# Comparative Genomics Reveal Accelerated Evolution in Conserved Pathways during the Diversification of Anole Lizards

## Supplementary Material

Marc Tollis, Elizabeth D. Hutchins, Jessica Stapley, Shawn M. Rupp, Walter L. Eckalbar, Inbar Maayan, Eris Lasku, Carlos R. Infante, Stuart Dennis, Joel A. Robertson, Catherine M. May, Michael R. Crusoe, Eldredge Bermingham, Dale F. DeNardo, S. Tonia Hsieh, Rob J. Kulathinal, W. Owen McMillan, Douglas B. Menke, Stephen C. Pratt, J. Alan Rawls, Oris Sanjur, Jeanne Wilson-Rawls, Melissa A. Wilson Sayres, Rebecca E. Fisher, Kenro Kusumi

## Supplementary Methods 1

## Mitochondrial Genome Assembly, Annotation and Divergence Time Estimation

*Marc Tollis, Kenro Kusumi*

We assembled the mitochondrial genomes of *Anolis frenatus* and *A. auratus* by mapping trimmed genomic reads to a reference iguanian mitochondrial consensus. To create this reference, we aligned the mitochondrial genomes of *A. carolinensis* (GenBank accession number NC_010972), *Polychrus marmoratus* (NC_012839), *Sceloporus occidentalis* (AB079242), and *Iguana iguana* (AJ278511) using MUSCLE (Edgar 2004) and extracted a majority consensus sequence in Geneious version 7 (http://www.geneious.com) (Kearse et al. 2012). To construct the *A. frenatus* and *A. auratus* mitochondrial genome assemblies, Illumina reads for each species were mapped to the iguanian reference using bowtie2 v2.2 (Langmead & Salzberg 2012) and the consensus sequences were extracted using mpileup and bcftools in samtools (Li et al. 2009). For each mitochondrial assembly, protein-coding genes were annotated by the mapping of *A. carolinensis* mitochondrial gene sequences in Geneious.

We obtained reference-guided mitochondrial genome assemblies that were 17,284 bp in length for *A. frenatus* and 17,246 bp for *A. auratus*, which compares to 17,223 bp for *A. carolinensis*. These are within the range of the complete mitochondrial genome sizes for closely related iguanian lizards, including the bush anole, *Polychrus marmoratus* (17,743 bp, GenBank accession number NC_012839) and western fence lizard, *Sceloporus occidentalis (*17,072 bp, AB079242). Of 13 mitochondrial protein-coding genes from *A. carolinensis*, we annotated 12 in *A. frenatus* and 10 in *A. auratus*.

After obtaining the homologous coding sequences from the mitochondrial genomes of seven acrodonts (extant squamates classified in the Agamidae and Chamaeleonidae) and four pleurodonts (extant squamates classified in the Iguanidae) from GenBank (Supplementary Table 6), we reconstructed the mitochondrial genome phylogeny of iguanian lizards (Iguania) and estimated the divergence times between the major iguanian lineages, including the *Anolis* radiation (i.e., the age of the common ancestor of *A. carolinensis* and *A. frenatus*), by applying an internal fossil calibration (Supplemental Fig. 1) using BEAST v1.8 (Drummond et al. 2012). Stationary mixing of the BEAST MCMC was not achieved using codon partitions; therefore, we used each gene as a partition with the HKY + Gamma + I substitution model. We used default settings for the uncorrelated lognormal relaxed clock, and a Yule process tree prior. As in Prates et al. (2015), we calibrated the age of the ancestral node for pleurodonts using the Late Cretaceous fossil taxon *Saichangurvel davidsoni* (Conrad & Norell 2007), and placed a lognormally distributed prior probability offset to 70my with a mean of 2 and a standard deviation of 0.8, in order to account for uncertainty in the fossil record. Two independent BEAST analyses were run for 60,000,000 generations, sampling every 6,000 generations for a total of 10,000 trees. To assess convergence between the two runs, we monitored the effective sample size values and consistency of parameter estimates using Tracer v1.6 (Drummond & Rambaut 2007). Once convergence was confirmed, the log and tree files from the two separate runs were combined using LogCombiner with BEAST in order to obtain the final parameter estimates.

## Supplementary Methods 2

## Enhancer Analysis

*Carlos R. Infante, Marc Tollis, Kenro Kusumi, Douglas B. Menke*

### *Identifying putative enhancers*

H3K27ac ChIP-seq datasets of reads aligned to the mouse genome (mm9) from two replicates each of 16 mouse adult and embryonic tissues were downloaded from the ENCODE project website (Shen et al. 2012). Regions of significant enrichment were determined using MACS2 with the default parameters (Zhang et al. 2008). For each replicate, significantly enriched regions within 1 kb were merged into a single region using BEDTools (Quinlan & Hall 2010). Enriched regions from each tissue replicate were then combined by merging regions with a minimum overlap of 1 bp. Putative enhancers were identified from the merged tissue replicates by excluding enriched regions that overlapped promoters and exons based on the UCSC genome browser Known Gene dataset (Speir et al. 2016).

### *K-means clustering of putative enhancers*

Putative enhancer regions from all 16 mouse tissues were combined by merging regions with a minimum overlap of 1 bp. The R package Rsubread was used to count the number of aligned reads from both replicates that overlapped regions in the combined enhancer list (R Core Team 2016; Liao et al. 2013). Reads per kilobase of transcript per million mapped reads (RPKM) values were calculated from the read counts in R using the edgeR package (Robinson et al. 2010). These RPKM values were then normalized based on the multi-IP normalization output across all tissue datasets calculated by CHANCE (Diaz et al. 2012). These values were then transformed into a matrix with rows as putative enhancer regions and columns as normalized RPKM for each tissue type. For each row, the data were standardized further by subtracting the mean and then dividing by the standard deviation for each value. This matrix was then clustered based on similar H3K27ac signal profiles by kmeans clustering.

### *Conservation of mouse enhancers in Anolis genomes*

Genomic coordinates of putative enhancers with similar enrichment based on kmeans clustering for mouse E14.5 embryonic brain, mouse E14.5 embryonic liver, mouse E14.5 embryonic heart, and mouse E14.5 embryonic limb were used to generate fasta sequence files. These mouse sequences were used as input for lastz alignments to the *A. carolinensis* genome (AnoCar2.0). The parameters used for lastz alignment were "--strand=both --scores=HoxD55.q --gap=400,30 --seed=12of19 --transition --hspthresh=3000 --entropy --ydrop=3400 --gappedthresh=6000 --inner=2000". Putative enhancers with multiple alignments within 2,000 bp in the *A. carolinensis* genome were merged into a single region and all alignments were converted to AnoCar2.0 genomic coordinates. Regions overlapping promoters and exons based on the Ensembl gene annotation (release 81) for AnoCar2.0 were excluded from further analysis. The AnoCar2.0 coordinates for each of the four putative mouse enhancer categories were used to compare the conservation across the four *Anolis* genomes using a multiple genome alignment file and the script maf_interval_alignability.py from the bx-python package (https://github.com/bxlab/bx-python) (Miller et al. 2007). For a given set of genomic coordinates, this script generates an alignability score by comparing the number of bases aligned for each species in a maf file with the total number of sites within the query region.

