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Historical contingency shapes adaptive radiation in Antarctic fishes

Jacob M. Daane^{1*}, Alex Dornburg², Patrick Smits³, Daniel J. MacGuigan⁴, M. Brent Hawkins^{3,5,6}, Thomas J. Near^{4,7}, H. William Detrich III^{1,†,*}, and Matthew P. Harris^{3,6,†,*}

Affiliations:

¹ Department of Marine and Environmental Sciences, Northeastern University Marine Science Center, Nahant, MA 01908, USA

² North Carolina Museum of Natural Sciences, Raleigh, NC 27601, USA

³ Orthopaedic Research Laboratories, Department of Orthopaedic Surgery, Boston Children's Hospital, Boston, MA 02115, USA

⁴ Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA

⁵ Museum of Comparative Zoology, Harvard University, Cambridge, MA 02138, USA

⁶ Department of Genetics, Harvard Medical School, Boston, MA 02115, USA

⁷ Peabody Museum of Natural History, Yale University, New Haven, CT 06520, USA

† Co-senior authors.

*Authors for correspondence. j.daane@northeastern.edu, w.detrich@northeastern.edu, harris@genetics.med.harvard.edu

Abstract:

Adaptive radiation illustrates links between ecological opportunity, natural selection, and the generation of biodiversity. Central to adaptive radiation is the association between a diversifying lineage and the evolution of phenotypic variation that facilitates the utilization of novel environments or resources. However, is not clear whether adaptive evolution or historical contingency is more important for the origin of key phenotypic traits in adaptive radiation. Here we use targeted sequencing of >250,000 loci across 46 species to examine hypotheses concerning the origin and diversification of key traits in the adaptive radiation of Antarctic notothenioid fishes. Contrary to expectations of adaptive evolution, we show that notothenioids experienced a punctuated burst of genomic diversification and evolved key skeletal modifications before the onset of polar conditions in the Southern Ocean. We show that diversifying selection in pathways associated with human skeletal dysplasias facilitates ecologically important variation in buoyancy among Antarctic notothenioid species, and demonstrate the sufficiency of altered *trip11*, *colla2* and *colla1* function in zebrafish

(*Danio rerio*) to phenocopy skeletal reduction in Antarctic notothenioids. Rather than adaptation being driven by the cooling of the Antarctic, our results highlight the role of historical contingency in shaping the adaptive radiation of notothenioids.

Understanding the historical and environmental context for the origin of key traits in adaptive radiations extends beyond reconstructing events that result in evolutionary innovation as it also provides a context in forecasting the effects of climate change on the stability and evolvability of natural populations.

During adaptive radiation, ecological opportunity and key phenotypic traits interact to facilitate the expansion of populations into new niches¹. Deciphering the genotype-phenotype relationships of these key traits provides important insight into the historical circumstances that result in phenotypic diversification^{7,8}. Advances in sequencing capabilities now allow for the efficient collection of genomic sequence data for scores of species. This potential for increased taxonomic, genome-wide sampling provides opportunities to investigate the macroevolutionary mechanisms driving evolution across clades. For example, a major question in the study of adaptive radiation is the relative role of trait novelty versus the modification of existing phenotypes in facilitating the diversification of species. Ecological opportunities, such as those brought about by environmental changes, can promote the origin of novel phenotypes that accelerate lineage diversification^{2,3}. However, trait novelty may arise at any time, and adaptive change can result from a shift in selection regimes that enable lineages to opportunistically explore new ecospace using a trait that had previously evolved under different circumstances⁹. These two hypotheses have long been central in the debate concerning the role of punctuated versus gradualistic evolution in the generation of biodiversity^{9,10}, and posit opposing views on how the genomic substrate of subsequent phenotypic diversification evolved. Here, we provide a genomic perspective of ecologically

important trait variation and phylogenetic origin in a species-rich adaptive radiation. Through consideration of closely related lineages that are not a part of the adaptive radiation, we can specifically investigate the relative importance of contingency in adaptive radiation and test whether the genomic basis of ecological trait variation coincides with or precedes the onset of radiation.

Results and Discussion

To test hypotheses concerning the origin of traits that facilitate adaptive radiation, we focused on Antarctic notothenioids (Cryonotothenioidea), an iconic example of adaptive radiation in marine vertebrates^{6,11}. The diversification of cryonotothenioids followed the progressive cooling of Antarctica that initiated ~33 million years ago⁶, and was coincident with the extinction of a phylogenetically diverse and cosmopolitan fish fauna¹². This combination of climate change and vacated niches presented notothenioids with the opportunity to diversify into a large range of benthic and water column habitats. All notothenioids lack a swim bladder, the primary buoyancy organ in most teleost fishes; however, there are substantial differences in buoyancy among notothenioid species that are correlated with habitat and resource utilization^{6,12}. These differences in buoyancy are achieved through the reduction of skeletal density coupled with the accumulation of corporeal lipids that provide static lift^{12–14}. In the narrative of the Antarctic cryonotothenioid adaptive radiation, these ecologically important traits are hypothesized to have arisen during the onset of polar conditions; however, the evolutionary origin of reduced skeletal density in the context of early-diverging non-Antarctic notothenioid lineages challenges this scenario¹⁴.

To develop a comprehensive phylogenomic perspective on the Antarctic notothenioid adaptive radiation, we employed a cross-species targeted sequence enrichment approach to specifically sequence conserved and functionally-annotated genetic loci¹⁵. We combined sequence information from the genome of *Notothenia coriiceps* and select other percomorph

teleost species to design a comparative DNA probe set for systematic targeted enrichment of ~250,000 coding and conserved non-coding (CNE) elements comprising over 40 Mb of genomic sequence (**Supplementary Figure 1**). CNEs were defined as miRNA hairpins, orthologs to human ultraconservative elements, and constrained genetic regions (GERP) identified in the Ensembl compara 11-way teleost genome alignment that did not overlap with protein-coding regions. Using this probe set, we captured coding and non-coding sequences from a phylogenetically-rich sampling of 46 notothenioid species that includes all three early-diverging non-Antarctic lineages and two species from the closely related Percidae (**Supplementary Figure 1**). Importantly, we used pooled population samples of individual species to permit identification of fixed and variable SNPs for each species.

We achieved 89-95% coverage of targeted regions for each sampled species (**Supplementary Figure 1; Supplementary Tables 1,2**). This permitted identification of an average of 95,000 fixed species-specific SNPs and 60,000 heterozygous SNPs for each lineage (**Supplementary Table 3**). As proof of the sensitivity of the dataset, we confirmed known cases of adult hemoglobin gene loss within the icefishes (**Supplementary Figure 2**)¹⁶. The power of this dataset was further illustrated by identification of a previously uncharacterized deletion of two putative embryonic hemoglobin genes (**Supplementary Figure 2**). Thus, this approach provides a robust and efficient method by which to characterize variation across taxonomically rich clades, even when separated by significant evolutionary distances.

Given expectations from the adaptive radiation of East African cichlids¹⁷, we tested whether shifts in the rate of nucleotide evolution correspond to the onset of rapid lineage diversification in the cryonotothenioid radiation. Using a time-calibrated phylogeny of most living notothenioids and a Bayesian framework to assess shifts in speciation rates^{11,18}, we confirmed a shift in lineage diversification at the origin of the antifreeze-bearing Antarctic

cryonotothenioids and an additional acceleration in diversification within the Plunderfishes (Artedidraconidae)⁶ (**Fig. 1a**). Intriguingly, these shifts do not correspond with accelerated rates of nucleotide evolution. Instead, we found that the majority of extant cryonotothenioid sequence diversity is derived from a period of significantly high rates of genomic evolution that occurred in the ancestral lineage that includes the most recent common ancestor of the non-Antarctic distributed *Eleginops maclovinus* and the Antarctic cryonotothenioids (Eleginopsioidea, **Fig. 1b, Supplementary Table 4**). This demonstrates that a major change in molecular rate variation preceded the onset of global cooling and adaptive radiation of cryonotothenioids by well over 10 million years (**Fig. 1c**). An assessment of the accumulation of genomic divergence through relaxed molecular clock models (**Fig. 1d**), along with an analysis of synonymous (dS) and nonsynonymous substitution (dN) rates (**Fig. 1e**), further substantiates a high rate of nucleotide evolution prior to the origin and diversification of cryonotothenioids. This acceleration of overall genomic change may have been critical for accumulating the genetic diversity that provided the substrate for phenotypic diversification during the early radiation of cryonotothenioids.

As buoyancy adaptations are key traits that facilitated notothenioid diversification into the water column during the adaptive radiation, we assessed patterns of skeletal density throughout the phylogeny using computerized tomography (**Fig. 2a, Supplementary Figure 3**). We find that, in parallel with the observed increase in nucleotide substitution rate, a broad reduction in bone density occurred prior to the recent common ancestor of *Eleginops maclovinus*¹⁹ and the Antarctic cryonotothenioids (Eleginopsioidea, **Fig. 2a**) and was retained throughout the clade. Consistent with these decreases in bone density, we found biased diversifying selection in genes associated with human skeletal dysplasias and mineralization defects in Eleginopsioidea relative to the well-ossified sister lineage, *Pseudaphritis urvillii* (**Fig. 2b,c**). This evolutionary reconstruction is contrary to the expectation of the emergence

of novel traits temporally coinciding with the onset of adaptive radiation and changing ecological opportunities, but rather indicates that prior to the shift to polar conditions, notothenioid lineages possessed key traits and may have begun to utilize new habitats in the water column through a modification of buoyancy enabled through a derived reduction in bone density.

To further explore the genetic basis of the evolved reduction in bone density, we assessed patterns of diversifying selection within the phylogeny. Among *Eleginops maclovinus* and all cryonotothenioids, we identified shared selective signatures in clinically relevant skeletal genes, such as *collagen1a1* and *collagen1a2* (**Fig. 3a, Supplementary Figures 4-6, Supplementary Table 5**). The function of these genes is conserved among disparate lineages of vertebrates²⁰ and nonsynonymous mutations in these collagens can lead to severe osteogenesis imperfecta in humans²¹. Notably, previous studies show that *collagen1* expression is reduced in the developing skeleton of notothenioid embryos, providing further evidence that broad changes at collagen loci are associated with skeletal variation²². In addition to selection on collagens, we identified diversifying selection in an unlikely gene candidate for skeletal variation, *trip11(gmap-210)* (**Fig 3a, Supplementary Figures 4-6, Supplementary Table 5**). This gene is conserved across eukaryotes and functions in vesicle tethering in the cis-Golgi membrane²³. In humans, mutations in *TRIP11* lead to severe skeletal deficiencies that lead to peri-natal lethality²⁴. Intriguingly, both *collagen1a1a* and *trip11* are also under diversifying selection and/or accelerated sequence evolution within the further diversification of the Channichthyidae, which have evolved an additional and significant reduction in bone density (**Fig. 2a; Fig 3a; Supplementary Figures 4-6, Supplementary Tables 5-8**)¹⁴. This parallelism suggests constraint in the types of mutations in evolution that can drive changes in skeletal density while maintaining viability.

To experimentally test the potential for alterations in *trip11*, *colla1* and *colla2* function to impart non-lethal skeletal phenotypes that are consistent with those observed in notothenioids, we analyzed mutants stemming from genetic screens and CRISPR-Cas9 gene editing in zebrafish (*Danio rerio*). Contrary to expectations from humans, zebrafish homozygous for loss-of-function alleles of *trip11* were viable with no obvious external morphological abnormalities (**Fig. 3b,c**). However, whereas heterozygous siblings had density patterns comparable to wild-type zebrafish, similarly size and age-matched homozygous *trip11* mutants had significantly reduced skeletal density (**Fig. 3b,c**). In affected individuals, all bones investigated, including those of the skull roof, vertebrae and operculum were reduced in density (**Fig. 3b,c**) and generally phenocopied the pattern observed in adult *Eleginops maclovinus* and cryonotothenioids (**Fig. 2a**). Notably, expressivity of the *trip11* skeletal phenotype was variable, which suggests the presence of background genetic modifiers. Similarly, we show mutant models of *colla1*^{dmh14/+} (G1144E) and *colla2*^{dmh15/+} (G882N) in zebrafish also cause a reduction in bone density (**Fig. 3c**)²⁰. These experiments confirm that changes to the loci under selection yield phenotypes consistent with those observed in *E. maclovinus* and species of the cryonotothenioid radiation.

Our results reveal that historical contingency was a major factor in shaping the adaptive radiation of notothenioids. As the onset of polar conditions ~33 mya decimated the teleost fauna of the Southern Ocean, notothenioids were the only surviving lineage poised to occupy newly open niche space in a range of benthic and water column habitats²⁵. Rather than being driven by directional selection to evolve extreme phenotypes in response to the onset of polar conditions^{26,27}, the genomic substrate for reduced ossifications and buoyancy modifications had long been established and was selected upon to facilitate the ecological diversification that characterizes the notothenioid adaptive radiation. These results provide an alternative view on the impact of climate change in driving extreme adaptations in the

Southern Ocean^{28,29}. As we march further into the Anthropocene, our results caution that as both ecosystems and climate continue to change worldwide, the expectation that rapid adaptation will be dominant factor in predicting the response of biodiversity requires careful consideration³⁰.

Methods

Custom targeted sequence enrichment design. We based the design of the DNA enrichment baits primarily on the *Notothenia coriiceps* genome, the most closely related species to those targeted for sequencing with a published reference assembly³¹. To account for regions that are unannotated, under drift, or not easily identified within the *N. coriiceps* assembly, we included targeted regions from several outgroup genomes as detailed below. By having the same element potentially represented by more than one genome, this strategy allowed us to mitigate against genome assembly and annotation artifacts while facilitating hybridization of diverse species to the capture probes. Elements were identified in the *N. coriiceps* genome using BLASTN (ncbi-blast-2.2.30+ ; parameters '-max_target_seqs 1 -outfmt 6'). If the BLASTN hit had a E-value < 0.0001 and covered >80% of the query sequence, we included this region from the *N. coriiceps* genome. If the region was not identified, or had <85% identity in *N. coriiceps*, we retained the version from the genome of origin.

