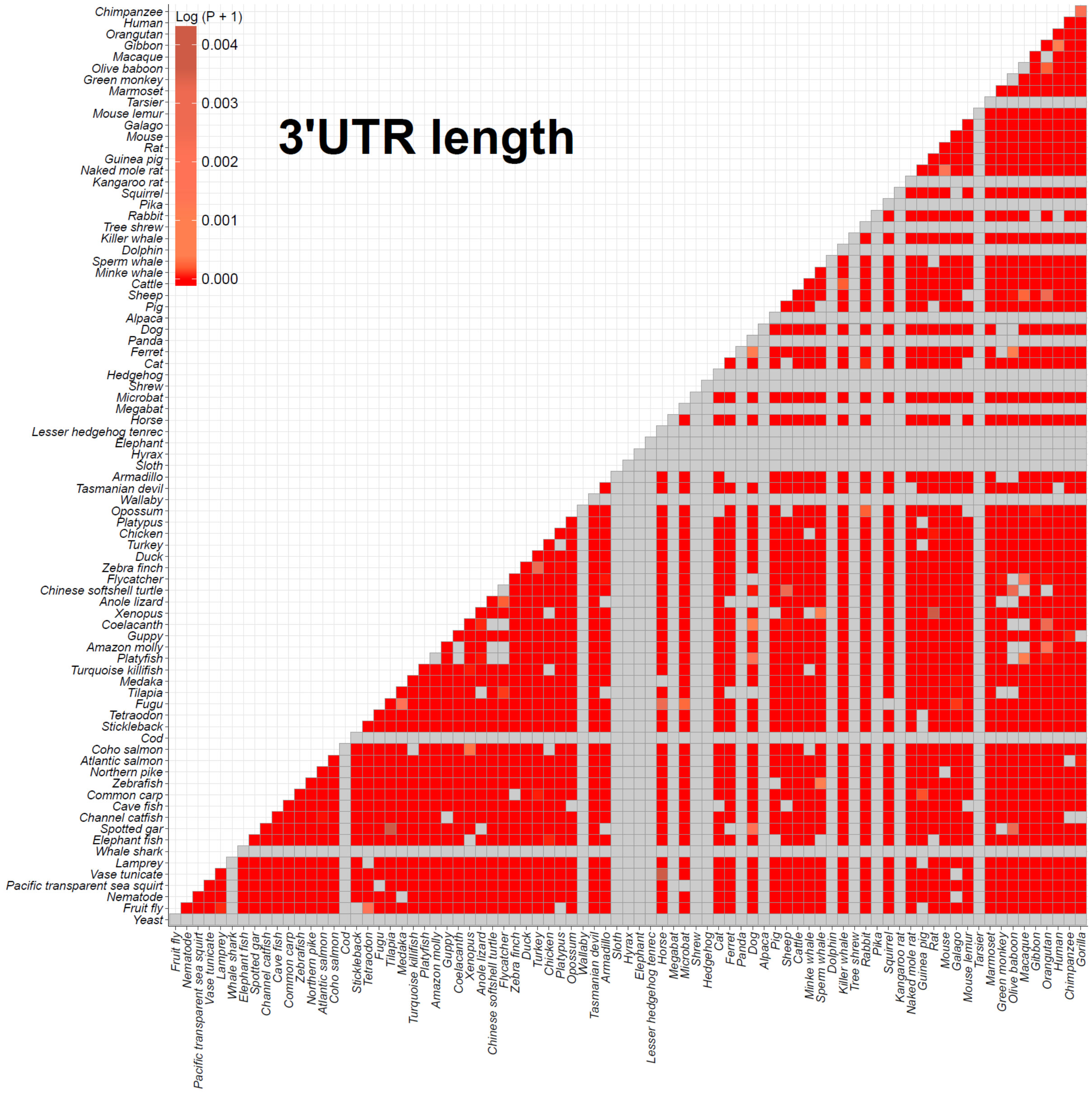


Figure S4L. Comparison of 3' UTR length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the 3' UTR length among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01 or NA values (species which have no 3' UTR information).

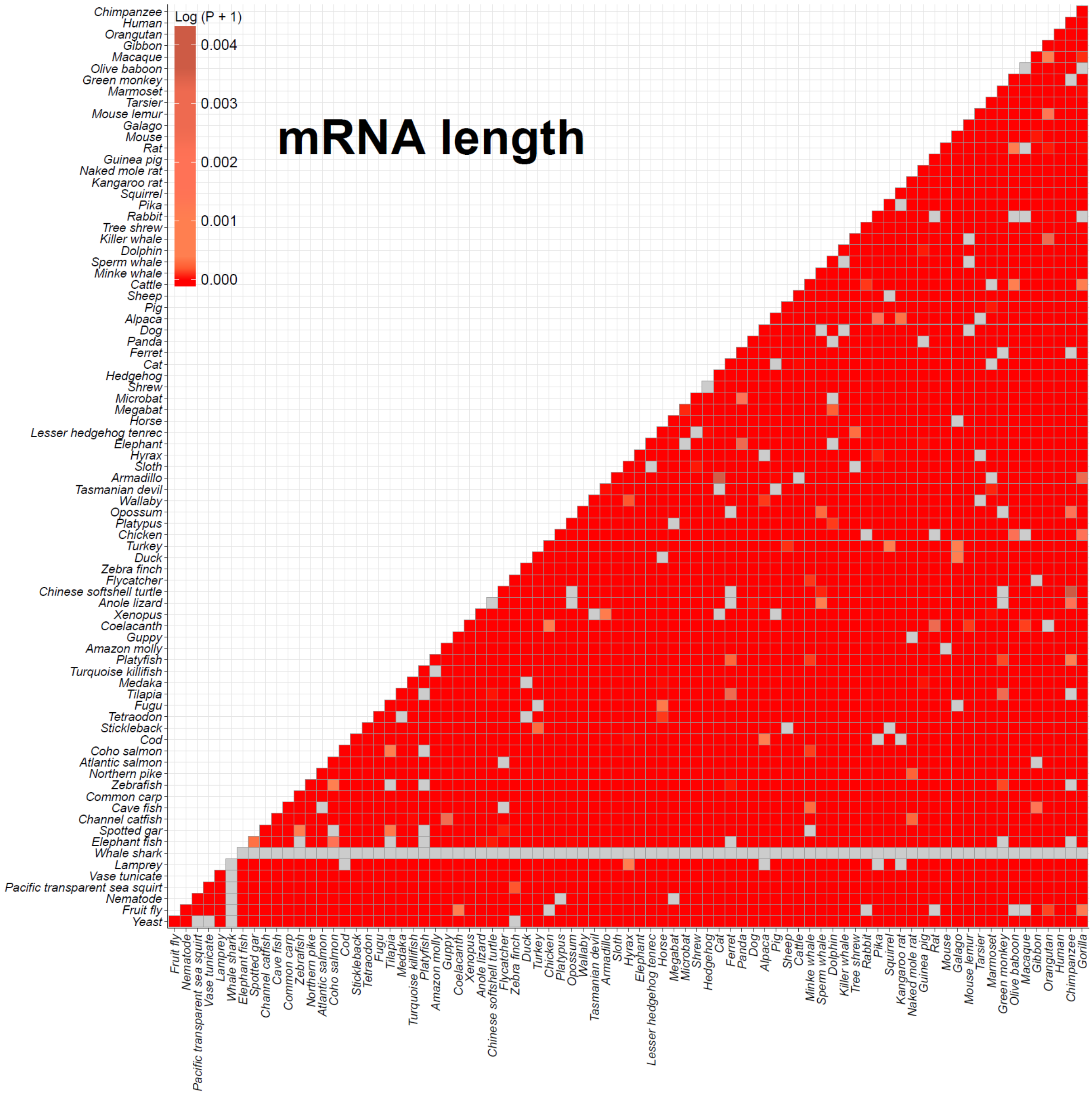


Figure S4M. Comparison of mRNA length by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the mRNA length among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01 or NA values (whale shark which have no mRNA information).

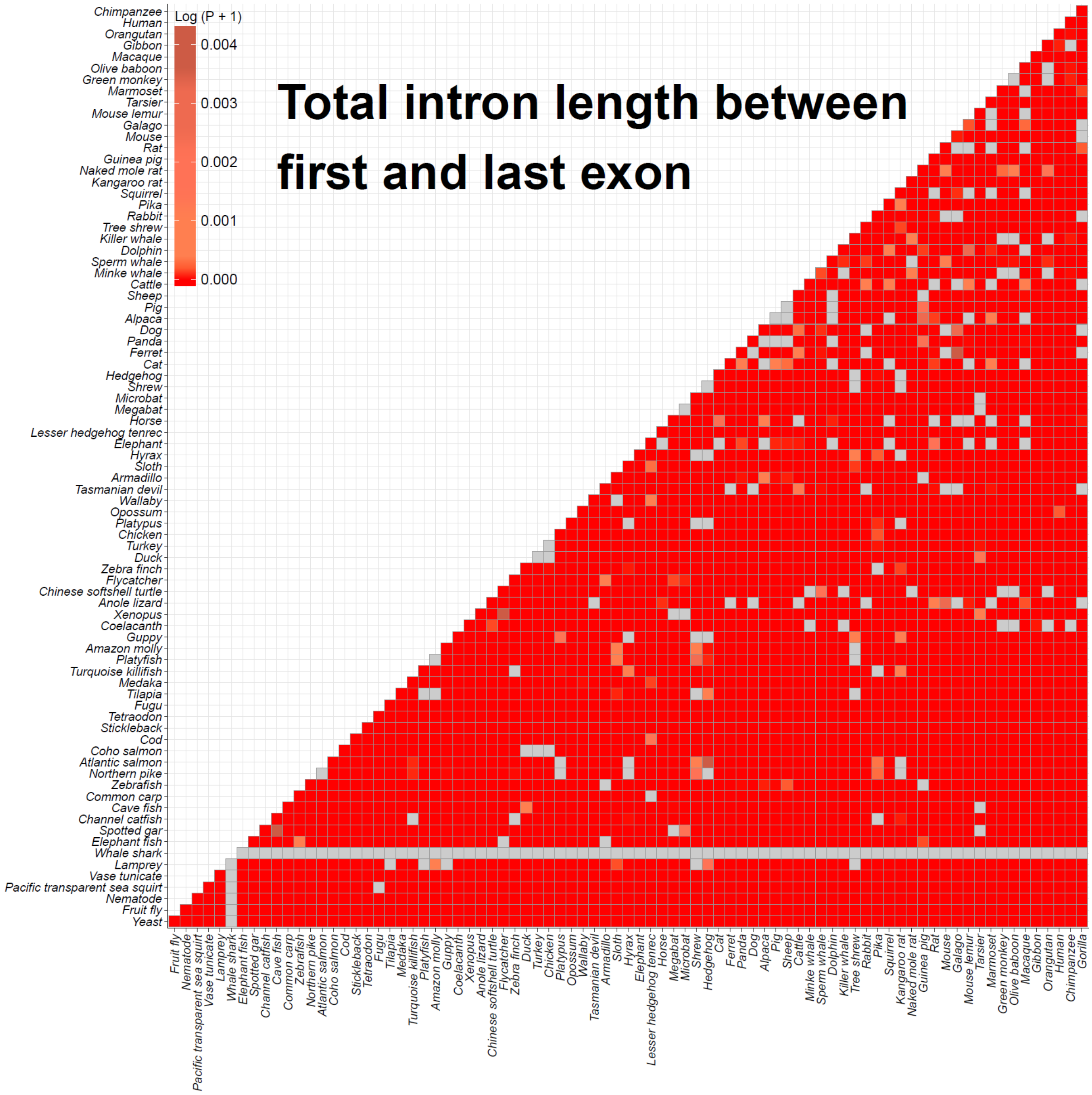


Figure S4N. Comparison of total intron length between first and last exon by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with the total length of introns between first and last exon among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01 or NA values (e.g., whale shark, which have no mRNA information).

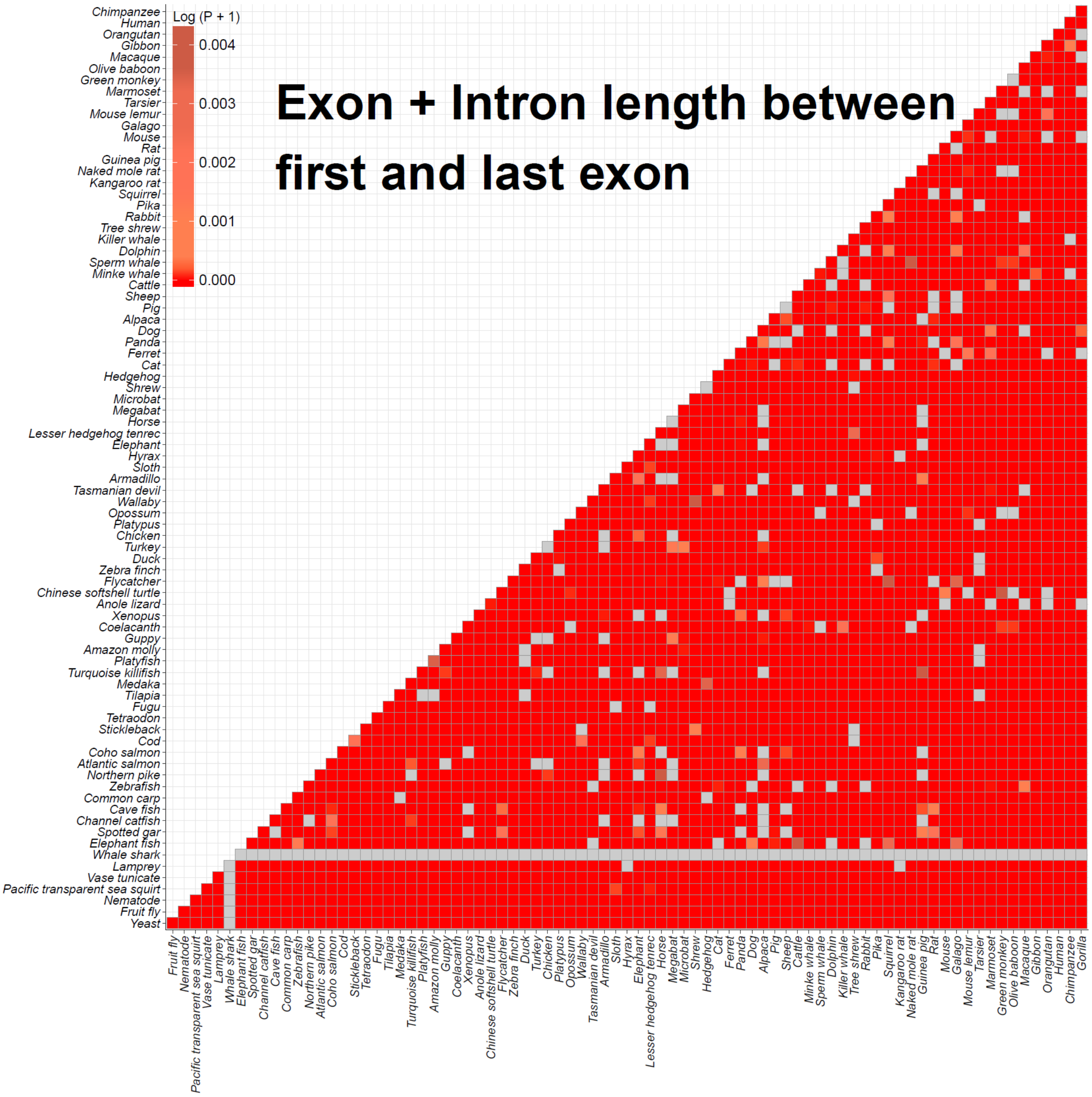


Figure S4O. Comparison of sum length of exons and introns between first and last exon by Wilcoxon rank sum test among 82 species. Two sided Wilcoxon rank sum tests were computed with sum length of exons and introns between first and last exon among 82 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01 or NA values (whale shark which have no mRNA information).

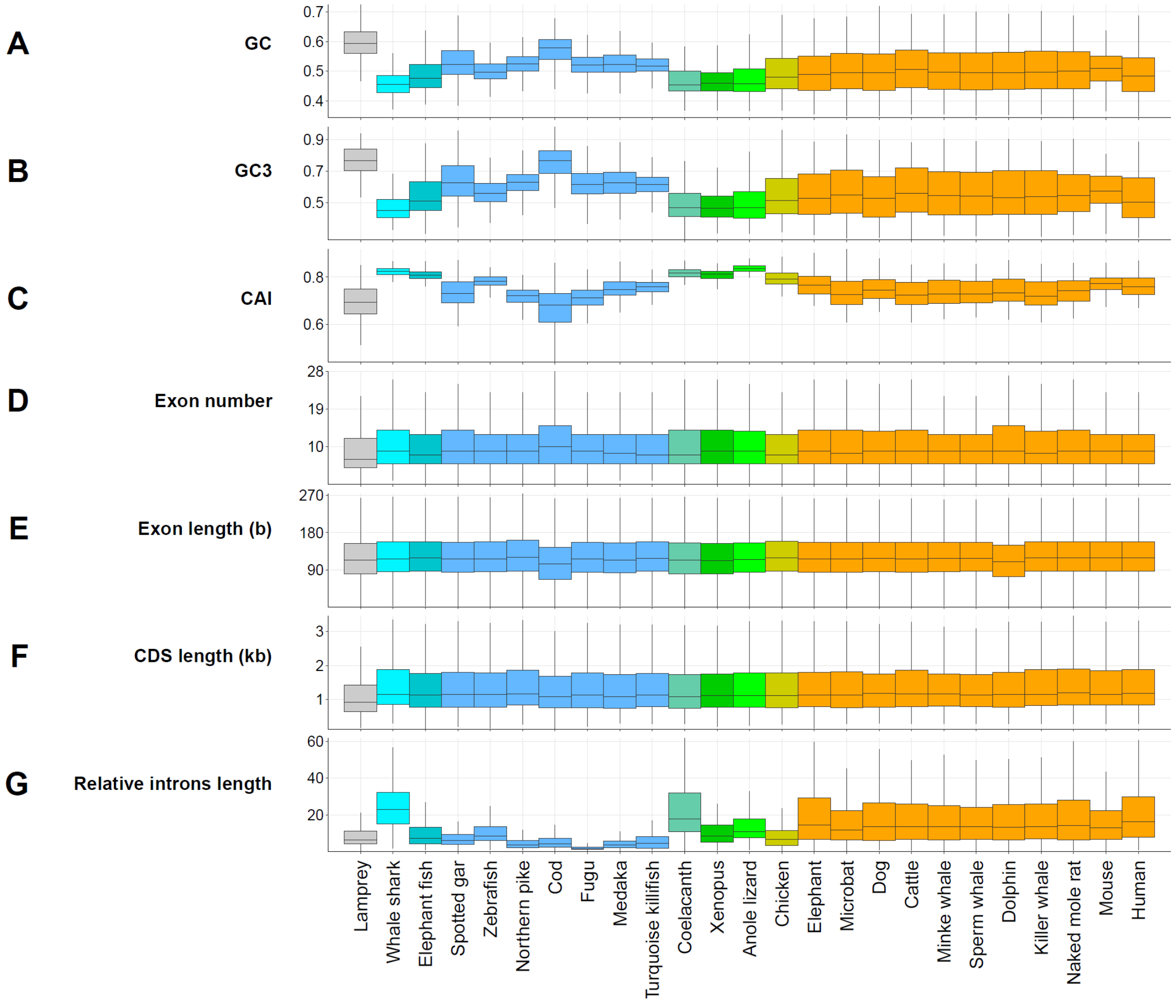


Figure S5. Comparison of genomic contexts in single-copy orthologous genes. 25 species were randomly selected from each class of 82 species. All comparisons (**A-G**) were performed using 275 single-copy gene families. The relative intron length (**G**) was calculated by dividing the total intron length between first coding exon and last coding exon by the CDS length. The nine colors indicate biological classifications (gray: Hyperoartia, cyan: whale shark, dark turquoise: elephant fish, light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

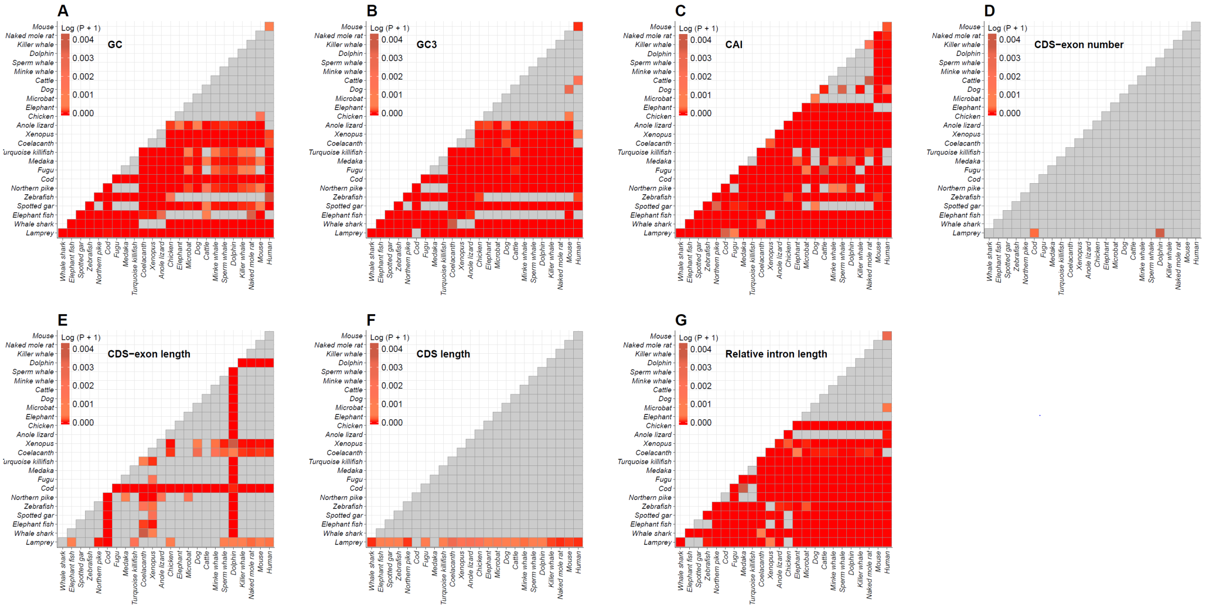


Figure S6. Comparison of seven genomic contexts by Wilcoxon rank sum test among 25 species. Two sided Wilcoxon rank sum tests were computed for each of the seven genomic properties in Figure S5 among 25 species. All *p*-values were adjusted using the Benjamini-Hochberg procedure and log-transformed. Deep red indicates significant adjusted *p*-values. Gray boxes indicate *p*-values higher than 0.01.

3. Maximum lifespan, body weight, basal metabolic rates association studies with gene size

3.1 Maximum lifespan data and maximum adult weight

Maximum lifespan, maximum adult weight and basal metabolic rates were downloaded from AnAge (http://genomics.senescence.info/species/), ADW (http://animaldiversity.org/), EOL (http://eol.org/), and aqW (https://www.theaquariumwiki.com/). The weight record of ten fishes were calculated by Froese, R., *et al.*'s methods, 'length-weight relationship' (Table S16).