## Supplementary Methods 3

## Comparative morphometric analysis of *Anolis* osteological preparations

*Elizabeth D. Hutchins, Inbar Maayan, Eris Lasku, Joel A. Robertson, Stephen C. Pratt, Rebecca E. Fisher, Kenro Kusumi*

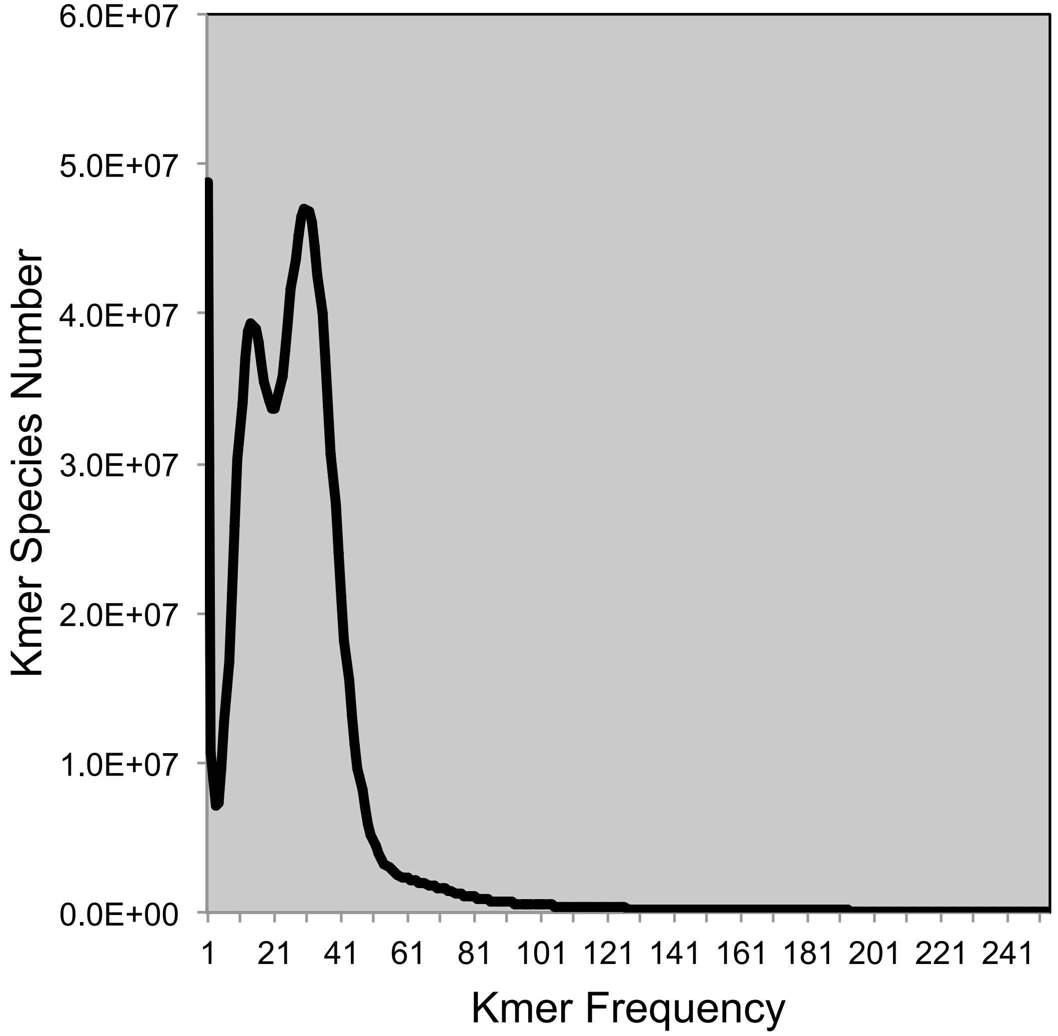
Specimens were weighed using a Mettler Toledo PG5002-S scale (0.01 g accuracy) and snout-vent length (SVL) and tail length were determined using digital calipers (0.01 mm accuracy). Specimens were prepared for osteological analysis using modifications to amphibian protocols (Hanken & Wassersug 1981) including: (1) incubation in 95% ethanol for greater than 5 days, instead of 10% neutral buffered formalin; (2) an Alcian blue concentration of 0.8%; (3) incubation with Alcian Blue carried out at 50°C; (4) specimens cleared over 5 weeks from 1% potassium hydroxide to 100% glycerol (weekly steps of 25%, 50%, 75%, 100% glycerol-1% potassium hydroxide).

Skeletal preparations were photodocumented using either a stereodissecting microscope (Nikon SMZ800 with Coolpix 995 digital camera) or camera (Nikon D60 with DX AF-S nikkor 18-55mm lens). Skeletal elements were quantified relative to a graticule (Lovins) using NIH ImageJ. Appendicular skeletal elements were measured from standardized proximal and distal anatomical landmarks. For the forelimb, the following elements were measured: humerus (head, trochlea), radius (head, distal tip), ulna (olecranon process, distal tip), carpus (proximal border of the radiale and ulnare, proximal border of metacarpals II and III), metacarpal IV (base, head), and cumulative length of digit IV phalanges (base, head). For the hindlimb, the following elements were measured: femur (head, medial condyle), tibia (midpoint of tibial plateau, distal tip), fibula (head, distal tip), tarsus (proximal-most medial point of the proximal tarsal between distal ends of the bones of the crus, distal articular surface with distal tarsals), metatarsal IV (base, head), and cumulative length of digit IV phalanges (base, head). For the manus and pes, digit IV was used, as this is typically the longest digit in anole lizards. Morphometric data were analyzed using one-way ANOVA and Tukey's HSD post hoc in the R statistical package (https://www.r-project.org).

