Supporting Information

Amemiya et al. 10.1073/pnas.0914312107

SI Materials and Methods

Library Construction, Screening, and Sequencing. High molecular weight genomic DNA was isolated from frozen heart tissue of the Indonesian coelacanth *L. menadoensis* (a gift from Mark Erdmann and Roy Caldwell, University of California, Berkeley). Two BAC genomic DNA libraries were constructed: the first was a pooled library and the second a previously described arrayed library (Danke J, et al. [2004] Genome resource for the Indonesian coelacanth, *Latimeria menadoensis. J Exp Zoolog A Comp Exp Biol* 301:228–234.). For the former, genomic DNA was cloned into the pBACe3.6 cloning vector and transformed into *Escherichia coli* DH10B cells. Transformants were then collected into 188 pools averaging 700 clones each. Genomic clones were obtained in a series of three steps. First, a genomic PCR survey of *Hox* sequences was performed via PCR amplification and sequencing of a portion of the homeobox using the universal Hox degenerate primer set ELEKEF and WFQNRR (primers 334 and 335; (Table S1), capable of amplifying the homeoboxes in *Hox* paralog groups PG1 through PG10. Second, the homeobox primers plus additional PG-specific primers (Table S1) were used in the isolation and identification of BAC clones from the BAC clone pools. Third, the arrayed library was screened using hybridization of PCR generated probes from the clone sets obtained in the PCR screens of the pooled library. The average insert size in the arrayed library is 170 kb, facilitating the isolation of complete HOX clusters. A minimal set of clones spanning the HOX clusters was then sent to the Stanford Human Genome Center (Palo Alto, CA) for complete DNA sequencing (Noonan JP, Grimwood J, Schmutz J, Dickson M, Myers RM (2004) Gene conversion and the evolution of protocadherin gene cluster diversity. *Genome Res* 14:354–366). Sequencing of BAC ends and PCR products was performed by the Benaroya Research Institute sequencing facility using the Prism DNA Sequencing Kit and the 3100 Genetic Analyzer (ABI).

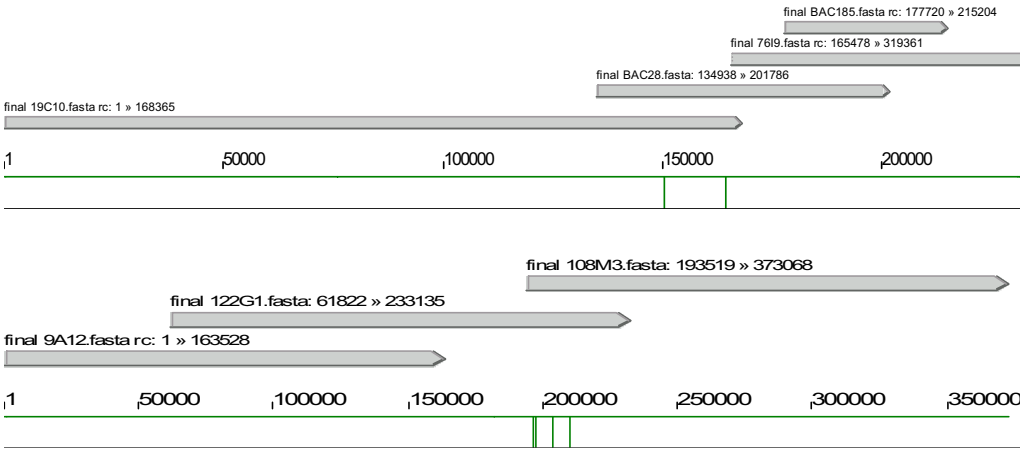
BAC Contig Assembly. Details of the HOX cluster BAC contig assembly for each respective cluster and description of the clone ordering for the final sequence analysis. The *Latimeria menadoensis* HOX contigs were assembled as follows.

A cluster: The coelacanth HOXA cluster sequence was assembled from sequenced BAC clones 19C10 and 76I9 from the arrayed BAC library. Clones 28 and 185 from the pooled BAC library were also sequenced. The final sequence was assembled using the complete sequence of clone 19C10 (base 1–168,364) and sequence from clone 76I9 beginning at base 2887 through base 153,884. Total length of the sequenced HOXA contig is 319,360 bases (GenBank FJ497005).

B cluster: The coelacanth HoxB cluster sequence was assembled from sequenced BAC clones 9A12, 122G1,and 108M3 from the arrayed BAC library. A contig was assembled using sequence from clone 9A12 from base 1 through base 61,821; the complete sequence of clone 122G1 (base 1–171,292) and sequence from clone 108M3 from base 39,616 through base 179,547. Total length of the sequenced HOXB contig is 373,046 bases (GenBank accession no. FJ497006).

C cluster: The coelacanth HOXC cluster sequence was assembled from sequenced BAC clones 181G6, 36M24,140H19, and 240M4 from the arrayed BAC library. A contig was assembled using sequence from clone 181G6 from base 1 through base 22,838; the complete sequence of clone 36M24 (base 1–178,466); sequence from clone 140H19 from base 1,282 through base 187,392; and sequence from clone 240M4 from base 163,213 through 179,105. Total length of the sequenced HOXC contig is 403,307 bases (GenBank accession no. FJ497007).

D cluster: The coelacanth HoxD cluster sequence was assembled from sequenced BAC clones 199G23,44I10,188D21, and 59J7 from the arrayed BAC library. A contig was assembled using sequence from clone 199G23 from base 1 through base 76,339; the complete sequence of clone 44I10 (base 1–160,742); the complete sequence of clone 118D21 (base 1 through base 146,465); and sequence from clone 59J7 from base 27,841 through base 161,339. Clone EVX3 from a coelacanth pooled BAC library was sequenced and confirmed that clones 44I10 and 118D21 are immediately juxtaposed at a single EcoRI restriction site in the genome. Thus clone EVX3 overlaps the ends of clones 44I10 and 118D21 forming a contiguous sequence, although sequence from clone EVX3 was not used in the final contig assembly. Total length of the sequenced HOXD contig is 517,039 bases (GenBank accession no. FJ497008).