Coding exons were identified from annotations of the *Notothenia coriiceps*³¹, stickleback (*Gasterosteus aculeatus*; BROADS1), and European sea bass (*Dicentrarchus labrax*) genomes³². Conserved non-coding elements (CNEs) were defined from the constrained elements identified in the stickleback and tilapia (*Oreochromis niloticus*; Orenil1.0) genomes from the Ensembl compara 11-way teleost whole genome alignment³³.

We also included predicted miRNA hairpins from miRbase³⁴ and ultraconservative non-coding (UCNE) elements from UCNEbase³⁵. miRNA hairpins were padded to be >100bp. CNEs, miRNAs, and UCNEs that overlapped coding exons were removed using Bedtools (v2.26.0) intersectBed³⁶, and CNEs <100bp were additionally excluded to facilitate space in the sequence capture design. Where the constrained regions that defined the CNEs

overlapped with annotations pertaining to specific miRNAs and UCNEs, the latter annotations were prioritized.

Targeted elements were submitted to Nimblegen for final probe design and the manufacturing of a Nimblegen SeqCap EZ Developer Library (Roche cat 06471684001) had 63,838,670 bp of capture space targeting 318,929 elements from four reference genomes (88.9% *Notothenia coriiceps*, 7.7% *Gasterosteus aculeatus*, 3.0% *Dicentrarchus labrax*, 0.4% *Oreochromis niloticus*). Accounting for redundancy of orthologous target regions between the genomes, the final design targeted 258,176 unique elements, of which 206,503 were predicted protein coding exons and 51,673 constrained non-coding regions with 85.0% coverage of targets not found in the *N. coriiceps* reference genome (47,097 elements; **Supplementary Table 2**).

Sample preparation and sequencing. Frozen tissue samples were acquired from the HWD and TJN labs, and from the Yale Peabody Museum frozen tissue collection (**Supplementary Table 9**). DNA from each species was isolated using Qiagen DNeasy Blood and Tissue kits, sequencing multiple individuals per species to account for population variation. For each species, equal amounts of DNA from each individual were pooled prior to shearing and library preparation. The pooled-population DNA was then sheared to an average size of 200bp using the Covaris E220 ultrasonicator in 130ul Covaris microTUBEs (Duty Cycle: 10%, Intensity: 5, Cycles/Burst: 200, Time: 300s, Temp: 4°C). Shearing was performed in shearing buffer: 10mM Tris, 0.1mM EDTA, pH8.3.

Targeted sequence enrichment and next generation sequencing. Sequencing libraries were constructed from 1µg of DNA using KAPA Library Prep kit (Roche cat# 07137923001), following standard protocol with barcoding and dual-SPRI size selection to generate libraries between 200-450bp. Sequencing libraries were hybridized, recovered, and amplified according to the standard protocol (Nimblegen SeqCap EZ Library SR User's

Guide v4.3) with the following changes: hybridization was performed at 45°C instead of 47°C to allow for more mismatches between sequencing libraries and probes, and we used SeqCap Developer Reagent (Roche cat# 06684335001) instead of Human CotI DNA to block non-specific hybridization as recommended by the protocol.

5 Since the majority of the capture probes were designed based on the sequence of a *Nothothenia coriiceps*, we hybridized species in groups to limit potential competition between sequencing libraries of varied relatedness to the capture baits. Captured libraries were pooled for 100bp single-end sequencing using Illumina HiSeq 2500. We targeted multiplexing of 8-9 species per HiSeq 2500 flow cell, totaling six flow cells.

10 **Reference contig assembly.** The contig assembly approach is modified from the previously defined Phylomapping pipeline for cross-species targeted sequence enrichment datasets¹⁵. Briefly, sequencing reads are grouped into bins by homology to a targeted element (exon, CNE, etc.) and then assembled into contigs *de novo* (**Supplementary Figure 7**)

15 **Processing of sequencing reads.** Prior to contig assembly, low quality bases within sequencing reads were masked using the FASTX-Toolkit (fastq_masker; parameters ‘-Q 33’) (http://hannonlab.cshl.edu/fastx_toolkit). Illumina adapter sequences were then trimmed using Trimmomatic v0.36³⁷. Identical sequencing reads were then collapsed using the fastx toolkit v0.0.13 (fastx_collapser; parameters ‘-Q 33’).

20 **Read binning into orthology groups by BLAST.** Reads were grouped by homology before contig assembly, using both blastn and dc-megablast (v2.6.0+, parameters ‘-max_target_seqs 2 -outfmt 6’). This dual-BLAST approach accounts for variation between sequencing reads and the reference genome from which the sequencing baits were defined³⁸. As short target exons and CNEs can produce disproportionately small blastn E-values, we
25 used an adaptive E-value cutoff based on the size of the target region. For target regions >25

bp, the cutoff was E-value $\leq 1e-05$. For targets that were ≤ 25 bp the E-value cutoff was $\leq 1e-04$ and for targets that were ≤ 20 bp the E-value cutoff was $\leq 1e-03$. Reads were further excluded if a substantial portion of the read (>10 bp) overlapped the target interval without being included in a blastn hit. The best resulting E-value from either blastn or dc-megablast was selected, with blastn selected in the event of a tie. As dc-megablast utilizes a mismatch-tolerant seed template, inclusion of dc-megablast resulted in the additional recovery of 30,000-50,000 sequencing reads per species (out of an average of 20,000,000 with total blastn hits) and the assembly of 50-150 more target regions than would be assembled by blastn alone.

de novo contig assembly. CAP3 was used to assemble contigs *de novo* from the bins of reads that have high homology to specific target regions (i.e. the same exon, CNE, etc.) that were identified by BLAST³⁹. Reads were reverse complemented if necessary in order to put everything into the same complement strand as the target region from the reference genome. To accelerate CAP3 assembly, overlapping reads were first merged into smaller contigs using Usearch and then mixed with original reads as input for CAP3 (parameters ‘-id 0.97 -fastq_maxdiffs 3 -fastq_minovlen 5’). For CAP3 assembly, we required a minimum read overlap of 16bp and 96% identity between reads during contig assembly (parameters ‘-o 16 -p 96’). This cutoff has an effect of separating the reads stemming from duplication events into separate contigs as long as there is $>4-6\%$ variance between the paralogous regions.

We simulated the ability of this pipeline to distinguish copy number variants (Supplementary Figure 8a), generating a 300bp random DNA sequence *in silico* and making a second copy of this sequence at with specific levels of variation from the original. Sequencing reads (100bp) were then generated *in silico* at a depth of one read every five base pairs. Reads were run through the assembly pipeline to assess whether the original DNA

sequences were reconstructed from the read data, or if the reads formed a chimeric sequence. This simulation was repeated 250 times. The current assembly approach reliably reproduced single copy exons at all levels of variation and was able to re-assemble the individual paralogs where there was >6% divergence between original paralogous sequences, with inconsistent results at <5% divergence (**Supplementary Figure 8b**).

Contig merging and filtering. Sequencing reads were aligned to the assembled contigs using NextGenMap (v0.5.5; parameters ‘-R 40’)⁴⁰. This alignment step allows for the recruitment of new reads to the contig that may have not been previously identified by blastn due to high degrees of variance relative to the reference blast database, large indels, or low amounts of overlapping sequence with the target region. This allows the contig to be elongated to include more of the flanking regions surrounding each target element. Reads were removed from the alignment to the contig if there were >3 mismatches with the exception of indels. To refine and extend the boundaries of the original contig, a second *de novo* assembly by CAP3 (parameters ‘-o 20 -p 85’) was performed using the aligned reads.

Multiple contigs were present in around 65% of target regions after CAP3 assembly. To remove misidentified contigs, we used blastn to compare each contig to the reference genome, removing contigs whose top hit did not match the original bin from which the reads were assembled. To correct for potential assembly artifacts, the multiple contigs that represent each target were compared to each other and the reference sequence in a multiple sequence alignment using Mafft v7.313⁴¹ (parameters ‘--maxiterate 1000 --localpair’), adding contigs as fragments (parameters ‘--addfragments’). Using the read support at each base in the alignment, we generated a consensus contig sequence for each target region. A mismatch between contigs was considered if the read support for the most common base was <80% of all bases present. Contigs were only merged if there were <3 mismatches, if the sequence identity between the contigs was >95%, or if the contigs did not overlap within the

target region. After this refinement step, <2% of target regions were represented by multiple contigs.

Comparison of assembled contigs to reference genome. In comparing our reference exome sequence assembly to the published *N. coriiceps* reference genome, we found 99.8% average percent identity of exome sequence to the reference target (**Supplementary Figure 9**), that included 98.5% of exons in the *N. coriiceps* reference genome (**Supplementary Figure 9**). The small differences in sequence identity and putative CNVs between this exome and the genome assembly may reflect meaningful biological variation in our independently sampled *N. coriiceps* populations. Both the sequence composition of the assembled exome and the predicted copy number data closely match the whole genome sequence data, providing confidence in our assembly.

Estimation of read coverage and depth of targeted regions. Coverage was estimated using BEDtools (2.23.0)³⁶. Reads were first aligned to the assembled contigs with NextGenMap⁴⁰. The coordinates of the read alignments were then lifted the corresponding position on the reference genome using information from a pairwise sequence alignment between the contig and the orthologous region on the reference genome. Pairwise alignments were performed using Biopython v1.70 (parameters ‘pairwise2; match = 5, mismatch = -4, gap_open = -15, gap_extend = -1’). Alignments were converted to BAM files, sorted, and indexed using SAMtools v1.9⁴². Reads alignments were manually inspected in the Integrative Genome Viewer (IGV) to verify accurate read alignment⁴³. Coverage is defined as the percentage of targeted bases in the primary reference genome having at least one read. Depth was estimated using coverageBed (-d).

Distribution of read coverage across the dataset. Most target regions had either 0% or 100% coverage (**Supplementary Figure 10**). Though the average depth is similar in the notothenioids compared to the outgroups, there is a wider distribution of depths in outgroup

species (**Supplementary Figure 10**). Though global coverage is >85% in all species, gene classes associated with the immune system, cell adhesion proteins and extracellular matrix were enriched among regions with relatively poor coverage (<25% coverage in >75% of exons; **Supplementary Table 10**). This suggests that these fast evolving gene classes are less likely to be highly represented in these datasets, and is similar to previous findings with cross-species targeted DNA enrichment⁶⁷.

Recovery of population variation. To determine the ability of this approach to recover population variation, we looked for heterozygous SNPs within the targeted regions of the dataset. Sequencing reads were aligned to the reference contigs for each species using NextGenMap⁴⁰. SAM files were converted to BAM files using SAMtools v1.9⁴². Variants were called using SAMtools mpileup and BCFtools v1.9 (parameters '*call -mv*'). We considered sites heterozygous in our small population samples if there is ≥ 2 reads showing the variant in at least 25% allele frequency within the sequencing reads.

Treatment of exons with a predicted history of duplication. We assembled a single exon/CNE copy for the majority of targets that were directly compared between species. However, less than 3% of targeted regions on average had assembled >1 contig per target region after assembly. For duplicated regions, both exon versions were ignored for that particular species in downstream analyses involving multiple groups, unless those analyses ask specific questions involving copy number.

Identification of orthologous sequences. For ortholog pairing, the contigs generated from the same reference target region were aligned using Mafft v7.313(parameters '*--op 10 --ep 10*')⁴¹, with a maximum likelihood tree topology estimated IQTree⁴⁴. Gene trees were reconciled with the species tree using Notung-2.9⁴⁵ (parameters '*--reconcile --rearrange --silent --threshold 90% --treeoutput nhx*') to infer patterns of duplication and loss. The total number of duplication and loss events inferred by Notung were then summed and compared

to a null scenario where all copies are local duplicates. If Notung inferred fewer gain/loss events, the duplicate exons were paired based on the reconciled gene tree.

Simulation of ortholog identification approach. To estimate the ability of this approach to parse copy number variation into orthologous groups, we performed a series of simulations (**Supplementary Figure 11a**) using a random DNA sequence generated *in silico*. This ancestral DNA sequence was duplicated, and mutations were added at defined levels to distinguish each paralog. Both paralogs were then evolved according to a specified phylogeny, with variation added to each paralog in increments at each branch point. We varied copy number, length of contig sequence and also simulated local losses of an individual paralog within downstream lineages. Each simulation was repeated 250 times. These results suggest that as long as there is $\geq 4\text{-}6\%$ variation between paralogous sequences, the approach can properly pair orthologous sequences (**Supplementary Figure 11b-d**). This coincides with the thresholds at which our pipeline can distinguish copy number variants during contig assembly (see above), meaning there will not be paralog sequences with $< 4\%$ divergence in the dataset.

Multiple sequence alignment. All paired orthologous sequences were aligned using Mafft v7.313 (parameters '`--maxiterate 1000 --localpair --op 10 --ep 10`'). For coding regions that had out-of-frame or frameshift-causing indels, these alignments were then refined into codon alignments using the frameshift-aware multiple sequence aligner MACSE v2.03 (parameters '`-prog alignSequences -seq -seq_lr -fs_lr 10 -stop_lr 15`')⁴⁶. The multiple sequence alignment was pruned using GUIDANCE v2.02 to mask residues with score < 0.6 (parameters '`--bootstrap 25 --mafft --maxiterate 100, --localpair --op 10 --ep 10`')⁴⁷.

Reconstruction of gene sequences from exon data. Single-copy coding exons with orthology to *Gasterosteus aculeatus* were concatenated into gene sequences using the annotations of the *G. aculeatus* genome. The exons for each gene were spliced together in the

same order in which they appear in the genome on the strand containing the gene. Transcript isoforms were merged into a non-redundant gene sequence containing all possible exons. A total of 18,600 gene sequences with orthology to *G. aculeatus* were reconstructed for each species.