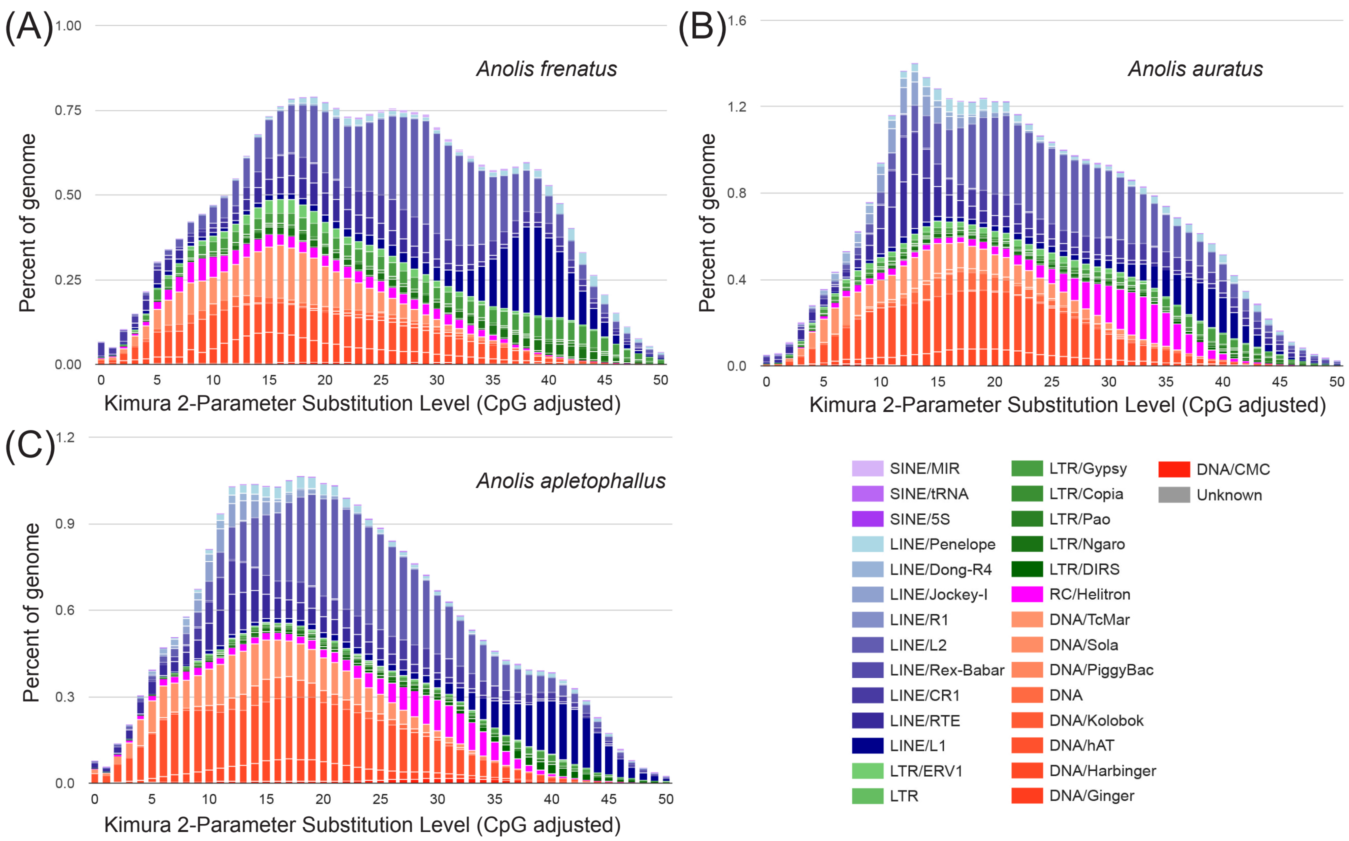
**Supplementary Methods 4. Guide tree used for the multiple whole genome alignment.**

(*Latimeria chalumnae*, ((*Ornithorhnychus anatinus*, (*Monodelphis domestica*, ((*Loxodonta africana*, *Dasypus novemcinctus*), ((*Homo sapiens*, *Mus musculus*), (*Bos taurus*, *Canis lupus familiaris*))))), (((*Chrysemys picta bellii*, *Pelodiscus sinensis*), ((*Alligator mississippiensis* (*Crocodylus porosus*, *Gavialis gangeticus*)), ((*Struthio camelus*), (((*Gallus gallus*, *Meleagris gallopavo*), *Anas platyrhyncos*), (*Melopsittacus undulatus*(*Taenopygia guttata*, *Geospiza fortis*)))))), (*Gekko japonicus*, (((*Python bivittatus, Boa constrictor*), (*Ophiophagus hannah*, *Crotalus mitchellii*)), (*Pogona vitticeps*, (*Anolis frenatus*, (*Anolis carolinensis*, (*Anolis auratus*, *Anolis apletophallus*)))))))))

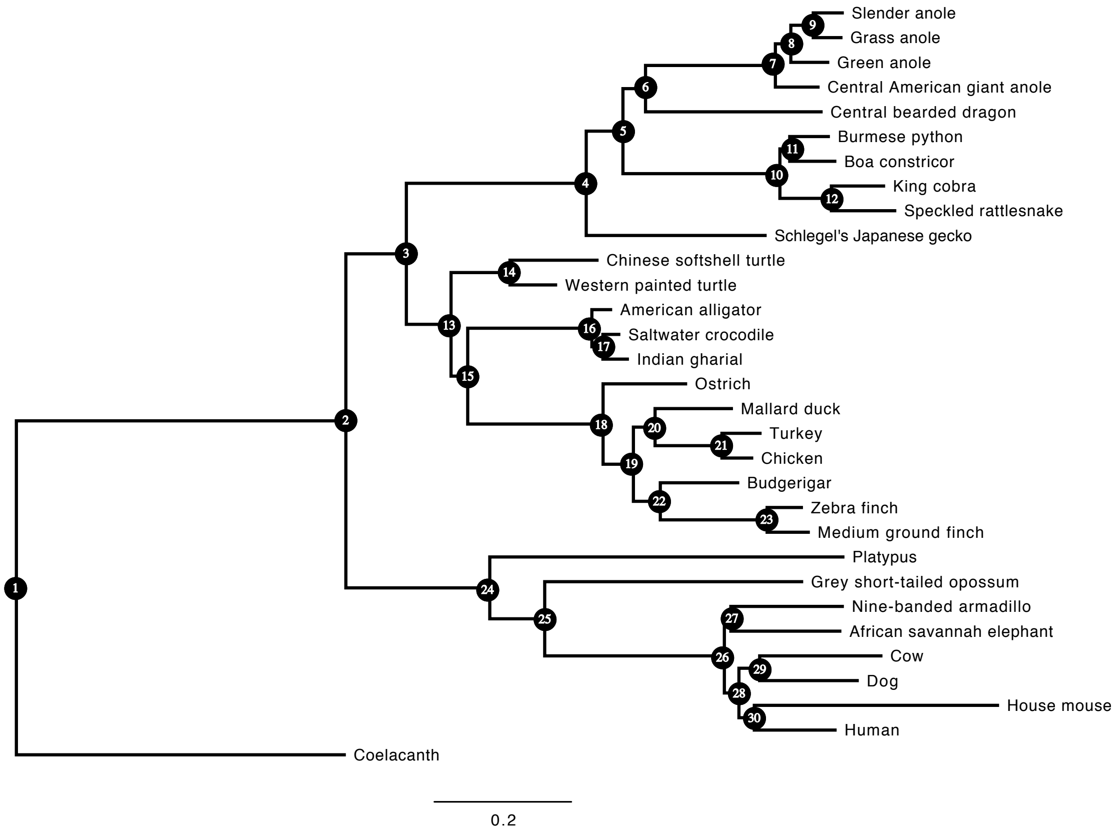
**Supplementary Figure 1. Kmer spectrum for the genome sequence of *Anolis apletophallus*.** Sequencing DNA from short insert libraries yielded 93.67 gigabases of trimmed data with an average read length of 90 bp, which generated 75.07x10E9 19-mer sequences. The solid line shows the frequency distribution of kmer species in the dataset. The two modes are for homozygous (peak height at frequency 29) and heterozygous (peak height at frequency 13) single copy sequences. Heterozygosity is high, which complicates genome assembly.