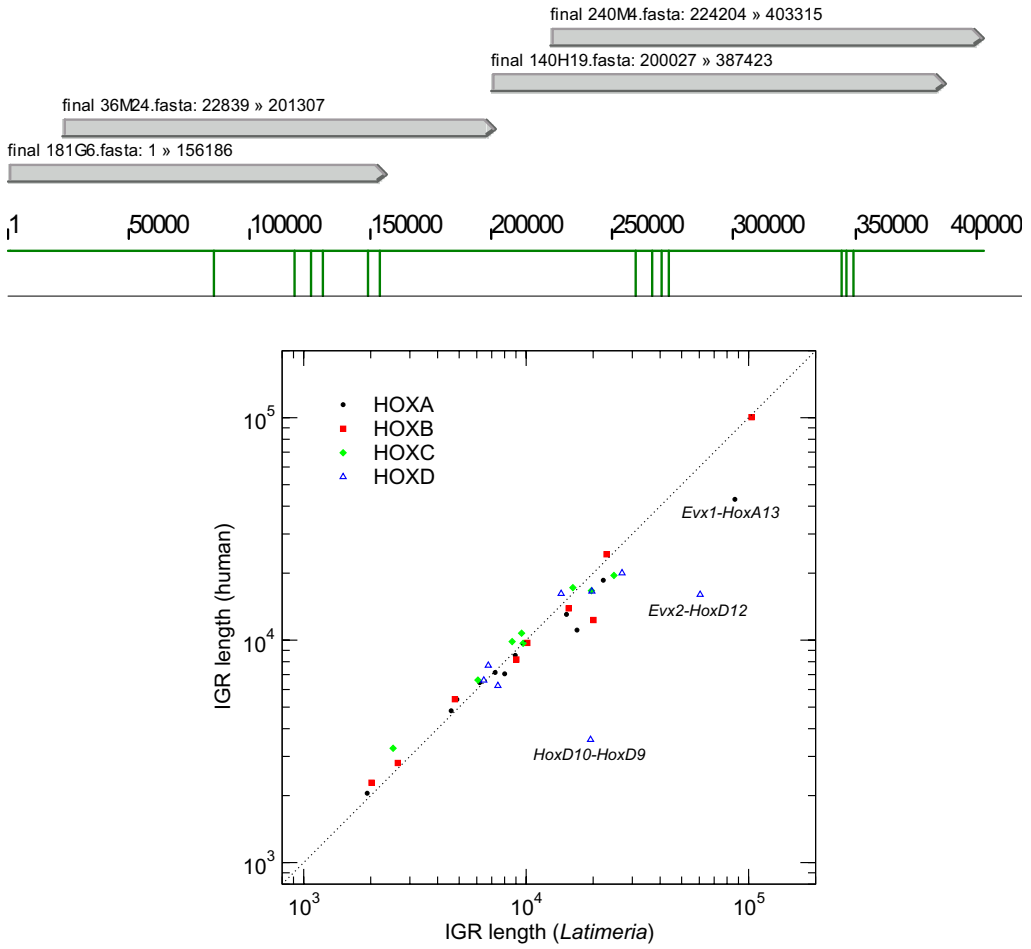


Fig. S1. Comparison of IGR lengths. Intergenic distances were computed as genomic distance from the last nucleotide of the 5′ coding sequence to the nucleotide before the start codon of the 3′ gene when genes are in the same orientation. For the distance between *Evx* and *Hox12* or *Hox13*, respectively, the distance between the start codons was used. With the exception of *Evx1-HoxA13*, *Exv2-HoxD12*, and *HoxD10-HoxD9* there is very little difference between the IGR lengths in the human and coelacanth HOX clusters (see Fig. 2 in the main text and Fig. S2).

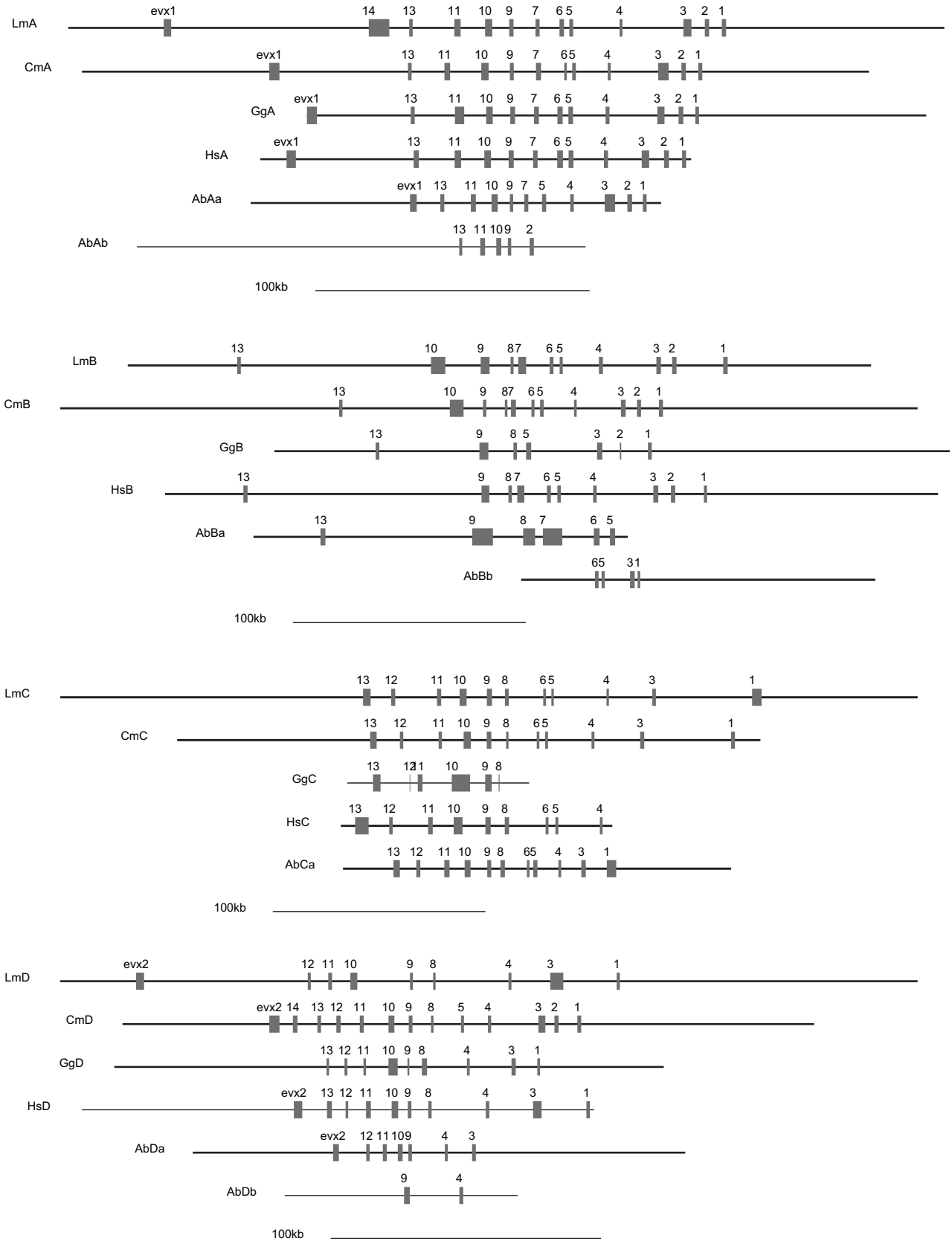


Fig. S2. Scale maps of the four HOX clusters of coelacanth (*Lm*), elephant shark (*Cm*), chicken (*Gg*), human (*Hs*), and the cichlid *A. burtoni* (*Ab*).