5 **Notothenioid phylogeny.** We used two approaches to infer a phylogeny for notothenioids. For both analyses, only single copy genes with >85% coverage in all species were included, resulting in a dataset of 11,627 genes. First, we individually partitioned each gene by codon position and used ModelFinder as implemented in IQTree v.1.6.3⁴⁸ to estimate the optimal partitioning scheme and molecular evolution model. Genes were concatenated all
10 a maximul likelihood tree was inferred using IQTree v.1.6.3⁴⁴. To assess support for the phylogenetic relationships, we performed 1,000 ultra-fast bootstrap replicates⁴⁹.

In order to account for the effects of incomplete lineage sorting and known issues with concatenation for phylogenetic inference^{50,51}, we also inferred a species tree. Full species tree inference is not computationally feasible with large genomic datasets, so we
15 relied on the summary species tree approach in ASTRAL v.5.6.2⁵². We first used IQTree v.1.6.3 to infer the maximum likelihood tree for each gene, apply partitioning schemes and molecular evolution models as described above. We then used ASTRAL to summarize the distribution of gene trees and estimate the species tree. To assess support for the species tree topology, we estimated local posterior probabilities for each quadpartition in the tree⁵³.

20 **Quantification of lineage diversification dynamics.** To test for changes in lineage diversification rates across the temporal history notothenioids, we used a Bayesian analysis of macroevolutionary mixtures implemented in BAMM v2.5 with a previously published time-tree that sampled all major lineages and 87 of ~120 species of notothenioids¹¹. Priors were defined using the function setBAMMpriors contained in the R package *BAMMtools*
25 v2.1.0⁵⁴, and missing species were accounted for based on currently described species, with

outgroup sampling capturing all major lineages of non-polar notothenioids. BAMM was run for 100 million generations and assessed for target sampling of the posterior distribution (Effective sample size >200). Results were visualized using functions available from the R-package *BAMMtools*⁵⁴. To assess the impact of phylogenetic uncertainty in topology and branch lengths on our estimates of speciation rates through time, we further replicated the above analysis across 500 topologies randomly selected from the posterior distribution of trees from the same prior work¹¹ (**Supplemental Figure 12**).

dS and dN estimates of substitution rate. dN, dS and dN/dS were calculated pairwise between each species and the outgroup *Percina caprodes*. This was performed for each reconstructed gene of at least 2,000 bp in both species using `cal_dn_ds` in the Biopython v1.70 `codonseq` module (parameters '`method="NG86"`'). The values of dN, dS and dN/dS were then averaged across all genes for each species.

Molecular clock models of substitution rate. The substitution rate was estimated on the reconstructed gene sequences using the random local clock model as implemented in BEAST v2.4.8 (codon partitioned, bModelTest, parameters '`chain length = 30M`')⁵⁵⁻⁵⁷. To simplify comparisons between gene trees, and as support for the relationships between the included species is high, we fixed the starting tree topology for each gene tree to match our ASTRAL-inferred species tree (**Supplementary Figure 13**). Only gene trees with ESS values ≥ 200 for all parameters were selected for downstream analysis (1,062 total). MRCA age priors were calibrated based on previous age estimates^{11,58}: *Psuedaphritis* + Elegendopsioidea 63.0my (52.6-73.4), Bovichtidae + all notothenioids 85.7my (69.5-102.6), *Harpagifer-Pogonophryne* 10.2my (7.7-13.0), *Bathhydraco-Chaenocephalus* 11.1my (9.4-13.3), *Notothenia* 17.7my (15.2-20.5), Cryonotothenioidea 21.6my (18.6-23.9), Elegendopsioidea 45.9my (37.2-53.2). The maximum clade credibility was constructed for each gene tree using TreeAnnotator.

Detection of diversifying selection. Positive selection was calculated using the adaptive branch-site random effects likelihood (aBSREL) implemented in HyPhy v2.3.9^{59,60}. Single copy exon alignments were concatenated into genes as input based on the gene order in the stickleback genome. The species tree was used for all comparisons (**Supplementary Figure 13**). Accelerated sequence evolution was assessed using phyloP⁶¹ as implemented in PHAST v1.4⁶² (parameters ‘*--method LRT --no-prune --features --mode ACC*’). The tree model for phyloP was derived separately for CNE and coding gene comparisons using phyloFit and the species (**Supplementary Figure 13**). Tree models for protein coding regions were based on 3,381 exons $\geq 1,000$ bp with $\geq 85\%$ coverage in all species. CNEs tree models based on 2,912 elements ≥ 250 bp with $\geq 85\%$ coverage in all species.

Ontology enrichment. As there are no gene ontology (GO) terms relating to *Notothenia coriiceps*, we generated a custom gene ontology database based on the combined GO data from multiple species. GO data was mined from chicken, mouse, rat, human, stickleback, medaka and zebrafish in Ensembl BioMart (downloaded December 2016)⁶³. All Ensembl gene IDs were converted to their Ensembl stickleback ortholog and a final merged GO list was created by combining stickleback orthologs for each species. As many of the evolved phenotypes in notothenioids are comparable to human pathologies, we further utilized Human Phenotype Ontology databases to characterize genetic trends within the fish dataset⁶⁴. This HPO database (downloaded April 2018) was then converted from human to stickleback orthologs using Ensembl BioMart.

Ontology enrichment was performed using Fisher’s Exact Test (SciPy v0.18.1; `fisher_exact`). We also assessed patterns of cumulative polygenic enrichment within ontologies using the SUMSTAT approach as implemented in Daub et al⁶⁵. This approach normalizes the distribution of log-likelihood ratio test values ($\Delta \ln L$) output from phyloP and HyPhy by taking the fourth root ($\Delta \ln L_4$). The $\Delta \ln L_4$ score is then summed for all genes

within an ontology and an enrichment p-value is estimated from the empirical sum($\Delta \ln L_4$) score through bootstrap resampling (1,500 replicates). For all enrichment analyses, p-values were corrected using FDR (python module statsmodels v0.6.1; fdr correction0).

Zebrafish husbandry and genetic lines. Zebrafish wild-type and mutant lines used were housed and maintained as previously described and in accordance with Boston Children's Hospital IACUC regulations⁶⁶. The *colla1a* (dmh14) and *colla2* (dmh15) mutants were derived from a forward genetic screen⁶⁷.

Zebrafish genome editing. The gRNA site GGTCAGAGTTTGGGTCAGGTCCGG in exon 1 of the zebrafish *trip11* gene was targeted. This site is 33 bp downstream of the ATG start codon of *trip11*. gRNA sequences were cloned in the BsaI site of the DR274 plasmid and *in vitro* transcribed with T7 RNAmix kit (ThermoFisher). Cas9 mRNA was obtained from SystemBio (CAS500A-1). Injections of fertilized zebrafish eggs were done with 50 ng/ul gRNA and 150 ng/ul Cas9 mRNA. Genotyping was performed using 5'-CCCTGGTCGGTGATTTAGGGTTAG-3' as forward primer and 5'-

CACCTCCCCTTCCTCGGCGCTTTCCAGCAGAATATCTTTGGTAAAATTAGAG-3' as reverse primer. PCRs using this primer pair yield a 178 bp wildtype band. Fish were identified with a 47 bp deletion spanning the gRNA target site and yielding a 131 bp genotyping band. The deleted sequence is 5' -

CTGGGTCAGAGTTTGGGTCAGGTCGGGGGAAGCTTGTCTTCATTTAC-3' and generates a frameshift at the 12th amino acid residue of *trip11*.

Analysis of skeletal density through computed tomography (CT). Adult notothenioid specimens were loaned from the Yale Peabody Museum and Harvard Museum of Comparative Zoology (**Supplementary Table 11**) and scanned using the Siemens Biograph at Boston Children's Hospital Department of Nuclear Medicine and Molecular

Imaging. Scan data was processed in Siemens PETsyngo VG60A software and analyzed in Amira (v 6.0.0 ; FEI Inc.).

5 Zebrafish were euthanized in 22% MS-222, fixed in 3.7% formaldehyde overnight, and rinsed in PBS. The fishes were embedded in 1% agarose to reduce movement during imaging. The fish skulls were scanned as in ref. 66, using a Skyscan 1173 (Bruker), 240-degree scan with 0.2 rotational step. X-ray source voltage set to 70 kV and current set to 80 μ A. Exposure time was 1500 ms. Resolution of scan was 7.14 microns per pixel. Volume renderings were reconstructed as maximum intensity projections in Amira software. Skeletal density was estimated based on the average pixel intensity measured from the maximum
10 intensity projection of the skull, vertebrae and operculum in ImageJ v1.51s (<https://imagej.nih.gov/ij/>). A total of n=6 *trip11*^{+/-} and n=10 *trip11*^{-/-} fish were scanned and quantified. The bones of the skull roof (parietal/frontal), operculum and vertebrae were measured from each individual.

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Supplementary Materials

Supplementary Figures 1-13

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5

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20 **Competing Interests.** The authors declare no competing interests.

Data Availability. The sequencing data has been deposited in the NCBI database as Bioproject PRJNA531677. Assembled contig data has been deposited in the Zenodo repository (10.5281/zenodo.2628936). Correspondence and requests for materials should be

addressed to j.daane@northeastern.edu, w.detrich@northeastern.edu or
harris@genetics.med.harvard.edu.

Figure Legends.

Fig. 1 | Punctuated elevation in genomic diversification prior to ecological change and adaptive radiation.

(a) Bayesian analysis of lineage diversification rate on time-calibrated notothenioid phylogeny^{12,18}. S indicates change in speciation rate. Colors on branches correspond to the mean of the marginal posterior density of estimated speciation rates, with a shift from low (0.04 lineages/Ma) to faster rates of speciation (0.046 lineages/Ma) at the onset of the notothenioid adaptive radiation. Colored squares at select tips in phylogeny refer to corresponding species in panel b. (b) Relaxed molecular clock model of nucleotide substitution rate (substitutions/site/Ma) during notothenioid evolution revealing a transient elevation in substitution rate prior to the increase in species diversification. Substitution rates estimated from 1,062 independent gene trees constructed using the random local clock model in BEAST2. λ_1 indicates change in substitution rate, from an ancestral baseline of 7.7×10^{-4} substitutions/site/Ma (95% highest posterior density interval (HPD): 5.0×10^{-4} , 1.1×10^{-3}) to 2.3×10^{-3} substitutions/site/Ma (95% HPD: 1.0×10^{-3} , 3.9×10^{-3}) observed on the branch leading to *Eleginops maclovinus* and all cryonotothenioids. λ_2 indicates return to baseline substitution rate in cryonototheniids, ranging from 4.2×10^{-4} substitutions/site/Ma in *Disosstichus mawsoni* (95% HPD: 1.0×10^{-4} , 9.0×10^{-4}) to 7.7×10^{-4} substitutions/site/Ma in *Chaenocephalus aceratus* (95% HPD: 2.0×10^{-4} , 2.2×10^{-3}). See Supplementary Table 4 for additional rates and 95% HPD. Colors on branches correspond to mean substitution rate. (c) Elevation in speciation rate well after increase in nucleotide substitution rate and climate change events that precipitated adaptive diversification. Shading indicates 10-90% Bayesian credible region across time. Error bars for age of cryonotothenioid most recent common ancestor (MRCA) and time of substitution rate shift at MRCA of eleginops and cryonotothenioids based on 95% Bayesian credible interval. (d) Distribution of substitution rates from select species in (b). (e) Average synonymous (dS) and non-synonymous (dN) substitution rate from over 4,000 pairwise alignments for each species relative to the outgroup, *Percina caprodes*. This shows an increase in overall substitution rate (dN and dS) without a genome-wide change in diversifying selection (dN/dS).

Fig. 2 | Skeletal reduction occurs prior to the cryonotothenioid radiation. (a) Computed tomography (CT) of notothenioid skulls showing relative skeletal density across the phylogeny and decrease in skeletal density prior to cryonotothenioid radiation. (b) Comparison of significance of enrichment for polygenic selection on representative bone-density associated HPO terms between Eleginopsioidea and well-ossified sister group *Pseudaphritis urvillii*. Dashed line indicates FDR q-value < 0.05 for SUMSTAT gene set enrichment test.

Fig. 3 | Skeletal genes under diversifying selection uncover genetic mechanisms regulating bone density.

(a) Comparison of genes under positive selection (aBSREL, $p < 0.05$) and/or accelerated sequence evolution (phyloP, $p < 0.05$) on the branch leading to *Eleginops maclovinus* and Antarctic cryonotothenioids (Eleginopsioidea) with the branch leading to the icefishes, Channichthyidae. (b) Quantitation of skeletal density from μ CT analysis of zebrafish mutants in *trip11*. Density is the average pixel intensity. n=6 scanned

trip11^{+/-} and n=10 *trip11*^{-/-} individuals. Center line is mean density, box bounds indicate lower and upper quartiles, whiskers extend to a maximum of 1.5 times the interquartile range.

*: one tailed t-test p-value <0.05. (c) Micro-computed tomography (μCT) of zebrafish showing reduction in bone density in the cranium of zebrafish mutant models of genes under selection in Euginopsioidea. Representative μCT images of zebrafish skulls from heterozygous and homozygous *trip11* mutants, showing reduced bone density and variable penetrance. Reduction in density in the cranium caused by mutations in *colla1a*^{dmh14/+} (G1144E) and *colla2*^{dmh15/+} (G882N).

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Supplementary Materials for

Historical contingency shapes adaptive radiation in Antarctic fishes

Jacob M. Daane, Alex Dornburg, Patrick Smits, Daniel J. MacGuigan, Michael Brent Hawkins,
Thomas J. Near, H. William Detrich III, and Matthew P. Harris

Correspondence to: j.daane@northeastern.edu, w.detrich@northeastern.edu,
harris@genetics.med.harvard.edu

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Supplementary Figure 1. Evolutionary history of notothenioids revealed through targeted sequence enrichment. (a) Designed ‘phylo-capture’ array demarcating regions targeted for cross-species sequence enrichment; total number of elements in parentheses. (b) Average coverage and sequencing read depth of the targeted regions for each major group within the notothenioids and close outgroups. The number of species within each group is shown in parenthesis.