Table S16. Maximum lifespan, weight, body temperature and basal metabolic rates of 82 species

Species	***Class***	***Maximum Lifespan***	***Reference***	***Maximum adult weight (g)***	***Reference***	***Average body temperature and growth optimum temperature (℃)***	***Reference***	***Basal metabolic rate (W)***	***Reference***
Gorilla gorilla	Mammalia	60.1	AnAge	275000	[16]	35.5	AnAge	NA	NA
Pan troglodytes	Mammalia	60	ADW	70000	AnAge	35.7	AnAge	NA	NA
Homo sapiens	Mammalia	122.5	AnAge	62035	AnAge	37	AnAge	82.78	AnAge
Pongo abelii	Mammalia	58	ADW	90000	ADW	37.6	[17]	NA	NA
Nomascus leucogenys	Mammalia	44.1	AnAge	7500	EOL	39	[18]	NA	NA
Macaca mulatta	Mammalia	40	AnAge	12000	ADW	37.3	AnAge	NA	NA
Papio anubis	Mammalia	45	The Animal Files	37000	The Animal Files	37.3	[18,19]	NA	NA
Chlorocebus sabaeus	Mammalia	13	ADW	8000	ADW	37.35	[20]	NA	NA
Callithrix jacchus	Mammalia	22.8	AnAge	360	ADW	36	AnAge	0.848	AnAge
Tarsius syrichta	Mammalia	16	AnAge	165	ADW	33.8	AnAge	0.43	AnAge
Microcebus murinus	Mammalia	18.2	AnAge	71.1	Primate Info Net	36.1	[18,19]	NA	NA
Otolemur garnettii	Mammalia	20	AnAge	1345	[21]	36	AnAge	3.927	AnAge
Mus musculus	Mammalia	4	AnAge	30	ADW	36.9	AnAge	0.271	AnAge
Rattus norvegicus	Mammalia	4	ADW	500	ADW	37.1	AnAge	1.404	AnAge
Cavia porcellus	Mammalia	14	ADW	1100	ADW	39	AnAge	2.13	AnAge
Heterocephalus glaber	Mammalia	31	AnAge	80	ADW	32.1	AnAge	0.128	AnAge
Dipodomys ordii	Mammalia	9.9	AnAge	96	ADW	34.6	AnAge	0.339	AnAge
Ictidomys tridecemlineatus	Mammalia	7.9	AnAge	220	Vertebrate Collection	35.7	AnAge	0.983	AnAge
Ochotona princeps	Mammalia	7	AnAge	180	Wildpro	40.1	AnAge	0.932	AnAge
Oryctolagus cuniculus	Mammalia	9	AnAge	2500	ADW	39	AnAge	7.395	AnAge
Tupaia belangeri	Mammalia	12	ADW	270	ADW	38.8	AnAge	NA	NA
Orcinus orca	Mammalia	90	AnAge	10000000	Seaworld	36	AnAge	NA	NA
Tursiops truncatus	Mammalia	53	ADW	650000	ACS	36.9	[22]	NA	NA
Physeter catodon	Mammalia	77	AnAge	57000000	MARINEBIO	38	WhaleForever	NA	NA
Balaenoptera acutorostrata scammoni	Mammalia	50	AnAge	13000000	Arkive	38	WhaleForever	NA	NA
Bos taurus	Mammalia	20	AnAge	1363000	ADW	38	AnAge	306.77	AnAge

Ovis aries	Mammalia	22.8	AnAge	200000	ADW	38.8	AnAge	NA	NA
Sus scrofa	Mammalia	27	AnAge	272000	ADW	39	AnAge	104.15	AnAge
Vicugna pacos	Mammalia	25.8	AnAge	84000	Facts about Animals	39.1	AnAge	NA	NA
Canis familiaris	Mammalia	29.5	ADW	70000	ADW	39	Circadian Rhythm Lab.	NA	NA
Ailuropoda melanoleuca	Mammalia	36.8	AnAge	125000	ADW	37	Panda facts	NA	NA
Mustela putorius furo	Mammalia	11.1	AnAge	2700	ADW	38.9	Wildpro	NA	NA
Felis catus	Mammalia	30	AnAge	5400	AnAge	38.1	AnAge	NA	NA
Erinaceus europaeus	Mammalia	11.7	AnAge	2000	Wildpro	34	AnAge	2.434	AnAge
Sorex araneus	Mammalia	3.2	AnAge	14	ADW	35	AnAge	0.348	AnAge
Myotis lucifugus	Mammalia	34	AnAge	14	ADW	32	AnAge	0.051	AnAge
Pteropus vampyrus	Mammalia	20.9	AnAge	1100	ADW	36.9	AnAge	4.486	AnAge
Equus caballus	Mammalia	57	AnAge	900000	ADW	38.3	AnAge	NA	NA
Echinops telfairi	Mammalia	19	AnAge	280	ADW	34.7	AnAge	NA	NA
Loxodonta africana	Mammalia	65	AnAge	6600000	ELASMO	36.2	AnAge	NA	NA
Procavia capensis	Mammalia	14.8	AnAge	4300	ADW	37	AnAge	4.954	AnAge
Choloepus hoffmanni	Mammalia	41	AnAge	12500	Slothsanctuary	34.4	AnAge	3.891	AnAge
Dasypus novemcinctus	Mammalia	22.3	AnAge	7700	ADW	34.5	AnAge	4.655	AnAge
Sarcophilus harrisii	Mammalia	13	AnAge	12000	ADW	35.8	AnAge	8.664	AnAge
Macropus eugenii	Mammalia	15.1	AnAge	9100	ADW	36.5	AnAge	7.78	AnAge
Monodelphis domestica	Mammalia	5.1	AnAge	155	ADW	32.6	AnAge	0.335	AnAge
Ornithorhynchus anatinus	Mammalia	22.6	AnAge	2500	AnAge	34	AnAge	1.931	AnAge
Gallus gallus	Aves	30	AnAge	1450	Arkive	41	[23]	6.005	AnAge
Meleagris gallopavo	Aves	13	AnAge	11000	ADW	42.4	[24]	NA	NA
Anas platyrhynchos	Aves	29.1	AnAge	1580	[25]	40.2	[26]	4.068	AnAge
Taeniopygia guttata	Aves	12	AnAge	19	FINCHINFO	41	AnAge	NA	NA
Ficedula albicollis	Aves	9.8	AnAge	16	Birds Natureguide	41	[27]	NA	NA
Pelodiscus sinensis	Reptilia	20	aqW	2247	EOL	26.5	INSECTIVORE	NA	NA
Anolis carolinensis	Reptilia	7.2	AnAge	6	ADW	25.5	The spruce	NA	NA
Xenopus tropicalis	Amphibia	20	Tropical-fish-keeping	26	[28]	25.5	Xenopus Express	NA	NA

Latimeria chalumnae	Sarcopterygii	48	AnAge	95000	Fishbase	19	VIMS	NA	NA
Poecilia reticulata	Actinopterygii	5	AnAge	4.13	Fishbase [29]	22.5	SERIOUSLY FISH	NA	NA
Poecilia formosa	Actinopterygii	3	[30]	11.3	Fishbase [29]	25	The aquarium guide	NA	NA
Xiphophorus maculatus	Actinopterygii	5	aqW	2	Fishbase [29]	22	Tropical Fish Site	NA	NA
Nothobranchius furzeri	Actinopterygii	1.1	AnAge	1.3	Fishbase [29]	27.8	WildNothos	NA	NA
Oryzias latipes	Actinopterygii	5	AnAge	0.2	Fishbase [29]	19	SERIOUSLY FISH	NA	NA
Oreochromis niloticus	Actinopterygii	9	AnAge	4300	Fishbase	33.5	FAO	NA	NA
Takifugu rubripes	Actinopterygii	9	IUCN	5754	Fishbase [29]	25	[31]	NA	NA
Tetraodon nigroviridis	Actinopterygii	10	aqW	96	Fishbase [29]	26	SERIOUSLY FISH	NA	NA
Gasterosteus aculeatus	Actinopterygii	8	AnAge	16	Fishbase [29]	21	[32]	NA	NA
Gadus morhua	Actinopterygii	25	AnAge	96000	Fishbase	12	[33]	NA	NA
Oncorhynchus kisutch	Actinopterygii	5	AnAge	15200	Fishbase	12	[34]	NA	NA
Salmo salar	Actinopterygii	13	AnAge	46800	Fishbase	8	USGS	NA	NA
Esox lucius	Actinopterygii	30	AnAge	28400	AnAge	20.5	[35]	NA	NA
Danio rerio	Actinopterygii	5.5	AnAge	1	Fishbase [29]	28.5	[36]	NA	NA
Cyprinus carpio	Actinopterygii	47	AnAge	40100	Fishbase	22.5	FAO	NA	NA
Astyanax mexicanus	Actinopterygii	8	aqW	21	Fishbase [29]	24	[37]	NA	NA
Ictalurus punctatus	Actinopterygii	16	Fishbase	13733	AnAge	27	FAO	NA	NA
Lepisosteus oculatus	Actinopterygii	18	AnAge	4400	Fishbase	16	SERIOUSLY FISH	NA	NA
Callorhinchus milii	Chondrichthyes	20	IUCN	4000	ADW	14	WHRHSmarin ebiology	NA	NA
Rhincodon typus	Chondrichthyes	80.4	[38]	42000000	IUCN	25	Arkive	NA	NA
Petromyzon marinus	Hyperoartia	9	AnAge	2500	AnAge	20	[39]	NA	NA
Ciona intestinalis	Ascidiacea	1	ADW	0	NA	NA	NA	NA	NA
Ciona savignyi	Ascidiacea	1	ADW	0	NA	NA	NA	NA	NA
Caenorhabditis elegans	Chromadorea	0.16	AnAge	0	NA	NA	NA	NA	NA
Drosophila melanogaster	Insecta	0.3	AnAge	0	NA	NA	NA	NA	NA
Saccharomyces cerevisiae	Saccharomycetes	0.04	AnAge	0	NA	NA	NA	NA	NA

AnAge: http://genomics.senescence.info/species/, **ADW**: http://animaldiversity.org/, **EOL**: http://eol.org/, **aqW**: https://www.theaquariumwiki.com/, **Fishbase**: http://www.fishbase.org/, **IUCN**: http://www.iucnredlist.org/, **PIN**: http://pin.primate.wisc.edu/, **Circadian Rhythm Lab.**: http://www.circadian.org/animal.html, **Panda facts**: http://www.chinadaily.com.cn/regional/2012-09/21/content_15774766.htm, **Wildpro**: http://wildpro.twycrosszoo.org/, **The spruce**: https://www.thespruce.com/keeping-green-anoles-as-pets-1236899, **Xenopus express**: http://www.xenopus.com/, **VIMS**: http://www.vims.edu/research/facilities/fishcollection/highlights/coelacanth.php, **Arkive**: http://www.arkive.org/, **SERIOUSLY FISH**: http://www.seriouslyfish.com/, **The aquarium guide**: http://www.theaquariumguide.com/, **FAO**: http://www.fao.org/, **USGS**: https://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=926, **WHRHSmarinebiology**: https://whrhsmarinebiology.wikispaces.com/, **INSECTIVORE**: http://www.insectivore.co.uk/, **WildNothos**: http://wildnothos.wixsite.com/wildnothos/furzeri, **The Animal Files**: http://www.theanimalfiles.com/, **Primate Info Net**: http://pin.primate.wisc.edu/, **Vertebrate Collection**: https://www.uwsp.edu/biology/VertebrateCollection/Pages/default.aspx, **Seaworld**: https://seaworld.org/, **ACS**: https://web.archive.org/web/20080725121057/http://acsonline.org/factpack/btlnose.htm, **MARINEBIO**: http://marinebio.org/, **WHALE FACTS**: http://www.whalefacts.org/, **Facts about Animals**: http://www.facts-about.info/, **ELASMO**: http://www.elasmo-research.org/, **Slothsanctuary**: http://www.slothsanctuary.com/about-sloths/choloepus-hoffmanni, **FINCHINFO**: http://www.finchinfo.com/birds/finches/species/zebra_finch.php, **Birds natureguide**: http://birds.natureguide.gr/, **Tropical-fish-keeping**: http://www.tropical-fish-keeping.com/western-clawed-frog-xenopus-tropicalis.html#sthash.FachDSO4.dpbs.

3.2 Basal metabolic rates calculation

We calculated basal metabolic rates (BMRs) of 82 species using Gillooly's equation[40] based on maximum adult weight and average body temperature (or growth optimum temperature for cold-blooded animal) (Table S16). The calculated BMRs were compared with published BMRs from the AnAge database (http://genomics.senescence.info/) using the Spearman's rank correlation coefficient (Figure S7). The Calculated Gillooly's BMRs[40] were significantly correlated with BMRs downloaded from the AnAge database.

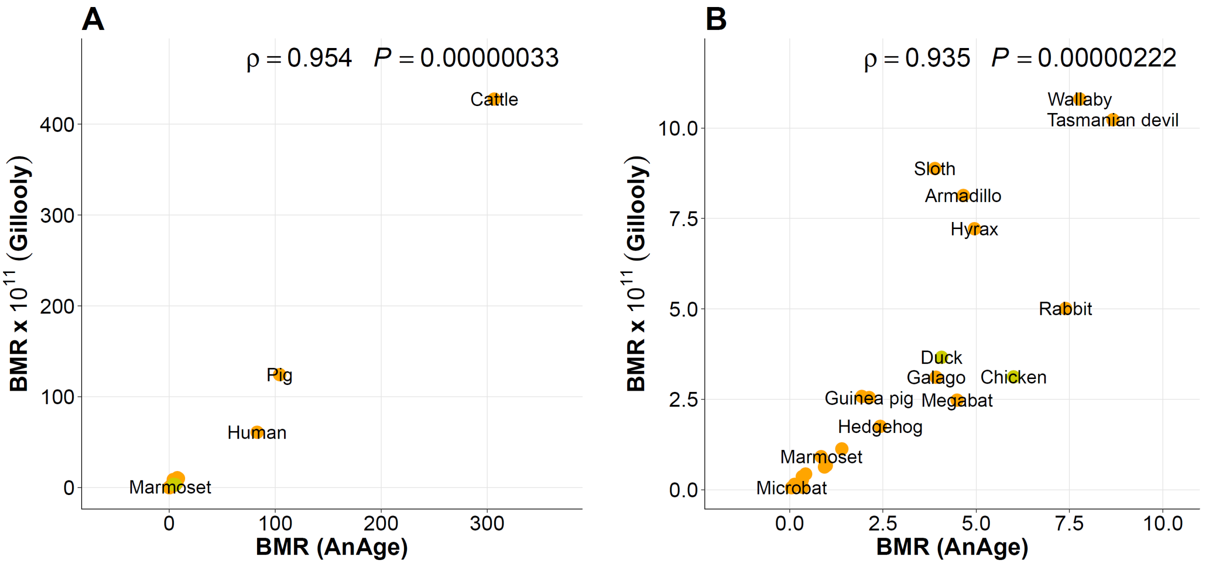


Figure S7. Correlation between AnAge's BMR and calculated BMR. We downloaded the BMRs of 27 species from AnAge (http://genomics.senescence.info/, Table S16) and also calculated BMRs using Gillooly's equation[40]. (**A**) The correlation test with 27 species. (**B**) The correlation test without cattle, pig, and human, which have extremely high BMR.

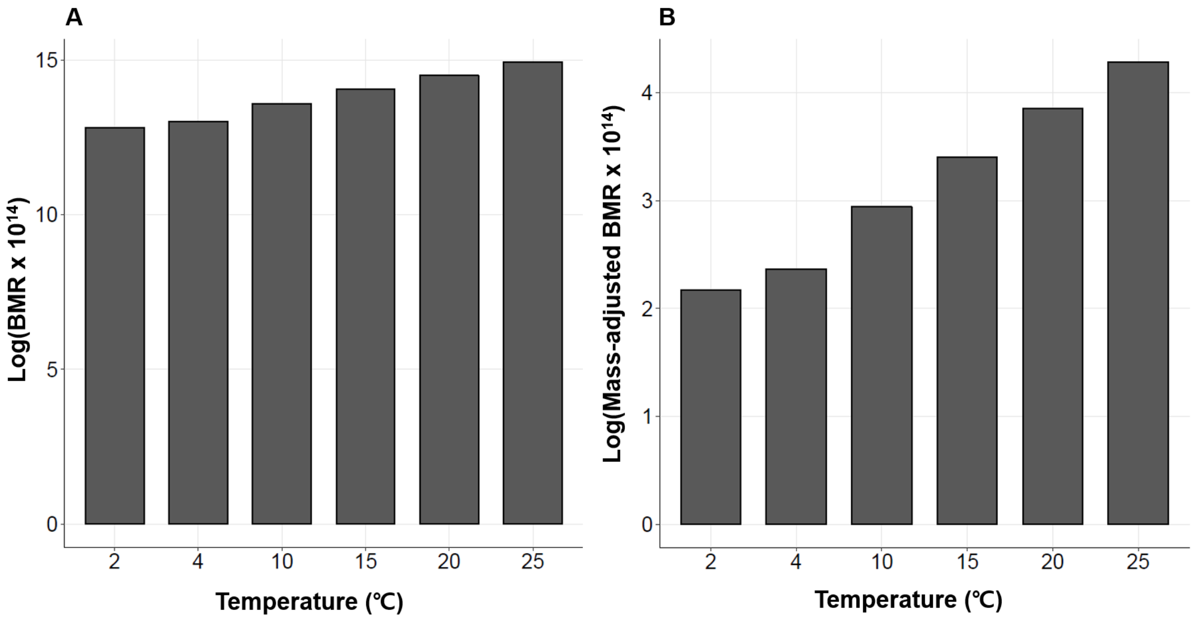


Figure S8. Changes of BMR and mass-adjusted BMR by temperature. Using the Gillooly's equation[40], we calculated the BMR (**A**) and the mass-adjusted BMR (**B**) using six temperatures selected within the range of temperatures at which whale shark (which dives to deep cold waters) is known to live. Both BMR and mass-adjusted BMR were multiplied by 10^{14} and log-transformed.

Table S17. Spearman's rho rank correlations between 22 properties in each *Eukaryota, Mammalia* and *Actinopterygii*

Property A	Property B	*Eukaryota*		*Mammalia*		*Actinopterygii*	
		Rho	*p*	Rho	*p*	Rho	*p*
Weight	Relative intron length	0.517	6.7E-07	0.385	7.5E-03	0.020	9.4E-01
Weight	mRNA length	0.094	4.0E-01	0.223	1.3E-01	0.042	8.7E-01
Weight	Intron length between first and last exons	0.482	5.2E-06	0.343	1.8E-02	-0.092	7.2E-01
Weight	Intron length between first and last coding exons	0.503	1.4E-06	0.351	1.6E-02	-0.084	7.4E-01
Weight	GC3	0.081	4.7E-01	0.067	6.5E-01	0.242	3.3E-01
Weight	GC contents	0.137	2.2E-01	0.079	6.0E-01	0.250	3.2E-01
Weight	Exon+Intron length between first and last exon	0.463	1.4E-05	0.342	1.9E-02	-0.080	7.5E-01
Weight	Exon+Intron length between first and last coding exon	0.510	1.0E-06	0.388	7.0E-03	-0.060	8.1E-01
Weight	Exon number	0.079	4.8E-01	0.183	2.2E-01	-0.071	7.8E-01
Weight	Exon length	-0.106	3.4E-01	0.174	2.4E-01	0.102	6.9E-01
Weight	Controlled intron length	-0.164	1.4E-01	0.309	3.5E-02	-0.344	1.6E-01
Weight	CDS length	0.074	5.1E-01	0.284	5.3E-02	-0.082	7.5E-01
Weight	CAI	0.109	3.3E-01	0.004	9.8E-01	-0.353	1.5E-01
Weight	5'UTR length	0.060	6.4E-01	-0.141	4.4E-01	-0.025	9.3E-01
Weight	3'UTR length	0.216	8.7E-02	0.029	8.7E-01	0.147	5.7E-01
Temperature	Weight	0.210	6.7E-02	0.392	6.5E-03	-0.409	9.2E-02
Temperature	Relative intron length	0.397	3.5E-04	0.161	2.8E-01	-0.064	8.0E-01
Temperature	mRNA length	-0.196	9.0E-02	0.181	2.2E-01	0.092	7.2E-01
Temperature	Intron length between first and last exons	0.399	3.5E-04	0.190	2.0E-01	0.033	9.0E-01
Temperature	Intron length between first and last coding exons	0.389	4.8E-04	0.187	2.1E-01	0.019	9.4E-01
Temperature	Genome size	0.381	6.4E-04	-0.028	8.5E-01	-0.206	4.1E-01
Temperature	GC3	-0.149	2.0E-01	0.169	2.6E-01	-0.495	3.7E-02
Temperature	GC contents	-0.069	5.5E-01	0.189	2.0E-01	-0.554	1.7E-02
Temperature	Exon+Intron length between first and last exon	0.289	1.1E-02	0.185	2.1E-01	0.054	8.3E-01
Temperature	Exon+Intron length between first and last coding exon	0.315	5.3E-03	0.187	2.1E-01	0.027	9.2E-01
Temperature	Exon number	-0.103	3.7E-01	0.172	2.5E-01	0.218	3.9E-01
Temperature	Exon length	-0.083	4.7E-01	0.106	4.8E-01	0.087	7.3E-01
Temperature	Controlled intron length	-0.155	1.8E-01	0.173	2.5E-01	0.327	1.9E-01
Temperature	CDS length	-0.155	1.8E-01	0.187	2.1E-01	0.423	8.0E-02
Temperature	CAI	0.249	2.9E-02	-0.152	3.1E-01	0.607	7.6E-03
Temperature	5'UTR length	-0.192	1.4E-01	0.077	6.8E-01	0.064	8.1E-01
Temperature	3'UTR length	0.022	8.7E-01	0.205	2.6E-01	-0.038	8.9E-01
Relative intron length	mRNA length	0.194	8.2E-02	0.737	3.4E-09	0.523	2.8E-02
Relative intron length	Intron length between first and last exons	0.944	0.0E+00	0.914	0.0E+00	0.975	8.4E-06
Relative intron length	GC3	-0.207	6.2E-02	-0.280	5.7E-02	-0.447	6.5E-02
Relative intron length	GC contents	-0.101	3.7E-01	-0.178	2.3E-01	-0.391	1.1E-01
Relative intron length	Exon+Intron length between first and last exon	0.874	0.0E+00	0.882	0.0E+00	0.959	5.6E-06
Relative intron length	Exon+Intron length between first and last coding exon	0.917	0.0E+00	0.891	0.0E+00	0.971	7.8E-06
Relative intron length	Exon number	-0.093	4.1E-01	0.197	1.9E-01	-0.379	1.2E-01
Relative intron length	Exon length	-0.032	7.8E-01	0.579	2.0E-05	0.556	1.7E-02
Relative intron length	Controlled intron length	-0.074	5.1E-01	0.713	9.6E-08	0.321	1.9E-01
Relative intron length	CDS length	0.076	5.0E-01	0.664	3.6E-07	0.259	3.0E-01
Relative intron length	CAI	0.497	2.8E-06	0.451	1.6E-03	0.302	2.2E-01
Relative intron length	5'UTR length	0.214	9.0E-02	0.368	3.8E-02	0.478	5.4E-02
Relative intron length	3'UTR length	0.407	8.4E-04	0.327	6.9E-02	0.495	4.5E-02
mRNA length	GC3	0.091	4.2E-01	0.011	9.4E-01	-0.292	2.4E-01
mRNA length	GC contents	0.085	4.5E-01	0.128	3.9E-01	-0.255	3.1E-01
mRNA length	Exon number	0.371	6.6E-04	0.294	4.5E-02	-0.122	6.3E-01
mRNA length	Exon length	0.618	8.2E-10	0.830	5.2E-13	0.783	1.2E-04
mRNA length	CDS length	0.836	2.7E-22	0.815	3.1E-12	0.724	6.8E-04
mRNA length	CAI	-0.021	8.5E-01	0.079	6.0E-01	0.230	3.6E-01
mRNA length	5'UTR length	0.705	7.9E-11	0.451	9.6E-03	0.583	1.6E-02
mRNA length	3'UTR length	0.615	6.6E-08	0.404	2.2E-02	0.581	1.6E-02