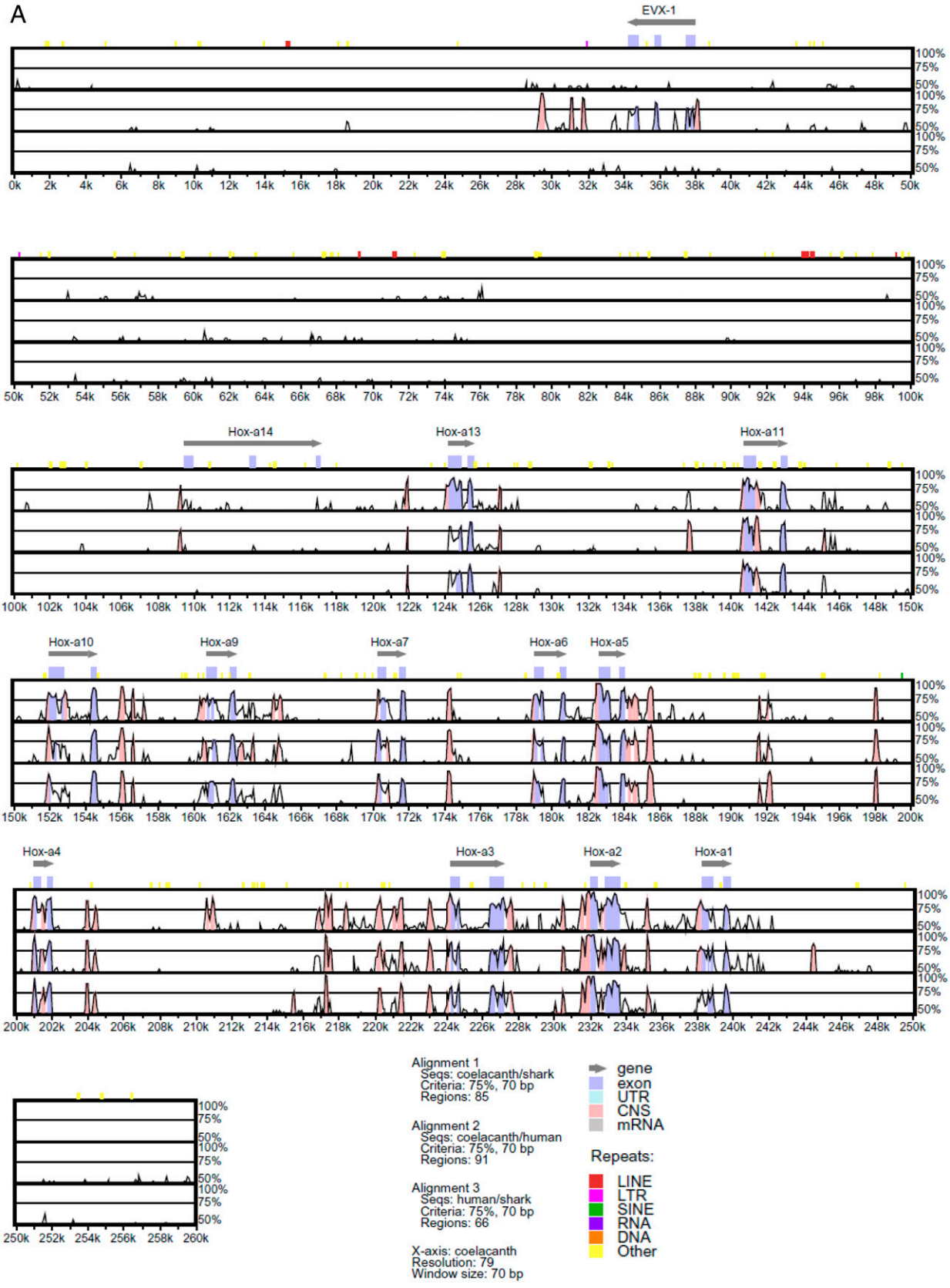


Fig. S3. (Continued)

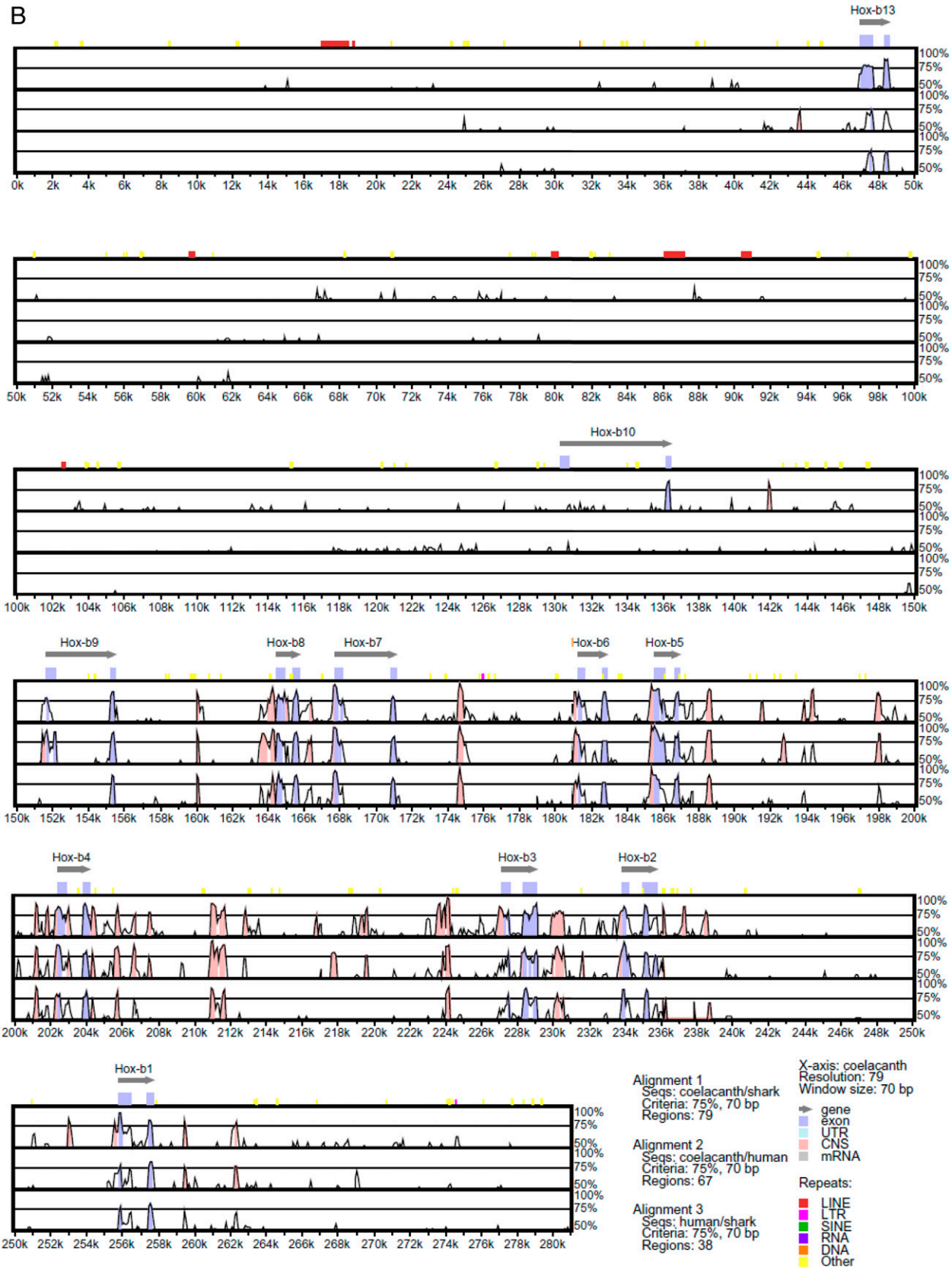


Fig. S3. (Continued)