Supplementary Figure 2. Sensitivity of targeted capture method in confirming and discovering variation in hemoglobin within Channichthyidae. Presence and lack of detection of individual hemoglobin exons in Channichthyidae and Bathydraconidae. Genomic analysis was sensitive in detecting loss of hemoglobins *hba1* and *hbb2* in the icefish clade as previously identified(16). The analysis was able to identify patterns of loss within two new hemoglobins *hbz1* and *hbae1* as well. Presence defined as > 25% coverage and > 5 sequencing reads per exon.

Supplementary Figure 3. Heatmap of skeletal density in replicate notothenioid CT scans. Dorsal and ventral views from each adult notothenioid specimen. Voucher information in Supplementary Table S11.

Supplementary Figure 4. Missense mutations in *trip11* shared among the common ancestor to Euginopsioidea and common ancestor to Channichthyidae. Above, lollipop plot of missense mutations mapped onto the orthologous *Gasterosteus aculeatus* transcript (ENSGACT00000016209.3). Below, example multiple sequence alignments. Variants were classified as predicted deleterious (SIFT ≤ 0.05) or predicted neutral (SIFT > 0.05) using SIFT 4G.

Supplementary Figure 5. Missense mutations in *coll1a1* shared among the common ancestor to Euginopsioidea and common ancestor to Channichthyidae. Above, lollipop plot of missense mutations mapped onto the *Notothenia coriiceps coll1a1* transcript (XP_010767913.1). Below, example multiple sequence alignments. Variants were classified as predicted deleterious (SIFT ≤ 0.05) or predicted neutral (SIFT > 0.05) using SIFT 4G.

Supplementary Figure 6. Missense mutations in *colla2* shared among the common ancestor to Eginopsioidea and common ancestor to Channichthyidae. Above, lollipop plot of missense mutations mapped onto the *Notothenia coriiceps colla2* transcript (XP_010772950.1). Below, example multiple sequence alignments. Variants were classified as predicted deleterious (SIFT ≤ 0.05) or predicted neutral (SIFT > 0.05) using SIFT 4G.

Supplementary Figure 7. Overview of contig assembly

Supplementary Figure 8. Detection of copy number variants through contig assembly. (a) Simulation of the limits of the contig assembly pipeline to reconstruct duplicate sequences from mixed read populations. (b) The mean number of contigs generated by the pipeline from 1, 2 and 3 input contigs put into the simulation. Data from 250 simulations of random 300-bp DNA fragments. Red line indicates input number of contigs. Error bars represent ± 1 sem.

Supplementary Figure 9. Comparison of reconstructed *Notothenia coriiceps* exome to published *N. coriiceps* genome. (a) Histogram of percent identity of the reference target regions relative to the corresponding region on the previously assembled *N. coriiceps* genome³⁰. (b) Histogram of predicted copy number for an exon in the exome reconstruction compared with the reference genome.

Supplementary Figure 10. Distribution of coverage as a function of evolutionary distance from hybridization baits. (a) Histogram of average depth per targeted region (exon, CNE, etc.), showing flattened distribution of read depth in *P. caprodes* suggesting that certain target regions in distantly related groups hybridize less efficiently. **(b)** Percent of bases covered with at least one read in both species, showing the majority of target regions were either highly covered or not covered at all.

Supplementary Figure 11. Assessing robustness of pairing of duplicated elements for comparative genomics. (a) Simulation to test the ability of this approach to properly identify orthologs among ancestrally duplicated sequences. As part of the pipeline for annotation, when there is more than one element per target region in the reference genome in at least two species, a gene tree is constructed with IQTree and reconciled with the species tree using Notung. Notung infers patterns of gain and loss throughout the phylogeny. Simulation was run 250 times. **b-d**) Effect of element length (**b**), copy number (**c**) and local losses within the phylogeny (**d**) on correct matching of duplicates relative to the percent divergence of the duplicated elements. Error bars represent ± 1 sem.

Supplementary Figure 12. Speciation rates through time estimated by BAMM across 500 random samples of the posterior distributions of timetrees. Blue color density shading corresponds to the 90% highest posterior density interval of rate estimates at a given time point across all trees. Red lines correspond with the mean speciation rates through time estimated from each tree of the posterior distribution. The black line corresponds to the mean speciation rate estimated from the maximum clade credibility tree that is also depicted in the primary text.

Supplementary Figure 13. Notothenioid species tree. Phylogenetic relationships of the sequenced species inferred as a species tree using ASTRAL and 11,627 gene trees. All nodes were supported with a quadpartition posterior probability of 1.00.

Supplementary Table 1. Coverage at various read depth thresholds

Species	Coverage at depth			
	>1X	>2X	>4X	>10X
<i>Percina caprodes</i>	88.0%	87.4%	84.5%	73.4%
<i>Percophis brasiliensis</i>	92.2%	92.0%	90.4%	83.7%
<i>Bovichtus diacanthus</i>	92.2%	91.9%	90.6%	82.8%
<i>Cottoperca trigloides</i>	93.9%	92.4%	88.6%	78.3%
<i>Pseudaphritis urvillii</i>	92.3%	91.8%	89.4%	79.1%
<i>Eleginops maclovinus</i>	93.4%	93.0%	90.9%	78.2%
<i>Pleurgramma antarctica</i>	95.9%	95.8%	95.3%	92.7%
<i>Aethotaxis mitopteryx</i>	96.4%	96.4%	96.1%	95.2%
<i>Dissostichus mawsoni</i>	95.6%	95.5%	94.7%	88.4%
<i>Dissostichus eleginoides</i>	95.9%	95.8%	95.4%	93.0%
<i>Nototheniops nudifrons</i>	95.9%	95.8%	95.4%	93.3%
<i>Lepidonotothen squamifrons</i>	95.9%	95.9%	95.5%	93.6%
<i>Patagonotothen squamiceps</i>	95.9%	95.9%	95.5%	93.6%
<i>Patagonotothen guntheri</i>	95.9%	95.8%	95.4%	93.3%
<i>Trematomus scotti</i>	95.7%	95.6%	95.1%	92.2%
<i>Trematomus newnesi</i>	95.8%	95.7%	95.2%	92.3%
<i>Trematomus eulepidotus</i>	95.9%	95.9%	95.5%	93.9%
<i>Trematomus bernacchii</i>	95.8%	95.6%	95.1%	91.6%
<i>Trematomus borchgrevinki</i>	95.7%	95.6%	95.1%	90.8%
<i>Trematomus hansonii</i>	95.6%	95.5%	95.1%	92.6%
<i>Gobionotothen gibberifrons</i>	96.0%	95.9%	95.4%	93.1%
<i>Notothenia coriiceps</i>	96.0%	95.9%	95.5%	93.3%
<i>Notothenia rossii</i>	95.7%	95.6%	95.1%	91.8%
<i>Paranotothenia angustata</i>	96.0%	95.9%	95.4%	92.2%
<i>Harpagifer antarcticus</i>	94.8%	94.6%	93.1%	81.9%
<i>Artedidraco skottsbergi</i>	95.3%	95.1%	94.2%	88.0%
<i>Histiodraco velifer</i>	95.8%	95.7%	95.3%	93.3%
<i>Dolloidraco longedorsalis</i>	95.8%	95.7%	95.4%	94.1%
<i>Pogonophryne scotti</i>	95.7%	95.6%	95.2%	92.8%
<i>Pogonophryne barsukovi</i>	95.8%	95.7%	95.3%	93.1%
<i>Gerlachea australis</i>	95.6%	95.4%	94.5%	88.9%
<i>Parachaenichthys charcoti</i>	94.2%	93.8%	92.0%	79.8%
<i>Vomeridens infuscipinnis</i>	95.8%	95.7%	95.1%	91.7%
<i>Akarotaxis nudiceps</i>	95.8%	95.7%	95.1%	91.9%
<i>Bathydraco marri</i>	95.7%	95.6%	95.2%	92.3%

<i>Gymnodraco acuticeps</i>	96.2%	96.1%	95.8%	94.4%
<i>Dacodraco hunteri</i>	95.4%	95.4%	94.9%	92.3%
<i>Champscephalus gunnari</i>	95.7%	95.5%	94.9%	92.0%
<i>Champscephalus esox</i>	95.4%	95.3%	94.7%	91.5%
<i>Pagetopsis macropterus</i>	95.4%	95.3%	94.7%	91.6%
<i>Neopagetopsis ionah</i>	95.5%	95.4%	94.8%	91.3%
<i>Pseudochaenichthys</i>				
<i>georgianus</i>	95.4%	95.3%	94.8%	92.1%
<i>Chaenodraco wilsoni</i>	95.5%	95.4%	95.0%	93.0%
<i>Chaenocephalus aceratus</i>	96.0%	95.9%	95.6%	93.9%
<i>Cryodraco antarcticus</i>	95.6%	95.5%	94.9%	91.5%
<i>Chionobathyscus dewitti</i>	95.2%	94.8%	93.7%	88.6%

Supplementary Table 2. Coverage of targeted regions in cross-species targeted sequence enrichment

species	total		<i>N. coriiceps</i> genome		non- <i>N. coriiceps</i> genome		CDS		CNE	
	cov.	depth	cov.	depth	cov.	depth	cov.	depth	cov.	depth
<i>Percina caprodes</i>	88.0%	40.2	87.6%	34.9	87.4%	60.3	86.6%	34.4	94.8%	64.7
<i>Percophis brasiliensis</i>	92.2%	52.9	92.3%	46.7	90.3%	76.3	91.3%	45.6	96.6%	84.7
<i>Bovichtus_diacanthus</i>	92.2%	54.3	92.3%	47.5	89.9%	78.7	91.3%	46.1	96.1%	90.4
<i>Cottoperca trigloides</i>	93.9%	40.7	94.4%	35.8	89.7%	59.4	93.4%	35.0	95.9%	66.4
<i>Pseudaphritis urvillii</i>	92.3%	36.2	92.5%	32.2	88.7%	51.4	91.5%	31.4	96.1%	57.5
<i>Eleginops maclovinus</i>	93.4%	24.7	94.3%	23.4	85.4%	28.9	92.9%	23.0	95.4%	32.2
<i>Aethotaxis mitopteryx</i>	95.9%	48.5	97.2%	48.8	86.3%	43.7	95.8%	48.8	96.4%	47.1
<i>Dissostichus mawsoni</i>	96.4%	89.1	97.7%	89.6	87.9%	81.3	96.4%	89.3	96.7%	87.8
<i>Dissostichus eleginoides</i>	95.6%	29.9	97.0%	30.3	85.4%	26.9	95.5%	30.3	96.0%	27.8
<i>Nototheniops nudifrons</i>	95.9%	50.1	97.2%	50.2	86.5%	46.6	95.8%	50.2	96.4%	49.7
<i>Lepidonotothen squamifrons</i>	95.9%	52.1	97.2%	52.1	86.5%	48.9	95.8%	52.0	96.4%	52.6
<i>Patagonotothen squamiceps</i>	95.9%	53.0	97.2%	53.2	86.9%	49.6	95.9%	53.1	96.4%	52.6
<i>Patagonotothen guntheri</i>	95.9%	54.5	97.2%	54.8	86.9%	50.5	95.8%	55.1	96.4%	51.8
<i>Trematomus scotti</i>	95.9%	54.6	97.2%	54.7	86.6%	51.0	95.8%	54.8	96.4%	53.6
<i>Trematomus newnesi</i>	95.7%	44.4	97.0%	44.6	86.1%	41.2	95.6%	44.5	96.2%	43.9
<i>Trematomus eulepidotus</i>	95.8%	47.1	97.1%	47.5	86.1%	42.5	95.7%	47.5	96.3%	45.4
<i>Trematomus bernacchii</i>	95.9%	54.6	97.2%	55.4	86.5%	47.8	95.8%	55.5	96.4%	50.3
<i>Trematomus borchgrevinki</i>	95.8%	44.7	97.1%	44.8	86.1%	41.8	95.6%	44.8	96.3%	43.9
<i>Trematomus hansonii</i>	95.7%	41.6	97.1%	42.1	85.9%	37.9	95.6%	42.2	96.2%	38.9
<i>Pleuragramma antarctica</i>	95.6%	61.3	96.9%	61.1	86.4%	58.2	95.5%	60.9	96.1%	62.9
<i>Gobionotothen gibberifrons</i>	96.0%	51.0	97.3%	51.3	86.3%	46.5	95.8%	51.2	96.5%	49.9
<i>Notothenia coriiceps</i>	96.0%	54.3	97.5%	54.7	85.0%	49.3	95.8%	54.6	96.8%	53.0
<i>Notothenia rossii</i>	95.7%	54.9	97.2%	57.0	84.5%	43.5	95.5%	57.1	96.4%	44.8
<i>Paranotothenia angustata</i>	96.0%	43.1	97.4%	44.0	85.6%	36.9	95.9%	43.7	96.6%	40.6
<i>Harpagifer antarcticus</i>	94.8%	24.2	96.4%	24.5	83.3%	21.4	94.7%	24.4	95.4%	23.1
<i>Artedidraco skottsbergi</i>	95.3%	32.1	96.8%	32.4	84.5%	29.2	95.2%	32.3	96.0%	30.9
<i>Histiodraco velifer</i>	95.8%	55.3	97.2%	56.0	85.8%	49.1	95.7%	55.9	96.5%	52.7
<i>Dolloidraco longedorsalis</i>	95.8%	68.2	97.2%	69.2	85.6%	59.4	95.6%	69.1	96.5%	64.0
<i>Pogonophryne scotti</i>	95.7%	52.6	97.1%	53.1	85.6%	47.0	95.6%	53.0	96.5%	50.5
<i>Pogonophryne barsukovi</i>	95.8%	56.5	97.2%	57.2	85.8%	50.5	95.7%	57.1	96.5%	54.0
<i>Gerlachea australis</i>	95.6%	35.2	97.0%	35.4	84.9%	32.4	95.4%	35.3	96.2%	34.6
<i>Parachaenichthys charcoti</i>	94.2%	27.8	95.8%	28.5	82.0%	23.6	94.1%	28.5	94.6%	24.4
<i>Vomeridens infuscipinnis</i>	95.9%	63.2	97.3%	64.6	85.8%	53.9	95.8%	64.7	96.5%	56.6
<i>Akarotaxis nudiceps</i>	95.8%	44.2	97.2%	44.7	85.5%	39.8	95.6%	44.6	96.4%	42.4
<i>Bathydraco marri</i>	95.8%	45.7	97.2%	46.3	85.6%	40.8	95.7%	46.2	96.4%	43.4