Maximum lifespan	Weight	0.791	1.0E-18	0.720	1.2E-08	0.801	6.4E-05
Maximum lifespan	Temperature	0.246	3.1E-02	0.049	7.4E-01	-0.239	3.4E-01
Maximum lifespan	Relative intron length	0.581	1.1E-08	0.399	5.5E-03	-0.076	7.7E-01
Maximum lifespan	mRNA length	0.054	6.3E-01	0.278	5.9E-02	-0.181	4.7E-01
Maximum lifespan	Mass adjusted BMR	-0.553	2.2E-07	-0.748	1.6E-09	-0.718	1.2E-03
Maximum lifespan	Intron length between first and last exons	0.523	5.3E-07	0.340	1.9E-02	-0.150	5.5E-01
Maximum lifespan	Intron length between first and last coding exons	0.554	6.7E-08	0.357	1.4E-02	-0.154	5.4E-01
Maximum lifespan	Genome size	0.407	1.5E-04	-0.151	3.1E-01	0.097	7.0E-01
Maximum lifespan	GC3	-0.023	8.4E-01	0.059	7.0E-01	-0.017	9.5E-01
Maximum lifespan	GC contents	0.056	6.2E-01	0.111	4.6E-01	-0.031	9.0E-01
Maximum lifespan	Exon+Intron length between first and last exon	0.494	2.8E-06	0.380	8.4E-03	-0.151	5.5E-01
Maximum lifespan	Exon+Intron length between first and last coding exon	0.557	5.6E-08	0.421	3.3E-03	-0.118	6.4E-01
Maximum lifespan	Exon number	0.064	5.7E-01	0.243	9.9E-02	-0.071	7.8E-01
Maximum lifespan	Exon length	-0.182	1.0E-01	0.160	2.8E-01	-0.121	6.3E-01
Maximum lifespan	Controlled maximum lifespan	0.349	1.3E-03	0.254	8.5E-02	0.162	5.2E-01
Maximum lifespan	Controlled intron length	-0.118	2.9E-01	0.362	1.3E-02	-0.161	5.2E-01
Maximum lifespan	CDS length	0.035	7.5E-01	0.329	2.4E-02	-0.196	4.4E-01
Maximum lifespan	CAI	0.253	2.2E-02	0.043	7.8E-01	-0.112	6.6E-01
Maximum lifespan	Basal metabolic rate	0.756	2.7E-15	0.709	2.5E-08	0.813	7.3E-05
Maximum lifespan	5'UTR length	-0.018	8.9E-01	-0.143	4.4E-01	-0.133	6.1E-01
Maximum lifespan	3'UTR length	0.145	2.5E-01	-0.098	5.9E-01	0.058	8.3E-01
Mass adjusted BMR	Weight	-0.863	1.3E-23	-0.981	9.3E-34	-0.957	1.8E-09
Mass adjusted BMR	Temperature	0.275	1.6E-02	-0.246	9.6E-02	0.672	3.2E-03
Mass adjusted BMR	Relative intron length	-0.142	2.2E-01	-0.378	9.2E-03	0.032	9.1E-01
Mass adjusted BMR	mRNA length	-0.090	4.4E-01	-0.209	1.6E-01	0.125	6.3E-01
Mass adjusted BMR	Intron length between first and last exons	-0.124	2.9E-01	-0.333	2.3E-02	0.091	7.3E-01
Mass adjusted BMR	Intron length between first and last coding exons	-0.149	2.0E-01	-0.341	2.0E-02	0.081	7.6E-01
Mass adjusted BMR	Genome size	-0.005	9.7E-01	0.064	6.7E-01	-0.289	2.6E-01
Mass adjusted BMR	GC3	-0.093	4.3E-01	-0.058	7.0E-01	-0.304	2.4E-01
Mass adjusted BMR	GC contents	-0.101	3.9E-01	-0.072	6.3E-01	-0.331	1.9E-01
Mass adjusted BMR	Exon+Intron length between first and last exon	-0.161	1.7E-01	-0.328	2.5E-02	0.100	7.0E-01
Mass adjusted BMR	Exon+Intron length between first and last coding exon	-0.201	8.2E-02	-0.376	9.6E-03	0.066	8.0E-01
Mass adjusted BMR	Exon number	0.012	9.2E-01	-0.159	2.9E-01	0.184	4.8E-01
Mass adjusted BMR	Exon length	-0.094	4.2E-01	-0.156	3.0E-01	-0.006	9.8E-01
Mass adjusted BMR	Controlled intron length	-0.063	5.9E-01	-0.290	4.9E-02	0.478	5.4E-02
Mass adjusted BMR	CDS length	-0.074	5.2E-01	-0.264	7.3E-02	0.275	2.9E-01
Mass adjusted BMR	CAI	0.091	4.3E-01	-0.006	9.7E-01	0.463	6.3E-02
Mass adjusted BMR	5'UTR length	0.062	6.4E-01	0.130	4.8E-01	0.109	6.9E-01
Mass adjusted BMR	3'UTR length	-0.011	9.3E-01	-0.027	8.9E-01	-0.062	8.2E-01
Intron length between first and last exons	mRNA length	0.424	7.8E-05	0.896	1.9E-17	0.554	1.9E-02
Intron length between first and last exons	GC3	-0.165	1.4E-01	-0.104	4.9E-01	-0.546	2.1E-02
Intron length between first and last exons	GC contents	-0.062	5.8E-01	0.005	9.7E-01	-0.494	3.9E-02
Intron length between first and last exons	Exon+Intron length between first and last exon	0.970	0.0E+00	0.966	0.0E+00	0.988	9.8E-06
Intron length between first and last exons	Exon+Intron length between first and last coding exon	0.979	0.0E+00	0.944	0.0E+00	0.994	1.0E-05
Intron length between first and last exons	Exon number	0.089	4.3E-01	0.339	2.0E-02	-0.363	1.4E-01
Intron length between first and last exons	Exon length	0.155	1.7E-01	0.765	3.9E-10	0.580	1.2E-02
Intron length between first and last exons	CDS length	0.337	2.1E-03	0.821	1.7E-12	0.336	1.7E-01
Intron length between first and last exons	CAI	0.440	4.8E-05	0.261	7.7E-02	0.401	1.0E-01
Intron length between first and last exons	5'UTR length	0.269	3.2E-02	0.407	2.1E-02	0.493	4.7E-02
Intron length between first and last exons	3'UTR length	0.465	1.1E-04	0.367	3.9E-02	0.490	4.8E-02
Intron length between first and last coding exons	Relative intron length	0.960	0.0E+00	0.930	0.0E+00	0.979	8.9E-06
Intron length between first and last coding exons	mRNA length	0.372	6.3E-04	0.862	7.7E-15	0.544	2.1E-02

Intron length between first and last coding exons	Intron length between first and last exons	0.995	0.0E+00	0.990	0.0E+00	0.998	1.1E-05
Intron length between first and last coding exons	GC3	-0.188	9.2E-02	-0.130	3.8E-01	-0.513	3.1E-02
Intron length between first and last coding exons	GC contents	-0.084	4.6E-01	-0.029	8.5E-01	-0.461	5.6E-02
Intron length between first and last coding exons	Exon+Intron length between first and last exon	0.954	0.0E+00	0.949	0.0E+00	0.986	9.6E-06
Intron length between first and last coding exons	Exon+Intron length between first and last coding exon	0.976	0.0E+00	0.945	0.0E+00	0.996	1.0E-05
Intron length between first and last coding exons	Exon number	0.039	7.3E-01	0.341	1.9E-02	-0.334	1.8E-01
Intron length between first and last coding exons	Exon length	0.127	2.6E-01	0.752	1.1E-09	0.572	1.3E-02
Intron length between first and last coding exons	Controlled intron length	0.076	5.0E-01	0.833	0.0E+00	0.397	1.0E-01
Intron length between first and last coding exons	CDS length	0.256	2.0E-02	0.821	1.7E-12	0.338	1.7E-01
Intron length between first and last coding exons	CAI	0.463	1.5E-05	0.289	4.9E-02	0.366	1.4E-01
Intron length between first and last coding exons	5'UTR length	0.240	5.6E-02	0.353	4.8E-02	0.493	4.7E-02
Intron length between first and last coding exons	3'UTR length	0.435	3.2E-04	0.309	8.6E-02	0.490	4.8E-02
Genome size	Weight	0.419	8.9E-05	-0.078	6.0E-01	0.193	4.4E-01
Genome size	Relative intron length	0.707	0.0E+00	0.132	3.7E-01	0.697	1.8E-03
Genome size	mRNA length	-0.123	2.7E-01	-0.122	4.1E-01	0.216	3.9E-01
Genome size	Intron length between first and last exons	0.572	4.5E-08	-0.007	9.6E-01	0.678	2.6E-03
Genome size	Intron length between first and last coding exons	0.595	6.5E-09	0.020	8.9E-01	0.662	3.6E-03
Genome size	GC3	-0.125	2.6E-01	-0.473	9.0E-04	-0.486	4.3E-02
Genome size	GC contents	-0.042	7.1E-01	-0.434	2.5E-03	-0.418	8.6E-02
Genome size	Exon+Intron length between first and last exon	0.450	3.0E-05	-0.083	5.8E-01	0.643	5.0E-03
Genome size	Exon+Intron length between first and last coding exon	0.489	4.3E-06	-0.106	4.8E-01	0.631	6.1E-03
Genome size	Exon number	-0.267	1.5E-02	-0.339	2.0E-02	-0.804	5.8E-05
Genome size	Exon length	-0.324	3.0E-03	-0.295	4.4E-02	0.401	9.9E-02
Genome size	Controlled intron length	-0.610	1.3E-09	-0.456	1.4E-03	-0.327	1.9E-01
Genome size	CDS length	-0.284	9.7E-03	-0.338	2.0E-02	-0.158	5.3E-01
Genome size	CAI	0.327	2.8E-03	0.356	1.4E-02	0.333	1.8E-01
Genome size	5'UTR length	0.238	5.8E-02	0.431	1.4E-02	0.238	3.6E-01
Genome size	3'UTR length	0.365	3.0E-03	0.407	2.2E-02	0.216	4.0E-01
GC3	Exon number	0.447	2.5E-05	0.530	1.3E-04	0.421	8.2E-02
GC3	Exon length	-0.121	2.8E-01	-0.002	9.9E-01	-0.248	3.2E-01
GC3	CDS length	0.216	5.1E-02	0.216	1.5E-01	-0.237	3.4E-01
GC3	CAI	-0.841	0.0E+00	-0.895	0.0E+00	-0.936	0.0E+00
GC3	5'UTR length	0.012	9.2E-01	-0.309	8.5E-02	-0.314	2.2E-01
GC3	3'UTR length	-0.093	4.7E-01	-0.250	1.7E-01	-0.265	3.0E-01
GC contents	GC3	0.975	0.0E+00	0.969	0.0E+00	0.992	1.0E-05
GC contents	Exon number	0.444	2.9E-05	0.598	8.9E-06	0.375	1.3E-01
GC contents	Exon length	-0.133	2.3E-01	0.046	7.6E-01	-0.216	3.9E-01
GC contents	CDS length	0.193	8.2E-02	0.297	4.3E-02	-0.264	2.9E-01
GC contents	CAI	-0.799	0.0E+00	-0.855	0.0E+00	-0.948	3.2E-06
GC contents	5'UTR length	0.013	9.2E-01	-0.195	2.9E-01	-0.277	2.8E-01
GC contents	3'UTR length	-0.050	7.0E-01	-0.131	4.7E-01	-0.223	3.9E-01
Exon+Intron length between first and last exon	mRNA length	0.579	1.5E-08	0.930	4.0E-21	0.600	9.9E-03
Exon+Intron length between first and last exon	GC3	-0.125	2.6E-01	-0.036	8.1E-01	-0.554	1.9E-02
Exon+Intron length between first and last exon	GC contents	-0.034	7.7E-01	0.078	6.0E-01	-0.505	3.5E-02
Exon+Intron length between first and last exon	Exon number	0.188	9.3E-02	0.368	1.1E-02	-0.354	1.5E-01
Exon+Intron length between first and last exon	Exon length	0.255	2.2E-02	0.776	1.5E-10	0.564	1.5E-02
Exon+Intron length between first and last exon	CDS length	0.490	3.4E-06	0.862	7.6E-15	0.352	1.5E-01

Exon+Intron length between first and last exon	CAI	0.383	4.5E-04	0.199	1.8E-01	0.408	9.4E-02
Exon+Intron length between first and last exon	5'UTR length	0.331	7.5E-03	0.383	3.1E-02	0.478	5.4E-02
Exon+Intron length between first and last exon	3'UTR length	0.501	2.4E-05	0.338	5.9E-02	0.480	5.3E-02
Exon+Intron length between first and last coding exon	mRNA length	0.452	2.3E-05	0.835	2.9E-13	0.558	1.8E-02
Exon+Intron length between first and last coding exon	GC3	-0.145	1.9E-01	-0.005	9.8E-01	-0.529	2.6E-02
Exon+Intron length between first and last coding exon	GC contents	-0.042	7.1E-01	0.102	4.9E-01	-0.480	4.6E-02
Exon+Intron length between first and last coding exon	Exon+Intron length between first and last exon	0.981	0.0E+00	0.964	0.0E+00	0.990	9.9E-06
Exon+Intron length between first and last coding exon	Exon number	0.148	1.9E-01	0.497	3.8E-04	-0.307	2.2E-01
Exon+Intron length between first and last coding exon	Exon length	0.165	1.4E-01	0.697	5.4E-08	0.564	1.5E-02
Exon+Intron length between first and last coding exon	CDS length	0.370	6.3E-04	0.887	1.1E-16	0.366	1.4E-01
Exon+Intron length between first and last coding exon	CAI	0.419	1.1E-04	0.215	1.5E-01	0.375	1.3E-01
Exon+Intron length between first and last coding exon	5'UTR length	0.246	5.0E-02	0.285	1.1E-01	0.488	4.9E-02
Exon+Intron length between first and last coding exon	3'UTR length	0.441	2.7E-04	0.256	1.6E-01	0.498	4.4E-02
Exon number	Exon length	-0.074	5.1E-01	0.220	1.4E-01	-0.265	2.9E-01
Exon number	CDS length	0.643	7.6E-11	0.604	7.1E-06	0.303	2.2E-01
Exon number	5'UTR length	0.215	8.8E-02	-0.038	8.4E-01	0.172	5.1E-01
Exon number	3'UTR length	0.219	8.2E-02	0.009	9.6E-01	0.193	4.6E-01
Exon length	CDS length	0.518	6.1E-07	0.801	1.4E-11	0.701	1.2E-03
Exon length	5'UTR length	0.170	1.8E-01	0.310	8.4E-02	0.334	1.9E-01
Exon length	3'UTR length	0.088	4.9E-01	0.260	1.5E-01	0.312	2.2E-01
Controlled maximum lifespan	Weight	-0.152	1.7E-01	-0.397	5.7E-03	-0.191	4.5E-01
Controlled maximum lifespan	Temperature	0.036	7.5E-01	-0.411	4.1E-03	0.223	3.7E-01
Controlled maximum lifespan	Relative intron length	0.178	1.1E-01	-0.077	6.1E-01	0.056	8.3E-01
Controlled maximum lifespan	mRNA length	0.077	5.0E-01	-0.060	6.9E-01	0.021	9.4E-01
Controlled maximum lifespan	Mass adjusted BMR	0.467	2.1E-05	0.351	1.6E-02	0.484	4.9E-02
Controlled maximum lifespan	Intron length between first and last exons	0.182	1.1E-01	-0.124	4.0E-01	0.007	9.8E-01
Controlled maximum lifespan	Intron length between first and last coding exons	0.158	1.6E-01	-0.124	4.1E-01	-0.009	9.7E-01
Controlled maximum lifespan	Genome size	0.148	1.8E-01	0.025	8.7E-01	-0.165	5.1E-01
Controlled maximum lifespan	GC3	-0.064	5.7E-01	-0.096	5.2E-01	-0.210	4.0E-01
Controlled maximum lifespan	GC contents	-0.029	8.0E-01	-0.022	8.8E-01	-0.212	4.0E-01
Controlled maximum lifespan	Exon+Intron length between first and last exon	0.183	1.0E-01	-0.059	6.9E-01	0.023	9.3E-01
Controlled maximum lifespan	Exon+Intron length between first and last coding exon	0.162	1.5E-01	-0.053	7.3E-01	0.011	9.6E-01
Controlled maximum lifespan	Exon number	0.187	9.2E-02	0.036	8.1E-01	0.086	7.4E-01
Controlled maximum lifespan	Exon length	-0.296	6.9E-03	-0.188	2.1E-01	-0.168	5.1E-01
Controlled maximum lifespan	Controlled intron length	-0.028	8.1E-01	-0.081	5.9E-01	0.402	9.9E-02
Controlled maximum lifespan	CDS length	0.076	5.0E-01	-0.058	7.0E-01	-0.066	7.9E-01
Controlled maximum lifespan	CAI	0.240	3.0E-02	0.107	4.7E-01	0.240	3.4E-01
Controlled maximum lifespan	Basal metabolic rate	-0.395	4.2E-04	-0.410	4.5E-03	-0.338	1.8E-01
Controlled maximum lifespan	5'UTR length	0.159	2.1E-01	0.075	6.9E-01	0.039	8.8E-01
Controlled maximum lifespan	3'UTR length	0.170	1.8E-01	-0.069	7.1E-01	0.093	7.2E-01
Controlled intron length	mRNA length	0.459	1.6E-05	0.791	3.7E-11	0.414	8.9E-02
Controlled intron length	Intron length between first and last exons	0.083	4.6E-01	0.842	0.0E+00	0.393	1.1E-01
Controlled intron length	GC3	-0.235	3.4E-02	0.139	3.5E-01	-0.302	2.2E-01
Controlled intron length	GC contents	-0.257	2.0E-02	0.201	1.8E-01	-0.321	1.9E-01
Controlled intron length	Exon+Intron length between first and last exon	0.198	7.6E-02	0.853	0.0E+00	0.430	7.6E-02
Controlled intron length	Exon+Intron length between first and last coding exon	0.173	1.2E-01	0.869	0.0E+00	0.439	7.0E-02
Controlled intron length	Exon number	0.296	6.9E-03	0.483	5.9E-04	0.499	3.5E-02
Controlled intron length	Exon length	0.513	8.1E-07	0.808	6.4E-12	0.186	4.6E-01
Controlled intron length	CDS length	0.512	8.9E-07	0.915	2.7E-19	0.578	1.2E-02
Controlled intron length	CAI	0.136	2.2E-01	0.061	6.8E-01	0.313	2.1E-01
Controlled intron length	5'UTR length	0.035	7.8E-01	0.074	6.9E-01	0.426	8.9E-02
Controlled intron length	3'UTR length	0.084	5.1E-01	0.053	7.7E-01	0.453	6.9E-02
CDS length	5'UTR length	0.407	8.5E-04	0.107	5.6E-01	0.511	3.6E-02

CDS length	3'UTR length	0.373	2.4E-03	0.140	4.5E-01	0.451	6.9E-02
CAI	Exon number	-0.301	6.1E-03	-0.336	2.1E-02	-0.293	2.4E-01
CAI	Exon length	-0.019	8.7E-01	0.063	6.7E-01	0.166	5.1E-01
CAI	CDS length	-0.118	2.9E-01	-0.010	9.5E-01	0.235	3.5E-01
CAI	5'UTR length	0.009	9.4E-01	0.279	1.2E-01	0.235	3.6E-01
CAI	3'UTR length	0.171	1.8E-01	0.249	1.7E-01	0.152	5.6E-01
Basal metabolic rate	Weight	0.958	6.8E-42	0.997	6.4E-52	0.920	1.7E-07
Basal metabolic rate	Temperature	0.401	3.4E-04	0.444	1.8E-03	-0.221	3.9E-01
Basal metabolic rate	Relative intron length	0.489	9.6E-06	0.386	7.7E-03	-0.184	4.8E-01
Basal metabolic rate	mRNA length	-0.092	4.3E-01	0.230	1.2E-01	-0.098	7.1E-01
Basal metabolic rate	Mass adjusted BMR	-0.709	0.0E+00	-0.970	0.0E+00	-0.811	1.0E-04
Basal metabolic rate	Intron length between first and last exons	0.428	1.5E-04	0.347	1.7E-02	-0.191	4.6E-01
Basal metabolic rate	Intron length between first and last coding exons	0.462	3.3E-05	0.355	1.5E-02	-0.186	4.7E-01
Basal metabolic rate	Genome size	0.356	1.7E-03	-0.082	5.9E-01	0.100	7.0E-01
Basal metabolic rate	GC3	-0.097	4.0E-01	0.079	6.0E-01	0.005	9.9E-01
Basal metabolic rate	GC contents	-0.024	8.4E-01	0.090	5.5E-01	-0.010	9.7E-01
Basal metabolic rate	Exon+Intron length between first and last exon	0.378	8.9E-04	0.346	1.8E-02	-0.154	5.5E-01
Basal metabolic rate	Exon+Intron length between first and last coding exon	0.452	5.0E-05	0.394	6.5E-03	-0.137	6.0E-01
Basal metabolic rate	Exon number	-0.121	3.0E-01	0.190	2.0E-01	-0.031	9.1E-01
Basal metabolic rate	Exon length	0.013	9.1E-01	0.177	2.3E-01	-0.058	8.3E-01
Basal metabolic rate	Controlled intron length	-0.108	3.5E-01	0.316	3.1E-02	-0.279	2.8E-01
Basal metabolic rate	CDS length	-0.075	5.2E-01	0.294	4.5E-02	-0.037	8.9E-01
Basal metabolic rate	CAI	0.164	1.6E-01	-0.006	9.7E-01	-0.103	6.9E-01
Basal metabolic rate	5'UTR length	-0.170	2.0E-01	-0.127	4.9E-01	-0.129	6.3E-01
Basal metabolic rate	3'UTR length	0.044	7.4E-01	0.043	8.2E-01	0.015	9.6E-01
5'UTR length	3'UTR length	0.887	1.6E-22	0.913	3.4E-13	0.956	0.0E+00

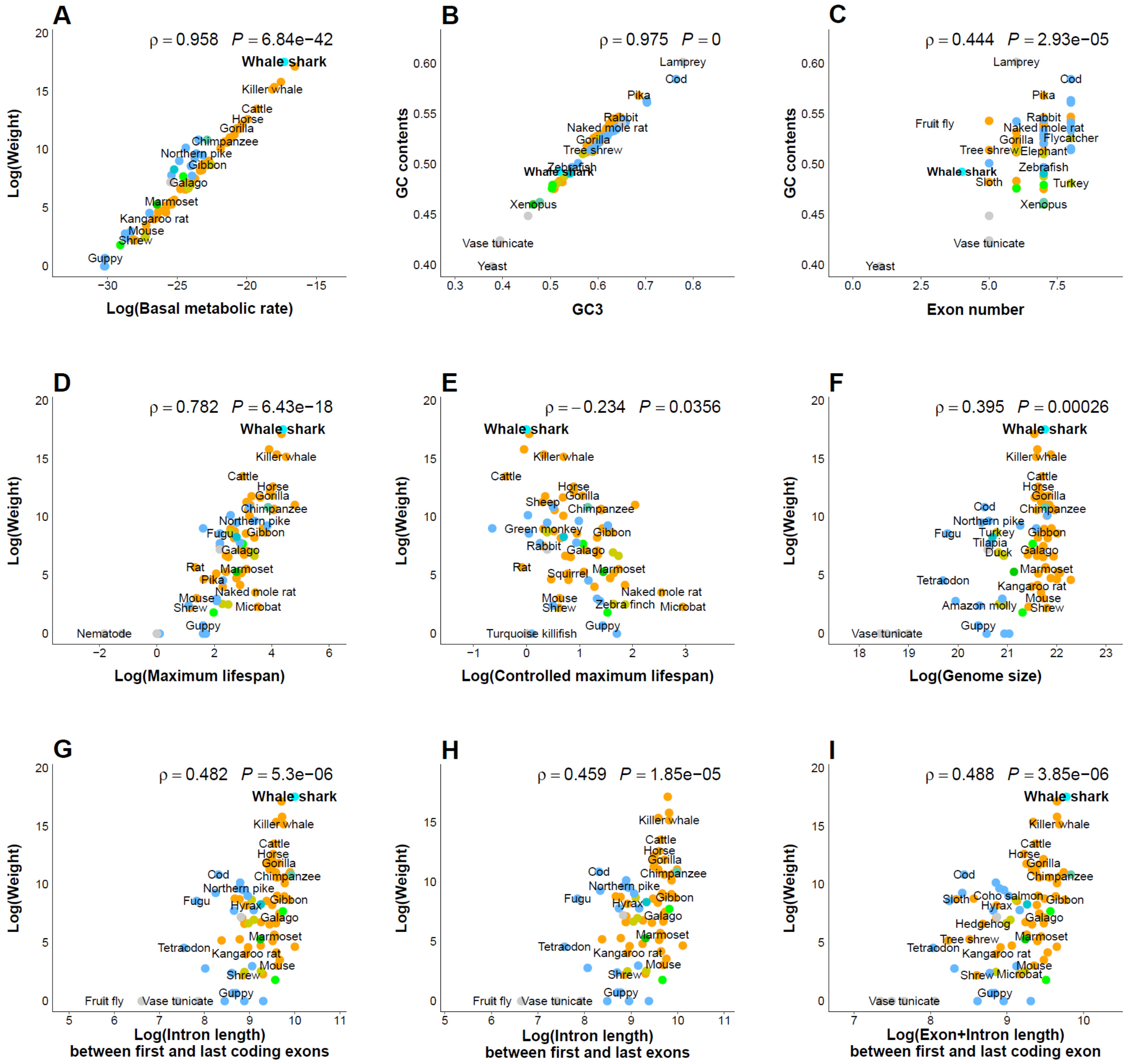


Figure S9. Scaling relationships between genomic and physiologic properties across 82 species. Extended data from Figure 2. The properties on both x-axis and y-axis were used to calculate Spearman's rank correlation coefficient for each plot. All *p*-values and rho values are shown in top of each plot. The general species names are centered over their dots. Overlapping species names in the same layer were not plotted. The nine colors of dots indicate biological classification (gray: Hyperoartia, Ascidiacea, Chromadorea, Insecta and Saccharomycetes, turquoise: Chondrichthyes (cyan: whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

3.3 Intron gain or loss

From the single-copy orthologous gene sets, CDS of each orthologous family were aligned using MUSCLE (version 3.8.31)[41]. Exon-exon boundaries as intron positions were marked on the CDS alignments. The intron positions within a permissible length (six bp), which are considered as alternative splice sites, were aligned. The aligned intron position was converted to a binary character matrix as an input table of the Malin program[42], which was used to calculate the "intron gain or loss" using Dollo parsimony (Figure S10).