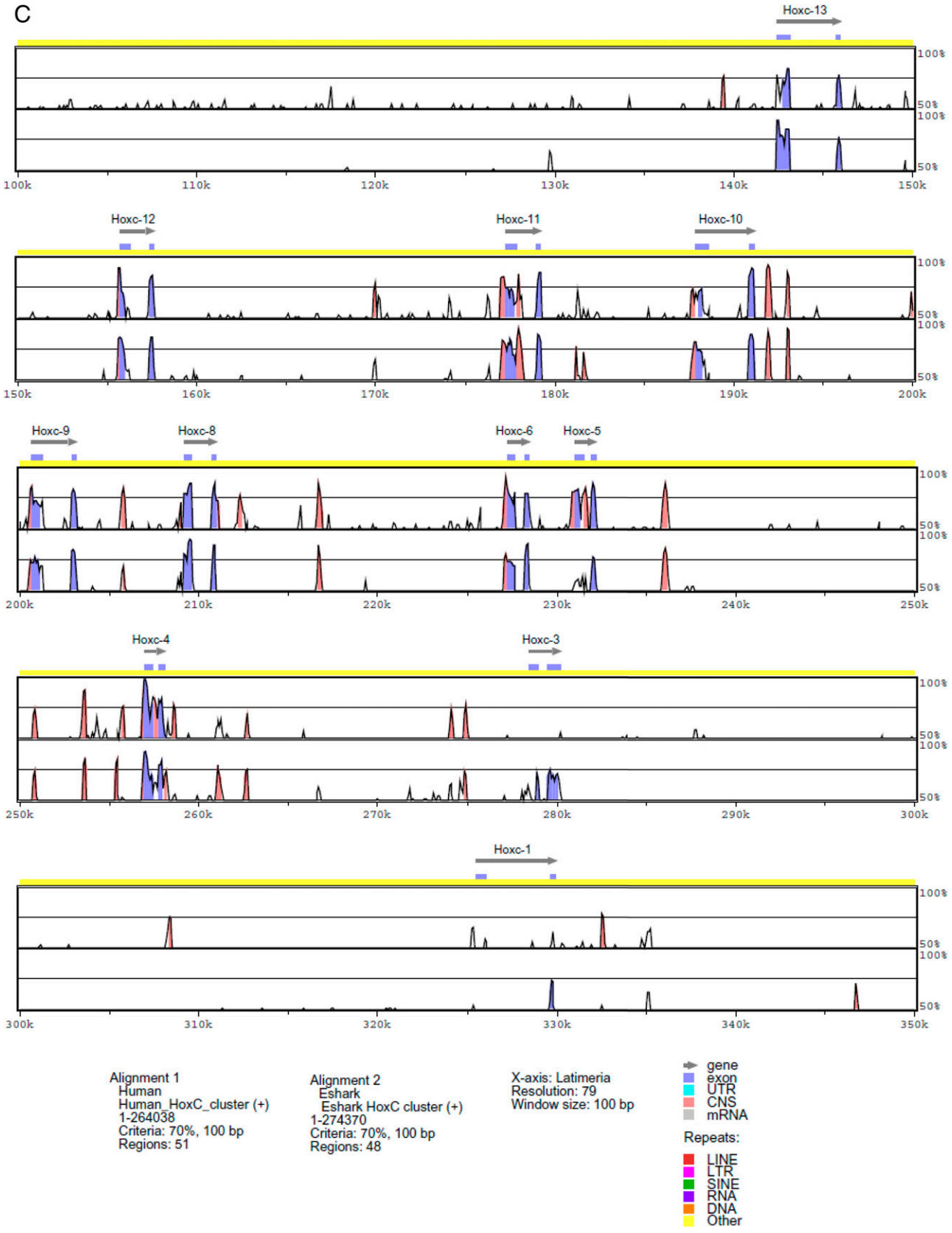


Fig. S3. (Continued)

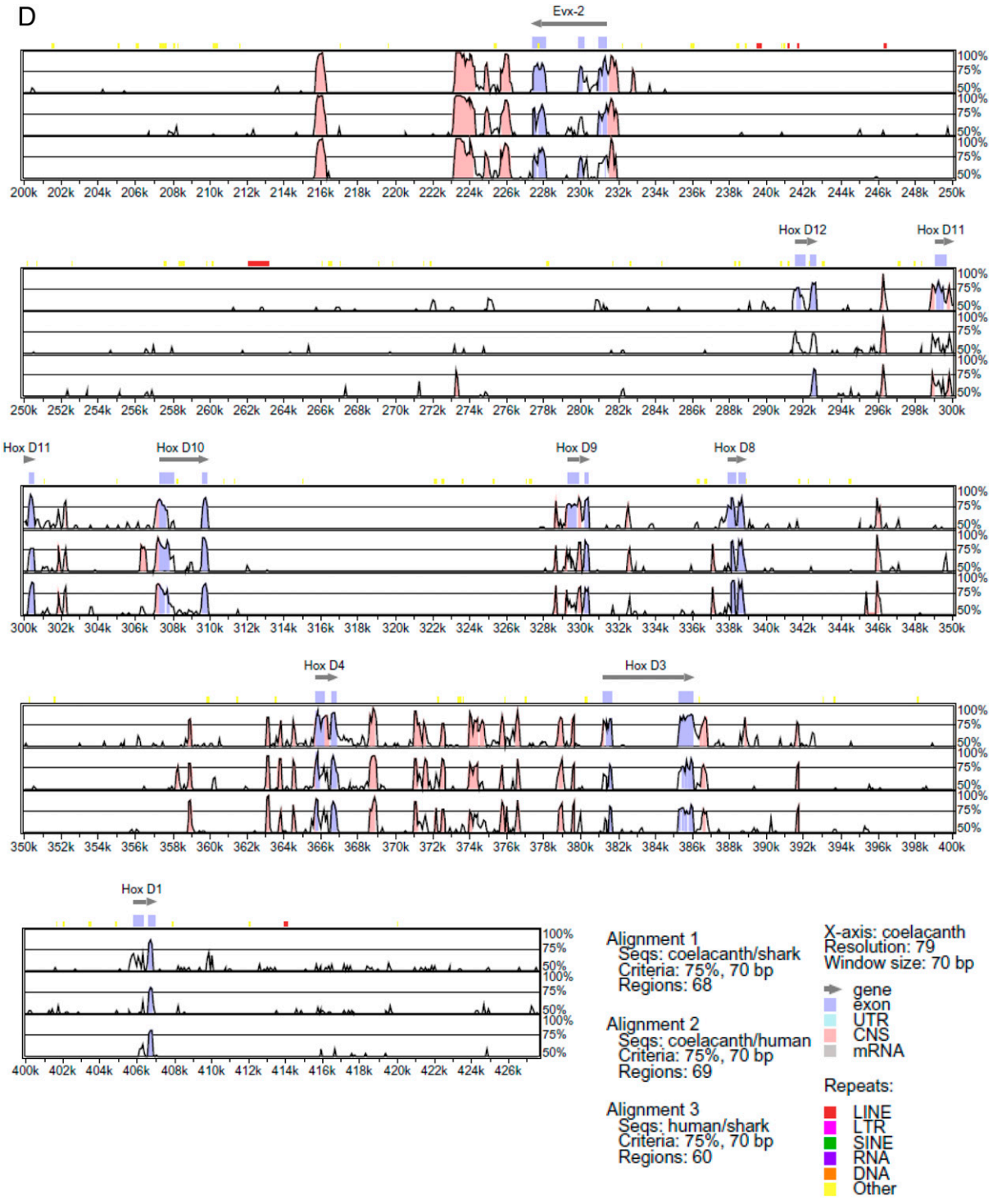


Fig. S3. (Continued)