<i>Gymnodraco acuticeps</i>	95.7%	43.4	97.2%	44.4	85.2%	37.1	95.6%	44.5	96.3%	38.5
<i>Champscephalus gunnari</i>	96.2%	74.8	97.5%	75.4	86.7%	67.5	96.0%	75.2	96.9%	72.6
<i>Champscephalus esox</i>	95.4%	50.3	96.9%	51.0	85.1%	43.3	95.3%	50.9	96.0%	47.1
<i>Dacodraco hunteri</i>	95.7%	47.8	97.1%	48.1	85.3%	43.3	95.6%	47.9	96.3%	47.1
<i>Pagetopsis macropterus</i>	95.4%	46.0	96.8%	46.7	84.9%	40.4	95.3%	46.5	96.0%	43.6
<i>Neopagetopsis ionah</i>	95.4%	48.0	96.8%	48.6	85.0%	42.5	95.3%	48.5	96.0%	45.7
<i>Pseudochaenichthys georgianus</i>	95.5%	44.6	96.9%	44.8	85.3%	41.5	95.4%	44.5	96.1%	45.3
<i>Chaenodraco wilsoni</i>	95.4%	48.3	96.8%	48.9	84.8%	43.0	95.2%	48.8	96.1%	45.9
<i>Chaenocephalus aceratus</i>	95.5%	53.6	96.9%	54.3	85.1%	47.3	95.4%	54.2	96.2%	50.8
<i>Cryodraco antarcticus</i>	96.0%	62.3	97.4%	63.2	86.3%	54.8	95.9%	63.1	96.6%	58.4
<i>Chionobathyscus dewitti</i>	95.6%	41.7	97.0%	42.3	85.3%	36.6	95.5%	42.3	96.1%	38.6

Supplementary Table 3. Recovery of heterozygous and fixed variation

Species	# Het SNPs	Het SNP/base	Percent of targets with Het	# Fixed species-specific SNPs	Fixed SNP/base	Percent of targets with fixed, species-specific SNP
<i>Percina caprodes</i>	48,777	0.0014	15.7%	300,691	0.0087	52.9%
<i>Percophis brasiliensis</i>	69,209	0.0019	19.7%	698,153	0.0187	77.4%
<i>Bovichtus diacanthus</i>	36,886	0.0010	11.6%	379,258	0.0096	54.1%
<i>Cottoperca trigloides</i>	30,261	0.0008	8.6%	428,614	0.0116	62.6%
<i>Pseudaphritis urvillii</i>	31,342	0.0009	10.2%	501,713	0.0137	69.9%
<i>Eleginops maclovinus</i>	53,559	0.0014	16.1%	599,422	0.0162	74.2%
<i>Pleuragramma antarctica</i>	154,174	0.0039	31.7%	223,284	0.0057	41.1%
<i>Aethotaxis mitopteryx</i>	77,172	0.0020	19.7%	69,895	0.0018	19.3%
<i>Dissostichus mawsoni</i>	42,867	0.0011	12.1%	40,827	0.0010	12.7%
<i>Dissostichus eleginoides</i>	53,849	0.0014	15.6%	44,805	0.0012	14.0%
<i>Nototheniops nudifrons</i>	58,203	0.0015	15.6%	41,278	0.0011	12.5%
<i>Lepidonotothen squamifrons</i>	62,976	0.0016	17.3%	26,827	0.0007	8.4%
<i>Patagonotothen squamiceps</i>	44,838	0.0011	12.4%	53,013	0.0014	16.0%
<i>Patagonotothen guntheri</i>	74,580	0.0019	19.7%	23,794	0.0006	7.5%
<i>Trematomus scotti</i>	66,169	0.0017	17.7%	25,783	0.0007	8.2%
<i>Trematomus newnesi</i>	53,985	0.0014	14.9%	39,385	0.0010	12.3%
<i>Trematomus eulepidotus</i>	47,855	0.0012	12.2%	25,282	0.0006	8.1%
<i>Trematomus bernacchii</i>	58,849	0.0015	16.1%	2,307	0.0001	0.4%
<i>Trematomus borchgrevinki</i>	37,388	0.0010	10.3%	33,673	0.0009	10.9%
<i>Trematomus hansonii</i>	61,373	0.0016	17.1%	2,041	0.0001	0.3%
<i>Gobionotothen gibberifrons</i>	64,191	0.0016	16.3%	111,759	0.0029	28.0%
<i>Notothenia coriiceps</i>	60,749	0.0015	16.2%	40,240	0.0010	12.6%
<i>Notothenia rossii</i>	66,220	0.0017	17.8%	45,190	0.0012	13.6%
<i>Paranotothenia angustata</i>	55,302	0.0014	15.5%	104,620	0.0027	27.7%
<i>Harpagifer antarcticus</i>	43,842	0.0012	13.1%	53,936	0.0014	16.4%
<i>Artedidraco skottsbergi</i>	64,811	0.0017	18.5%	11,638	0.0003	3.9%
<i>Histiodraco velifer</i>	42,299	0.0011	12.1%	8,164	0.0002	2.7%
<i>Dolloidraco longedorsalis</i>	52,428	0.0013	15.0%	5,526	0.0001	1.7%
<i>Pogonophryne scotti</i>	45,650	0.0012	13.2%	3,446	0.0001	1.0%
<i>Pogonophryne barsukovi</i>	44,453	0.0011	12.8%	3,061	0.0001	0.9%

<i>Gerlachea australis</i>	73,269	0.0019	20.5%	21,708	0.0006	7.0%
<i>Parachaenichthys charcoti</i>	30,350	0.0009	9.2%	46,682	0.0013	14.4%
<i>Vomeridens infuscipectus</i>	41,820	0.0011	11.0%	36,750	0.0009	11.8%
<i>Akarotaxis nudiceps</i>	56,254	0.0014	15.6%	12,176	0.0003	4.0%
<i>Bathyraco marri</i>	48,433	0.0012	13.2%	19,462	0.0005	6.4%
<i>Gymnodraco acuticeps</i>	58,027	0.0015	16.5%	41,434	0.0011	12.9%
<i>Dacodraco hunteri</i>	52,739	0.0013	14.8%	23,605	0.0006	7.8%
<i>Champscephalus gunnari</i>	37,061	0.0010	10.9%	14,024	0.0004	4.5%
<i>Champscephalus esox</i>	35,548	0.0009	10.3%	40,568	0.0010	12.7%
<i>Pagetopsis macropterus</i>	41,887	0.0011	12.1%	20,670	0.0005	6.9%
<i>Neopagetopsis ionah</i>	58,368	0.0015	15.9%	17,087	0.0004	5.6%
<i>Pseudochaenichthys georgianus</i>	14,921	0.0004	3.7%	29,936	0.0008	9.8%
<i>Chaenodraco wilsoni</i>	108,985	0.0028	27.6%	4,751	0.0001	1.4%
<i>Chaenocephalus aceratus</i>	26,447	0.0007	7.1%	18,353	0.0005	6.2%
<i>Cryodraco antarcticus</i>	115,004	0.0029	26.9%	2,531	0.0001	0.4%
<i>Chionobathyscus dewitti</i>	44,558	0.0011	11.9%	8,258	0.0002	2.7%

Supplementary Table 4. Substitution rates in notothenioids and outgroups

Branch	Substitution Rate	95% HPD
<i>Percina caprodes</i>	0.00078	0.0005-0.0011
<i>Percophis brasiliensis</i>	0.00077	0.0005-0.0011
<i>Bovichtus diacanthus</i>	0.00077	0.0005-0.0011
<i>P. urvillii</i> + Elegendopsioidea	0.00098	0.0004-0.0023
<i>Pseudaphritis urvillii</i>	0.00079	0.0005-0.0011
Elegendopsioidea	0.00228	0.0010-0.0039
<i>Elegendops maclovinus</i>	0.00168	0.0009-0.0024
Cryonotothenioidea	0.00218	0.0008-0.0038
<i>Dissostichus mawsoni</i>	0.00042	0.0001-0.0009
<i>Trematomus scottii</i>	0.00061	0.0002-0.0010
<i>Gobionotothen gibberifrons</i>	0.00078	0.0004-0.0012
<i>Notothenia coriiceps</i>	0.00076	0.0005-0.0012
<i>Pogonophryne scotti</i>	0.00071	0.0004-0.0011
<i>Harpagifer antarcticus</i>	0.00076	0.0005-0.0012
<i>Chaenocephalus aceratus</i>	0.00092	0.0003-0.0022
<i>Bathyraco marri</i>	0.00069	0.0003-0.0011

Supplementary Table 5. Positive selection in bone-associated[†] genes in Eleginopsioidea

Ensembl Ortholog	Gene	P	P (Bonferroni)	LRT
ENSGACG00000014066	LRRK1	1.46E-03	2.93E-03	10.922
ENSGACG00000001740	prdm1a	5.90E-03	1.18E-02	8.170
ENSGACG00000014774	cahz	4.37E-02	8.73E-02	4.251
ENSGACG00000011819	col12a1b	4.44E-02	8.88E-02	4.218
ENSGACG00000010282	minpp1b	1.11E-02	2.21E-02	6.935
ENSGACG00000013609	tacr1a	3.90E-02	7.81E-02	4.469
ENSGACG00000016847	sparc	1.69E-03	3.39E-03	10.635
ENSGACG00000013381	GABBR1	2.69E-02	5.38E-02	5.192
ENSGACG00000014923	col5a2b	1.25E-05	2.49E-05	20.389
ENSGACG00000008814	tmem119a	2.14E-02	4.28E-02	5.640
ENSGACG00000007303	ctnnbip1	8.15E-03	1.63E-02	7.534
ENSGACG00000018120	IGFBP4	7.24E-05	1.45E-04	16.889
ENSGACG00000005830	prpsap2	2.48E-02	4.95E-02	5.354
ENSGACG00000012442	notch2	3.74E-02	7.47E-02	4.553
ENSGACG00000013102	mapk3	1.42E-03	2.83E-03	10.990
ENSGACG00000003838	si:ch211-285f17.1	8.50E-03	1.70E-02	7.451
ENSGACG00000005488	phex	1.17E-02	2.34E-02	6.819
ENSGACG00000004564	hoxd10a	2.44E-03	4.88E-03	9.911
ENSGACG00000007087	LIMD1	4.55E-03	9.11E-03	8.680
ENSGACG00000006673	rbp4	4.58E-04	9.17E-04	13.225
ENSGACG00000017924	ghrb	2.67E-02	5.34E-02	5.208
ENSGACG00000019885	slc8a1a	1.36E-02	2.72E-02	6.530
ENSGACG00000011433	sun1	8.17E-03	1.63E-02	7.529
ENSGACG00000007343	col9a2	2.79E-02	5.58E-02	5.120
ENSGACG00000006287	ufl1	1.88E-02	3.76E-02	5.892
ENSGACG00000008639	pkdcc	1.49E-02	2.99E-02	6.343
ENSGACG00000010609	ptk2bb	2.00E-02	4.00E-02	5.772
ENSGACG00000010218	gata1a	1.51E-03	3.03E-03	10.856
ENSGACG00000015542	LOCENSGACG00000015542	8.65E-03	1.73E-02	7.416
ENSGACG00000005143	col1a1a	5.37E-04	1.07E-03	12.912
ENSGACG00000018619	il6st	3.97E-02	7.94E-02	4.435
ENSGACG00000014732	penka	3.16E-02	6.33E-02	4.876
ENSGACG00000016426	LOCENSGACG00000016426	4.59E-02	9.18E-02	4.153
ENSGACG00000011376	plxnb1b	1.07E-03	2.15E-03	11.537
ENSGACG00000006690	TNFRSF11B	2.58E-02	5.15E-02	5.277