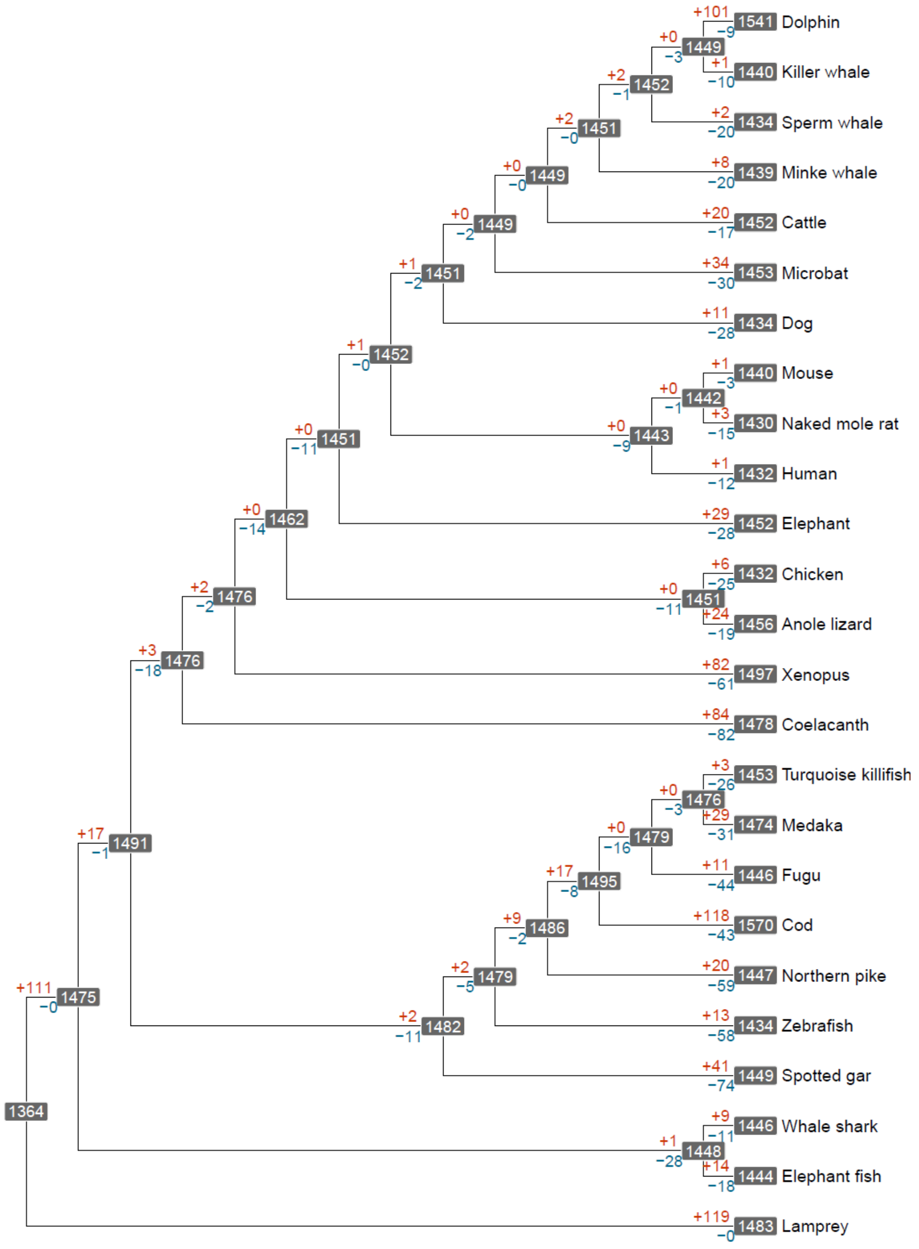


Figure S10. Intron gain or loss in the single-copy orthologous gene group. The phylogenetic tree was derived from Figure 3D. The intron gains and losses were computed by Dollo parsimony using Malin[42]. The numbers in the gray boxes indicate the number of introns. Red and blue numbers indicate the number of gained and lost introns from the most common ancestor, respectively.

3.4 Prediction of repetitive elements within introns

To compare intronic repetitive elements in each species, we constructed consensus models of putative interspersed repeats by RepeatModeler (version 1.0.10)[7]. Using RepeatMasker (version 4.0.5)[6], we then predicted repeat elements in the introns of 81 species (yeast is excluded from our 82 species set) with the '-no_is -cutoff 255 -frag 20000' options. The predicted repetitive elements containing domains that overlapped with other repeats (higher-scoring match), which were denoted by asterisk in the RepeatMasker result file, were filtered out.

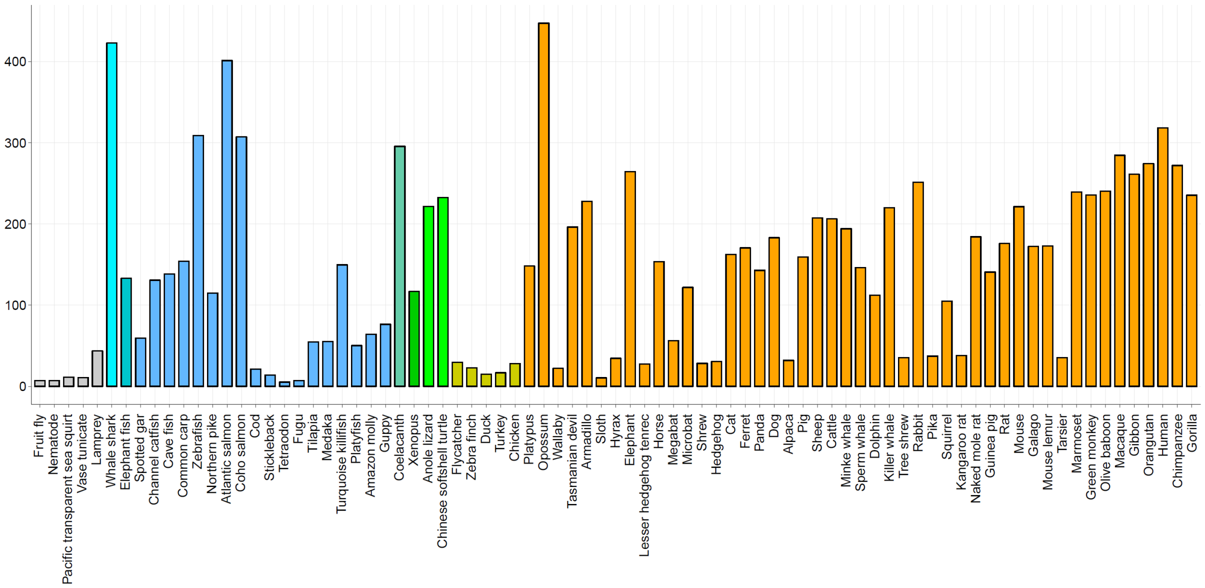


Figure S11. Total length of repetitive elements in the introns of 81 species. The total length was summed across ten repetitive elements: SINEs, LINEs, LTR elements, DNA elements, unclassified elements, interspersed repeats, small RNA, satellites, simple repeats, and low complexity region. Colors indicate the species class as in Figure 1. Yeast is excluded from 82 species.

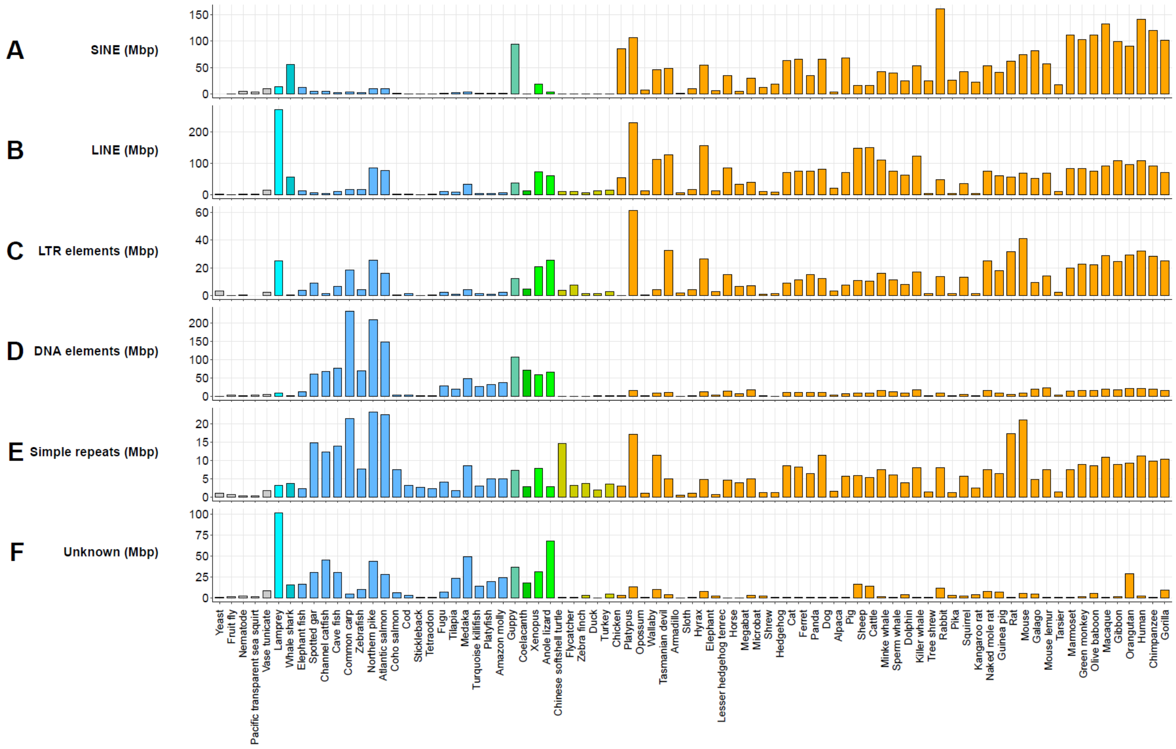


Figure S12. Total length of six repetitive elements in the introns of 81 species. The bold text to the left of the plots indicates the type of repeat element. Colors indicate the species class as in Figure 1. Yeast is excluded from 82 species.

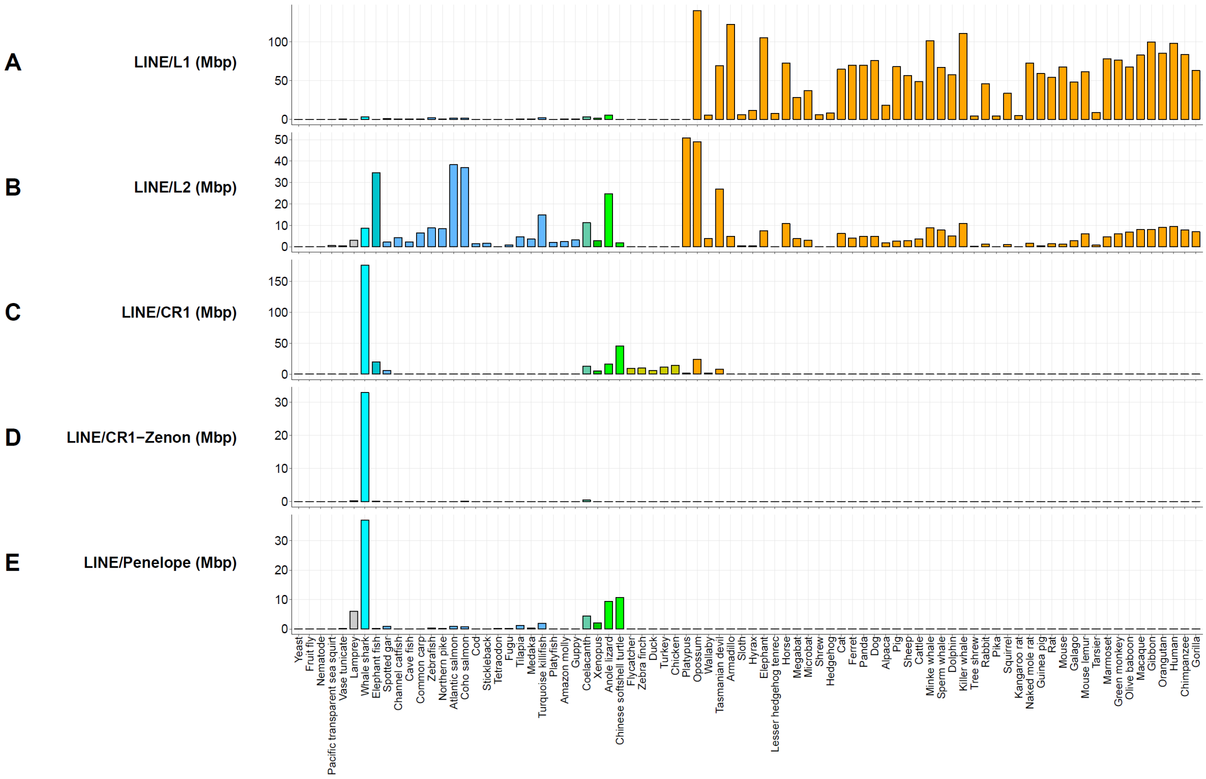


Figure S13. Total length of five LINEs in the introns of 81 species. The bold text to the left of the plots indicates the type of repeat elements. Colors indicate the species class as in Figure 1. Yeast is excluded from 82 species.

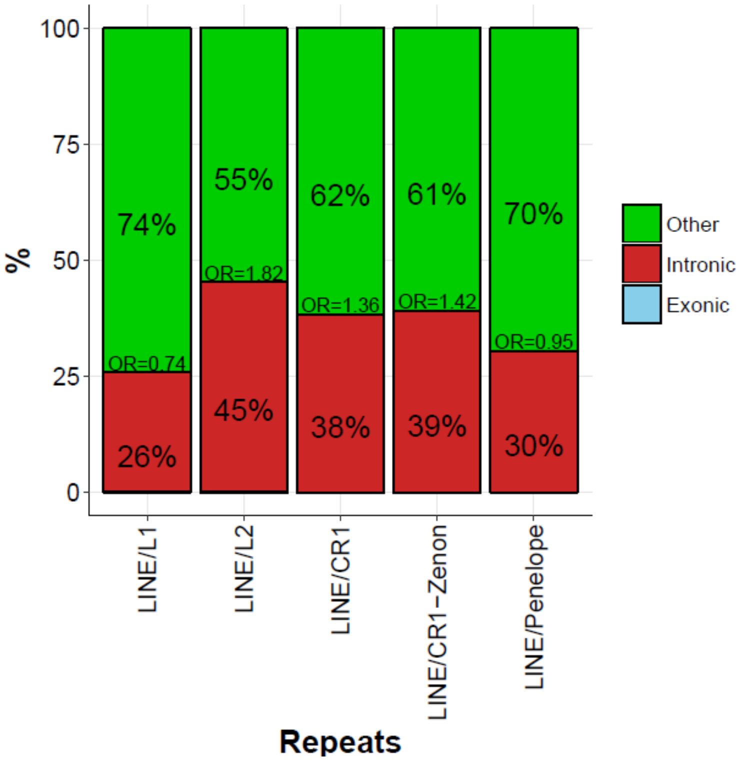


Figure S14. Distribution of LINEs in the whale shark genome. Using the non-redundant predicted gene model, we analyzed the proportion of LINE elements across the intronic, exonic, and intergenic regions. The bar plots show the percentage of five repetitive elements in each of the three regions. The actual percentages are shown in middle of the bars. Odds ratio analyses of the five LINE elements across exonic, intronic, and intergenic regions within the whale shark showed a slightly higher representation of these elements within introns, though these findings lacked statistical significance (*p*-value calculated by chi-square test > 0.05, Figure S14). The odd ratios are listed in the middle of the bars, and are the ratio of the proportion of repetitive elements in the intronic region to the proportion of the intronic region in the whale shark genome.

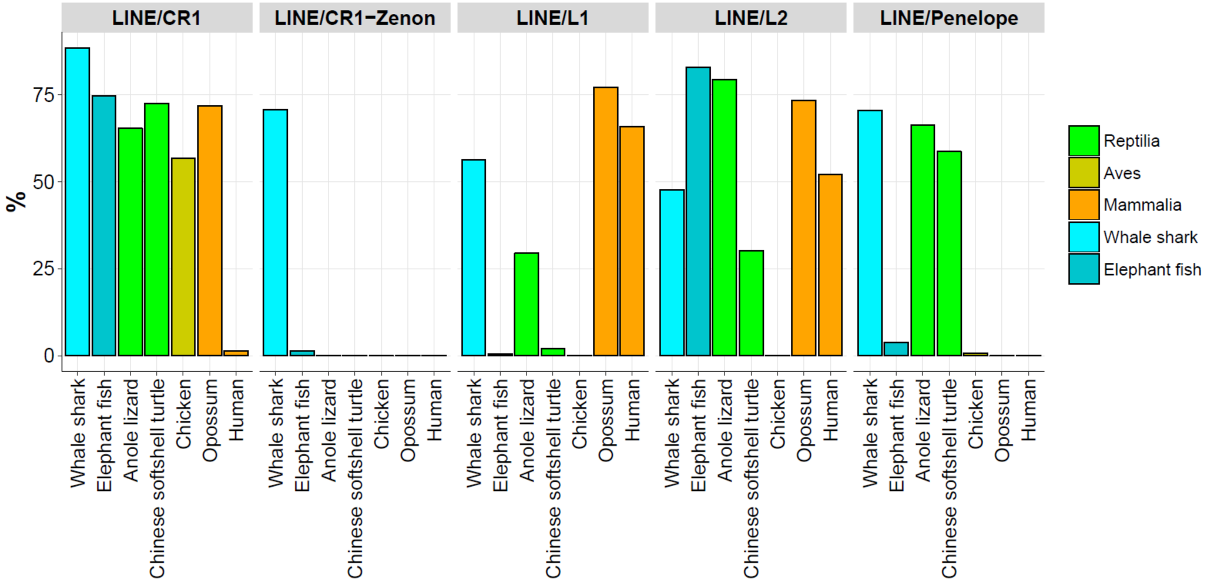


Figure S15. Proportion of genes containing LINEs in their introns. Each bar plot shows the proportion of genes which have repetitive elements (element types are shown in gray boxes) in the genome of each species (x-axis).

3.5 Synonymous codon usage comparison

We measured relative synonymous codon usage (RSCU) using Sharp *et. al.*'s method[43] in each of the 82 species. A principal component analysis (PCA) on the RSCU was performed using the R packages (version 3.3.0)[44] ggplot2[45] and ggfortify[46] (Figure S16).

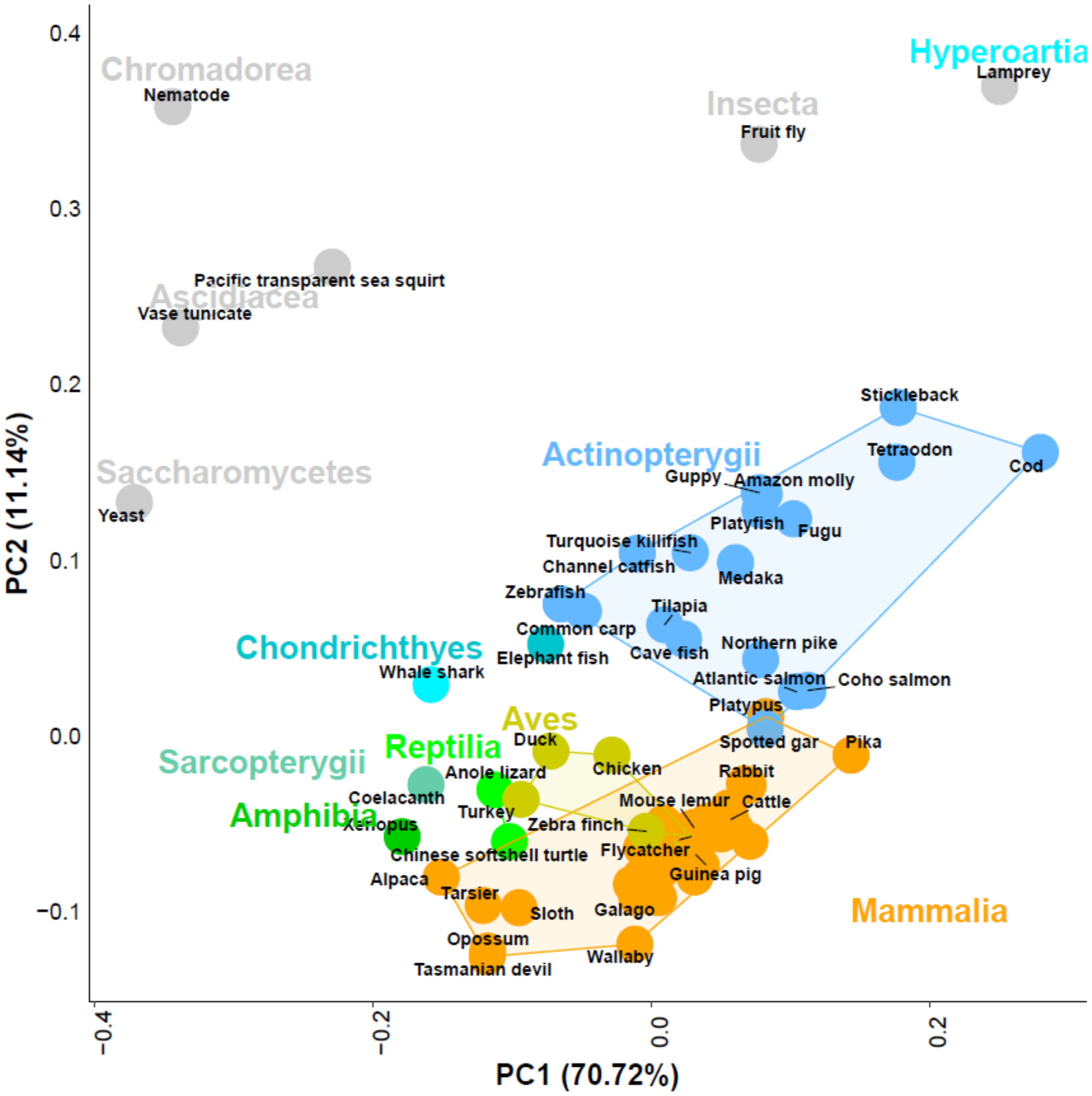


Figure S16A. Principal component analysis of relative synonymous codon usage of 82 species. The common species name is shown in black text over a colored circle. Each color shows the class of 82 species (gray: Hyperoartia, Ascidiacea, Chromadorea, Insecta and Saccharomycetes, turquoise: Chondrichthyes (cyan: whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

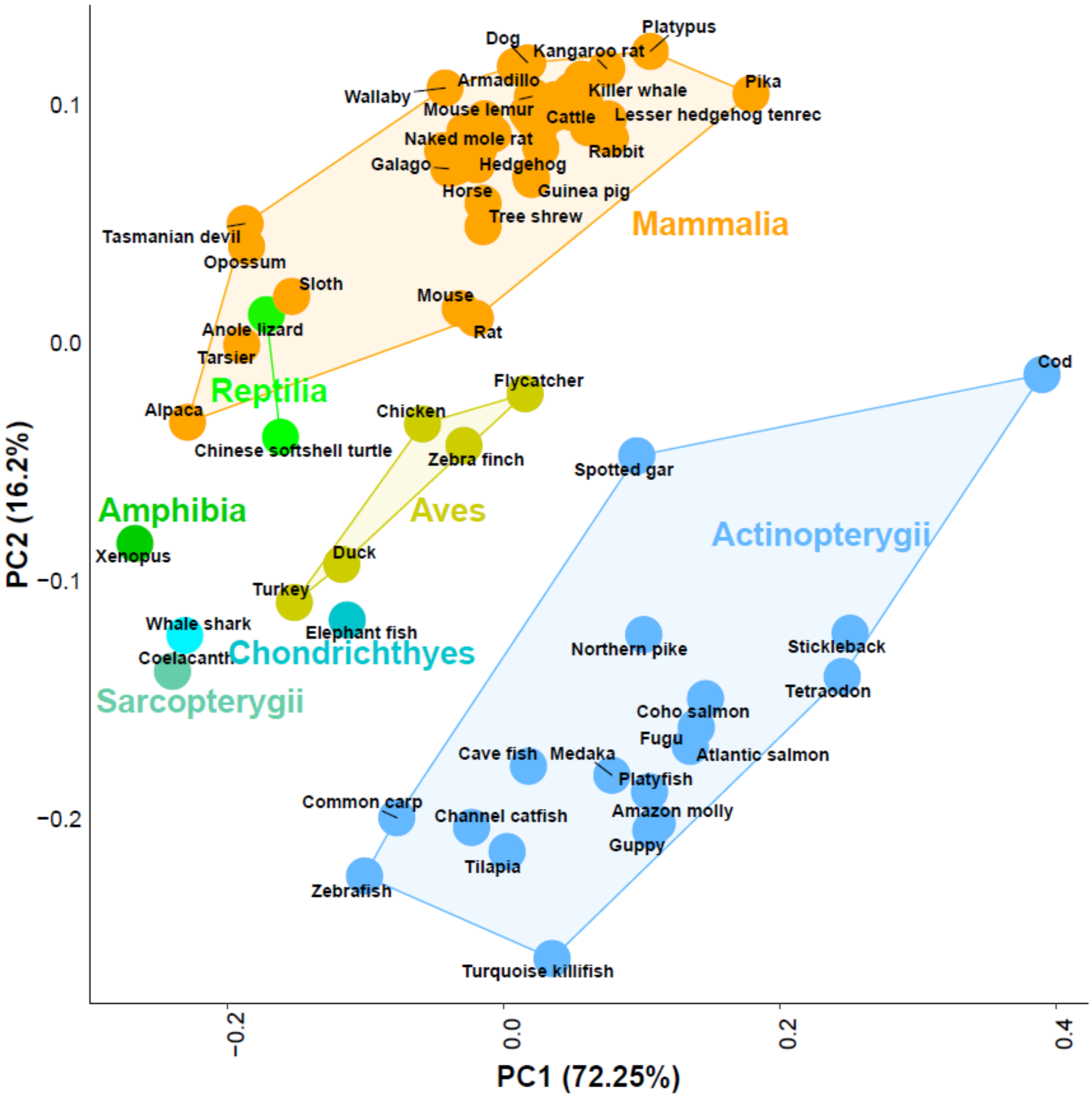


Figure S16B. Principal component analysis of relative synonymous codon usage of 76 species. Six species having distant codon usage pattern were excluded from comparison of 82 species to investigate the evolutionary history of codon usage from whale shark to human. Common species name is shown in black text over a colored circle. Each color of circles shows the class of 76 species (turquoise: Chondrichthyes (cyan: whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

4. Evolutionary studies of whale shark

4.1 Phylogeny construction

We constructed a phylogenetic tree of 25 species including whale shark (*Anolis carolinensis, Balaenoptera acutorostrata scammoni, Bos taurus, Callorhinchus milii, Canis familiaris, Danio rerio, Esox lucius, Gadus morhua, Gallus gallus, Heterocephalus glaber, Homo sapiens, Latimeria chalumnae, Lepisosteus oculatus, Loxodonta africana, Mus musculus, Myotis lucifugus, Nothobranchius furzeri, Orcinus orca, Oryzias latipes, Petromyzon marinus, Physeter catodon, Rhincodon typus, Takifugu rubripes, Tursiops truncatus, Xenopus tropicalis*). We first extracted 275 single-copy gene families of 25 species from the orthologous gene family table of 82 species. We filtered out clusters with average GC3 below 0.45 to prevent bias[47], leaving 255 clusters. We performed multiple sequence alignment (MSA) of each remaining single copy gene family using MUSCLE 3.8.31[41] and concatenated the MSA results without gap regions. The phylogenetic tree was constructed using RAxML 8.2[48] with maximum likelihood (1,000 bootstrapping), using the PROTCATLG amino acid substitution model (Figure 3D).