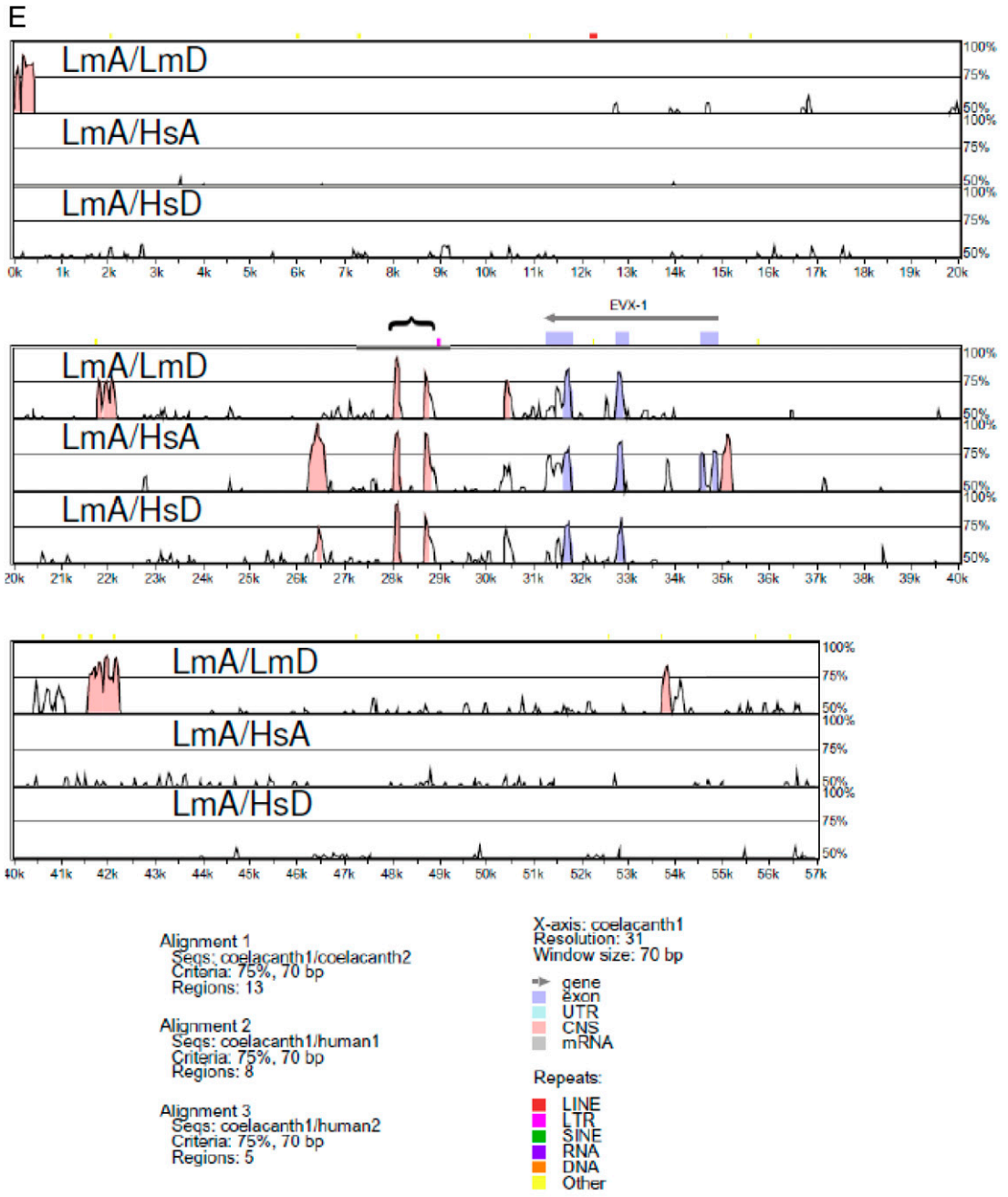


Fig. S3. Representative VISTA plots of HOX clusters. (*A*) HOXA cluster; (*B*) HOXB cluster; (*C*) HOXC cluster; (*D*) HOXD cluster; (*E*) the upstream regions of the HOXA and HOXD clusters. The alignments were constructed using the multiple VISTA server at the Lawrence Berkeley National Laboratory (http://genome.lbl.gov/vista/mvista/submit.shtml). All alignments in were done with the respective coelacanth HOX cluster sequence as the reference. Coding sequences are indicated by blue boxes with an arrow above; pink peaks denote non-coding conserved stretches that are potential *cis*-regulatory elements. For *E*, comparisons are shown for just the upstream *Evx* regions, which are highly conserved within and among the HOXA and HOXD clusters. *Top* lane: LmHOXA/LmHOXD; *Middle* lane: LmHOXA/HsHOXA; *Bottom* lane: LmHOXA/HsHOXD. The brace marks a bipartite neuronal enhancer previously demonstrated to be a functional enhancer element (1).

1. Suster ML, et al. (2009) A novel conserved *evx1* enhancer links spinal interneuron morphology and cis-regulation from fish to mammals. *Dev Biol* 325:422–433.

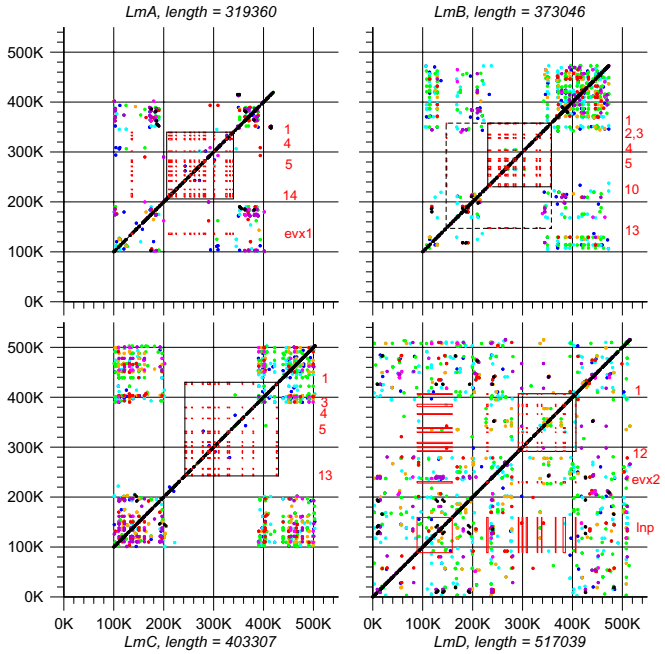


Fig. S4. Dot plots of repetitive elements created by comparing the four coelacanth HOX clusters against themselves using BlastN. The BlastN hits are shown color-coded by their *E*-value: black, 0; violet, 10^{-70}; magenta, 10^{-50}; red, 10^{-30}; orange, 10^{-20}; green, 10^{-10}; cyan, 1; blue, 10. Regions of repetitive sequence can be clearly seen outside the HOX clusters and between *evx* and the most posterior *Hox* gene. Red rectangles indicate the pairs of coding regions; BlastN hits within *Hox* genes correspond to the homeobox sequences. In the HOXD cluster, many blast hits lie inside the lnp gene (large red box at lower left), indicating that the introns of lnp also contain a large amount of repetitive sequence.