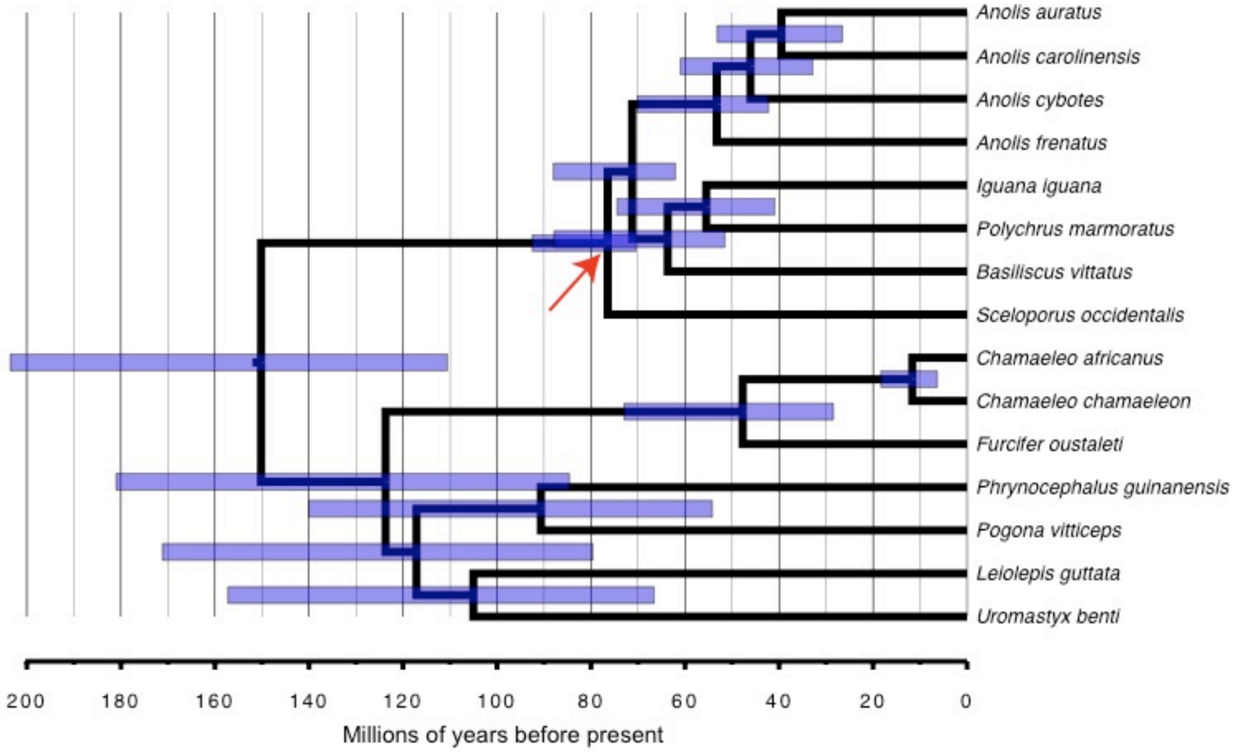
**Supplementary Figure 2. Repeat landscapes of three *de novo* anole lizard genomes.**



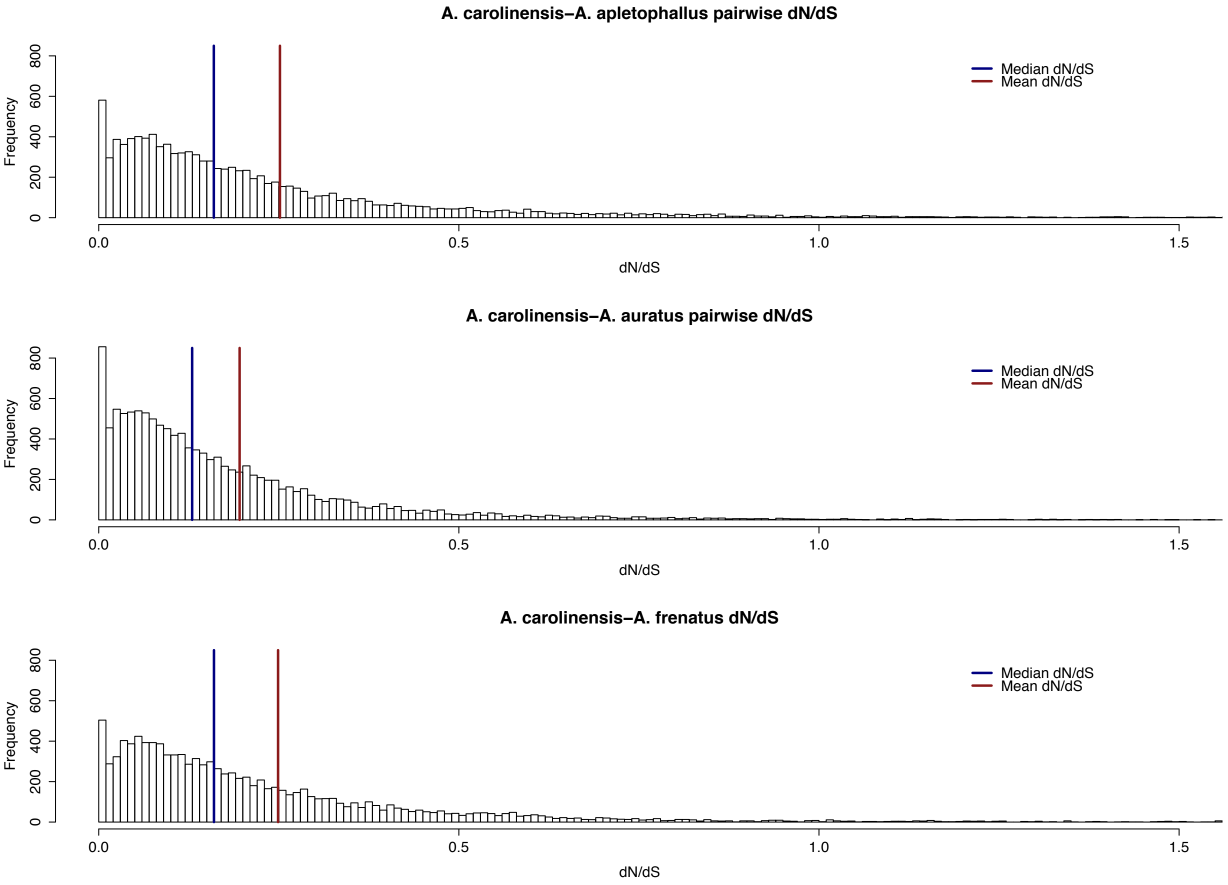
**Supplementary Figure 3. Phylogeny of 31 vertebrates with sequenced genomes.** Branc lengths are in terms of substitutions per site derived from fourfold degenerate sites. Nodes a labeled in reference to Supplementary Table 10.