4.2 Divergence time estimation

We estimated that the common ancestor of the whale shark and elephant fish diverged roughly 268 million years ago (MYA) (Figure 3D). Divergence times were estimated using the MCMCtree program in PAML package 4.8[49] with the independent rates model (clock=2). The date of the node between *O. orca-L. chalumnae* was constrained to 401-425 MYA and *O. latipes-R. typus* was constrained to 450-497 MYA based on the TimeTree database[50].

4.3 Whale shark evolutionary rate

We compared the molecular evolutionary rate of the whale shark and other 23 species with sea lamprey as an outgroup. We found that the whale shark had the shortest distance to the outgroup (sea lamprey) indicating slowest evolutionary rate (Table S18). We also performed a relative rate test using MEGA7[51], and found that the whale shark protein coding genes are evolving more slowly than any 81 species (Table S19). We also performed the Two-Cluster test with LINTRE[52]. The distances between nodes in the phylogenetic tree (Figure 3D and Figure S17) used as the pairwise distances for the Two-Cluster test. The distances from the sea lamprey as an outgroup were calculated using the 'ape' R-package[53]. The two-cluster test also supported that the whale shark has a slower evolutionary rate than the elephant fish (Table S20).

Table S18. Pairwise distance to the outgroup for 24 species

The pairwise distances were calculated using the R-package ‘ape’[53] with an outgroup (sea lamprey).

Species	Distance to *Petromyzon marinus*
Rhincodon typus	**0.61505568**
Callorhinchus milii	0.62512995
Latimeria chalumnae	0.63722456
Lepisosteus oculatus	0.6546978
Gallus gallus	0.66767913
Anolis carolinensis	0.68388984
Loxodonta africana	0.68996989
Canis familiaris	0.69043867
Homo sapiens	0.69124695
Balaenoptera acutorostrata scammoni	0.69823335
Orcinus orca	0.69905548
Tursiops truncatus	0.70085231
Physeter catodon	0.70232111
Bos taurus	0.70553639
Myotis lucifugus	0.70839722
Heterocephalus glaber	0.71537803
Esox lucius	0.71747889
Xenopus tropicalis	0.71869153
Mus musculus	0.72308379
Danio rerio	0.72473367
Nothobranchius furzeri	0.77434503
Gadus morhua	0.77479036
Takifugu rubripes	0.77698009
Oryzias latipes	0.78572188

Table S19. Results of relative rate test of whale shark versus other vertebrates

The ‘Identical’ and ‘Divergent’ columns indicate the number of sites where the amino acid is same or different in all three groups, respectively.

Ingroup1	Ingroup2	Outgroup	Genes	Identical	Divergent	Ingroup1 Specific	Ingroup2 Specific	Outgroup Specific	CHI^2	P-value
Anolis carolinesis	*Rhincodon typus*	*Petromyzon marinus*	255	38,421	6,307	3,864	3,473	10,043	20.84	5.00E-06
Balaenoptera acutorostrata	*Rhincodon typus*	*Petromyzon marinus*	255	38,315	6,267	3,962	3,662	9,888	11.8	5.91E-04
Bos Taurus	*Rhincodon typus*	*Petromyzon marinus*	255	38,305	6,315	3,978	3,658	9,850	13.41	2.50E-04
Callorhinchus milii	*Rhincodon typus*	*Petromyzon marinus*	255	39,726	4,660	2,559	2,321	12,842	11.61	6.57E-04
Canis familiaris	*Rhincodon typus*	*Petromyzon marinus*	255	38,329	6,269	3,956	3,617	9,937	15.18	9.80E-05
Danio rerio	*Rhincodon typus*	*Petromyzon marinus*	255	38,056	6,865	4,229	3,775	9,183	25.75	3.88E-07
Esox Lucius	*Rhincodon typus*	*Petromyzon marinus*	255	38,148	6,734	4,137	3,738	9,350	20.22	6.12E-06
Gadus morhua	*Rhincodon typus*	*Petromyzon marinus*	255	37,587	7,026	4,661	3,951	8,805	58.53	2.00E-14
Gallus gallus	*Rhincodon typus*	*Petromyzon marinus*	255	38,606	6,149	3,679	3,510	10,164	3.97	4.62E-02
Heterocephalus glaber	*Rhincodon typus*	*Petromyzon marinus*	255	38,167	6,394	4,118	3,658	9,771	27.21	1.82E-07
Homo sapiens	*Rhincodon typus*	*Petromyzon marinus*	255	38,354	6,292	3,931	3,612	9,919	13.49	2.40E-04
Latimeria chalumnae	*Rhincodon typus*	*Petromyzon marinus*	255	38,522	5,824	3,763	3,305	10,694	29.68	5.10E-08
Lepisosteus oculatus	*Rhincodon typus*	*Petromyzon marinus*	255	38,550	6,122	3,734	3,591	10,109	2.79	9.48E-02
Loxodonta Africana	*Rhincodon typus*	*Petromyzon marinus*	255	38,214	6,311	4,070	3,623	9,889	25.97	3.46E-07
Mus Musculus	*Rhincodon typus*	*Petromyzon marinus*	255	38,268	6,371	4,017	3,672	9,780	15.48	8.34E-05
Myotis lucifugus	*Rhincodon typus*	*Petromyzon marinus*	255	38,192	6,352	4,092	3,645	9,826	25.83	3.74E-07
Nothobranchius furzeri	*Rhincodon typus*	*Petromyzon marinus*	255	37,790	6,935	4,494	3,749	9,139	67.33	2.29E-16
Orcinus orca	*Rhincodon typus*	*Petromyzon marinus*	255	38,353	6,269	3,932	3,654	9,900	10.19	1.41E-03
Oryzias latipes	*Rhincodon typus*	*Petromyzon marinus*	255	37,568	6,979	4,707	3,773	9,070	102.87	3.58E-24
Physeter catodon	*Rhincodon typus*	*Petromyzon marinus*	255	38,154	6,348	4,131	3,654	9,821	29.23	6.44E-08
Takifugu rubripes	*Rhincodon typus*	*Petromyzon marinus*	255	37,685	7,043	4,600	3,808	8,972	74.6	5.76E-18
Tursiops truncates	*Rhincodon typus*	*Petromyzon marinus*	255	38,094	6,215	3,971	3,615	9,815	16.71	4.36E-05
Xenopus tropicalis	*Rhincodon typus*	*Petromyzon marinus*	255	37,519	6,735	4,765	3,523	9,564	186.12	2.23E-42

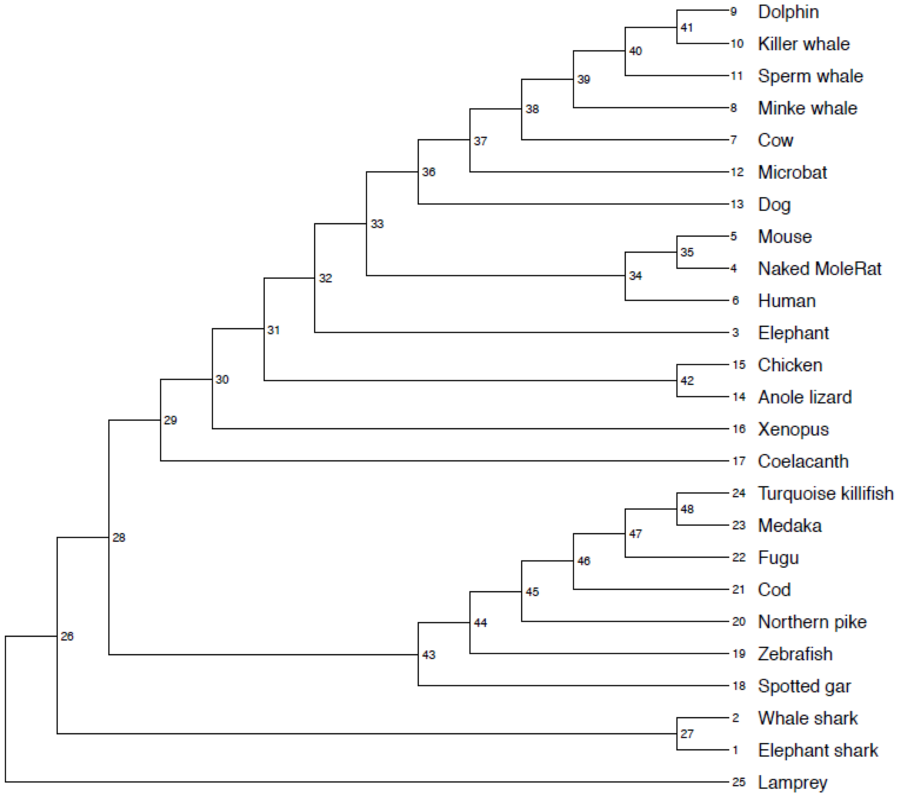


Figure S17. The phylogenetic tree used in the two-cluster test. Numbers indicate the nodes, the left, and the right in Table S20.

Table S20. The results of two cluster test of whale shark versus other vertebrates

Node	Left		Right	delta	s.e.	Z	CP	height	s.e.	bA	bB	bC
27	2	<	1	0.066988	0.001331	50.327722	99.96%	0.104797	0.001132	0.071303	0.138291	0.128126
42	14	<	15	0.007774	0.000899	8.645688	99.96%	0.041636	0.000634	0.037749	0.045523	0.1763
35	4	>	5	0.113236	0.00173	65.466742	99.96%	0.148789	0.001454	0.205407	0.092171	0.106489
34	35	<	6	0.047336	0.001573	30.091571	99.96%	0.161306	0.001385	0.137638	0.184974	0.11458
41	10	<	9	0.037975	0.0013	29.221827	99.96%	0.100237	0.001098	0.08125	0.119225	0.13499
40	41	>	11	0.029928	0.000826	36.233157	99.96%	0.063647	0.000669	0.078611	0.048683	0.161523
39	40	<	8	0.090038	0.0016	56.27053	99.96%	0.171928	0.001515	0.126909	0.216947	0.097834
38	39	<	7	0.049123	0.001291	38.043271	99.96%	0.140867	0.001195	0.116305	0.165428	0.129223
37	38	<	12	0.040654	0.001526	26.636184	99.96%	0.142865	0.001167	0.122538	0.163191	0.131217
36	37	<	13	0.025798	0.001283	20.105648	99.96%	0.129603	0.001044	0.116704	0.142502	0.1439
33	36	<	34	0.005831	0.000777	7.501552	99.96%	0.131407	0.000932	0.128492	0.134322	0.136093
32	33	>	3	0.077327	0.000853	90.663726	99.96%	0.112226	0.000887	0.150889	0.073562	0.118436
31	32	>	42	0.048181	0.000708	68.032487	99.96%	0.106587	0.000812	0.130678	0.082497	0.139804
30	31	>	16	0.03557	0.000815	43.664202	99.96%	0.096445	0.000766	0.11423	0.07866	0.15522
29	30	<	17	0.04978	0.001324	37.594456	99.96%	0.147465	0.001199	0.122575	0.172355	0.141532
48	24	<	23	0.112731	0.001606	70.206746	99.96%	0.169157	0.001603	0.112792	0.225523	0.081835
47	48	<	22	0.054553	0.001641	33.250362	99.96%	0.156946	0.001331	0.129669	0.184222	0.118464
46	47	>	21	0.065644	0.000942	69.650583	99.96%	0.110532	0.000931	0.143354	0.07771	0.125119
45	46	<	20	0.255255	0.003121	81.792064	99.96%	0.246672	0.001959	0.119045	0.3743	0.120952
44	45	<	19	0.014926	0.001527	9.775209	99.96%	0.169814	0.001295	0.162351	0.177277	0.126141
43	44	>	18	0.11091	0.000979	113.273282	99.96%	0.123299	0.000928	0.178754	0.067844	0.11469
28	43	>	29	0.038478	0.000685	56.169011	99.96%	0.137945	0.000922	0.157184	0.118706	0.128968
26	28	>	27	0.043569	0.001146	38.020739	99.96%	0.115526	0.00083	0.13731	0.093741	0.180338

Q=17759.984030

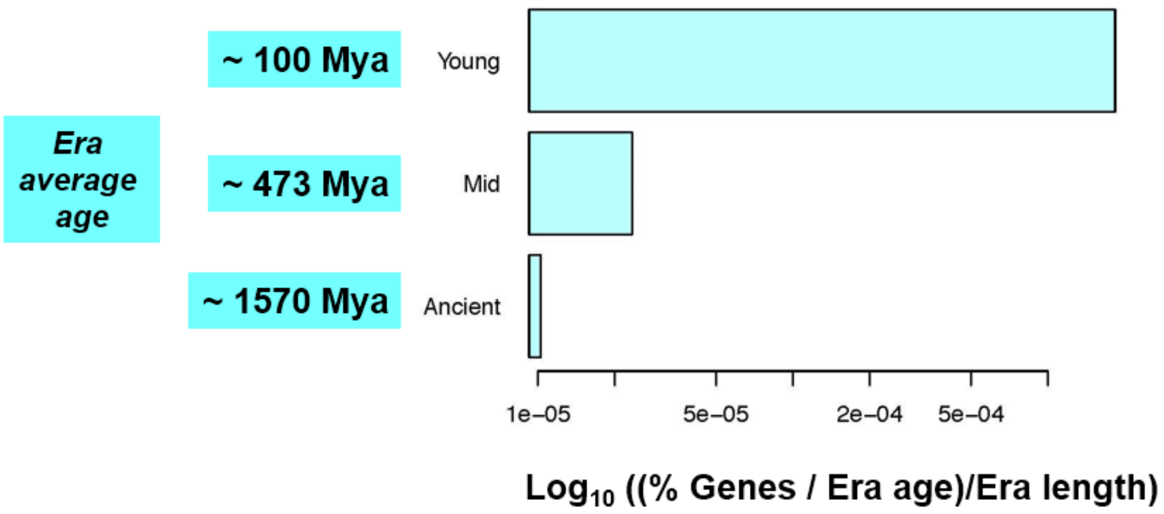


Figure S18. Supplementary figure linked to Figure 3C. When accounting for the age and length (duration) of evolutionary eras (average era ages: ancient, ~1,570 Mya; mid, ~473 Mya; young, ~100 Mya), the number of genes in every era increases steadily as the genes are more recent, which suggests that gene turnover is highest in recent ages.

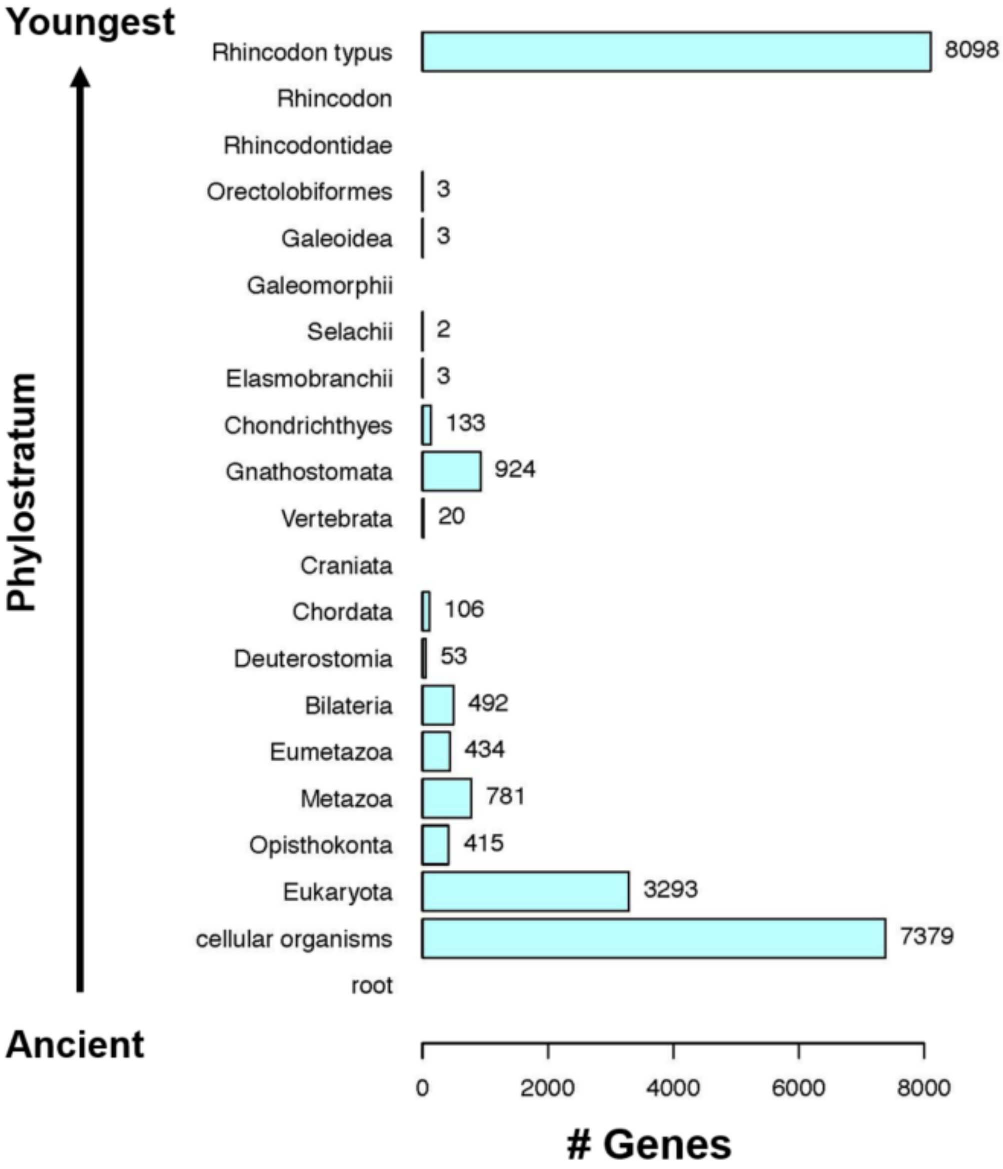


Figure S19. The number of genes in every phylostratum from most ancient to the youngest shows that most whale shark genes are ancient (7,379 genes in PS 1 and 3,293 in Eukaryota). The large number of genes that appear species-specific (8,098 genes) likely reflects the scarcity of sequenced genomes since the emergence of Chondrichthyes.

4.4 Gene family expansion and contraction analyses

Gene family expansion/contraction analyses were performed by using CAFÉ software[54] (v3.1) with phylogenetic tree and p-value cut-off <0.05 demonstrating significantly changed the number of genes in the family (Figure 3D). 32 gene families were expanded and 233 gene families were contracted in the whale shark genome. We performed gene ontology (GO) enrichment test using ClueGO[55]. Expanded gene families were enriched in pattern specification involved in kidney development (GO:0061004) and nephron tubule formation (GO:0072079). Contracted gene families were enriched in nucleosome assembly (GO:0006334) and chromatin assembly (GO:0031497). We also found smaller number of histone 1 (H1), histone 2A (H2A) and histone 2Bs (H2Bs) in the whale shark than in other bony fishes and mammals (Figure S20).

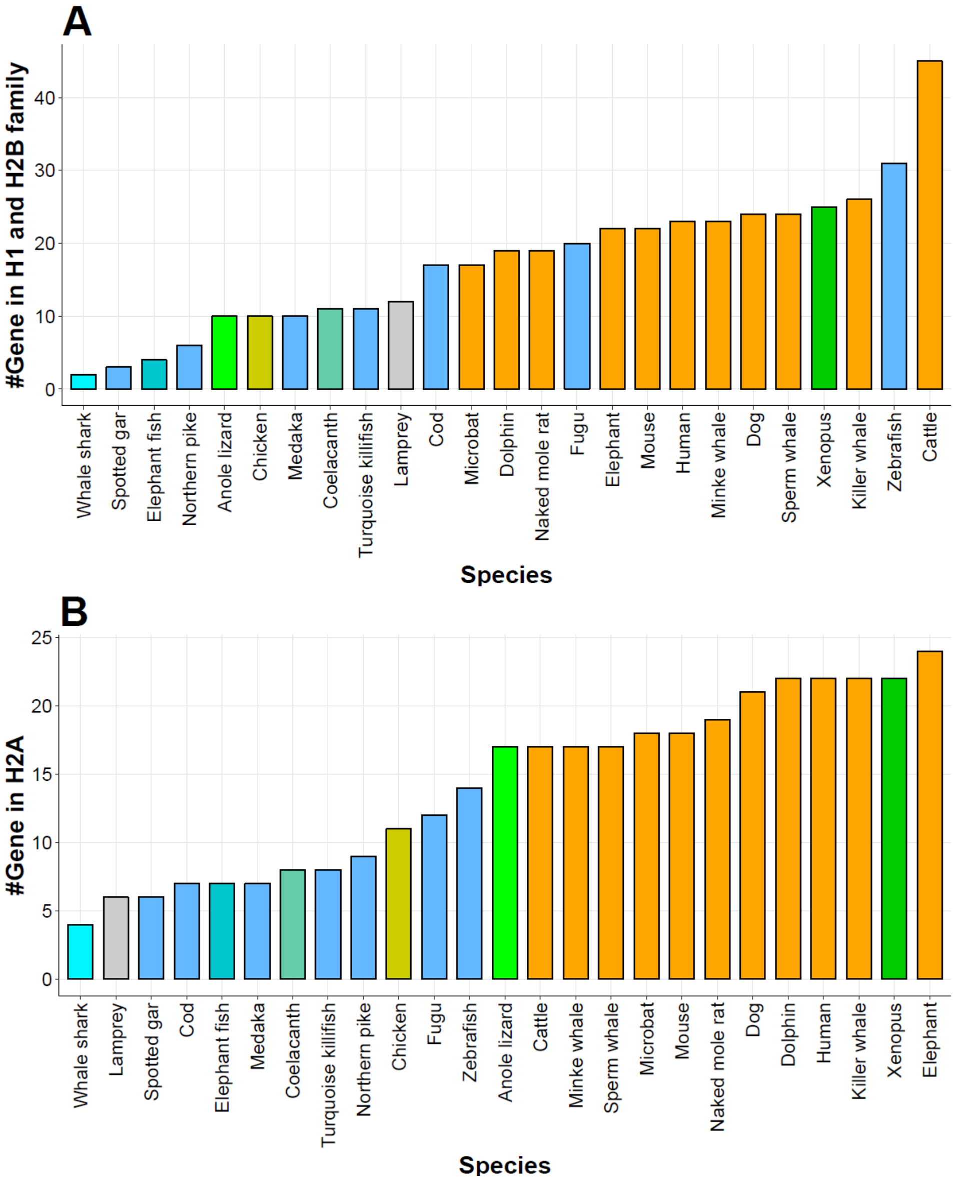


Figure S20. Contracted Histone gene families in whale shark. The OG000022 cluster contains the H1 and H2B classes. The OG000023 cluster contains the H2A class. The Y-axis indicates the number of histone genes in the cluster for each species. Each color shows the class of 82 species (gray: Hyperoartia, turquoise: Chondrichthyes (cyan: whale shark), light blue: Actinopterygii, aquamarine: Sarcopterygii, dark green: Amphibia, light green: Reptilia, dark yellow: Aves, orange: Mammalia).