ENSGACG00000003940	ufd1l	3.28E-02	6.56E-02	4.807
ENSGACG00000009412	LOCENSGACG00000009412	1.94E-04	3.87E-04	14.935
ENSGACG00000016814	slc26a2	4.17E-03	8.34E-03	8.853
ENSGACG00000012223	trip11	1.35E-02	2.71E-02	6.537
ENSGACG00000008029	PCSK5	4.34E-02	8.68E-02	4.262
ENSGACG00000011806	LOCENSGACG00000011806	2.28E-02	4.57E-02	5.512
ENSGACG00000004355	EIF2AK2	6.96E-04	1.39E-03	12.397
ENSGACG00000006819	col2a1b	1.50E-02	3.00E-02	6.337
ENSGACG00000004632	KLF10	4.31E-06	8.63E-06	22.504
ENSGACG00000019023	actn3a	2.03E-02	4.06E-02	5.740
ENSGACG00000010790	six2a	4.38E-03	8.76E-03	8.757
ENSGACG00000014956	eng1b	2.25E-02	4.51E-02	5.539
ENSGACG00000018018	NPR2	9.96E-03	1.99E-02	7.139
ENSGACG00000000260	itgav	1.89E-02	3.79E-02	5.877
ENSGACG00000002837	sulf1	1.84E-02	3.69E-02	5.930
ENSGACG00000009713	matn3a	1.35E-02	2.69E-02	6.548
ENSGACG00000006853	usp1	2.92E-02	5.83E-02	5.035
ENSGACG00000006537	mcph1	1.49E-02	2.97E-02	6.353
ENSGACG00000017894	spp1	2.59E-02	5.19E-02	5.264
ENSGACG00000008948	itgb3a	1.01E-03	2.01E-03	11.666
ENSGACG00000014564	hdac4	3.97E-02	7.93E-02	4.437
ENSGACG00000016287	CSGALNACT1	3.48E-02	6.95E-02	4.694
ENSGACG00000012231	LOCENSGACG00000012231	4.13E-04	8.27E-04	13.430
ENSGACG00000017458	lef1	3.11E-02	6.22E-02	4.909
ENSGACG00000018036	slc34a1a	4.85E-02	9.71E-02	4.046
ENSGACG00000020663	ryr1a	1.72E-02	3.45E-02	6.064
ENSGACG00000018814	ano6	1.44E-02	2.88E-02	6.416
ENSGACG00000016719	col11a1b	3.84E-03	7.69E-03	9.014
ENSGACG00000004416	eif2ak3	2.15E-02	4.30E-02	5.629
ENSGACG00000015182	NCAN (1 of many)	9.69E-03	1.94E-02	7.193
ENSGACG00000016060	ddx21	2.79E-02	5.59E-02	5.119
ENSGACG00000016773	FBN1	1.15E-02	2.30E-02	6.857
ENSGACG00000015511	jund	4.29E-02	8.59E-02	4.284
ENSGACG00000012007	mst1rb	1.62E-02	3.24E-02	6.184
ENSGACG00000004964	nf1b	3.70E-03	7.40E-03	9.090
ENSGACG00000020221	spns2	8.60E-03	1.72E-02	7.428
ENSGACG00000011567	LOCENSGACG00000011567	2.97E-02	5.94E-02	4.999
ENSGACG00000003402	foxp1a	4.78E-02	9.57E-02	4.074
ENSGACG00000008971	isg15	2.29E-07	4.58E-07	28.358
ENSGACG00000010238	igsf10	3.86E-02	7.71E-02	4.491

ENSGACG00000016670	mmp2	1.69E-02	3.38E-02	6.103
ENSGACG00000008267	nog2	1.56E-03	3.12E-03	10.795
ENSGACG00000006511	col1a2	7.06E-04	1.41E-03	12.367
ENSORLGG00000002037	LOCENSORLGG00000002037	5.35E-03	1.07E-02	8.363

†Select gene ontology IDs associated with bone development and mineralization (637 genes total)

GO:0001501,GO:0001503,GO:0001649,GO:0001957,GO:0001958,GO:0002051,GO:0002076
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GO:0030501,GO:0030502,GO:0031214,GO:0033687,GO:0033688,GO:0033689,GO:0033690
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GO:0045668,GO:0045669,GO:0045670,GO:0045671,GO:0045672,GO:0045778,GO:0048706
GO:0060351,GO:0070168,GO:0072674,GO:0072675,GO:0090290,GO:0090291,GO:1900158
GO:1900159

Supplementary Table 6. Accelerated sequence evolution in bone-associated[†] genes in Eleinopsioidea

Ensembl Ortholog	Gene	P	P (Bonferroni)	LRT
ENSGACG00000014564	hdac4	0.00E+00	0.00E+00	45.267
ENSGACG00000002204	fasn	0.00E+00	0.00E+00	37.930
ENSGACG00000003893	shhb	0.00E+00	0.00E+00	28.550
ENSGACG00000003678	zic1	0.00E+00	0.00E+00	21.956
ENSGACG00000008942	deaf1	0.00E+00	0.00E+00	20.953
ENSGACG00000014956	eng1b	0.00E+00	0.00E+00	20.861
ENSGACG00000003838	si:ch211-285f17.1	0.00E+00	0.00E+00	18.399
ENSGACG00000014897	CHD7	0.00E+00	0.00E+00	17.048
ENSGACG00000011376	plxnb1b	0.00E+00	0.00E+00	15.932
ENSGACG00000017481	gtpbp4	0.00E+00	0.00E+00	15.515
ENSGACG00000003906	npr3	0.00E+00	0.00E+00	14.273
ENSGACG00000016083	notch1a	0.00E+00	0.00E+00	12.690
ENSGACG00000020196	wnt11r	0.00E+00	0.00E+00	11.440
ENSGACG00000020771	zbtb16a	1.00E-05	2.00E-05	9.754
ENSGACG00000005297	rarab	1.00E-05	2.00E-05	9.003
ENSGACG00000020663	ryr1a	1.00E-05	2.00E-05	8.860
ENSGACG00000016835	nipblb	5.00E-05	1.00E-04	7.520
ENSGACG00000011665	syncrip	5.00E-05	1.00E-04	7.517
ENSGACG00000007999	RARB	7.00E-05	1.40E-04	7.286
ENSORLG00000009503	chd	1.70E-04	3.40E-04	6.433
ENSGACG00000016814	slc26a2	1.70E-04	3.40E-04	6.426
ENSGACG00000005810	ACVR1	2.30E-04	4.60E-04	6.129
ENSGACG00000020010	tmem178	2.50E-04	5.00E-04	6.047
ENSGACG00000010046	id1	3.10E-04	6.20E-04	5.863
ENSGACG00000008814	tmem119a	4.00E-04	8.00E-04	5.626
ENSGACG00000016310	sh3pxd2aa	4.50E-04	9.00E-04	5.520
ENSGACG00000006853	usp1	4.60E-04	9.20E-04	5.488
ENSGACG00000016468	foxc1a	4.70E-04	9.40E-04	5.465
ENSGACG00000005279	gdf6a	4.80E-04	9.60E-04	5.445
ENSGACG00000008289	trim45	7.10E-04	1.42E-03	5.088
ENSGACG00000010282	minpp1b	8.50E-04	1.70E-03	4.924
ENSGACG00000006287	ufl1	9.50E-04	1.90E-03	4.820
ENSGACG00000020147	osr1	1.11E-03	2.22E-03	4.678
ENSGACG00000018575	hmgcs1	1.43E-03	2.86E-03	4.448
ENSGACG00000005508	sfrp1a	1.46E-03	2.92E-03	4.425

ENSGACG00000010238	igsf10	2.06E-03	4.12E-03	4.116
ENSGACG00000004632	KLF10	2.07E-03	4.14E-03	4.113
ENSGACG00000012411	SMOC1	2.15E-03	4.30E-03	4.076
ENSGACG00000020486	pafah1b1b	2.18E-03	4.36E-03	4.066
ENSGACG00000014186	fgf3	2.31E-03	4.62E-03	4.010
ENSGACG00000003402	foxp1a	2.45E-03	4.90E-03	3.960
ENSGACG00000009557	alx1	2.62E-03	5.24E-03	3.899
ENSGACG00000004931	dlx1a	2.82E-03	5.64E-03	3.831
ENSGACG00000004834	gli3	3.34E-03	6.68E-03	3.680
ENSGACG00000011493	si:dkey-42p8.3	3.39E-03	6.78E-03	3.666
ENSGACG00000014119	acp2	3.63E-03	7.26E-03	3.604
ENSGACG00000005488	phex	3.90E-03	7.80E-03	3.540
ENSGACG00000007912	smurf1	3.94E-03	7.88E-03	3.531
ENSGACG00000012407	ercc2	4.26E-03	8.52E-03	3.461
ENSGACG00000004764	DNAJC13	4.62E-03	9.24E-03	3.387
ENSGACG00000015083	acvr2ab	4.80E-03	9.60E-03	3.354
ENSGACG00000008948	itgb3a	4.96E-03	9.92E-03	3.325
ENSGACG00000009396	hoxc9a	7.28E-03	1.46E-02	2.984
ENSGACG00000007341	ireb2	7.42E-03	1.48E-02	2.968
ENSGACG00000009872	kat7b	7.75E-03	1.55E-02	2.930
ENSGACG00000013817	BMPR2	8.04E-03	1.61E-02	2.897
ENSGACG00000012967	MATN3	8.53E-03	1.71E-02	2.845
ENSGACG00000012822	LOCENSGACG00000012822	8.89E-03	1.78E-02	2.809
ENSGACG00000007795	inpp1a	9.54E-03	1.91E-02	2.747
ENSGACG00000004680	sema7a	9.61E-03	1.92E-02	2.741

†Select gene ontology IDs associated with bone development and mineralization (637 genes total)

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GO:0002158,GO:0003433,GO:0030278,GO:0030279,GO:0030282,GO:0030316,GO:0030500
GO:0030501,GO:0030502,GO:0031214,GO:0033687,GO:0033688,GO:0033689,GO:0033690
GO:0035630,GO:0036035,GO:0036179,GO:0043931,GO:0043932,GO:0044339,GO:0045667
GO:0045668,GO:0045669,GO:0045670,GO:0045671,GO:0045672,GO:0045778,GO:0048706
GO:0060351,GO:0070168,GO:0072674,GO:0072675,GO:0090290,GO:0090291,GO:1900158
GO:1900159

Supplementary Table 7. Positive selection in bone-associated[†] genes in Channichthyidae

Ensembl Ortholog	Gene	P	P (Bonferroni)	LRT
ENSGACG00000014066	LRRK1	0.00E+00	0.00E+00	101.842
ENSGACG00000010790	six2a	2.65E-10	5.29E-10	41.853
ENSGACG00000010565	tgfb3	7.14E-06	1.43E-05	21.500
ENSGACG00000016783	tbx3a	8.11E-06	1.62E-05	21.248
ENSGACG00000017894	spp1	2.50E-04	5.00E-04	14.429
ENSGACG00000011014	aak1a	4.19E-04	8.38E-04	13.403
ENSGACG00000005143	col1a1a	7.30E-04	1.46E-03	12.301
ENSGACG00000012822	LOCENSGACG00000012822	1.37E-03	2.75E-03	11.048
ENSGACG00000007087	LIMD1	1.85E-03	3.71E-03	10.455
ENSGACG00000010042	BCAP29	3.20E-03	6.40E-03	9.376
ENSGACG00000016860	mfge8a	4.62E-03	9.23E-03	8.654
ENSGACG00000017255	inpp4b	4.76E-03	9.52E-03	8.593
ENSGACG00000012007	mst1rb	5.49E-03	1.10E-02	8.310
ENSGACG00000016468	foxc1a	6.33E-03	1.27E-02	8.030
ENSGACG00000013533	tfa	8.34E-03	1.67E-02	7.487
ENSGACG00000000393	il19l	1.19E-02	2.39E-02	6.783
ENSGACG00000003946	gpm6bb	1.24E-02	2.47E-02	6.714
ENSGACG00000016773	FBN1	1.33E-02	2.65E-02	6.577
ENSGACG00000020771	zbtb16a	1.82E-02	3.64E-02	5.958
ENSGACG00000011694	axin2	1.95E-02	3.89E-02	5.824
ENSGACG00000008971	isg15	2.40E-02	4.80E-02	5.416
ENSGACG00000011781	ocstamp	3.01E-02	6.02E-02	4.975
ENSGACG00000009662	NA	3.13E-02	6.26E-02	4.898
ENSORLG00000002037	LOCENSORLG00000002037	3.85E-02	7.70E-02	4.495
ENSGACG00000004134	chad	4.95E-02	9.89E-02	4.009

[†]Select gene ontology IDs associated with bone development and mineralization (637 genes total)

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GO:0030501,GO:0030502,GO:0031214,GO:0033687,GO:0033688,GO:0033689,GO:0033690
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GO:0045668,GO:0045669,GO:0045670,GO:0045671,GO:0045672,GO:0045778,GO:0048706
GO:0060351,GO:0070168,GO:0072674,GO:0072675,GO:0090290,GO:0090291,GO:1900158
GO:1900159

Supplementary Table 8. Accelerated sequence evolution in bone-associated[†] genes in Channichthyidae

Ensembl Ortholog	Gene	P	P (Bonferroni)	LRT
ENSGACG00000018025	gdf2	0.00E+00	0.00E+00	44.466
ENSGACG00000014066	LRRK1	0.00E+00	0.00E+00	18.742
ENSGACG00000010790	six2a	0.00E+00	0.00E+00	16.153
ENSGACG00000020111	BBX	0.00E+00	0.00E+00	16.044
ENSGACG00000012007	mst1rb	0.00E+00	0.00E+00	10.213
ENSGACG00000012223	trip11	1.00E-05	2.00E-05	9.635
ENSGACG00000005143	col1a1a	1.00E-05	2.00E-05	9.225
ENSGACG00000011839	FOXP1	1.00E-05	2.00E-05	8.772
ENSGACG00000003352	sec22c	2.00E-05	4.00E-05	8.542
ENSGACG00000018575	hmgcs1	4.00E-05	8.00E-05	7.720
ENSGACG00000014838	ptk2ba	6.00E-05	1.20E-04	7.335
ENSGACG00000018814	ano6	7.00E-05	1.40E-04	7.308
ENSGACG00000005830	prpsap2	1.40E-04	2.80E-04	6.617
ENSGACG00000016287	CSGALNACT1 (2 of 2)	1.60E-04	3.20E-04	6.447
ENSGACG00000014536	xpr1b	1.70E-04	3.40E-04	6.423
ENSGACG00000011843	cdh11	6.30E-04	1.26E-03	5.194
ENSGACG00000007087	LIMD1	6.50E-04	1.30E-03	5.165
ENSGACG00000014186	fgf3	8.90E-04	1.78E-03	4.878
ENSGACG00000018497	AXIN2 (1 of many)	1.16E-03	2.32E-03	4.640
ENSGACG00000013007	skia	1.82E-03	3.64E-03	4.227
ENSGACG00000016062	col27a1a	2.59E-03	5.18E-03	3.907
ENSGACG00000016083	notch1a	2.80E-03	5.60E-03	3.838
ENSGACG00000011349	src	3.32E-03	6.64E-03	3.684
ENSGACG00000019404	psmc2	3.69E-03	7.38E-03	3.589
ENSGACG00000001547	gli2a	3.96E-03	7.92E-03	3.525
ENSGACG00000014550	txlng	4.58E-03	9.16E-03	3.395
ENSGACG00000007522	jag2b	6.46E-03	1.29E-02	3.090
ENSGACG00000016016	fam73b	6.48E-03	1.30E-02	3.087