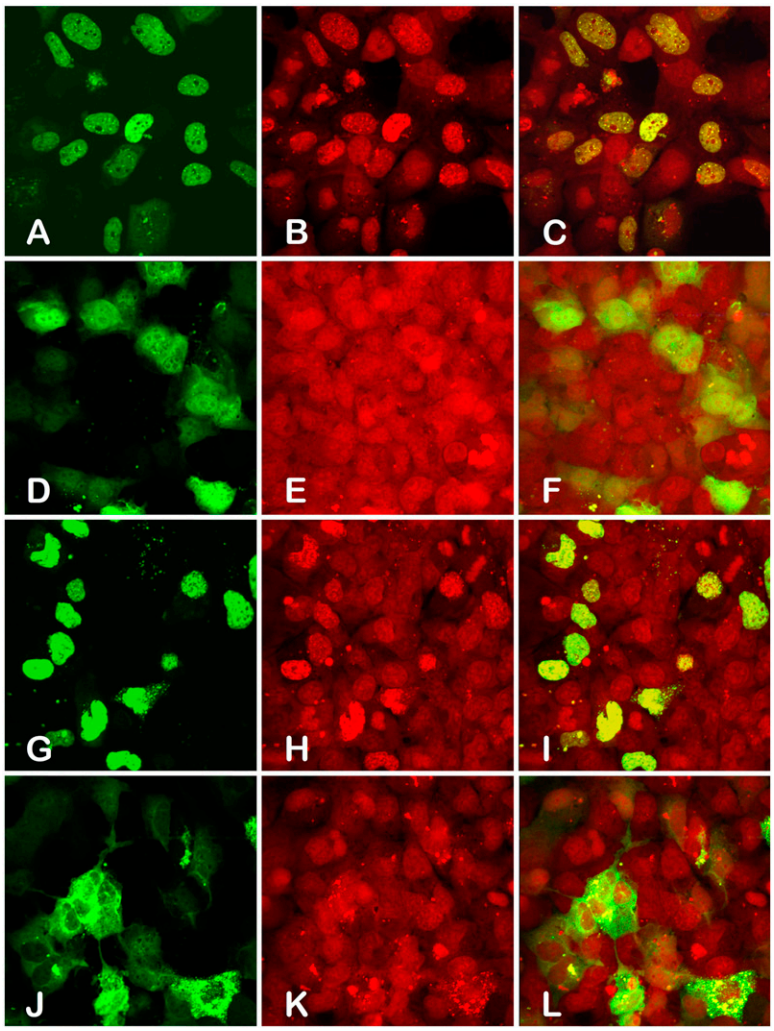


Fig. S5. Determination of potential functionality of coelacanth *HoxA14* via mammalian cell transfection assay using fusion constructs of *Hox* and *GFP*. First column, GFP; second column, DNA stain (TOTO-3-iodide; Molecular Probes); third column, superimposed images. First row (*A*–C): fusion of coelacanth *HoxA14* and *GFP* showing that expression is localized to the nucleus as predicted for a homeodomain-containing transcription factor. Second row (*D*–*F*): nonspecific expression of GFP only (empty vector control). Third row (*G*–*I*): fusion construct of *HoxA11* and *GFP* showing nuclear localization. Fourth row (*J*–*L*): *HoxA11-GFP* fusion construct in which the *HoxA11* has a large deletion in the homeodomain and hence cannot import the GFP into the nucleus. The coelacanth *HoxA14* results suggest that the *HoxA14* gene is potentially functional.

Table S1. List of primers used in this study

CTA ID	Oligo name	Date	Sequence	Notes
334	ELEKEF	1/18/2001	GARYTIGARAARGARTTY	Degenerate homeobox PCR primer; used in HB clone screens
335	WFQNRR	1/18/2001	ICKICKRTTYTGRAACCA	Degenerate homeobox PCR primer; used in HB clone screens.
336	CH11U	3/27/2001	CGTTTCGGGTCCCGATTTCTCCAG	Coelacanth Hoxa11 exon 1 PCR primer; Used in Hoxa11 screens
337	CH11D	3/27/2001	TGCCGGAAGAAGACTCTGGGCTACTGC	Coelacanth Hoxa11 exon 1 PCR primer; used in Hoxa11 screens (Chi-Hua Chu)
379	SP62	11/13/2001	TGTTCATGTTCATGTCTCC	Vector SP6 BAC end sequencing primer
380	T72	11/13/2001	TACGAAGTTATCTAGTAGAC	Vector T7 BAC end sequencing primer
454	A13F1	4/26/2002	CTYCATYCCCGCTGGATYGA	Degenerate Hoxa13 PCR primer
455	A13R1	4/26/2002	CTTTKACYCKYCTGTTYTGRAACC	Degenerate Hoxa13 PCR primer
456	28T7U	4/26/2002	GTCTGGTATAAGCAGTTCGAG	Clone LmHoxA1(BAC28) PCR primer
457	28T7D	4/26/2002	GAGCCGATAGTGTACCCTTGG	Clone LmHoxA1(BAC28) PCR primer
458	VKIWFQNRR	4/26/2002	ICKICKRTTYTGRAACCADATYTTIAC	Degenerate homeobox PCR primer
459	KRARTA	4/26/2002	AARMGIGCIMGIACIGCI	Degenerate homeobox PCR primer
460	A5U	4/26/2002	CATCAGGCAGGATTTACGAC	Hoxa5 PCR primer; used in Hoxa5 screens
461	A5D	4/26/2002	CTCACCGAGGTATGATCTCC	Hoxa5 PCR primer; used in Hoxa5 screens
462	A7U	4/26/2002	AGGACGCTTCAGTGTCGG	Hoxa7 PCR primer
463	A7D	4/26/2002	CTGAAGTGGGATGAGTAC	Hoxa7 PCR primer
464	PCEE	4/26/2002	CCNAARTTYCCNCCNTGYGARG	Degenerate Hox4 PCR primer; used in Hox4 clone screens
465	YPWMR	4/26/2002	TGNACYTTYTTCATCCANGGRTA	Degenerate Hox4 PCR primer; used in Hox4 clone screens
466	SFO	4/26/2002	CGAAAGAAGGCGNTGTCCNTACAC	Degenerate Hox4 PCR primer
467	EVXU	4/26/2002	CARAAYMGNMGNATGAARGAYAA	Degenerate EVX PCR primer; used in EVX clone screens
468	EVXD	4/26/2002	GTRTGNCATCATRTANGTRTARAA	Degenerate EVX PCR primer; used in EVX clone screens
504	C1SP6U	10/11/2002	ATGGTTTTCAATTGAGCTCC	Clone C1 PCR primer
505	C1SP6D	10/11/2002	GTGGTCTGATGCGAAATCATG	Clone C1 PCR primer
506	185T7U	10/11/2002	ACATACTAGATACTGAATTACATG	Clone 185 PCR primer
507	185T7D	10/11/2002	GCAACTGAACTTGCATACACTAC	Clone 185 PCR primer
508	A14D	10/11/2002	CTATTATGTATCAGAACTGCG	Hoxa14 PCR primer
509	A14U	10/11/2002	TACCTGTCGTTCAGTTAACCCAAG	Hoxa14 PCR primer
510	B1T7U	10/11/2002	GCAAGCGGCGAATTGAGGTG	Clone B1 PCR primer
511	B1T7D	10/11/2002	ATTCCTGCCTTACATTAATC	Clone B1 PCR primer
512	B1SP6U	10/11/2002	TCGCTTAGGGTCCTGTACTG	Clone B1 PCR primer
513	B1SP6D	10/11/2002	ACTATAGACTTACAGCACTTG	Clone B1 PCR primer
514	8ET7U	10/11/2002	ACACGTGCATAGTGCCACTATC	Clone 8E PCR primer
515	8ET7D	10/11/2002	CCAAGTTGCTGGTGAAATGTTGCG	Clone 8E PCR primer
516	8ESP6U	10/11/2002	GTGTAGTTACAGTTTTTGTCATG	Clone 8E PCR primer
517	8ESP6D	10/11/2002	GAGCCAACCTTCTGCCTCCAGTG	Clone 8E PCR primer
518	18HT7D	10/11/2002	GCTGCCATCCTTTCTGCATC	Clone 18H PCR primer
519	18HT7U	10/11/2002	GCTCCTGCACTGGCTGATGGTC	Clone 18H PCR primer
520	18HSP6D	10/11/2002	GGTGAGTGTCGTATTCTGGAG	Clone 18H PCR primer
521	18HSP6U	10/11/2002	CAATCTCCCAGCAGAAGTCC	Clone 18H PCR primer
522	20CSP6U	10/11/2002	TGGATTGCACACTGGACCTGG	Clone 20C PCR primer
523	20CSP6D	10/11/2002	CTATAGCCAAAAGTAGGTATGGC	Clone 20C PCR primer
524	7BSP6U	10/11/2002	GCATTCGTGAATCATTAGCTTCAG	Clone 7B PCR primer
525	7BSP6D	10/11/2002	ACCTCAGCTGGTAGCAATCG	Clone 7B PCR primer
526	C1T7U	10/11/2002	AACAGCACCAGGGTATGC	Clone C1 PCR primer
527	C1T7D	10/11/2002	CACTACAAGGTGGATTTGGCTTG	Clone C1 PCR primer
529	KRRPYSK	10/11/2002	AARMGNMGNCCNTAYWSNAAR	Degenerate posterior group PCR primer
564	COELA1	1/20/2003	GCAGTGCTTATGGAGCTGCTGC	HoxD regulatory element PCR primer. Used in HoxD screens
565	COELA2	1/20/2003	GGATAATGATGTCAAAGGCAGAAAC	HoxD regulatory element PCR primer. Used in HoxD screens
566	COELB1	1/20/2003	GAGCACACAGTTAGCCTAGGTCC	HoxD regulatory element PCR primer. Used in HoxD screens