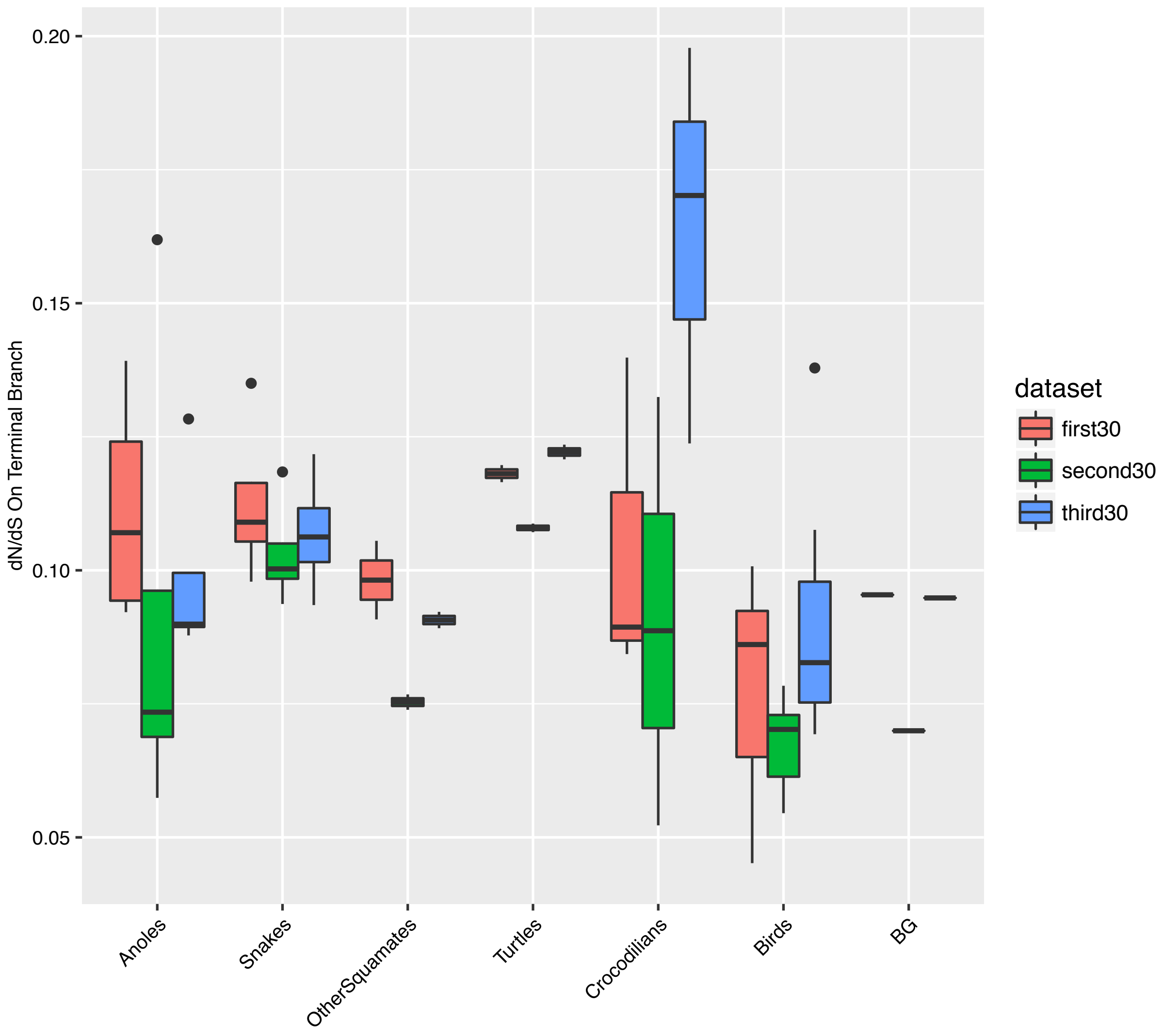
**Supplementary Figure 4. Divergence time estimation between iguanian lizards.** We included four species of anoles and based the analysis on 13 mitochondrial gene partitions (11,363 bp total) using BEAST. Blue bars indicate the 95% highest posterior density for each node. Red arrow indicates the fossil-calibrated node. *Anolis apletophallus* was not included in this analysis.



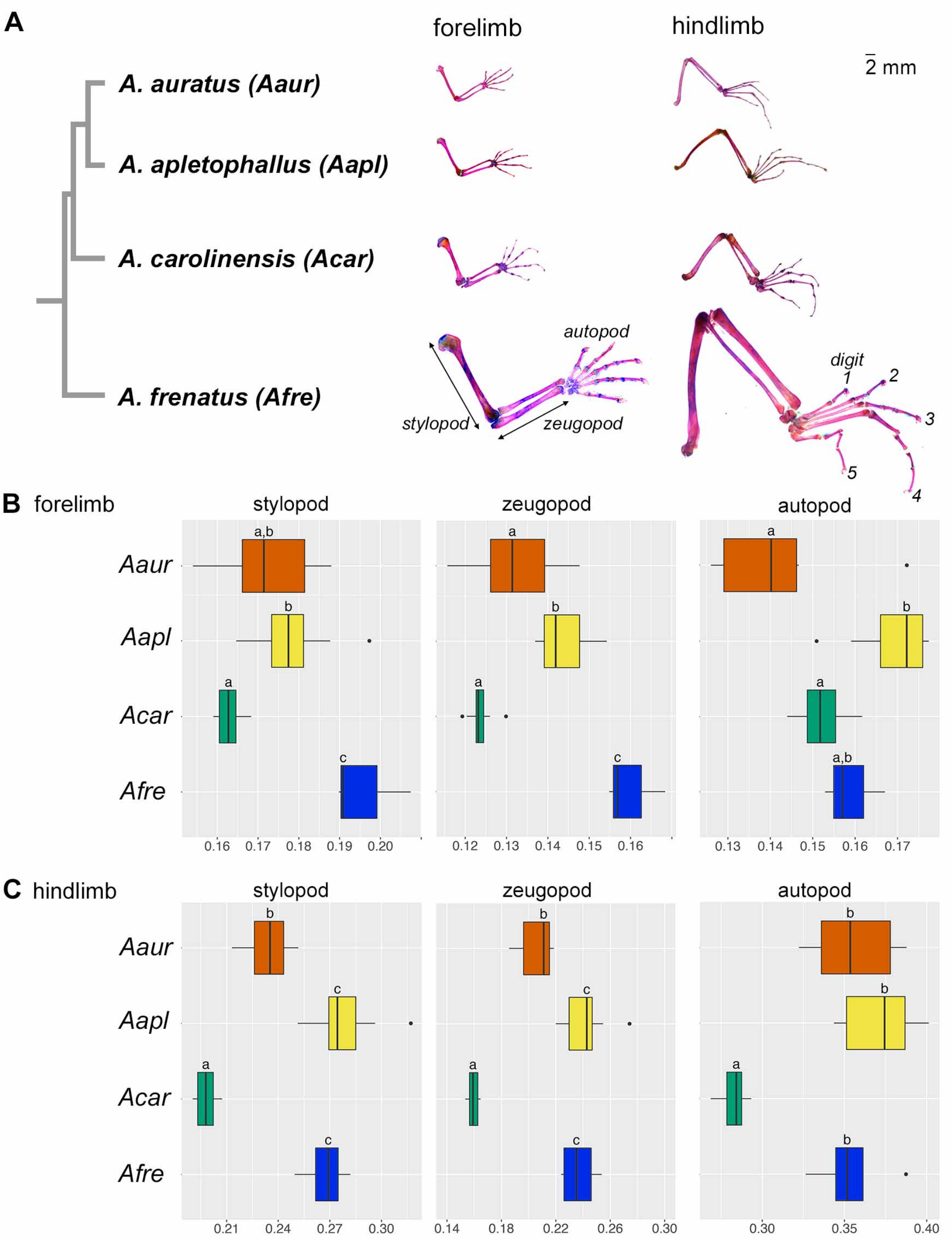
**Supplementary Figure 5. Distribution of $d_N/d_S$ values across pairwise genome comparisons between *A. carolinensis* and either *A. apletophallus*, *A. auratus*, or *A. frenatus*.** The median $d_N/d_S$ (blue vertical line) and mean $d_N/d_S$ (red vertical line) are plotted.