[†]Select gene ontology IDs associated with bone development and mineralization (637 genes total)

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GO:0045668,GO:0045669,GO:0045670,GO:0045671,GO:0045672,GO:0045778,GO:0048706
GO:0060351,GO:0070168,GO:0072674,GO:0072675,GO:0090290,GO:0090291,GO:1900158
GO:1900159

Supplementary Table 9. Tissue samples used in this study

Family	Species	Source	ID#
Artedidraconidae	<i>Artedidraco skottsbergi</i>	No voucher	YFTC 4131
Artedidraconidae	<i>Artedidraco skottsbergi</i>	No voucher	YFTC 4132
Artedidraconidae	<i>Artedidraco skottsbergi</i>	YPM ICH 016389	YFTC 7804
Artedidraconidae	<i>Artedidraco skottsbergi</i>	YPM ICH 016389	YFTC 7805
Artedidraconidae	<i>Artedidraco skottsbergi</i>	YPM ICH 022479	YFTC 15048
Artedidraconidae	<i>Dolloidraco longedorsalis</i>	Detrich lab	HWD 53
Artedidraconidae	<i>Dolloidraco longedorsalis</i>	Detrich lab	HWD 54
Artedidraconidae	<i>Dolloidraco longedorsalis</i>	Detrich lab	HWD 55
Artedidraconidae	<i>Dolloidraco longedorsalis</i>	Detrich lab	HWD 56
Artedidraconidae	<i>Dolloidraco longedorsalis</i>	Detrich lab	HWD 57
Artedidraconidae	<i>Histiodraco velifer</i>	No voucher	YFTC 2049
Artedidraconidae	<i>Histiodraco velifer</i>	No voucher	YFTC 4135
Artedidraconidae	<i>Histiodraco velifer</i>	No voucher	YFTC 4136
Artedidraconidae	<i>Histiodraco velifer</i>	No voucher	YFTC 13801
Artedidraconidae	<i>Histiodraco velifer</i>	No voucher	YFTC 14514
Artedidraconidae	<i>Pogonophryne barsukovi</i>	YPM ICH 022357	YFTC 15530
Artedidraconidae	<i>Pogonophryne barsukovi</i>	YPM ICH 022357	YFTC 15531
Artedidraconidae	<i>Pogonophryne barsukovi</i>	YPM ICH 022357	YFTC 15532
Artedidraconidae	<i>Pogonophryne barsukovi</i>	YPM ICH 022357	YFTC 15533
Artedidraconidae	<i>Pogonophryne barsukovi</i>	YPM ICH 022357	YFTC 15534
Artedidraconidae	<i>Pogonophryne scotti</i>	YPM ICH 022357	YFTC 15105
Artedidraconidae	<i>Pogonophryne scotti</i>	YPM ICH 022553	YFTC 15106
Artedidraconidae	<i>Pogonophryne scotti</i>	YPM ICH 022553	YFTC 15107
Artedidraconidae	<i>Pogonophryne scotti</i>	YPM ICH 022553	YFTC 15108
Artedidraconidae	<i>Pogonophryne scotti</i>	YPM ICH 022553	YFTC 15109
Bathydraconidae	<i>Akarotaxis nudiceps</i>	YPM ICH 024046	YFTC 20912
Bathydraconidae	<i>Akarotaxis nudiceps</i>	YPM ICH 024238	YFTC 20924
Bathydraconidae	<i>Akarotaxis nudiceps</i>	YPM ICH 024118	YFTC 20928
Bathydraconidae	<i>Akarotaxis nudiceps</i>	YPM ICH 024118	YFTC 20929
Bathydraconidae	<i>Akarotaxis nudiceps</i>	YPM ICH 024118	YFTC 20930
Bathydraconidae	<i>Bathyraco marri</i>	NMNZ 043393	YFTC 13884
Bathydraconidae	<i>Bathyraco marri</i>	NMNZ 043394	YFTC 13885
Bathydraconidae	<i>Bathyraco marri</i>	NMNZ 043553	YFTC 13886
Bathydraconidae	<i>Bathyraco marri</i>	NMNZ 043634	YFTC 13887
Bathydraconidae	<i>Bathyraco marri</i>	NMNZ 043635	YFTC 13888
Bathydraconidae	<i>Gerlachea australis</i>	NMNZ 043338	YFTC 13898

Bathydraconidae	<i>Gerlachea australis</i>	NMNZ 043495	YFTC 13899
Bathydraconidae	<i>Gerlachea australis</i>	NMNZ 043517	YFTC 13900
Bathydraconidae	<i>Gerlachea australis</i>	NMNZ 043518	YFTC 13901
Bathydraconidae	<i>Gerlachea australis</i>	NMNZ 043545	YFTC 13902
Bathydraconidae	<i>Gymnodraco acuticeps</i>	Detrich lab	HWD 6
Bathydraconidae	<i>Gymnodraco acuticeps</i>	Detrich lab	HWD 7
Bathydraconidae	<i>Gymnodraco acuticeps</i>	Detrich lab	HWD 8
Bathydraconidae	<i>Gymnodraco acuticeps</i>	Detrich lab	HWD 9
Bathydraconidae	<i>Gymnodraco acuticeps</i>	Detrich lab	HWD 10
Bathydraconidae	<i>Gymnodraco acuticeps</i>	Detrich lab	HWD 11
Bathydraconidae	<i>Parachaenichthys charcoti</i>	Detrich lab	HWD 24
Bathydraconidae	<i>Parachaenichthys charcoti</i>	Detrich lab	HWD 25
Bathydraconidae	<i>Parachaenichthys charcoti</i>	Detrich lab	HWD 26
Bathydraconidae	<i>Vomeridens infuscipinnis</i>	YPM ICH 020008	YFTC 12880
Bathydraconidae	<i>Vomeridens infuscipinnis</i>	YPM ICH 020009	YFTC 12881
Bathydraconidae	<i>Vomeridens infuscipinnis</i>	YPM ICH 020041	YFTC 12884
Bathydraconidae	<i>Vomeridens infuscipinnis</i>	YPM ICH 020025	YFTC 12882
Bathydraconidae	<i>Vomeridens infuscipinnis</i>	YPM ICH 020061	YFTC 12919
Bovichtidae	<i>Bovichtus diacanthus</i>	YPM ICH 021534	YFTC 3482
Bovichtidae	<i>Bovichtus diacanthus</i>	YPM ICH 021534	YFTC 3483
Bovichtidae	<i>Bovichtus diacanthus</i>	YPM ICH 021534	YFTC 3484
Bovichtidae	<i>Bovichtus diacanthus</i>	YPM ICH 021534	YFTC 3485
Bovichtidae	<i>Bovichtus diacanthus</i>	YPM ICH 021534	YFTC 3486
Bovichtidae	<i>Cottoperca trigloides</i>	Detrich lab	HWD 71
Bovichtidae	<i>Cottoperca trigloides</i>	Detrich lab	HWD 72
Bovichtidae	<i>Cottoperca trigloides</i>	Detrich lab	HWD 73
Bovichtidae	<i>Cottoperca trigloides</i>	Detrich lab	HWD 74
Bovichtidae	<i>Cottoperca trigloides</i>	Detrich lab	HWD 75
Channichthyidae	<i>Chaenocephalus aceratus</i>	Detrich lab	HWD 1
Channichthyidae	<i>Chaenocephalus aceratus</i>	Detrich lab	HWD 2
Channichthyidae	<i>Chaenocephalus aceratus</i>	Detrich lab	HWD 3
Channichthyidae	<i>Chaenocephalus aceratus</i>	Detrich lab	HWD 4
Channichthyidae	<i>Chaenocephalus aceratus</i>	Detrich lab	HWD 5
Channichthyidae	<i>Chaenodraco wilsoni</i>	Detrich lab	HWD 76
Channichthyidae	<i>Chaenodraco wilsoni</i>	Detrich lab	HWD 77
Channichthyidae	<i>Chaenodraco wilsoni</i>	Detrich lab	HWD 78
Channichthyidae	<i>Chaenodraco wilsoni</i>	Detrich lab	HWD 79
Channichthyidae	<i>Chaenodraco wilsoni</i>	Detrich lab	HWD 80
Channichthyidae	<i>Champscephalus esox</i>	Detrich lab	HWD 41
Channichthyidae	<i>Champscephalus esox</i>	Detrich lab	HWD 42

Channichthyidae	<i>Champscephalus gunnari</i>	YPM ICH 022626	YFTC 14750
Channichthyidae	<i>Champscephalus gunnari</i>	YPM ICH 022626	YFTC 14751
Channichthyidae	<i>Champscephalus gunnari</i>	YPM ICH 022626	YFTC 14752
Channichthyidae	<i>Champscephalus gunnari</i>	YPM ICH 022626	YFTC 14753
Channichthyidae	<i>Champscephalus gunnari</i>	YPM ICH 022626	YFTC 14754
Channichthyidae	<i>Chionobathyscus dewitti</i>	Detrich lab	HWD 32
Channichthyidae	<i>Chionobathyscus dewitti</i>	Detrich lab	HWD 33
Channichthyidae	<i>Chionobathyscus dewitti</i>	Detrich lab	HWD 34
Channichthyidae	<i>Chionobathyscus dewitti</i>	Detrich lab	HWD 35
Channichthyidae	<i>Cryodraco antarcticus</i>	No voucher	YFTC 11045
Channichthyidae	<i>Cryodraco antarcticus</i>	No voucher	YFTC 11046
Channichthyidae	<i>Cryodraco antarcticus</i>	No voucher	YFTC 11047
Channichthyidae	<i>Cryodraco antarcticus</i>	Detrich lab	HWD 81
Channichthyidae	<i>Cryodraco antarcticus</i>	Detrich lab	HWD 82
Channichthyidae	<i>Cryodraco antarcticus</i>	Detrich lab	HWD 83
Channichthyidae	<i>Dacodraco hunteri</i>	NMNZ 043399	YFTC 16112
Channichthyidae	<i>Dacodraco hunteri</i>	NMNZ 043430	YFTC 16113
Channichthyidae	<i>Dacodraco hunteri</i>	NMNZ 043450	YFTC 16114
Channichthyidae	<i>Dacodraco hunteri</i>	NMNZ 043496	YFTC 16115
Channichthyidae	<i>Neopagetopsis ionah</i>	NMNZ 043314	YFTC 16116
Channichthyidae	<i>Neopagetopsis ionah</i>	NMNZ 043315	YFTC 16117
Channichthyidae	<i>Neopagetopsis ionah</i>	NMNZ 043316	YFTC 16118
Channichthyidae	<i>Neopagetopsis ionah</i>	NMNZ 043358	YFTC 16119
Channichthyidae	<i>Neopagetopsis ionah</i>	NMNZ 043378	YFTC 16120
Channichthyidae	<i>Pagetopsis macropterus</i>	YPM ICH 016490	YFTC 7775
Channichthyidae	<i>Pagetopsis macropterus</i>	YPM ICH 016490	YFTC 7776
Channichthyidae	<i>Pagetopsis macropterus</i>	YPM ICH 016490	YFTC 7777
Channichthyidae	<i>Pagetopsis macropterus</i>	YPM ICH 016490	YFTC 7778
Channichthyidae	<i>Pagetopsis macropterus</i>	YPM ICH 016490	YFTC 7779
Channichthyidae	<i>Pseudochaenichthys georgianus</i>	Detrich lab	HWD 43
Channichthyidae	<i>Pseudochaenichthys georgianus</i>	Detrich lab	HWD 44
Channichthyidae	<i>Pseudochaenichthys georgianus</i>	Detrich lab	HWD 45
Channichthyidae	<i>Pseudochaenichthys georgianus</i>	Detrich lab	HWD 46
Channichthyidae	<i>Pseudochaenichthys georgianus</i>	Detrich lab	HWD 47
Eleginopsidae	<i>Eleginops maclovinus</i>	Detrich lab	HWD 58
Eleginopsidae	<i>Eleginops maclovinus</i>	Detrich lab	HWD 59
Eleginopsidae	<i>Eleginops maclovinus</i>	Detrich lab	HWD 60
Eleginopsidae	<i>Eleginops maclovinus</i>	Detrich lab	HWD 61
Eleginopsidae	<i>Eleginops maclovinus</i>	Detrich lab	HWD 62
Eleginopsidae	<i>Eleginops maclovinus</i>	Detrich lab	HWD 63