Table S1. Cont.

CTA ID	Oligo name	Date	Sequence	Notes
567	COELB2	1/20/2003	GAAGCAGTCAGCAGCAAATCAAG	HoxD regulatory element PCR primer. Used in HoxD screens
582	ELEREY	1/20/2003	GARYTNGARMGNGARTAY	Degenerate posterior group PCR primer
583	KKRVPY	1/20/2003	AARAARMGNGTNCCNTAY	Degenerate posterior group PCR primer
584	D1TD	1/20/2003	GGCAACTGAACCTACTTAGCAACCAATG	Clone D1 PCR primer
585	D1TU	1/20/2003	TTTAGCCTTTAACAGGACCACAGTAG	Clone D1 PCR primer
586	D1SD	1/20/2003	TGGTCCAACTTGCGGATGTC	Clone D1 PCR primer
587	D1SU	1/20/2003	GTTCTGGCCAAGCTCATGGAG	Clone D1 PCR primer
598	pCC1BACfp	1/29/2003	GGATGTGCTGCAAGGCGATTAAGTTGG	Vector sequencing primer
599	pCC1BACrp	1/29/2003	CTCGTATGTTGTGTGGAATTGTGAGC	Vector sequencing primer
645	140H19fu	6/2/2003	GTAAGCATATCCTTCAAGCAG	Clone 140H19 PCR primer
646	140H19fd	6/2/2003	AGTCGATGGGACAGAGGTATG	Clone 140H19 PCR primer
647	140H19ru	6/2/2003	ATAGCCTCTACCAATAGGCT	Clone 140H19 PCR primer
648	140H19rd	6/2/2003	TAGTAGTGGTGAACCAGGCT	Clone 140H19 PCR primer
649	122G1fu	6/2/2003	GTATTCTACTTGCTGTTCAC	Clone 122G1 PCR primer
650	122G1fd	6/2/2003	TGCTACCACCGCTTCCACCG	Clone 122G1 PCR primer
651	122G1ru	6/2/2003	GACTAGAGTGAGTGGGCGAT	Clone 122G1 PCR primer
652	122G1rd	6/2/2003	TCTGTCCGCTGTAAAAGTAG	Clone 122G1 PCR primer
653	118D21fu	6/2/2003	CTTCCTTGAATCCTGGATAC	Clone 118D21 PCR primer
654	118D21fd	6/2/2003	TGTTCGTTACTTATATCTGC	Clone 118D21 PCR primer
655	118D21ru	6/2/2003	GGCTGTTCAGTGGCCCATGT	Clone 118D21 PCR primer
656	118D21rd	6/2/2003	GCACCAGAGACTGAAGTCCTC	Clone 118D21 PCR primer
657	44I10fu	6/2/2003	CAGAGGTGATGCTCTCAGCTG	Clone 44I10 PCR primer
658	44I10fd	6/2/2003	ATACCACTGCTGTCCAGTTG	Clone 44I10 PCR primer
659	44I10ru	6/2/2003	TTTAAGTCTATGGGCTGTTG	Clone 44I10 PCR primer
660	44I10rd	6/2/2003	CTGTGACCTTTGGTACTTTG	Clone 44I10 PCR primer
661	6B14rpU	6/2/2003	TACTTGAAATAGACAGCGCTC	Clone 6B14 PCR primer
662	6B14rpD	6/2/2003	CCTTGGTATAGGTCATAGAACAC	Clone 6B14 PCR primer
663	6B14fpU	6/2/2003	CACTCAAGGATTGCATGACCATG	Clone 6B14 PCR primer
664	6B14fpD	6/2/2003	ATAGAGGCCAGTCGACTGATC	Clone 6B14 PCR primer
665	59J7rpU	6/2/2003	TGCTCCACACCAACTACTGAAATCCG	Clone 59J7 PCR primer
666	59J7rpD	6/2/2003	GTGTTTACTCACTTTTATGGATCTTTG	Clone 59J7 PCR primer
667	59J7fpU	6/2/2003	GATCTTCGGCAGCTGGACAGG	Clone 59J7 PCR primer
668	59J7fpD	6/2/2003	CGAACAGCTGTGATGCCTCAG	Clone 59J7 PCR primer
791	Pst Lm A14 Ex1	5/10/2004	AACTGCAGATGATCTTCCCTGGAAATTGG	Forward (5′; N-terminal) primer for PCR amplification of *L. menadoensis* Hox A14 exon 1. Includes 2 "spacer" nts (AA) and PstI restriction palindrome at the 5′-end
792	3′ Lm A14 Ex1	5/10/2004	ATGAAAACTCAAAGCCTAAAGAGTGTGGGGAATTAC	Reverse primer for PCR amplification of *L. menadoensis* Hox A14 exon 1. Includes 15 nts from the 5′-end of exon 2 at the end
793	5′ Lm A14 Ex2	5/10/2004	CCCCACACTCTTTAGGCTTTGAGTTTTCATTCCTCTTG	Forward primer for PCR amplification of *L. menadoensis* Hox A14 exon 2. Includes 15 nts from the 3′ end of exon 1 at the beginning
794	3′ Lm A14 Ex2	5/10/2004	GAACCAGATTTTTACCTGTCGTTCAGTTAACCCAAG	Reverse primer for PCR amplification of *L. menadoensis* Hox A14 exon 2. Includes 15 nts from the 5′-end of exon 3 at the end
795	5′ Lm A14 Ex3	5/10/2004	TTAACTGAACGACAGGTAAAAATCTGGTTCCAAAACCAACG	Forward primer for PCR amplification of *L. menadoensis* Hox A14 exon 3. Includes 15 nts from the 3′-end of exon 2 at the beginning
796	Xba Lm A14 Ex3	5/10/2004	GCTCTAGATTACGTTTGTCCAGCTCCAATAG	Reverse (3′; C-terminal) primer for PCR amplification of L. menadoensis Hox A14 exon 3. Includes 2 "spacer" nts (GC) and XbaI restriction palindrome at the end