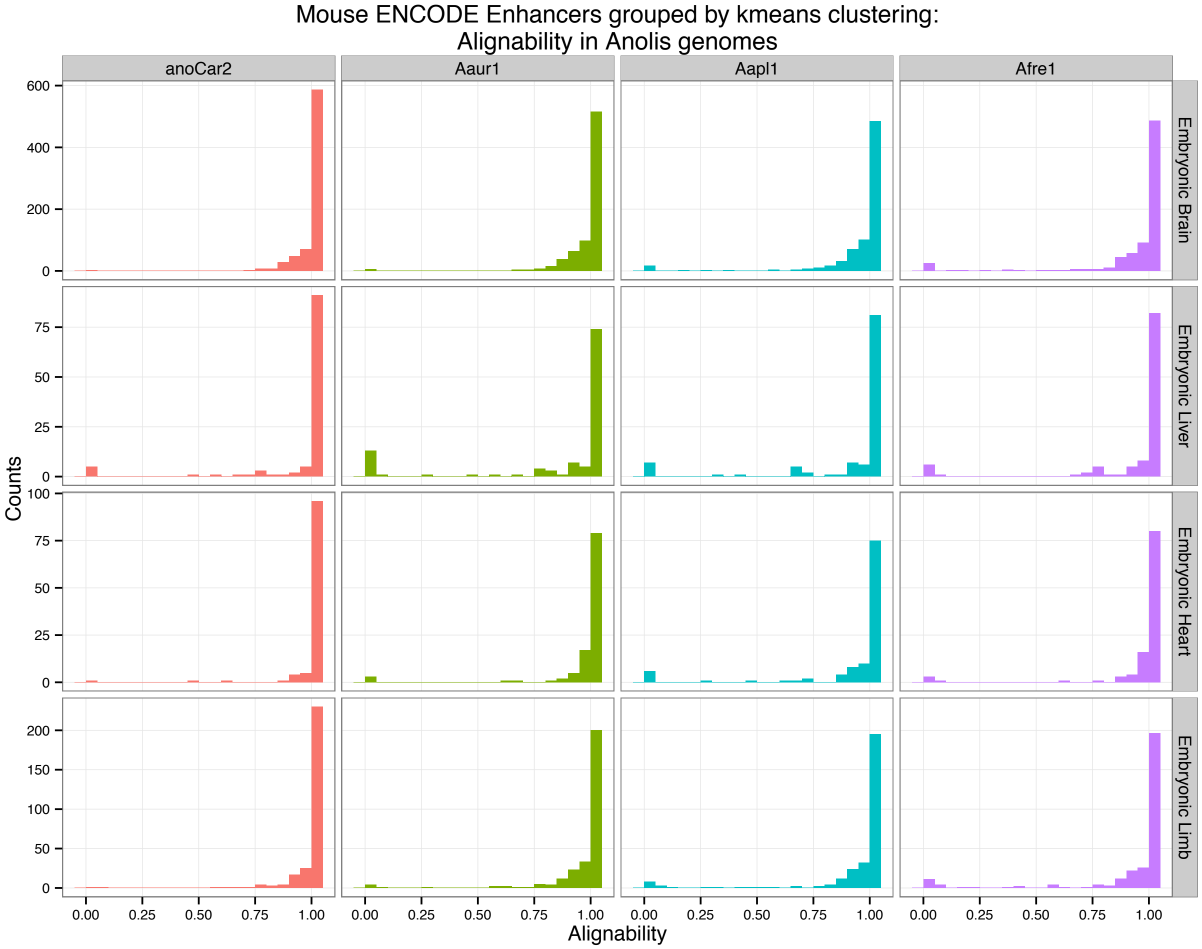
**Supplementary Figure 6. Ratio of nonsynonymous (dN) to synonymous (dS) substitutions in three separate randomized sets of 30 protein-coding genes across clades of reptiles.** Within each analyzed set of genes, we could not reject a null hypothesis of lack of difference in dN/dS between species groups (Kruskal-Wallis rank sum test; first 30 p-value = 0.1028, second 30 p-value = 0.1168, third 30 p-value = 0.0815). BG = dN/dS estimated for the background branches.



**Supplementary Figure 7. Osteological analysis of anole limbs.** (A) Replicates of the grass anole *A. auratus* (Aaur), the slender anole *A. apletophallus* (Aapl), the green anole *A. carolinensis* (Acar), and the Central American giant anole *A. frenatus* (Afre). Limb elements from the proximal stylopod (humerus or femur), middle zeugopod (radius + ulna or tibia + fibula), and distal autopod (carpus + metacarpal IV + digit IV phalanges or tarsus + metatarsal IV + digit IV phalanges) were measured in forelimb and hindlimb osteological preparations, respectively. Osteological preparations are shown to scale (2 mm bar). Distributions of the ratio of individual elements in the stylopod, zeugopod, or autopod length for the forelimb (B) and hindlimb (C) are shown. Significant groups at $p<0.05$ identified by ANOVA and Tukey HSD post-hoc analysis are indicated (a, b, or c).



**Supplementary Figure 8. Histogram of alignability scores of mouse embryonic brain, embryonic liver, embryonic heart, and embryonic limb enhancers in *Anolis* genomes.** Most mouse regions detected in *A. carolinensis* are highly conserved across anoles.



**Supplementary Table 1. Specimens used for genomic and morphological analysis.**

| **Species** | **Identifier** | **Sex** | **Collection Location (GPS Coordinates or Location)** |
|---|---|---|---|
| *Specimens for Genomic Sequencing* | | | |
| *A. apletophallus* | *Aapl224* | M | 9° 04' 46" N 79° 39' 36" W |
| *A. auratus* | *Aaur221* | F | 9° 07' 14" N 79° 41' 48" W |
| *A. frenatus* | *Afre204* | F | 9° 09' 40" N 79° 44' 43" W |
| *Specimens for Morphological Analysis* | | | |
| *A. apletophallus* | Aapl201 | F | 9° 4' 59" N 79° 39' 54" W |
| *A. apletophallus* | Aapl202 | F | 9° 9' 43" N -79° 44' 42" W |
| *A. apletophallus* | Aapl203 | F | Parque Nacional Soberanía, Panama |
| *A. apletophallus* | Aapl204 | M | 9° 5' 6" N 79° 39' 54" W |
| *A. apletophallus* | Aapl205 | M | Parque Nacional Soberanía, Panama |
| *A. apletophallus* | Aapl206 | M | 9° 9' 47" N 79° 44' 42" W |
| *A. apletophallus* | Aapl207 | M | 9° 4' 44.4" N 79° 39' 32" W |
| *A. apletophallus* | Aapl208 | F | Parque Nacional Soberanía, Panama |
| *A. apletophallus* | Aapl901 | M | Parque Nacional Soberanía, Panama |
| *A. apletophallus* | Aapl225 | M | 9° 9' 22" N 79° 44' 35" W |
| *A. apletophallus* | Aapl103 | M | 9° 4' 45" N 79° 39' 36" W |
| *A. apletophallus* | Aapl104 | F | 9° 5' 5" N 79° 39' 53" W |
| *A. apletophallus* | Aapl105 | F | 9° 4' 40" N 79° 39' 35" W |
| *A. auratus* | Aaur201 | F | 9° 3' 54" N 79° 39' 0" W |

| | | | |
|---|---|---|---|
| *A. auratus* | Aaur202 | M | 9° 3' 54" N 79° 39' 0" W |
| *A. auratus* | Aaur204 | F | 9° 3' 54" N 79° 39' 0" W |
| *A. auratus* | Aaur205 | F | 9° 3' 54" N 79° 39' 0" W |
| *A. auratus* | Aaur206 | F | 9° 3' 54" N 79° 39' 0" W |
| *A. auratus* | Aaur209 | M | 9° 3' 54" N 79° 39' 0" W |
| *A. auratus* | Aaur210 | F | 9° 3' 54" N 79° 39' 0" W |
| *A. frenatus* | Afre901 | F | Parque Nacional Soberanía, Panama |
| *A. frenatus* | Afre902 | M | Parque Nacional Soberanía, Panama |
| *A. carolinensis* | F121 | F | |
| *A. carolinensis* | F123 | F | |
| *A. carolinensis* | F125 | F | |
| *A. carolinensis* | F126 | F | |
| *A. carolinensis* | E008 | M | |
| *A. carolinensis* | E012 | M | |
| *A. carolinensis* | E018 | M | |
| *A. carolinensis* | E024 | M | |
| *A. carolinensis* | E083 | M | |
| *A. carolinensis* | E124 | M | |