Table S21A. The GO terms enriched in contracted single-copy orthologous gene families in the whale shark from MRCA

GO IDs	GO terms	# Genes	*p*-values	Adjusted *p*-values
GO:0000122	negative regulation of transcription from RNA polymerase II promoter	91	2.00E-14	1.54E-11
GO:0001654	eye development	43	1.45E-08	1.09E-05
GO:0001704	formation of primary germ layer	20	1.28E-06	9.38E-04
GO:0003151	outflow tract morphogenesis	14	7.80E-06	5.61E-03
GO:0003231	cardiac ventricle development	18	1.10E-05	7.86E-03
GO:0006069	ethanol oxidation	7	2.67E-07	1.98E-04
GO:0006323	DNA packaging	31	5.88E-10	4.46E-07
GO:0006333	chromatin assembly or disassembly	33	7.10E-13	5.43E-10
GO:0006334	nucleosome assembly	31	1.37E-15	1.06E-12
GO:0006342	chromatin silencing	20	7.14E-09	5.41E-06
GO:0006351	transcription, DNA-templated	309	3.80E-26	3.02E-23
GO:0006355	regulation of transcription, DNA-templated	306	2.42E-28	1.94E-25
GO:0006357	regulation of transcription from RNA polymerase II promoter	194	2.08E-23	1.65E-20
GO:0006366	transcription from RNA polymerase II promoter	203	4.20E-22	3.31E-19
GO:0006821	chloride transport	21	1.95E-08	1.47E-05
GO:0007214	gamma-aminobutyric acid signaling pathway	9	8.94E-07	6.58E-04
GO:0007274	neuromuscular synaptic transmission	9	2.67E-06	1.94E-03
GO:0007417	central nervous system development	84	2.11E-08	1.58E-05
GO:0007420	brain development	61	8.13E-06	5.84E-03
GO:0007423	sensory organ development	65	2.14E-12	1.63E-09
GO:0007517	muscle organ development	39	5.71E-06	4.12E-03
GO:0008016	regulation of heart contraction	28	6.44E-06	4.64E-03
GO:0009887	animal organ morphogenesis	95	4.87E-11	3.70E-08
GO:0009913	epidermal cell differentiation	65	7.31E-22	5.75E-19
GO:0010557	positive regulation of macromolecule biosynthetic process	147	5.44E-13	4.16E-10
GO:0010558	negative regulation of macromolecule biosynthetic process	146	7.85E-18	6.12E-15
GO:0010628	positive regulation of gene expression	165	2.53E-16	1.96E-13
GO:0010629	negative regulation of gene expression	154	8.72E-14	6.71E-11
GO:0015698	inorganic anion transport	34	5.34E-13	4.09E-10
GO:0016070	RNA metabolic process	314	2.01E-13	1.54E-10
GO:0017187	peptidyl-glutamic acid carboxylation	6	1.18E-05	8.42E-03
GO:0018214	protein carboxylation	6	1.18E-05	8.42E-03
GO:0021781	glial cell fate commitment	8	1.96E-08	1.48E-05
GO:0021953	central nervous system neuron differentiation	25	1.11E-06	8.11E-04
GO:0022008	neurogenesis	117	1.15E-07	8.57E-05
GO:0030182	neuron differentiation	103	1.26E-07	9.36E-05
GO:0030216	keratinocyte differentiation	57	1.07E-19	8.40E-17
GO:0030219	megakaryocyte differentiation	14	2.36E-06	1.72E-03
GO:0030574	collagen catabolic process	15	5.97E-07	4.41E-04
GO:0030856	regulation of epithelial cell differentiation	21	1.41E-06	1.03E-03
GO:0031269	pseudopodium assembly	7	8.42E-06	6.03E-03
GO:0031272	regulation of pseudopodium assembly	7	1.92E-06	1.40E-03
GO:0031274	positive regulation of pseudopodium assembly	7	1.07E-06	7.83E-04
GO:0031327	negative regulation of cellular biosynthetic process	148	3.13E-16	2.42E-13
GO:0031328	positive regulation of cellular biosynthetic process	154	2.49E-12	1.90E-09
GO:0031424	keratinization	49	1.71E-19	1.34E-16
GO:0031497	chromatin assembly	31	2.08E-13	1.60E-10
GO:0032774	RNA biosynthetic process	309	2.13E-25	1.69E-22
GO:0034728	nucleosome organization	32	3.68E-13	2.82E-10
GO:0035881	amacrine cell differentiation	5	9.94E-06	7.12E-03
GO:0043010	camera-type eye development	37	1.90E-07	1.41E-04
GO:0043588	skin development	71	8.23E-22	6.47E-19
GO:0045814	negative regulation of gene expression, epigenetic	20	9.93E-08	7.43E-05
GO:0045892	negative regulation of transcription, DNA-templated	135	3.89E-23	3.08E-20
GO:0045893	positive regulation of transcription, DNA-templated	142	7.31E-17	5.68E-14
GO:0045934	negative regulation of nucleobase-containing compound metabolic process	145	1.56E-18	1.22E-15
GO:0045935	positive regulation of nucleobase-containing compound metabolic process	155	6.89E-14	5.31E-11
GO:0045944	positive regulation of transcription from RNA polymerase II promoter	123	3.96E-18	3.09E-15
GO:0048013	ephrin receptor signaling pathway	17	8.09E-07	5.96E-04
GO:0048568	embryonic organ development	45	7.30E-07	5.39E-04
GO:0048663	neuron fate commitment	17	9.21E-09	6.96E-06
GO:0048665	neuron fate specification	9	1.21E-05	8.63E-03
GO:0048699	generation of neurons	113	3.45E-08	2.58E-05
GO:0051252	regulation of RNA metabolic process	313	2.73E-26	2.17E-23

GO:0051253	negative regulation of RNA metabolic process	140	4.26E-21	3.34E-18
GO:0051254	positive regulation of RNA metabolic process	147	3.66E-17	2.85E-14
GO:0060485	mesenchyme development	29	5.33E-06	3.86E-03
GO:0061337	cardiac conduction	21	2.82E-06	2.05E-03
GO:0065004	protein-DNA complex assembly	31	3.07E-08	2.30E-05
GO:0070268	cornification	48	1.25E-33	1.00E-30
GO:0071624	positive regulation of granulocyte chemotaxis	10	3.30E-06	2.39E-03
GO:0090022	regulation of neutrophil chemotaxis	10	5.65E-06	4.08E-03
GO:0090023	positive regulation of neutrophil chemotaxis	10	9.89E-07	7.27E-04
GO:0090084	negative regulation of inclusion body assembly	7	1.16E-07	8.64E-05
GO:0090131	mesenchyme migration	5	1.99E-07	1.48E-04
GO:0090596	sensory organ morphogenesis	32	4.30E-07	3.18E-04
GO:0097264	self proteolysis	5	9.94E-06	7.12E-03
GO:0097659	nucleic acid-templated transcription	309	1.13E-25	8.96E-23
GO:0098656	anion transmembrane transport	36	2.63E-08	1.97E-05
GO:0098661	inorganic anion transmembrane transport	27	2.53E-11	1.92E-08
GO:0099133	ATP hydrolysis coupled anion transmembrane transport	7	5.55E-07	4.11E-04
GO:1902476	chloride transmembrane transport	20	8.66E-09	6.56E-06
GO:1902622	regulation of neutrophil migration	10	1.18E-05	8.43E-03
GO:1902624	positive regulation of neutrophil migration	10	2.48E-06	1.81E-03
GO:1902679	negative regulation of RNA biosynthetic process	138	6.39E-23	5.04E-20
GO:1902680	positive regulation of RNA biosynthetic process	142	7.50E-17	5.82E-14
GO:1903506	regulation of nucleic acid-templated transcription	306	6.13E-28	4.90E-25
GO:1903507	negative regulation of nucleic acid-templated transcription	138	5.70E-23	4.50E-20
GO:1903508	positive regulation of nucleic acid-templated transcription	142	7.31E-17	5.68E-14
GO:1903779	regulation of cardiac conduction	14	5.63E-06	4.07E-03
GO:2000112	regulation of cellular macromolecule biosynthetic process	315	5.04E-23	3.99E-20
GO:2000113	negative regulation of cellular macromolecule biosynthetic process	142	3.81E-18	2.98E-15
GO:2001141	regulation of RNA biosynthetic process	306	1.04E-27	8.30E-25

Functional enrichment tests were performed using ClueGO with options as below[55]

Options: 'Min GO Level = 6, Max GO Level = 13, Number of Genes = 2, Min Percentage = 5.0, GO Fusion = false, GO Group = true, Kappa Score Threshold = 0.4, Over View Term = SmallestPValue, Group By Kappa Statistics = true, Initial Group Size = 1, Sharing Group Percentage = 50.0'

Table S21B. The GO terms enriched in expanded single-copy orthologous gene families in the whale shark from MRCA

GO IDs	GO terms	# Genes	*p*-values	Adjusted *p*-values
GO:0001676	long-chain fatty acid metabolic process	14	1.48E-15	1.67E-13
GO:0003095	pressure natriuresis	2	1.05E-04	7.58E-03
GO:0003148	outflow tract septum morphogenesis	5	4.98E-07	5.18E-05
GO:0003151	outflow tract morphogenesis	6	6.06E-06	5.76E-04
GO:0003161	cardiac conduction system development	3	3.28E-05	2.82E-03
GO:0003164	His-Purkinje system development	3	8.21E-07	8.29E-05
GO:0003166	bundle of His development	3	2.06E-07	2.18E-05
GO:0003197	endocardial cushion development	4	8.36E-05	6.36E-03
GO:0003206	cardiac chamber morphogenesis	7	1.09E-05	1.00E-03
GO:0003207	cardiac chamber formation	4	5.70E-07	5.87E-05
GO:0003279	cardiac septum development	7	2.38E-06	2.30E-04
GO:0006069	ethanol oxidation	3	4.36E-05	3.58E-03
GO:0006690	icosanoid metabolic process	17	5.16E-19	5.98E-17
GO:0006721	terpenoid metabolic process	6	7.47E-05	5.83E-03
GO:0007368	determination of left/right symmetry	7	7.91E-06	7.36E-04
GO:0007379	segment specification	3	1.32E-04	9.36E-03
GO:0009258	10-formyltetrahydrofolate catabolic process	2	3.52E-05	2.96E-03
GO:0009397	folic acid-containing compound catabolic process	2	1.05E-04	7.58E-03
GO:0009855	determination of bilateral symmetry	8	1.15E-06	1.15E-04
GO:0009954	proximal/distal pattern formation	4	4.28E-05	3.55E-03
GO:0010002	cardioblast differentiation	5	7.45E-08	8.12E-06
GO:0016098	monoterpenoid metabolic process	3	7.09E-06	6.66E-04
GO:0019369	arachidonic acid metabolic process	13	6.53E-18	7.51E-16
GO:0019373	epoxygenase P450 pathway	13	1.08E-24	1.26E-22
GO:0021510	spinal cord development	6	3.48E-05	2.96E-03
GO:0021515	cell differentiation in spinal cord	6	7.11E-07	7.25E-05
GO:0021517	ventral spinal cord development	6	3.42E-07	3.60E-05
GO:0021520	spinal cord motor neuron cell fate specification	3	5.64E-05	4.52E-03
GO:0021522	spinal cord motor neuron differentiation	6	3.78E-08	4.16E-06
GO:0021912	regulation of transcription from RNA polymerase II promoter involved in spinal cord motor neuron fate specification	2	1.05E-04	7.58E-03
GO:0021953	central nervous system neuron differentiation	11	1.27E-08	1.41E-06
GO:0030157	pancreatic juice secretion	3	8.90E-05	6.68E-03
GO:0031016	pancreas development	6	1.16E-05	1.05E-03
GO:0033559	unsaturated fatty acid metabolic process	15	1.46E-16	1.67E-14
GO:0035050	embryonic heart tube development	5	9.62E-05	7.12E-03
GO:0035051	cardiocyte differentiation	7	1.27E-05	1.13E-03
GO:0035115	embryonic forelimb morphogenesis	4	4.28E-05	3.55E-03
GO:0035136	forelimb morphogenesis	4	1.02E-04	7.45E-03
GO:0035282	segmentation	7	2.38E-06	2.30E-04
GO:0036100	leukotriene catabolic process	3	8.21E-07	8.29E-05
GO:0036101	leukotriene B4 catabolic process	3	8.21E-07	8.29E-05
GO:0036102	leukotriene B4 metabolic process	3	8.21E-07	8.29E-05
GO:0042196	chlorinated hydrocarbon metabolic process	2	3.52E-05	2.96E-03
GO:0042197	halogenated hydrocarbon metabolic process	2	3.52E-05	2.96E-03
GO:0042361	menaquinone catabolic process	2	3.52E-05	2.96E-03
GO:0042376	phylloquinone catabolic process	2	1.05E-04	7.58E-03
GO:0042377	vitamin K catabolic process	2	3.52E-05	2.96E-03
GO:0042471	ear morphogenesis	6	7.83E-05	6.03E-03
GO:0042758	long-chain fatty acid catabolic process	3	2.40E-05	2.09E-03
GO:0048663	neuron fate commitment	8	7.53E-09	8.43E-07
GO:0048665	neuron fate specification	5	1.42E-06	1.41E-04
GO:0055011	atrial cardiac muscle cell differentiation	2	1.05E-04	7.58E-03
GO:0055014	atrial cardiac muscle cell development	2	1.05E-04	7.58E-03
GO:0060037	pharyngeal system development	4	1.61E-05	1.42E-03
GO:0060043	regulation of cardiac muscle cell proliferation	4	1.02E-04	7.45E-03
GO:0060411	cardiac septum morphogenesis	7	1.74E-07	1.86E-05
GO:0060413	atrial septum morphogenesis	3	1.32E-04	9.36E-03
GO:0060579	ventral spinal cord interneuron fate commitment	3	8.90E-05	6.68E-03
GO:0060596	mammary placode formation	2	1.05E-04	7.58E-03
GO:0060926	cardiac pacemaker cell development	2	1.05E-04	7.58E-03
GO:0060932	His-Purkinje system cell differentiation	2	3.52E-05	2.96E-03
GO:0061004	pattern specification involved in kidney development	3	2.40E-05	2.09E-03

GO:0061371	determination of heart left/right asymmetry	5	4.58E-05	3.71E-03
GO:0072047	proximal/distal pattern formation involved in nephron development	3	4.07E-06	3.91E-04
GO:0072048	renal system pattern specification	3	2.40E-05	2.09E-03
GO:0072070	loop of Henle development	3	5.64E-05	4.52E-03
GO:0072081	specification of nephron tubule identity	3	4.07E-06	3.91E-04
GO:0072086	specification of loop of Henle identity	3	8.21E-07	8.29E-05
GO:0072272	proximal/distal pattern formation involved in metanephric nephron development	2	3.52E-05	2.96E-03
GO:0090186	regulation of pancreatic juice secretion	3	1.13E-05	1.03E-03
GO:0090188	negative regulation of pancreatic juice secretion	3	2.04E-06	2.00E-04
GO:0097267	omega-hydroxylase P450 pathway	4	1.47E-07	1.59E-05
GO:1901213	regulation of transcription from RNA polymerase II promoter involved in heart development	3	7.15E-05	5.65E-03
GO:1901523	icosanoid catabolic process	3	2.04E-06	2.00E-04
GO:1901662	quinone catabolic process	2	1.05E-04	7.58E-03

Functional enrichment tests were performed using ClueGO with options as below [55]

Options: ‘Min GO Level = 6, Max GO Level = 13, Number of Genes = 2, Min Percentage = 5.0, GO Fusion = false, GO Group = true, Kappa Score Threshold = 0.4, Over View Term = SmallestPValue, Group By Kappa Statistics = true, Initial Group Size = 1, Sharing Group Percentage = 50.0’

4.5 Neural genes

We downloaded and corrected the neuronal genes with ten categories from GO and public databases as below.

1) Neuronal connectivity genes:

GO:0071526	(BP)	semaphorin-plexin signaling pathway (25 genes)
GO:0030215	(MF)	semaphorin receptor binding (10 genes)
GO:0017154	(MF)	semaphorin receptor activity (11 genes)
GO:0002116	(CC)	semaphorin receptor complex (7 genes)
GO:0038189	(BP)	neuropilin signaling pathway (4 genes)
GO:0038191	(MF)	neuropilin binding (12 genes)
GO:0048013	(BP)	ephrin receptor signaling pathway (87 genes)
GO:0005003	(MF)	ephrin receptor activity (19 genes)
GO:0046875	(MF)	ephrin receptor binding (28 genes)
GO:0038007	(BP)	netrin-activated signaling pathway (5 genes)
GO:0005042	(MF)	netrin receptor activity (2 genes)
GO:0035385	(BP)	Roundabout signaling pathway (7 genes)
GO:0048495	(MF)	Roundabout binding (5 genes)
GO:0007219	(BP)	Notch signaling pathway (169 genes)
GO:0005112	(MF)	Notch binding (21 genes)

2) Cell adhesion:

MCAM (http://app1.unmc.edu/mcam/index.cfm) (181 genes)		
GO:0007158	(BP)	neuron cell-cell adhesion (15 genes)
GO:0071253	(MF)	connexin binding (6 genes)
GO:0005922	(CC)	connexin complex (20 genes)
GO:1905071	(BP)	occluding junction disassembly (3 genes)
GO:0070160	(CC)	occluding junction
GO:0044331	(BP)	cell-cell adhesion mediated by cadherin (15 genes)
GO:0045296	(MF)	cadherin binding (304 genes)
GO:1904886	(BP)	beta-catenin destruction complex disassembly (22 genes)
GO:1904885	(BP)	beta-catenin destruction complex assembly (5 genes)
GO:1904837	(BP)	beta-catenin-TCF complex assembly (44 genes)
GO:0008013	(MF)	beta-catenin binding (81 genes)
GO:1904713	(MF)	beta-catenin destruction complex binding (2 genes)
GO:1990907	(CC)	beta-catenin-TCF complex (5 genes)
GO:0030877	(CC)	beta-catenin destruction complex (11 genes)

3) Olfactory receptors:

- HORDE (https://genome.weizmann.ac.il/horde/) (834 genes)
- GO:0004984 (MF) olfactory receptor activity (426 genes)
- GO:0031849 (MF) olfactory receptor binding (6 genes)

4) Ion channel:

- GO:0045161 (BP) neuronal ion channel clustering (12 genes)
- GO:0072578 (BP) neurotransmitter-gated ion channel clustering (8 genes)
- GO:0005216 (MF) ion channel activity (425 genes)
- GO:0099106 (MF) ion channel regulator activity (93 genes)
- GO:0034702 (CC) ion channel complex (288 genes)

5) Unfolded protein response associated genes:

- GO:0030968 (BP) endoplasmic reticulum unfolded protein response (130 genes)

6) Neuronal activity and memory:

- NADtranscriptomics (http://nadtranscriptomics.in.umh-csic.es/) (p value <= 0.01)
  - ✓ BDNF-regulated genes BDNF.txt
  - ✓ Forskolin-regulated genes forskolin.txt
  - ✓ Bicuculline-regulated genes bicuculline.txt
  - ✓ CREB-regulated genes CREB_regulon.txt
  - ✓ SRF-regulated genes SRF_regulon.txt
  - ✓ EGR1-regulated genes EGR1_regulon.txt
  - ✓ FOS-regulated genes FOS_regulon.txt
- GO:0007611 (BP) learning or memory (234 genes)

7) Neuropeptides:

- Neuropeptide database (http://www.neuropeptides.nl/tabel%20neuropeptides%20linked.htm) (96 genes)
- Two genes, CCAP and AstA (Allatostatin)

8) Homeobox genes:

- HGNC database (https://www.genenames.org/) (319 genes)

9) Synaptic genes:

- SynaptomeDB (http://metamoodics.org/SynaptomeDB/index.php) (1,886 genes)

10) Neurodegeneration:

- KEGG Human diseases (http://www.genome.jp/)
  - ✓ Neurodegenerative diseases (236 genes)

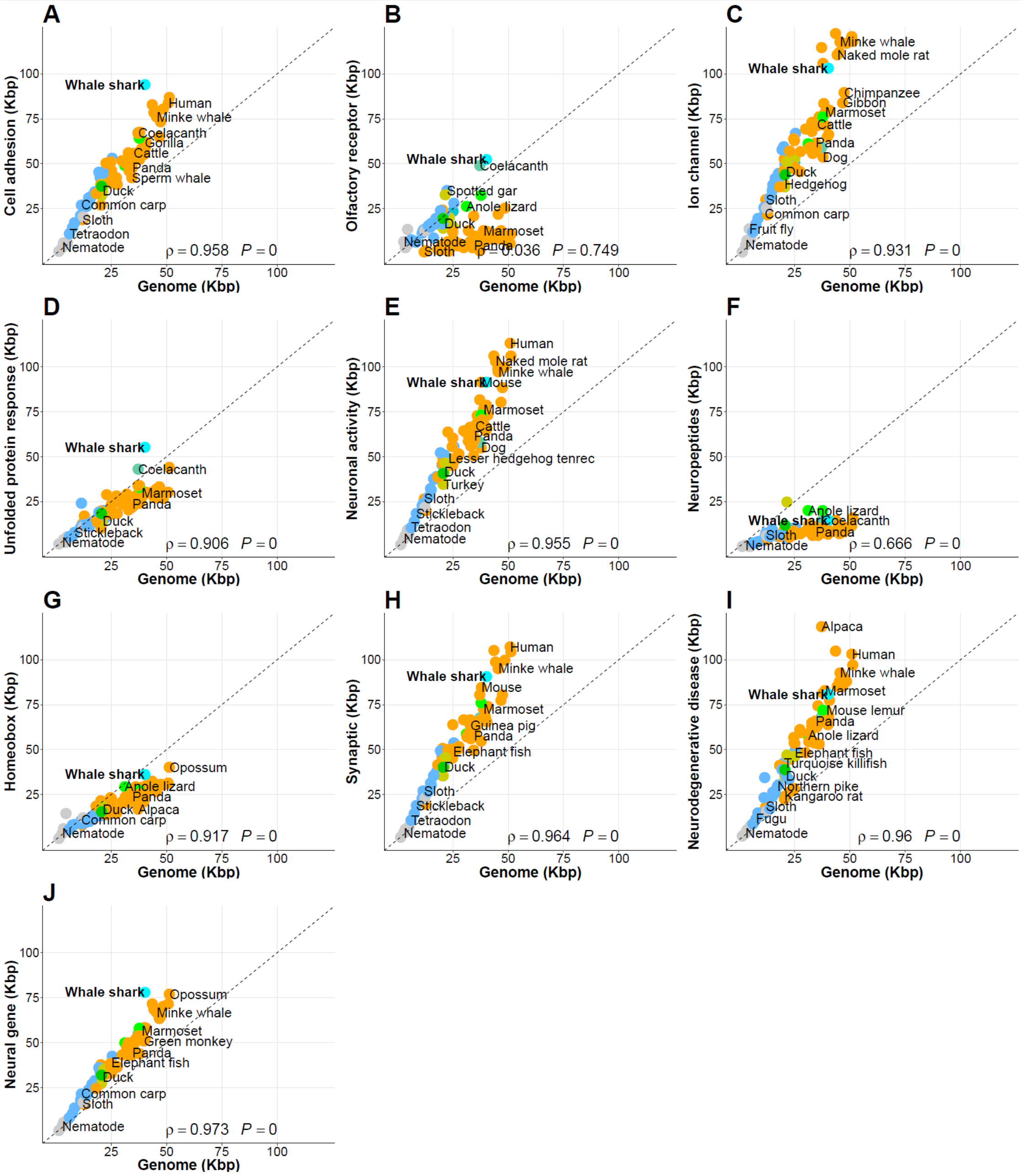


Figure S21. Supplementary figure linked to Figure 4 – All ten other scatter plots. Neuronal connectivity genes are longer in 81 species except yeast. The x- and y-axes correspond to average gene length (exon + intron) and the gene length of neuronal-related genes, respectively.