Harpagiferidae	<i>Harpagifer antarcticus</i>	YPM ICH 016640	YFTC 4399
Harpagiferidae	<i>Harpagifer antarcticus</i>	YPM ICH 016640	YFTC 4400
Harpagiferidae	<i>Harpagifer antarcticus</i>	YPM ICH 016640	YFTC 4401
Harpagiferidae	<i>Harpagifer antarcticus</i>	YPM ICH 016640	YFTC 4402
Harpagiferidae	<i>Harpagifer antarcticus</i>	YPM ICH 016640	YFTC 4403
Nototheniidae	<i>Aethotaxis mitopteryx</i>	YPM ICH 022552	YFTC 15291
Nototheniidae	<i>Aethotaxis mitopteryx</i>	YPM ICH 022552	YFTC 15292
Nototheniidae	<i>Aethotaxis mitopteryx</i>	YPM ICH 022552	YFTC 15293
Nototheniidae	<i>Aethotaxis mitopteryx</i>	YPM ICH 022552	YFTC 15294
Nototheniidae	<i>Aethotaxis mitopteryx</i>	YPM ICH 022552	YFTC 15295
Nototheniidae	<i>Dissostichus eleginoides</i>	Detrich lab	HWD 36
Nototheniidae	<i>Dissostichus eleginoides</i>	Detrich lab	HWD 37
Nototheniidae	<i>Dissostichus eleginoides</i>	Detrich lab	HWD 38
Nototheniidae	<i>Dissostichus eleginoides</i>	Detrich lab	HWD 39
Nototheniidae	<i>Dissostichus eleginoides</i>	Detrich lab	HWD 40
Nototheniidae	<i>Dissostichus mawsoni</i>	YPM ICH 022349	YFTC 15462
Nototheniidae	<i>Dissostichus mawsoni</i>	YPM ICH 022397	YFTC 15346
Nototheniidae	<i>Dissostichus mawsoni</i>	YPM ICH 022397	YFTC 15347
Nototheniidae	<i>Dissostichus mawsoni</i>	YPM ICH 022397	YFTC 15348
Nototheniidae	<i>Dissostichus mawsoni</i>	YPM ICH 022397	YFTC 15349
Nototheniidae	<i>Gobionotothen gibberifrons</i>	No voucher	YFTC 12759
Nototheniidae	<i>Gobionotothen gibberifrons</i>	No voucher	YFTC 12760
Nototheniidae	<i>Gobionotothen gibberifrons</i>	No voucher	YFTC 12761
Nototheniidae	<i>Gobionotothen gibberifrons</i>	No voucher	YFTC 12762
Nototheniidae	<i>Gobionotothen gibberifrons</i>	No voucher	YFTC 12763
Nototheniidae	<i>Nototheniops nudifrons</i>	YPM ICH 020081	YFTC 3809
Nototheniidae	<i>Nototheniops nudifrons</i>	YPM ICH 020081	YFTC 3810
Nototheniidae	<i>Nototheniops nudifrons</i>	YPM ICH 020081	YFTC 3811
Nototheniidae	<i>Nototheniops nudifrons</i>	YPM ICH 020081	YFTC 3812
Nototheniidae	<i>Nototheniops nudifrons</i>	YPM ICH 020081	YFTC 3813
Nototheniidae	<i>Lepidonotothen squamifrons</i>	YPM ICH 028210	YFTC 14785
Nototheniidae	<i>Lepidonotothen squamifrons</i>	YPM ICH 028210	YFTC 14786
Nototheniidae	<i>Lepidonotothen squamifrons</i>	YPM ICH 022548	YFTC 15065
Nototheniidae	<i>Lepidonotothen squamifrons</i>	YPM ICH 022548	YFTC 15066
Nototheniidae	<i>Lepidonotothen squamifrons</i>	YPM ICH 022548	YFTC 15067
Nototheniidae	<i>Paranotothenia angustata</i>	Detrich lab	HWD 12
Nototheniidae	<i>Paranotothenia angustata</i>	Detrich lab	HWD 13
Nototheniidae	<i>Paranotothenia angustata</i>	Detrich lab	HWD 14
Nototheniidae	<i>Paranotothenia angustata</i>	Detrich lab	HWD 15
Nototheniidae	<i>Paranotothenia angustata</i>	Detrich lab	HWD 16

Nototheniidae	<i>Notothenia coriiceps</i>	Detrich lab	HWD 27
Nototheniidae	<i>Notothenia coriiceps</i>	Detrich lab	HWD 28
Nototheniidae	<i>Notothenia coriiceps</i>	Detrich lab	HWD 29
Nototheniidae	<i>Notothenia coriiceps</i>	Detrich lab	HWD 30
Nototheniidae	<i>Notothenia coriiceps</i>	Detrich lab	HWD 31
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 64
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 65
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 66
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 67
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 68
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 69
Nototheniidae	<i>Notothenia rossii</i>	Detrich lab	HWD 70
Nototheniidae	<i>Trematomus borchgrevinki</i>	No voucher	YFTC 12070
Nototheniidae	<i>Trematomus borchgrevinki</i>	No voucher	YFTC 12071
Nototheniidae	<i>Trematomus borchgrevinki</i>	No voucher	YFTC 12072
Nototheniidae	<i>Trematomus borchgrevinki</i>	No voucher	YFTC 13638
Nototheniidae	<i>Patagonotothen squamiceps</i>	No voucher	YFTC 24046
Nototheniidae	<i>Patagonotothen squamiceps</i>	No voucher	YFTC 24047
Nototheniidae	<i>Patagonotothen squamiceps</i>	No voucher	YFTC 24048
Nototheniidae	<i>Patagonotothen squamiceps</i>	No voucher	YFTC 24049
Nototheniidae	<i>Patagonotothen squamiceps</i>	No voucher	YFTC 24050
Nototheniidae	<i>Patagonotothen guntheri</i>	No voucher	YFTC 24057
Nototheniidae	<i>Patagonotothen guntheri</i>	No voucher	YFTC 24058
Nototheniidae	<i>Patagonotothen guntheri</i>	No voucher	YFTC 24059
Nototheniidae	<i>Patagonotothen guntheri</i>	No voucher	YFTC 24060
Nototheniidae	<i>Patagonotothen guntheri</i>	No voucher	YFTC 24061
Nototheniidae	<i>Pleuragramma antarctica</i>	YPM ICH 022586	YFTC 15002
Nototheniidae	<i>Pleuragramma antarctica</i>	YPM ICH 022586	YFTC 15003
Nototheniidae	<i>Pleuragramma antarctica</i>	YPM ICH 022586	YFTC 15004
Nototheniidae	<i>Pleuragramma antarctica</i>	YPM ICH 022586	YFTC 15005
Nototheniidae	<i>Pleuragramma antarctica</i>	YPM ICH 022586	YFTC 15006
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 17
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 18
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 19
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 20
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 21
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 22
Nototheniidae	<i>Trematomus bernacchii</i>	Detrich lab	HWD 23
Nototheniidae	<i>Trematomus eulepidotus</i>	YPM ICH 022564	YFTC 15403
Nototheniidae	<i>Trematomus eulepidotus</i>	YPM ICH 022564	YFTC 15404

Nototheniidae	<i>Trematomus eulepidotus</i>	YPM ICH 022564	YFTC 15405
Nototheniidae	<i>Trematomus eulepidotus</i>	YPM ICH 022564	YFTC 15406
Nototheniidae	<i>Trematomus eulepidotus</i>	YPM ICH 022564	YFTC 15407
Nototheniidae	<i>Trematomus hansonii</i>	Detrich lab	HWD 48
Nototheniidae	<i>Trematomus hansonii</i>	Detrich lab	HWD 49
Nototheniidae	<i>Trematomus hansonii</i>	Detrich lab	HWD 50
Nototheniidae	<i>Trematomus hansonii</i>	Detrich lab	HWD 51
Nototheniidae	<i>Trematomus hansonii</i>	Detrich lab	HWD 52
Nototheniidae	<i>Trematomus newnesi</i>	YPM ICH 016464	YFTC 7708
Nototheniidae	<i>Trematomus newnesi</i>	YPM ICH 016464	YFTC 7709
Nototheniidae	<i>Trematomus newnesi</i>	YPM ICH 016464	YFTC 7710
Nototheniidae	<i>Trematomus newnesi</i>	YPM ICH 016464	YFTC 7711
Nototheniidae	<i>Trematomus newnesi</i>	YPM ICH 016464	YFTC 7712
Nototheniidae	<i>Trematomus scottii</i>	YPM ICH 024115	YFTC 20940
Nototheniidae	<i>Trematomus scottii</i>	YPM ICH 024115	YFTC 20941
Nototheniidae	<i>Trematomus scottii</i>	YPM ICH 024115	YFTC 20942
Nototheniidae	<i>Trematomus scottii</i>	YPM ICH 024115	YFTC 20943
Nototheniidae	<i>Trematomus scottii</i>	YPM ICH 024115	YFTC 20944
Percidae	<i>Percina caprodes</i>	YPM ICH 027375	YFTC 24610
Percidae	<i>Percina caprodes</i>	YPM ICH 027375	YFTC 24610
Percidae	<i>Percina caprodes</i>	YPM ICH 027375	YFTC 24611
Percidae	<i>Percina caprodes</i>	YPM ICH 027375	YFTC 24612
Percidae	<i>Percina caprodes</i>	YPM ICH 027375	YFTC 24613
Percophidae	<i>Percophis brasiliensis</i>	LBP 8668	35303
Percophidae	<i>Percophis brasiliensis</i>	LBP 8654	35304
Percophidae	<i>Percophis brasiliensis</i>	LBP 8654	35305
Percophidae	<i>Percophis brasiliensis</i>	LBP 8668	35307
Percophidae	<i>Percophis brasiliensis</i>	LBP 19527	73365
Percophidae	<i>Percophis brasiliensis</i>	LBP 19527	73366
Percophidae	<i>Percophis brasiliensis</i>	LBP 19527	73367
Pseudaphritidae	<i>Pseudaphritis urvillii</i>	CSIRO 6892-01	YFTC 16619
Pseudaphritidae	<i>Pseudaphritis urvillii</i>	CSIRO 6892-01	YFTC 16620
Pseudaphritidae	<i>Pseudaphritis urvillii</i>	CSIRO 7637-05	YFTC 16621

Supplementary Table 10. Enrichment of genes with missing coverage in dataset

GO Term	Term Name	GO type	# genes	# genes low cov. †	fold enrichment	p-value	FDR qvalue
GO:0043410	positive regulation of MAPK cascade	bp	92	15	4.02	4.22E-06	1.72E-03
GO:0030198	extracellular matrix organization	bp	209	30	3.54	1.89E-09	5.41E-06
GO:0016925	protein sumoylation	bp	111	15	3.33	4.27E-05	1.36E-02
GO:0006955	immune response	bp	268	29	2.67	1.70E-06	8.07E-04
GO:0006954	inflammatory response	bp	308	33	2.64	4.19E-07	2.99E-04
GO:0007166	cell surface receptor signaling pathway	bp	297	31	2.57	1.63E-06	8.07E-04
GO:0018108	peptidyl-tyrosine phosphorylation	bp	211	21	2.45	1.47E-04	3.81E-02
GO:0002376	immune system process	bp	251	24	2.36	9.61E-05	2.75E-02
GO:0016032	viral process	bp	377	35	2.29	5.23E-06	1.87E-03
GO:0007155	cell adhesion	bp	615	53	2.12	2.36E-07	2.25E-04
GO:0016504	peptidase activator activity	mf	13	5	9.48	1.07E-04	1.01E-02
GO:0005031	tumor necrosis factor-activated receptor activity	mf	23	6	6.43	2.45E-04	2.08E-02
GO:0005201	extracellular matrix structural constituent	mf	86	21	6.02	2.27E-11	9.65E-09
GO:0004714	transmembrane receptor protein tyrosine kinase activity	mf	89	13	3.60	6.03E-05	6.41E-03
GO:0005198	structural molecule activity	mf	230	21	2.25	4.76E-04	3.38E-02
GO:0003779	actin binding	mf	464	38	2.02	3.64E-05	4.43E-03
GO:0000800	lateral element	cc	13	4	7.58	1.44E-03	2.48E-02
GO:0005771	multivesicular body	cc	22	6	6.72	1.87E-04	5.02E-03
GO:0005640	nuclear outer membrane	cc	22	5	5.60	1.61E-03	2.70E-02
GO:0005581	collagen trimer	cc	111	18	4.00	5.16E-07	2.51E-05
GO:0005604	basement membrane	cc	92	14	3.75	1.98E-05	6.23E-04
GO:0030175	filopodium	cc	69	10	3.57	4.47E-04	9.58E-03
GO:0005578	proteinaceous extracellular matrix	cc	371	46	3.06	1.80E-11	2.41E-09
GO:0005814	centriole	cc	107	13	2.99	3.97E-04	8.87E-03
GO:0072562	blood microparticle	cc	91	11	2.98	1.13E-03	2.09E-02
GO:0009897	external side of plasma membrane	cc	166	20	2.97	1.40E-05	5.01E-04
GO:0005923	bicellular tight junction	cc	158	18	2.81	7.78E-05	2.32E-03

GO:0031225	anchored component of membrane	cc	88	10	2.80	2.98E-03	4.20E-02
GO:0043235	receptor complex	cc	116	13	2.76	8.64E-04	1.78E-02
GO:0005911	cell-cell junction	cc	193	19	2.43	3.43E-04	7.98E-03
GO:0031012	extracellular matrix	cc	322	31	2.37	8.63E-06	3.30E-04
GO:0005788	endoplasmic reticulum lumen	cc	194	17	2.16	2.45E-03	3.75E-02
GO:0009986	cell surface	cc	498	42	2.08	7.11E-06	2.93E-04
GO:0005874	microtubule	cc	357	29	2.00	3.35E-04	7.98E-03

bp – biological process, mf – molecular function, cc – cellular component

Supplementary Table 11. Notothenioid specimens for CT scan

Species	Catelog Number	Specimen #	STL (cm)
Bovichtus variegatus	YPM ICH 005976	5976	13.5
	MCZ ICH 25558	NA	13.0
Eleginops maclovinus	YPM ICH 009315	NA	32.0
	YPM ICH 009315	NA	25.0
Dissostichus mawsoni	YPM ICH 020040	12848	27.5
	MCZ ICH 195009	NA	35.0
Trematomus bernacchii	YPM ICH 020469	01-46	19.5
	YPM ICH 020469	01-45	15.0
Gobionotothen gibberifrons	YPM ICH 024029	20745	19.0
	YPM ICH 024029	20746	16.0
Gymnodraco acuticeps	YPM ICH 022337	15454	24.0
	YPM ICH 022337	15449	25.5
Champsocephalus gunnari	YPM ICH 016478	07959	31.0
	YPM ICH 016478	07958	23.0