**Supplementary Table 2. Publicly available genome assemblies used in the whole genome alignment.**

| **Species** | **Common Name** | **Genome Build** | **Source** |
|---|---|---|---|
| *Latimeria chalumnae* | African coelacanth | LatCha1 | UCSC |
| *Ornithorhynchus anatinus* | Platypus | ornAna1 | UCSC |
| *Monodelphis domestica* | Gray opossum | monDom5 | UCSC |
| *Dasypus novemcinctus* | Armadillo | dasNov3 | UCSC |
| *Loxodonta africana* | African elephant | loxAfr3 | UCSC |
| *Bos taurus* | Cow | bosTau8 | UCSC |
| *Canis lupus familiaris* | Dog | canFam3 | UCSC |
| *Mus musculus* | Mouse | mm10 | UCSC |
| *Homo sapiens* | Human | hg19 | UCSC |
| *Chrysemys picta bellii* | Western painted turtle | chrPic1 | UCSC |
| *Pelodiscus sinensis* | Chinese softshell turtle | PelSin_1.0 | Ensembl |
| *Alligator mississippiensis* | American alligator | allMiss1 | UCSC |
| *Crocodylus porosus* | Saltwater crocodile | v2.0 | crocgenomes.org |
| *Gavialis gangeticus* | Indian gharial | v2.0 | crocgenomes.org |
| *Struthio camelus* | Common ostrich | v1 | GigaDB |
| *Gallus gallus* | Chicken | galGal3 | UCSC |
| *Anas platyrhyncos* | Duck | anaPla1 | UCSC |
| *Meleagris gallopavo* | Turkey | melGal1 | UCSC |
| *Melopsittacus undulatus* | Budgerigar | melUnd1 | UCSC |
| *Taenopygia guttata* | Zebra finch | taeGut2 | UCSC |
| *Geospiza fortis* | Medium ground finch | geoFor1 | UCSC |
| *Gekko japonica* | Japanese gecko | v1 | GigaDB |
| *Python bivittatus* | Burmese python | Python_molurus_bivittatus-5.0.2 | NCBI |

| | | | |
|---|---|---|---|
| *Boa constrictor* | Boa constrictor | 7 | GigaDB |
| *Ophiophagus hannah* | King cobra | OphHan1.0 | NCBI |
| *Crotalus mitchelli* | Speckled rattlesnake | CrotMitch1.0 | NCBI |
| *Pogona vitticeps* | Central bearded dragon | | GigaDB |
| *Anolis carolinensis* | Green anole | anoCar2.0 | UCSC |
| *Anolis frenatus* | Central American giant anole | Afre1.0 | This study |
| *Anolis auratus* | Grass anole | Aaur1.0 | |
| *Anolis apletophallus* | Slender anole | Aapl1.0 | |

**Supplementary Table 3. Comparisons of candidate assemblies generated during this study.**

| *Anolis apletophallus* contigs using paired-end information | | | |
|---|---|---|---|
| | **Platanus** | **ABySS contigs (k=63)** | **SOAP contigs (k=63)** |
| Number contigs | 2.92E+06 | 1.76E+07 | 2.74E+07 |
| Contig N50 | 2,999 bp | 2,534 bp | 991 bp |
| Longest contig | 110,494 bp | 110,998 bp | 58,123 bp |
| Assembly length | 8.38E+08 | 1.89E+09 | 1.01E+09 |

| *Anolis apletophallus* scaffolding of contigs | | | |
|---|---|---|---|
| | **ABySS contigs and scaffolds** | **ABySS contings with SOAP scaffolds** | **SOAP all** |
| Number scaffolds (>1000 bp) | 454,194 | 531,014 | 435,144 |
| Scaffold N50 | 9,520 bp | 4,956 bp | 5,068 bp |
| Longest scaffold | 217,008 bp | 156,272 bp | 245,967 |
| Assembly length | 2.18+09 | 1.78E+09 | 1.53E+09 |

**Supplementary Table 4. Comparison of candidate assemblies for *Anolis frenatus.***

| *Anolis frenatus* contigs using paired-end information | | | |
|---|---|---|---|
| | **Platanus** | **ABySS contigs (k=83)** | **SOAP contigs (k=83)** |
| Number contigs | 2.12E+06 | 1.61E+07 | 1.97E+07 |
| Contig N50 | 11,317 bp | 21,942 bp | 15,331 bp |
| Longest contig | 268,901 bp | 448,330 bp | 462,521 bp |
| Assembly length | 9.12E+08 | 1.919E+09 | 1.88E+09 |

| *Anolis frenatus* scaffolding of contigs | | | |
|---|---|---|---|
| | **ABySS contigs and scaffolds** | **ABySS contings with SOAP scaffolds** | **SOAP all** |
| Number scaffolds (>1000 bp) | 531,276 | 649,467 | 587,977 |
| Scaffold N50 | 24,799 bp | 36,910 bp | 31,063 bp |
| Longest scaffold | 512,709 bp | 649,467 bp | 712,131 bp |
| Assembly length | 1.88+09 | 2.02E+09 | 1.97E+09 |

**Supplementary Table 5. Comparison of candidate assemblies for *Anolis auratus*.**

| *Anolis auratus* contigs using paired-end information | | |
|---|---|---|
| | **ABySS contigs (k=51)** | **SOAP contigs (k=51)** |
| Number contigs | 6.60E+07 | 5.87E+07 |
| Contig N50 | 19,858 bp | 14,331 bp |
| Longest contig | 304,182 bp | 278,457 bp |
| Assembly length | 1.94E+09 | 1.89E+09 |

| *Anolis auratus* scaffolding of contigs | | |
|---|---|---|
| | **ABySS contigs and scaffolds** | **ABySS contings with SOAP scaffolds** |
| Number scaffolds (>1000 bp) | 191,021 | 141,345 |
| Scaffold N50 | 39,412 bp | 48,995 bp |
| Longest scaffold | 491,711 bp | 563,800 bp |
| Assembly length | 1.90+09 | 2.02E+09 |