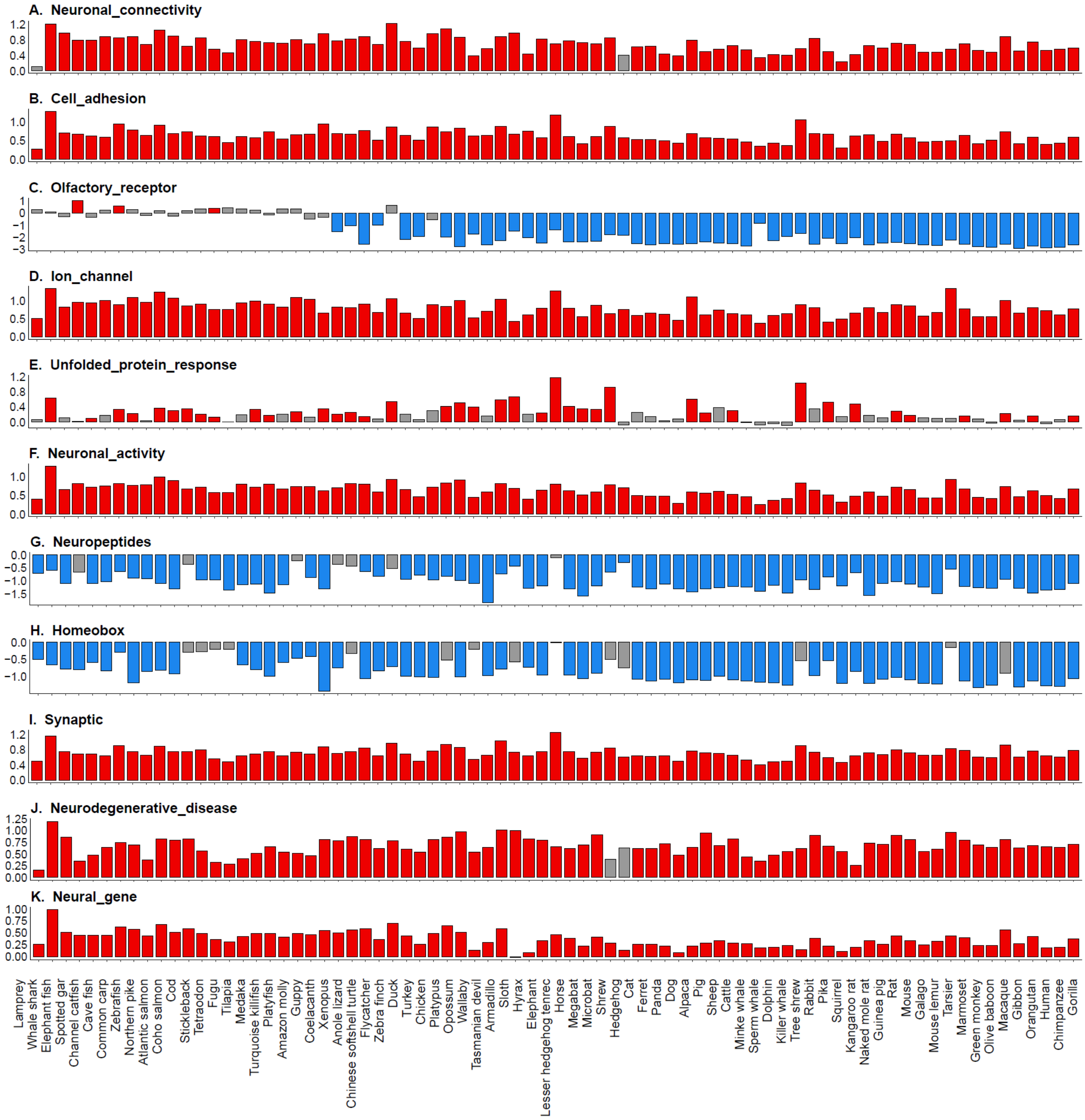
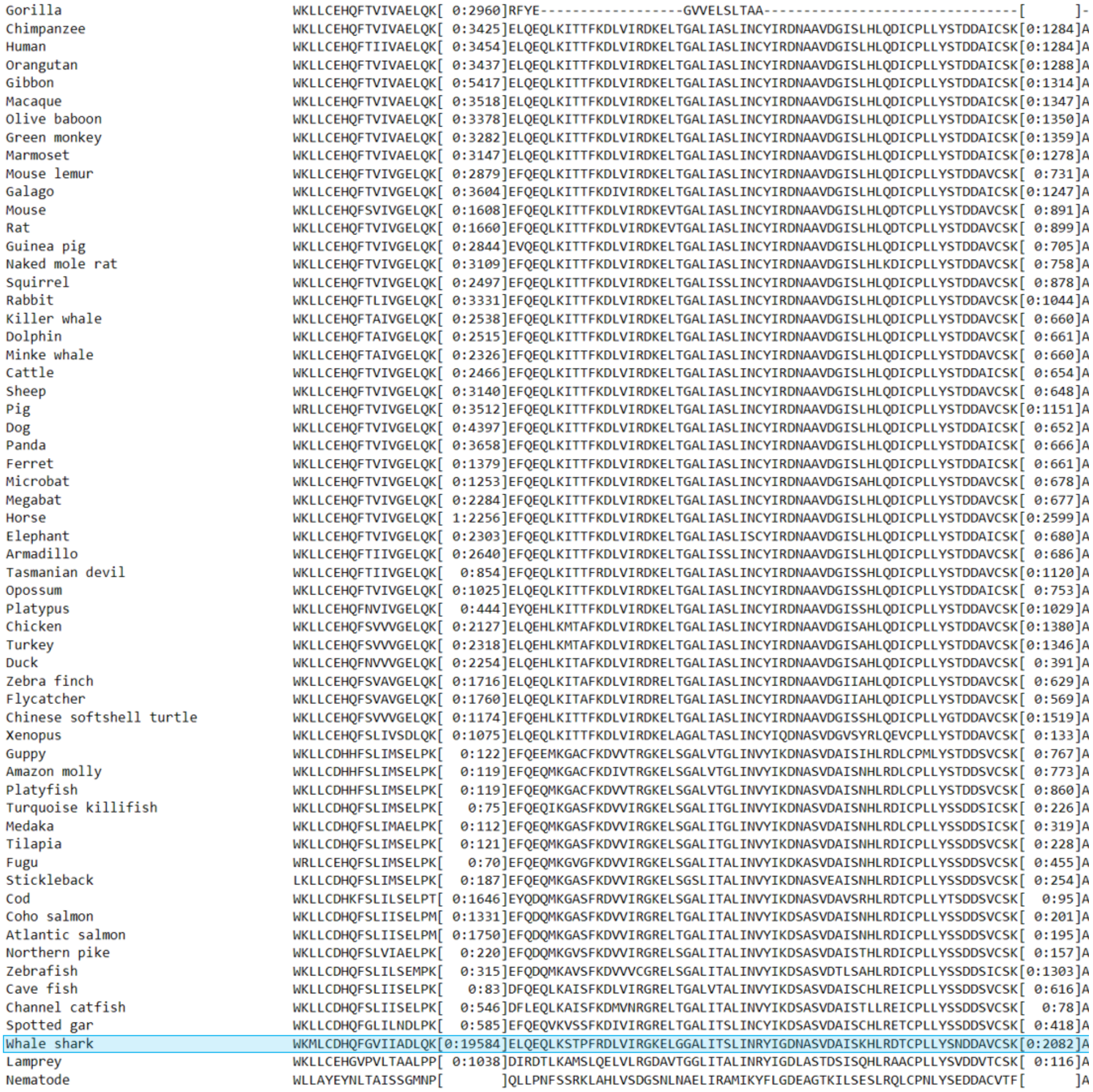


Figure S22. Relative median gene size of each neural subsets to median of gene size of genome. The Y-axis shows log transformed relative median value. The relative median values were calculated by dividing median of gene length (exon + intron) in each neuronal subset by median of gene length in genome. Red (or blue) bars indicate significantly higher (or lower) median gene length in the neuronal subset compared to the median genome-wide gene length by Wilcoxon-rank sum test.

4.6 Gene set enrichment analysis with gene size

Gene Set Enrichment Analysis (GSEA)[56] was used to calculate statistically significant differences between short and long gene in 82 species using the clusterProfiler package[57] with Gene Ontology. All genes were assigned to human gene symbols in order to use human-GO. Finally, we obtained the results of 77 species (Figure 4C and Additional File 1).



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Gorilla                             WKLLCEHQFTVIVAELQK[ 0:2960]RFYE------------------GVVELSLTAA--------------------------------[      ]-
Chimpanzee                          WKLLCEHQFTVIVAELQK[ 0:3425]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1284]A
Human                               WKLLCEHQFTIIVAELQK[ 0:3454]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1284]A
Orangutan                           WKLLCEHQFTVIVAELQK[ 0:3437]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1288]A
Gibbon                              WKLLCEHQFTVIVAELQK[ 0:5417]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1314]A
Macaque                             WKLLCEHQFTVIVAELQK[ 0:3518]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1347]A
Olive baboon                        WKLLCEHQFTVIVAELQK[ 0:3378]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1350]A
Green monkey                        WKLLCEHQFTVIVAELQK[ 0:3282]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1359]A
Marmoset                            WKLLCEHQFTVIVAELQK[ 0:3147]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1278]A
Mouse lemur                         WKLLCEHQFTVIVGELQK[ 0:2879]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:731]A
Galago                              WKLLCEHQFTVIVGELQK[ 0:3604]EFQEQLKITTFKDIVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[0:1247]A
Mouse                               WKLLCEHQFSVIVGELQK[ 0:1608]EFQEQLKITTFKDLVIRDKEVTGALIASLINCYIRDNAAVDGISLHLQDTCPLLYSTDDAVCSK[ 0:891]A
Rat                                 WKLLCEHQFTVIVGELQK[ 0:1660]EFQEQLKITTFKDLVIRDKEVTGALIASLINCYIRDNAAVDGISLHLQDTCPLLYSTDDAVCSK[ 0:899]A
Guinea pig                          WKLLCEHQFTVIVGELQK[ 0:2844]EVQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:705]A
Naked mole rat                      WKLLCEHQFTVIVGELQK[ 0:3109]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLKDICPLLYSTDDAVCSK[ 0:758]A
Squirrel                            WKLLCEHQFTVIVGELQK[ 0:2497]EFQEQLKITTFKDLVIRDKELTGALISSLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:878]A
Rabbit                              WKLLCEHQFTLIVGELQK[ 0:3331]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[0:1044]A
Killer whale                        WKLLCEHQFTAIVGELQK[ 0:2538]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:660]A
Dolphin                             WKLLCEHQFTAIVGELQK[ 0:2515]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:661]A
Minke whale                         WKLLCEHQFTAIVGELQK[ 0:2326]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:660]A
Cattle                              WKLLCEHQFTVIVGELQK[ 0:2466]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[ 0:654]A
Sheep                               WKLLCEHQFTVIVGELQK[ 0:3140]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:648]A
Pig                                 WRLLCEHQFTVIVGELQK[ 0:3512]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[0:1151]A
Dog                                 WKLLCEHQFTVIVGELQK[ 0:4397]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[ 0:652]A
Panda                               WKLLCEHQFTVIVGELQK[ 0:3658]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[ 0:666]A
Ferret                              WKLLCEHQFTVIVGELQK[ 0:1379]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[ 0:661]A
Microbat                            WKLLCEHQFTVIVGELQK[ 0:1253]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISAHLQDICPLLYSTDDAVCSK[ 0:678]A
Megabat                             WKLLCEHQFTVIVGELQK[ 0:2284]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:677]A
Horse                               WKLLCEHQFTVIVGELQK[ 1:2256]EFQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[0:2599]A
Elephant                            WKLLCEHQFTVIVGELQK[ 0:2303]EFQEQLKITTFKDLVIRDKELTGALIASLISCYIRDNAAVDGISLHLQDICPLLYSTDDAICSK[ 0:680]A
Armadillo                           WKLLCEHQFTIIVGELQK[ 0:2640]EFQEQLKITTFKDLVIRDKELTGALISSLINCYIRDNAAVDGISLHLQDICPLLYSTDDAVCSK[ 0:686]A
Tasmanian devil                     WKLLCEHQFTIIVGELQK[  0:854]EFQEQLKITTFRDLVIRDKELTGALIASLINCYIRDNAAVDGISSHLQDICPLLYSTDDAVCSK[0:1120]A
Opossum                             WKLLCEHQFTVIVGELQK[ 0:1025]ELQEQLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISSHLQDICPLLYSTDDAICSK[ 0:753]A
Platypus                            WKLLCEHQFNVIVGELQK[  0:444]EYQEHLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISSHLQDICPLLYSTDDAVCSK[0:1029]A
Chicken                             WKLLCEHQFSVVVGELQK[ 0:2127]ELQEHLKMTAFKDLVIRDKELTGALIASLINCYIRDNAAVDGISAHLQDICPLLYSTDDAVCSK[0:1380]A
Turkey                              WKLLCEHQFSVVVGELQK[ 0:2318]ELQEHLKMTAFKDLVIRDKELTGALIASLINCYIRDNAAVDGISAHLQDICPLLYSTDDAVCSK[0:1346]A
Duck                                WKLLCEHQFNVVVGELQK[ 0:2254]ELQEHLKITAFKDLVIRDRELTGALIASLINCYIRDNAAVDGISAHLQDICPLLYSTDDAVCSK[ 0:391]A
Zebra finch                         WKLLCEHQFSVAVGELQK[ 0:1716]ELQEQLKITAFKDLVIRDRELTGALIASLINCYIRDNAAVDGIIAHLQDICPLLYSTDDAVCSK[ 0:629]A
Flycatcher                          WKLLCEHQFSVAVGELQK[ 0:1760]ELQEQLKITAFKDLVIRDRELTGALIASLINCYIRDNAAVDGIIAHLQDICPLLYSTDDAVCSK[ 0:569]A
Chinese softshell turtle            WKLLCEHQFSVVVGELQK[ 0:1174]EFQEHLKITTFKDLVIRDKELTGALIASLINCYIRDNAAVDGISSHLQDICPLLYGTDDAVCSK[0:1519]A
Xenopus                             WKLLCEHQFSLIVSDLQK[ 0:1075]ELQEQLKITTFKDLVIRDKELAGALTASLINCYIQDNASVDGVSYRLQEVCPLLYSTDDAVCSK[ 0:133]A
Guppy                               WKLLCDHHFSLIMSELPK[  0:122]EFQEEMKGACFKDVVTRGKELSGALVTGLINVYIKDNASVDAISIHLRDLCPMLYSTDDSVCSK[ 0:767]A
Amazon molly                        WKLLCDHHFSLIMSELPK[  0:119]EFQEQMKGACFKDIVTRGKELSGALVTGLINVYIKDNASVDAISNHLRDLCPLLYSTDDSVCSK[ 0:773]A
Platyfish                           WKLLCDHHFSLIMSELPK[  0:119]EFQEQMKGACFKDVVTRGKELSGALVTGLINVYIKDNASVDAISNHLRDLCPLLYSTDDSVCSK[ 0:860]A
Turquoise killifish                 WKLLCDHQFSLIMSELPK[   0:75]EFQEQIKGASFKDVVIRGKELSGALITGLINVYIKDNASVDAISNHLRDICPLLYSSDDSICSK[ 0:226]A
Medaka                              WKLLCDHQFSLIMAELPK[  0:112]EFQEQMKGASFKDVVIRGKELSGALITGLINVYIKDNASVDAISNHLRDLCPLLYSSDDSICSK[ 0:319]A
Tilapia                             WKLLCDHQFSLIMSELPK[  0:121]EFQEQMKGASFKDVVIRGKELSGALITGLINVYIKDNASVDAISNHLRDICPLLYSSDDSVCSK[ 0:228]A
Fugu                                WRLLCEHQFSLIMSELPK[   0:70]EFQEQMKGVGFKDVVIRGKELSGALITALINVYIKDKASVDAISNHLRDICPLLYSSDDSVCSK[ 0:455]A
Stickleback                         LKLLCDHQFSLIMSELPK[  0:187]EFQEQMKGASFKDVVIRGKELSGSLITALINVYIKDNASVEAISNHLRDICPLLYSSDDSVCSK[ 0:254]A
Cod                                 WKLLCDHKFSLILSELPT[ 0:1646]EYQDQMKGASFRDVVIRGKELSGALITALINVYIKDNASVDAVSRHLRDTCPLLYTSDDSVCSK[  0:95]A
Coho salmon                         WKLLCDHQFSLIISELPM[ 0:1331]EFQDQMKGASFKDVVIRGRELTGALITALINVYIKDSASVDAISNHLRDICPLLYSSDDSVCSK[ 0:201]A
Atlantic salmon                     WKLLCDHQFSLIISELPM[ 0:1750]EFQDQMKGASFKDVVIRGRELTGALITALINVYIKDSASVDAISNHLRDICPLLYSSDDSVCSK[ 0:195]A
Northern pike                       WKLLCDHQFSLVIAELPK[  0:220]EFQDQMKGVSFKDVVIRGRELSGALITALINVYIKDSASVDAISTHLRDICPLLYSSDDSVCSK[ 0:157]A
Zebrafish                           WKLLCDHQFSLILSEMPK[  0:315]EFQDQMKAVSFKDVVVCGRELSGALITALINVYIKDSASVDTLSAHLRDICPLLYSSDDSICSK[0:1303]A
Cave fish                           WKLLCDHQFSLIISELPK[   0:83]DFQEQLKAISFKDLVIRGRELTGALVTALINVYIKDSASVDAISCHLREICPLLYSSDDSVCSK[ 0:616]A
Channel catfish                     WKLLCDHQFSLIISELPK[  0:546]DFLEQLKAISFKDMVNRGRELTGALITALINVYIKDSASVDAISTLLREICPLLYSSDDSVCSK[  0:78]A
Spotted gar                         WKLLCDHQFGLILNDLPK[  0:585]EFQEQVKVSSFKDIVIRGRELTGALITSLINCYIKDSASVDAISCHLRETCPLLYSSDDSVCSK[ 0:418]A
Whale shark                         WKMLCDHQFGVIIADLQK[0:19584]ELQEQLKSTPFRDLVIRGKELGGALITSLINRYIGDNASVDAISKHLRDTCPLLYSNDDAVCSK[0:2082]A
Lamprey                             WKLLCEHGVPVLTAALPP[ 0:1038]DIRDTLKAMSLQELVLRGDAVTGGLITALINRYIGDLASTDSISQHLRAACPLLYSVDDVTCSK[ 0:116]A
Nematode                            WLLAYEYNLTAISSGMNP[       ]QLLPNFSSRKLAHLVSDGSNLNAELIRAMIKYFLGDEAGTKILSESLRQLCPNLYSEDDACVTF[      ]A
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Figure S23. Portion of the sequence alignment of the NUP155 cluster of single copy orthologous genes. Intron position and length are shown in the square brackets. The cyan box shows the partially aligned sequence of the whale shark.

Table S22. GO enrichment of correlated single-copy orthologous gene families between gene length and the maximum lifespan, body weight, and BMR simultaneously

GO ID	GO terms	# Genes	*p*-value	Adjusted *p*-value	Associated Genes Found
GO:0006405	RNA export from nucleus	7	2.E-04	6.E-03	[ABCE1, ENY2, NUP155, RAE1, SARNP, SDAD1, XPO5]
GO:0006611	protein export from nucleus	9	3.E-05	8.E-04	[ABCE1, CSE1L, ENY2, NUP155, RAE1, SARNP, SDAD1, STYX, XPO5]
GO:0007004	telomere maintenance via telomerase	3	2.E-02	2.E-02	[MAPKAPK5, NAT10, XRCC5]
GO:0008209	androgen metabolic process	3	2.E-03	4.E-02	[CYP19A1, HSD17B3, SGPL1]
GO:0008210	estrogen metabolic process	3	2.E-03	3.E-02	[CYP19A1, SGPL1, STAR]
GO:0042537	benzene-containing compound metabolic process	3	1.E-03	2.E-02	[AADAT, KMO, STAR]
GO:0043648	dicarboxylic acid metabolic process	5	3.E-03	5.E-02	[AADAT, DLD, KMO, NMNAT2, STAR]
GO:0051168	nuclear export	9	6.E-05	2.E-03	[ABCE1, CSE1L, ENY2, NUP155, RAE1, SARNP, SDAD1, STYX, XPO5]
GO:0051439	regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	3	2.E-02	5.E-02	[ANAPC5, PSMA1, PSMD14]
GO:0060632	regulation of microtubule-based movement	3	1.E-03	3.E-02	[CFAP20, CNIH4, TCTEX1D2]
GO:0061370	testosterone biosynthetic process	3	5.E-05	1.E-03	[CYP19A1, HSD17B3, STAR]
GO:0065002	intracellular protein transmembrane transport	4	1.E-03	3.E-02	[AGK, PEX3, PEX7, SRP54]
GO:0071166	ribonucleoprotein complex localization	6	9.E-04	2.E-02	[ABCE1, ENY2, NUP155, RAE1, SARNP, SDAD1]
GO:0071426	ribonucleoprotein complex export from nucleus	6	8.E-04	2.E-02	[ABCE1, ENY2, NUP155, RAE1, SARNP, SDAD1]
GO:0071806	protein transmembrane transport	4	2.E-03	4.E-02	[AGK, PEX3, PEX7, SRP54]

Functional enrichment tests were performed using ClueGO with options as below [55]

Options: 'Min GO Level = 3, Max GO Level = 8, Number of Genes = 3, Min Percentage = 4.0, GO Fusion = false, GO Group = true, Kappa Score Threshold = 0.4, Over View Term = SmallestPValue, Group By Kappa Statistics = true, Initial Group Size = 1, Sharing Group Percentage = 50.0'

Table S23. Representative human gene list in the single-copy orthologous gene families having correlated gene length with the maximum lifespan, the body weight, and the BMR simultaneously

Gene symbol	Entrez ID	Gene name
RPL31	6160	ribosomal protein L31
CYP19A1	1588	cytochrome P450 family 19 subfamily A member 1
PRPF38A	84950	pre-mRNA processing factor 38A
MPC2	25874	mitochondrial pyruvate carrier 2
NUDT21	11051	nudix hydrolase 21
TMEM67	91147	transmembrane protein 67
SAP18	10284	Sin3A associated protein 18
ABCE1	6059	ATP binding cassette subfamily E member 1
MED27	9442	mediator complex subunit 27
UBR4	23352	ubiquitin protein ligase E3 component n-recognin 4
DNAJC8	22826	DnaJ heat shock protein family (Hsp40) member C8
FCF1	51077	FCF1, rRNA-processing protein
IDE	3416	insulin degrading enzyme
DPH3	285381	diphthamide biosynthesis 3
ATP5O	539	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit
PCGF5	84333	polycomb group ring finger 5
PSMA1	5682	proteasome subunit alpha 1
XPO5	57510	exportin 5
DLD	1738	dihydrolipoamide dehydrogenase
SRP54	6729	signal recognition particle 54
WASHC5	9897	WASH complex subunit 5
TBCD	6904	tubulin folding cofactor D
SARNP	84324	SAP domain containing ribonucleoprotein
UFD1	7353	ubiquitin recognition factor in ER associated degradation 1
NUP155	9631	nucleoporin 155
ERGIC2	51290	ERGIC and golgi 2
GALC	2581	galactosylceramidase
NAA35	60560	N(alpha)-acetyltransferase 35, NatC auxiliary subunit
CCNC	892	cyclin C
KMO	8564	kynurenine 3-monooxygenase
SNX4	8723	sorting nexin 4
ITGB1BP1	9270	integrin subunit beta 1 binding protein 1
DHDDS	79947	dehydrodolichyl diphosphate synthase subunit
SCGN	10590	secretagogin, EF-hand calcium binding protein
TRAIP	10293	TRAF interacting protein
MINDY3	80013	MINDY lysine 48 deubiquitinase 3
SCFD1	23256	sec1 family domain containing 1
CDK7	1022	cyclin dependent kinase 7
VAMP4	8674	vesicle associated membrane protein 4
DENR	8562	density regulated re-initiation and release factor
CFAP20	29105	cilia and flagella associated protein 20
LARS2	23395	leucyl-tRNA synthetase 2, mitochondrial
ENY2	56943	ENY2, transcription and export complex 2 subunit
EIF2B1	1967	eukaryotic translation initiation factor 2B subunit alpha
MRPS14	63931	mitochondrial ribosomal protein S14
C6orf62	81688	chromosome 6 open reading frame 62
C11orf54	28970	chromosome 11 open reading frame 54
EFTUD2	9343	elongation factor Tu GTP binding domain containing 2
RTCB	51493	RNA 2',3'-cyclic phosphate and 5'-OH ligase
IFT81	28981	intraflagellar transport 81
MAPKAPK5	8550	mitogen-activated protein kinase-activated protein kinase 5
CNEP1R1	255919	CTD nuclear envelope phosphatase 1 regulatory subunit 1
COTL1	23406	coactosin like F-actin binding protein 1
MRPL13	28998	mitochondrial ribosomal protein L13
CSE1L	1434	chromosome segregation 1 like
SGPL1	8879	sphingosine-1-phosphate lyase 1
LIN52	91750	lin-52 DREAM MuvB core complex component
VPS53	55275	VPS53, GARP complex subunit
TMEM243	79161	transmembrane protein 243
PSMD14	10213	proteasome 26S subunit, non-ATPase 14
C11orf49	79096	chromosome 11 open reading frame 49