Table S2. Summary of Tajima RRTs for exon-1 protein sequences

Gene/outgroup	Ingroup1	Ingroup2	*P* value
Hoxa1			
Hf	Hs	Lm	0.876
Hf	Lm	**DrA1a**	0**
Hf	Hs	**DrA1a**	0**
Hoxa2			
Hf	**Hs**	Lm	0.021*
Hf	Lm	**DrA2b**	0.02*
Hf	Hs	DrA2b	0.796
Hoxa3			
Hf	**Hs**	Lm	0.02*
Hf	Lm	**DrA3a**	0**
Hf	Hs	**DrA3a**	0**
Hoxa4			
Hf	**Hs**	Lm	0**
Hf	Lm	**DrA4a**	0.002**
Hf	**Hs**	DrA4a	0.013*
Hoxa5			
Hf	**Hs**	Lm	0.04*
Hf	Lm	**DrA5a**	0**
Hf	Hs	**DrA5a**	0.004**
Hoxa6			
Hf	Hs	Lm	0.866
Hoxa7			
Hf	Hs	Lm	0.67
Hoxa9			
Hf	Hs	Lm	0.645
Hf	Lm	**DrA9a**	0**
Hf	Lm	**DrA9b**	0**
Hf	Hs	**DrA9a**	0**
Hf	Hs	**DrA9b**	0**
Hoxa10			
Hf	Hs	Lm	0.21
Hf	Lm	**DrA10b**	0.001**
Hf	Hs	**DrA10b**	0.024*
Hoxa11			
Hf	Hs	Lm	0.072
Hf	Lm	**DrA11a**	0.001**
Hf	Lm	**DrA11b**	0.001**
Hf	Hs	DrA11a	0.069
Hf	Hs	DrA11b	0.114
Hoxa13			
Hf	**Hs**	Lm	0.003**
Hf	Lm	**DrA13a**	0**
Hf	Lm	**DrA13b**	0**
Hf	Hs	DrA13a	0.086
Hf	Hs	DrA13b	0.105
Hoxb1			
Hf	**Hs**	Lm	0**
Hf	Lm	**DrB1a**	0**
Hf	Lm	**DrB1b**	0**
Hf	Hs	DrB1a	0.873
Hf	Hs	**DrB1b**	0.01**
Hoxb2			
Hf	**Hs**	Lm	0.034*
Hf	Lm	**DrB2a**	0.035*
Hf	Hs	DrB2a	0.763
Hoxb3			
Hf	Hs	Lm	0.002**
Hf	Lm	DrB3a	0.683
Hf	Hs	DrB3a	0.013*
Hoxb4			
Hf	**Hs**	Lm	0**
Hf	Lm	**DrB4a**	0.05*

Table S2. Cont.

Gene/outgroup	Ingroup1	Ingroup2	*P* value
Hf	Hs	DrB4a	0.068
Hoxb5			
Hf	Hs	Lm	0.134
Hf	Lm	**DrB5a**	0.019*
Hf	Lm	**DrB5b**	0**
Hf	Hs	DrB5a	0.273
Hf	Hs	**DrB5b**	0.002**
Hoxb6			
Hf	**Hs**	Lm	0.041*
Hf	Lm	DrB6a	0.102
Hf	Lm	**DrB6b**	0.012*
Hf	Hs	DrB6a	0.577
Hf	Hs	DrB6b	0.739
Hoxb7			
Hf	**Hs**	Lm	0.003**
Hf	Lm	**DrB7a**	0.001**
Hf	Hs	DrB7a	0.398
Hoxb8			
Hf	Hs	Lm	0.564
Hf	Lm	DrB8a	0.275
Hf	Lm	**DrB8b**	0**
Hf	Hs	DrB8a	0.162
Hf	Hs	**DrB8b**	0**
Hoxb9			
Hf	Hs	Lm	0.467
Hf	Lm	DrB9a	0.433
Hf	Hs	DrB9a	0.178
Hoxb13			
Hf	**Hs**	Lm	0**
Hf	Lm	Dr13a	0.0771
Hf	**Hs**	Dr13a	0.00022**
Hoxc4			
Eshark	Lm	Hs	0.251
Eshark	Lm	DrC4a	0.257
Eshark	Hs	DrC4a	0.835
Hoxc5			
Eshark	Lm	Hs	0.285
Eshark	Lm	DrC5a	0.223
Eshark	Hs	DrC5a	0.622
Hoxc6			
Eshark	Lm	Hs	0.346
Eshark	Lm	**DrC6a**	0.006**
Eshark	Lm	**DrC6b**	0.004**
Eshark	Hs	Drc6a	0.061
Eshark	Hs	**Drc6b**	0.033*
Hoxc8			
Eshark	Lm	Hs	0.248
Eshark	Lm	**Drc8a**	0.008**
Eshark	Hs	Drc8a	0.071
Hoxc9			
Eshark	Lm	**Hs**	0.004**
Eshark	Lm	**Drc9a**	0.002**
Eshark	Hs	Drc9a	0.631
Hoxc10			
Eshark	Lm	**Hs**	0.015*
Eshark	Lm	**Drc10a**	0.0001**
Eshark	Hs	Drc10a	0.118
Hoxc11			
Eshark	Lm	**Hs**	0.00001**
Eshark	Lm	Drc11a	0.071
Eshark	Lm	**Drc11b**	0.00002**
Eshark	**Hs**	Drc11a	0.004**

Table S2. Cont.

Gene/outgroup	Ingroup1	Ingroup2	*P* value
Eshark	Hs	Drc11b	0.884
Hoxc12			
Eshark	Lm	**Hs**	0.00006**
Eshark	Lm	**Drc12a**	0.007**
Eshark	Lm	**Drc12b**	0**
Eshark	Hs	Drc12a	0.105
Eshark	Hs	**Drc12b**	0.005**
Hoxc13			
Eshark	Lm	**Hs**	0.005**
Eshark	Lm	Drc13a	0.248
Eshark	Lm	**Drc13b**	0.00006**
Eshark	**Hs**	Drc13a	0.039*
Eshark	Hs	Drc13b	0.258
Hoxd1			
Hf	**Hs**	Lm	0.033*
Hoxd3			
Hf	**Hs**	Lm	0.007**
Hf	Lm	Dr	0.182
Hf	Hs	Dr	0.166
Hoxd4			
Hf	**Hs**	Lm	0.001**
Hf	Lm	Dr	0.683
Hf	**Hs**	Dr	0.001**
Hoxd8			
Hf	Hs	Lm	0.746
Hoxd9			
Hf	**Hs**	Lm	0.003**
Hf	Lm	**Dr**	0.017*
Hf	Hs	Dr	0.26
Hoxd10			
Hf	**Lm**	Hs	0.017*
Hf	Lm	Dr	0.354
Hf	Hs	**Dr**	0**
Hoxd11			
Hf	**Hs**	Lm	0**
Hf	Lm	Dr	0.149
Hf	**Hs**	Dr	0.022*
Hoxd12			
Hf	**Hs**	Lm	0**
Hf	Lm	**Dr**	0.007**
Hf	**Hs**	Dr	0.027*

Genes that are evolving significantly faster are shown in boldface. Alignments and trees for the individual RRTs are given in Dataset S1. Hf, *Heterodontus francisci* (horn shark), Hs, *Homo sapiens* (human); Dr, *Danio rerio* (zebrafish); Lm, *Latimeria menadoensis* (coelacanth); Eshark, *Callorhinchus milii* (elephant shark).

Other Supporting Information Files

Dataset S1(PDF)