CNIH4	29097	cornichon family AMPA receptor auxiliary protein 4
WASHC3	51019	WASH complex subunit 3
STAR	6770	steroidogenic acute regulatory protein
SLC10A7	84068	solute carrier family 10 member 7
MAP2K5	5607	mitogen-activated protein kinase kinase 5
AVL9	23080	AVL9 cell migration associated
AGK	55750	acylglycerol kinase
RAE1	8480	ribonucleic acid export 1
TTC37	9652	tetratricopeptide repeat domain 37
C10orf76	79591	chromosome 10 open reading frame 76
GPCPD1	56261	glycerophosphocholine phosphodiesterase 1
SDAD1	55153	SDA1 domain containing 1
POLR3F	10621	RNA polymerase III subunit F
PRPF18	8559	pre-mRNA processing factor 18
TBC1D19	55296	TBC1 domain family member 19
PPP4R4	57718	protein phosphatase 4 regulatory subunit 4
RWDD4	201965	RWD domain containing 4
AADAT	51166	aminoadipate aminotransferase
EIF3K	27335	eukaryotic translation initiation factor 3 subunit K
POLE2	5427	DNA polymerase epsilon 2, accessory subunit
GATM	2628	glycine amidinotransferase
COG6	57511	component of oligomeric golgi complex 6
NUDT5	11164	nudix hydrolase 5
FAF1	11124	Fas associated factor 1
TMEM38A	79041	transmembrane protein 38A
USP37	57695	ubiquitin specific peptidase 37
ACER3	55331	alkaline ceramidase 3
TTC38	55020	tetratricopeptide repeat domain 38
ATP6AP2	10159	ATPase H+ transporting accessory protein 2
NMNAT2	23057	nicotinamide nucleotide adenylyltransferase 2
GTF2H3	2967	general transcription factor IIH subunit 3
EED	8726	embryonic ectoderm development
COG2	22796	component of oligomeric golgi complex 2
BDH2	56898	3-hydroxybutyrate dehydrogenase 2
UTP20	27340	UTP20, small subunit processome component
MBIP	51562	MAP3K12 binding inhibitory protein 1
NPL	80896	N-acetylneuraminate pyruvate lyase
NAT10	55226	N-acetyltransferase 10
PEX7	5191	peroxisomal biogenesis factor 7
PEX3	8504	peroxisomal biogenesis factor 3
PNPT1	87178	polyribonucleotide nucleotidyltransferase 1
UBR2	23304	ubiquitin protein ligase E3 component n-recognin 2
ANAPC5	51433	anaphase promoting complex subunit 5
JKAMP	51528	JNK1/MAPK8 associated membrane protein
SUPT4H1	6827	SPT4 homolog, DSIF elongation factor subunit
RARS2	57038	arginyl-tRNA synthetase 2, mitochondrial
TMEM144	55314	transmembrane protein 144
DYNC2LI1	51626	dynein cytoplasmic 2 light intermediate chain 1
ITIH2	3698	inter-alpha-trypsin inhibitor heavy chain 2
CCDC93	54520	coiled-coil domain containing 93
RNASEH2B	79621	ribonuclease H2 subunit B
FANCI	55215	Fanconi anemia complementation group I
ADGRD1	283383	adhesion G protein-coupled receptor D1
KRIT1	889	KRIT1, ankyrin repeat containing
SLC37A3	84255	solute carrier family 37 member 3
C1orf112	55732	chromosome 1 open reading frame 112
MRPS10	55173	mitochondrial ribosomal protein S10
SCARB2	950	scavenger receptor class B member 2
UBA6	55236	ubiquitin like modifier activating enzyme 6
APPBP2	10513	amyloid beta precursor protein binding protein 2
SLC35A1	10559	solute carrier family 35 member A1
ITGA9	3680	integrin subunit alpha 9
POLB	5423	DNA polymerase beta
RTTN	25914	rotatin
MTTP	4547	microsomal triglyceride transfer protein
NAAA	27163	N-acylethanolamine acid amidase
STYX	6815	serine/threonine/tyrosine interacting protein
DNTTIP1	116092	deoxynucleotidyltransferase terminal interacting protein 1
POLA2	23649	DNA polymerase alpha 2, accessory subunit
VPS41	27072	VPS41, HOPS complex subunit
NSUN6	221078	NOP2/Sun RNA methyltransferase family member 6
CWF19L1	55280	CWF19 like 1, cell cycle control (S. pombe)
MIGA2	84895	mitoguardin 2

RFX4	5992	regulatory factor X4
ACAD11	84129	acyl-CoA dehydrogenase family member 11
XRCC5	7520	X-ray repair cross complementing 5
CFAP69	79846	cilia and flagella associated protein 69
AAGAB	79719	alpha and gamma adaptin binding protein
HSD17B3	3293	hydroxysteroid 17-beta dehydrogenase 3
RMC1	29919	regulator of MON1-CCZ1
PPP1R21	129285	protein phosphatase 1 regulatory subunit 21
GDA	9615	guanine deaminase
NCAPG2	54892	non-SMC condensin II complex subunit G2
PQLC3	130814	PQ loop repeat containing 3
NARS2	79731	asparaginyl-tRNA synthetase 2, mitochondrial
CENPW	387103	centromere protein W
C17orf67	339210	chromosome 17 open reading frame 67
TCTEX1D2	255758	Tctex1 domain containing 2
FAAH2	158584	fatty acid amide hydrolase 2
ODR4	54953	odr-4 GPCR localization factor homolog
TXNDC16	57544	thioredoxin domain containing 16
SMIM7	79086	small integral membrane protein 7
MTCP1	4515	mature T-cell proliferation 1
TRPM8	79054	transient receptor potential cation channel subfamily M member 8

Table S24. Representative human gene list of single-copy orthologous gene families with correlations between gene length and only maximum lifespan, only the body mass, or only the BMR, respectively

Groups	Gene symbol	Entrez ID	Gene name
Maximum lifespan	PARG	8505	poly(ADP-ribose) glycohydrolase
Maximum lifespan	HECTD4	283450	HECT domain E3 ubiquitin protein ligase 4
Maximum lifespan	TM9SF2	9375	transmembrane 9 superfamily member 2
Maximum lifespan	PI4KA	5297	phosphatidylinositol 4-kinase alpha
Maximum lifespan	UBL3	5412	ubiquitin like 3
Maximum lifespan	NUP210	23225	nucleoporin 210
Maximum lifespan	SFXN5	94097	sideroflexin 5
Maximum lifespan	IARS	3376	isoleucyl-tRNA synthetase
Maximum lifespan	FRG1	2483	FSHD region gene 1
Maximum lifespan	POLR2H	5437	RNA polymerase II subunit H
Maximum lifespan	TTC26	79989	tetratricopeptide repeat domain 26
Maximum lifespan	ZBTB8OS	339487	zinc finger and BTB domain containing 8 opposite strand
Maximum lifespan	SRP19	6728	signal recognition particle 19
Maximum lifespan	GINS4	84296	GINS complex subunit 4
Maximum lifespan	ELP1	8518	elongator complex protein 1
Maximum lifespan	FRA10AC1	118924	FRA10A associated CGG repeat 1
Maximum lifespan	LRPPRC	10128	leucine rich pentatricopeptide repeat containing
Maximum lifespan	VWF	7450	von Willebrand factor
Maximum lifespan	LAMTOR3	8649	late endosomal/lysosomal adaptor, MAPK and MTOR activator 3
Maximum lifespan	CRIPT	9419	CXXC repeat containing interactor of PDZ3 domain
Maximum lifespan	VIPAS39	63894	VPS33B interacting protein, apical-basolateral polarity regulator, spe-39 homolog
Maximum lifespan	RPN2	6185	ribophorin II
Maximum lifespan	LIN37	55957	lin-37 DREAM MuvB core complex component
Maximum lifespan	AP4M1	9179	adaptor related protein complex 4 mu 1 subunit
Maximum	GPLD1	2822	glycosylphosphatidylinositol specific phospholipase D1

lifespan			
BMR	SNX14	57231	sorting nexin 14
BMR	TADA2A	6871	transcriptional adaptor 2A
BMR	TXNL4B	54957	thioredoxin like 4B
BMR	CCDC134	79879	coiled-coil domain containing 134
BMR	HMCN1	83872	hemicentin 1
BMR	BORCS7	119032	BLOC-1 related complex subunit 7
Body weight	COX5B	1329	cytochrome c oxidase subunit 5B

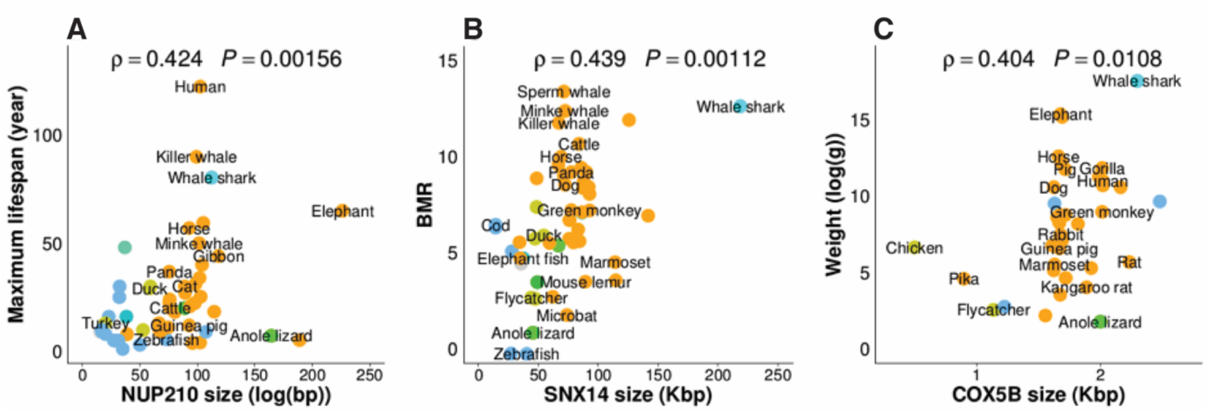

Figure S24. Single-copy orthologous gene families with correlations between gene length and maximum lifespan, weight, and BMR. Three instances of correlation between gene lengths of NUP210 (A), SNX14 (B), and COX5B (C) single-copy orthologous gene families and maximum lifespan, BMR, and body weight respectively. Dot colors represent the class as in Figure 1.

5. Scaling relationships

5.1 Scaling between genomic traits, physiological traits, and ecological parameters

We set out to determine whether the statistically significant correlations between genomic traits, physiological traits, and ecological parameters that we observed (Figure 2) in our array of species (centered on Chordates) reflect scaling relationships that may be formalized as power laws written as $Y = A{*}X^{B}$ [40]. Consistent with previous work[40], we found that the Basal Metabolic Rate (BMR) correlates with mass (B = 0.68, Figure S25). Furthermore, genome size, measured as golden path length, scales with gene size, measured as summed length of exons and intron per gene (B = 1.32, Figure S26), consistent with the observed lengthening of the whale shark genome by expanded CR1 repetitive elements (Figure 1A). Additionally, unlike in bacteria[58] and crustaceans[59], genome size in Chordates scales positively with temperature (B = 0.77, Figure S27).

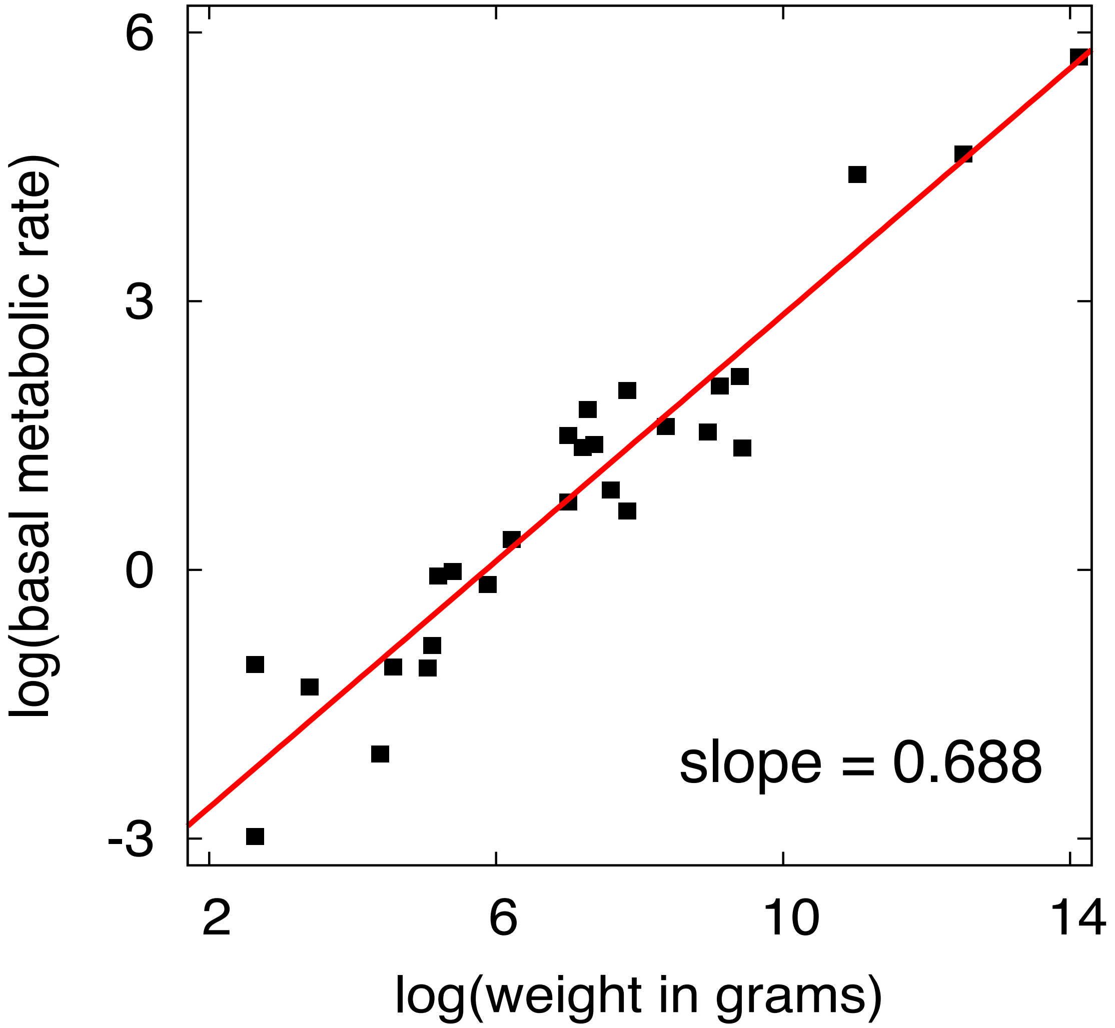

Figure S25. Scaling of basal metabolic rate to body size. Our regression analysis shows that, in 27 animal species, experimentally-determined BMR is positively scaled with body mass with an exponent B that is smaller than 1 (B = 0.688). Thus, BMRs are increased less than expected from body size.

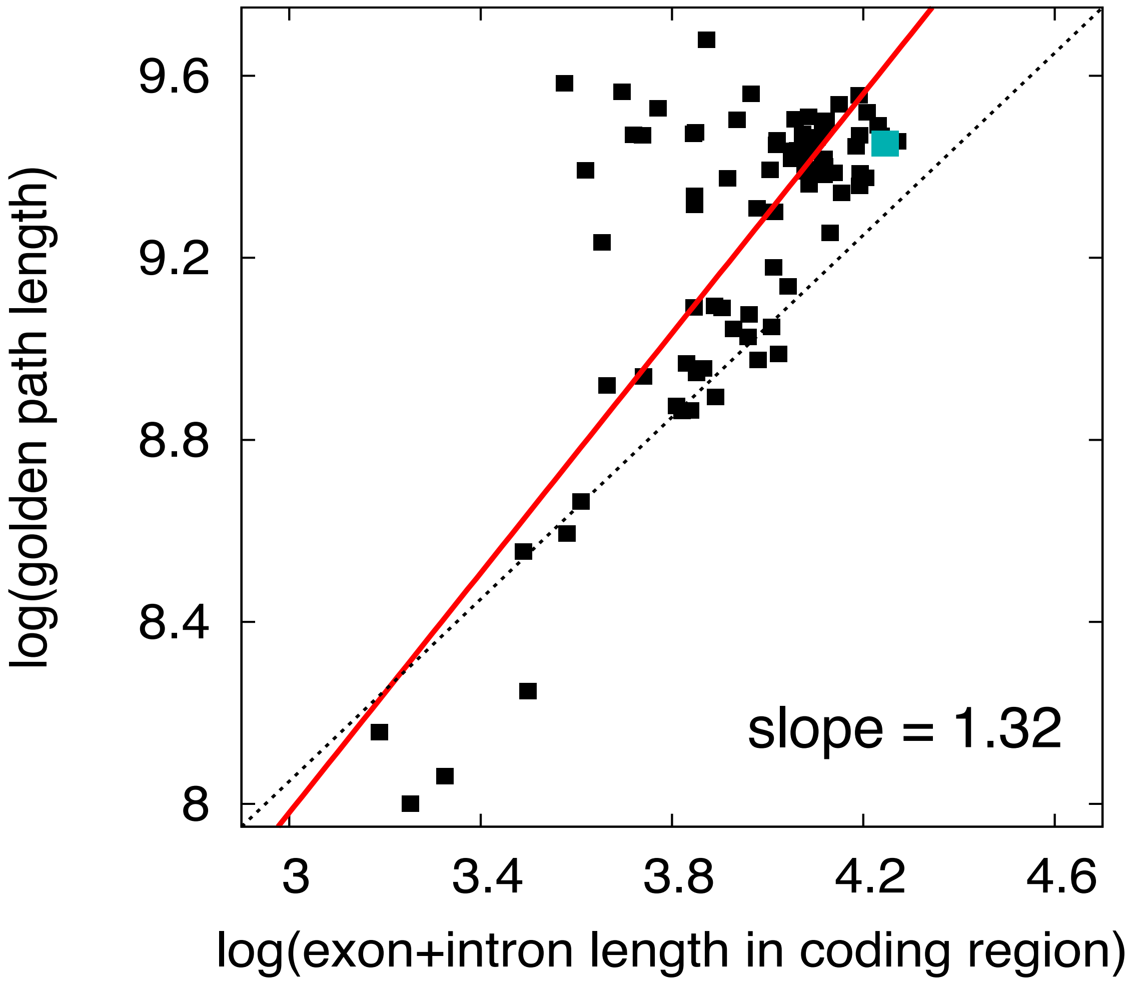

Figure S26. Scaling of genome size to gene size. Our regression analysis shows that, in 81 animal species, genome size (measured as golden path length) is positively scaled with gene size (measured for every gene as the sum of exons and introns) with an exponent B that is bigger than 1 (B = 1.32). Thus, genome size is significantly longer than expected from gene size alone.

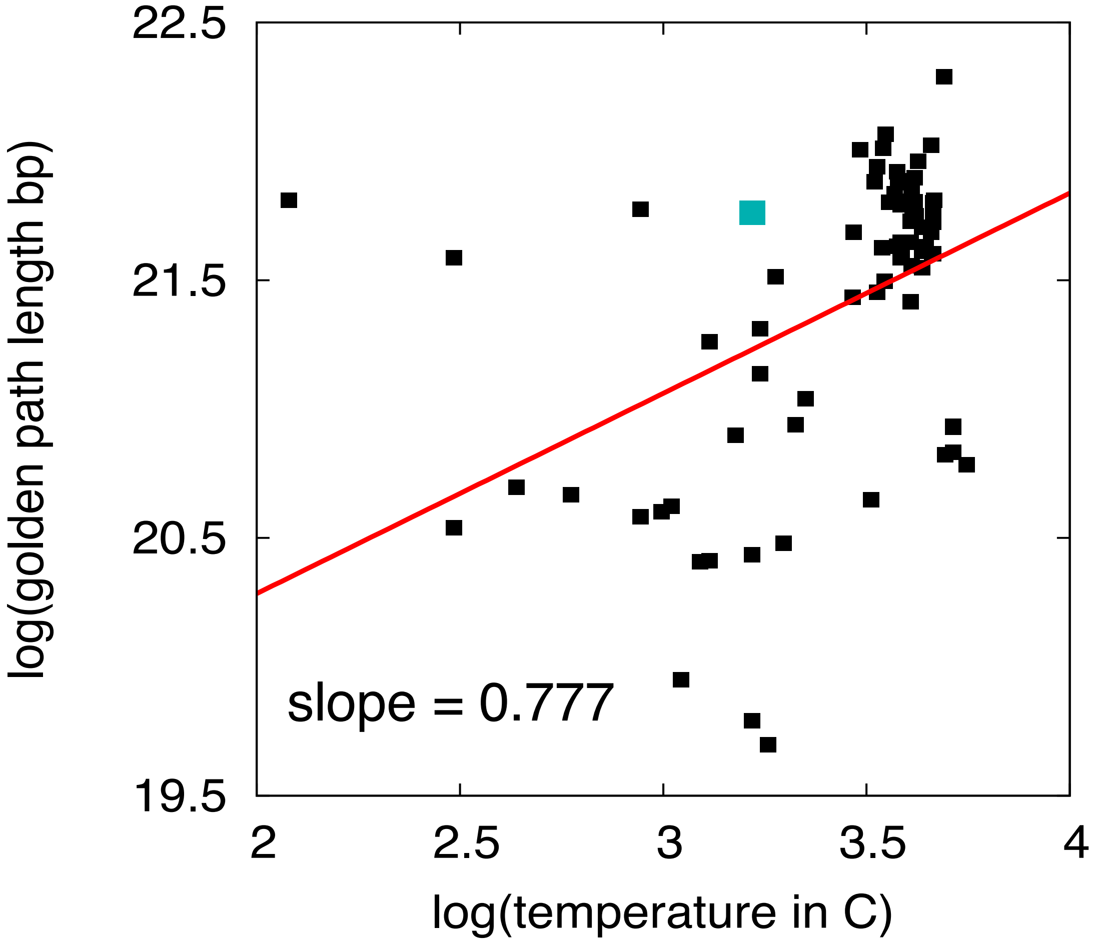

Figure S27. Scaling of genome size to temperature. Our regression analysis shows that, in 81 animal species, genome size (measured as golden path length) is positively scaled with temperature with an exponent B that is smaller than 1 (B = 0.777). Thus, genome size increases with temperature.

5.2 Scaling of neural genes to average gene lengths

Since several categories of neural genes are longer than average genes (Figure 4A, Figure S21), we examined whether their neural and average lengths obey a scaling relationship. Surprisingly, we found that neural genes are scaled to average genes with an exponent greater than 1 (B = 1.038, Figure S28), with the whale shark showing an extreme lengthening of neural genes.

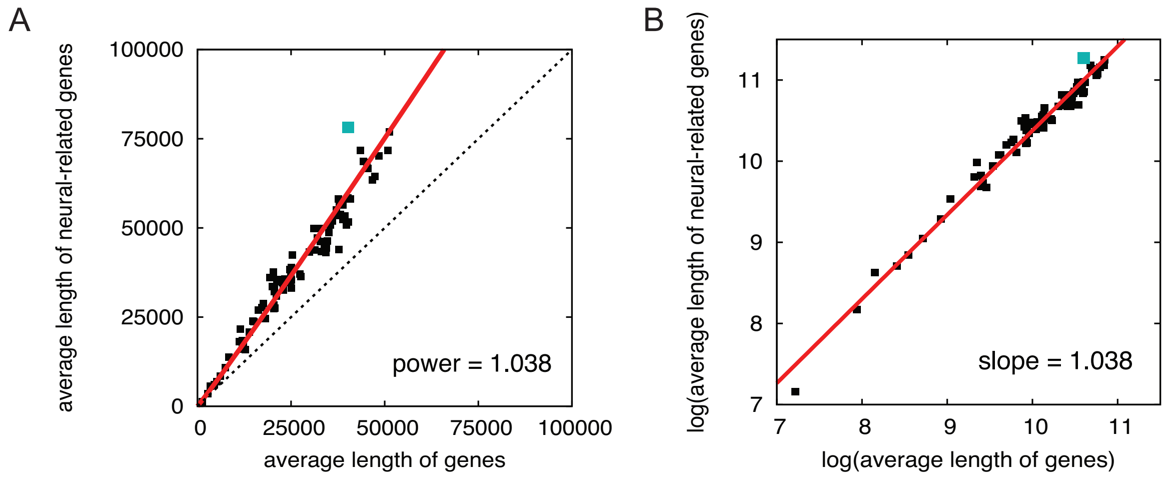

Figure S28. Scaling of neural genes to average gene size. Our regression analysis shows that, in 81 animal species, neural gene size is positively scaled with gene length (measured for every gene as the sum of exons and introns) with an exponent B that is bigger than 1 (B = 1.038). Thus, neural genes are longer than expected from gene size alone.

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