**Supplementary Table 6. Classification and abundance of repetitive elements in the *Anolis carolinensis* genome (AnoCar2.0).**

| | *Anolis carolinensis* | | |
|---|---|---|---|
| | Number of Elements | Length Occupied | Percentage of Sequence |
| Retroelements | 1,073,611 | 388,214,793 | 21.58 |
| SINEs: | 369,191 | 75,178,765 | 4.18 |
| Penelope | 73,604 | 14,461,881 | 0.8 |
| LINEs: | 629,604 | 229,226,809 | 12.74 |
| CRE/SLACS | | | |
| L2/CR1/Rex | 359,759 | 121,373,487 | 6.75 |
| R1/LOA/Jockey | 23,648 | 11,550,406 | 0.64 |
| R2/R4/NeSL | 58,783 | 24,566,369 | 1.37 |
| RTE/Bov-B | 56,289 | 28,845,930 | 1.6 |
| L1/CIN4 | 57,422 | 28,309,554 | 1.57 |
| LTR elements | 74,816 | 83,809,219 | 4.66 |
| BEL/Pao | 3,645 | 6,722,196 | 0.37 |
| Ty1/Copia | 11,487 | 5,709,575 | 0.32 |
| Gypsy/DIRS1 | 53,365 | 64,220,973 | 3.57 |
| Retroviral | 3,521 | 5,543,473 | 0.31 |
| DNA Transposons | 954,525 | 153,377,709 | 8.53 |
| hobo-Activator | 447,082 | 68,087,697 | 3.78 |
| Tc1-IS630-Pogo | 213,830 | 45,287,573 | 2.52 |
| En-Spm | | | |
| MuDR-IS905 | | | |
| PiggyBac | | | |
| Tourist/Harbinger | 148,028 | 19,360,784 | 1.08 |
| Other (Mirage, P-element, Transib) | | | |
| Rolling circles | | | |
| Unclassified | 140,799 | 34,141,611 | 1.9 |
| Total interspersed repeats | | 575,734,113 | 32 |
| Small RNA | 11,338 | 993,039 | 0.06 |
| Satellites | 5,313 | 560,792 | 0.03 |
| Simple Repeats | 562,190 | 25,458,981 | 1.42 |
| Low complexity | 49,903 | 2,752,659 | 0.15 |

**Supplementary Table 7. Classification and abundance of repetitive elements in the *Anolis frenatus* genome (Afre1.0).**

| | *Anolis frenatus* | | |
|---|---|---|---|
| | Number of Elements | Length Occupied | Percentage of Sequence |
| Retroelements | 1,236,888 | 306,307,808 | 15.11 |
| SINEs: | 300,316 | 32,865,714 | 1.62 |
| Penelope | 54,554 | 11,585,157 | 0.57 |
| LINEs: | 782,749 | 202,624,363 | 9.99 |
| CRE/SLACS | 0 | 0 | 0 |
| L2/CR1/Rex | 543,139 | 128,634,170 | 6.34 |
| R1/LOA/Jockey | 1,362 | 625,800 | 0.03 |
| R2/R4/NeSL | 2,573 | 836,211 | 0.04 |
| RTE/Bov-B | 89,796 | 24,069,475 | 1.19 |
| L1/CIN4 | 87,269 | 36,446,917 | 1.8 |
| LTR elements | 153,823 | 70,817,731 | 3.49 |
| BEL/Pao | 7,858 | 3,344,009 | 0.16 |
| Ty1/Copia | 22,188 | 4,157,333 | 0.21 |
| Gypsy/DIRS1 | 62,433 | 32,945,783 | 1.62 |
| Retroviral | 28,033 | 15,098,573 | 0.74 |
| DNA Transposons | 1,019,692 | 139,516,415 | 6.88 |
| hobo-Activator | 368,139 | 45,281,119 | 2.23 |
| Tc1-IS630-Pogo | 268,492 | 46,079,764 | 2.27 |
| En-Spm | 0 | 0 | 0 |
| MuDR-IS905 | 0 | 0 | 0 |
| PiggyBac | 560 | 140,251 | 0.01 |
| Tourist/Harbinger | 241,244 | 27,450,638 | 1.35 |
| Other (Mirage, P-element, Transib) | 0 | 0 | 0 |
| Rolling circles | 0 | 0 | 0 |
| Unclassified | 106738 | 18,295,928 | 0.9 |
| Total interspersed repeats | | 464,120,151 | 22.89 |
| Small RNA | 21,215 | 1,438,370 | 0.07 |
| Satellites | 10 | 471 | 0 |
| Simple Repeats | 981,230 | 290,510,612 | 14.33 |
| Low complexity | 67,535 | 5,830,316 | 0.29 |

**Supplementary Table 8. Classification and abundance of repetitive elements in the *Anolis auratus* genome (Aaur1.0).**

| | *Anolis auratus* | | |
|---|---|---|---|
| | Number of Elements | Length Occupied | Percentage of Sequence |
| Retroelements | 1,648,045 | 430,454,491 | 21.33 |
| SINEs: | 419,746 | 64,982,172 | 3.22 |
| Penelope | 87,573 | 20,822,627 | 1.03 |
| LINEs: | 1,087,570 | 308,143,389 | 15.27 |
| CRE/SLACS | 0 | 0 | 0 |
| L2/CR1/Rex | 680,553 | 175,681,230 | 8.71 |
| R1/LOA/Jockey | 52,869 | 18,600,999 | 0.92 |
| R2/R4/NeSL | 31,929 | 9,965,755 | 0.49 |
| RTE/Bov-B | 158,178 | 50,041,142 | 2.48 |
| L1/CIN4 | 76,337 | 32,992,282 | 1.64 |
| LTR elements | 140,729 | 57,328,930 | 2.84 |
| BEL/Pao | 9,242 | 4,124,453 | 0.2 |
| Ty1/Copia | 13,061 | 5,148,831 | 0.26 |
| Gypsy/DIRS1 | 55,649 | 23,141,494 | 1.15 |
| Retroviral | 34,383 | 14,873,326 | 0.74 |
| DNA Transposons | 1,436,556 | 201,463,523 | 9.99 |
| hobo-Activator | 650,488 | 94,974,261 | 4.71 |
| Tc1-IS630-Pogo | 337,201 | 51,597,063 | 2.56 |
| En-Spm | 0 | 0 | 0 |
| MuDR-IS905 | 0 | 0 | 0 |
| PiggyBac | 232 | 46,655 | 0 |
| Tourist/Harbinger | 213,393 | 24,164,254 | 1.2 |
| Other (Mirage, P-element, Transib) | 0 | 0 | 0 |
| Rolling circles | 0 | 0 | 0 |
| Unclassified | 127,906 | 27,972,190 | 1.39 |
| Total interspersed repeats | | 659,890,204 | 32.71 |
| Small RNA | 38,428 | 5,197,242 | 0.26 |
| Satellites | 4,491 | 1,321,832 | 0.07 |
| Simple Repeats | 881,006 | 120,191,955 | 5.96 |
| Low complexity | 74,283 | 6,961,908 | 0.35 |

**Supplementary Table 9. Classification and abundance of repetitive elements in the *Anolis apletophallus* genome (Aapl1.0).**

| | *Anolis apletophallus* | | |
|---|---|---|---|
| | Number of Elements | Length Occupied | Percentage of Sequence |
| Retroelements | 1,743,069 | 369,731,371 | 16.95 |
| SINEs: | 501,821 | 71,673,610 | 3.28 |
| Penelope | 88,327 | 16,080,596 | 0.74 |
| LINEs: | 1,151,430 | 266,780,076 | 12.23 |
| CRE/SLACS | 0 | 0 | 0 |
| L2/CR1/Rex | 728,644 | 154,176,123 | 7.07 |
| R1/LOA/Jockey | 43,071 | 13,622,968 | 0.62 |
| R2/R4/NeSL | 26,373 | 6,789,411 | 0.31 |
| RTE/Bov-B | 185,405 | 39,313,128 | 1.8 |
| L1/CIN4 | 79,474 | 36,761,842 | 1.68 |
| LTR elements | 89,818 | 31,277,685 | 1.43 |
| BEL/Pao | 7,971 | 2,046,288 | 0.09 |
| Ty1/Copia | 7,375 | 2,284,710 | 0.1 |
| Gypsy/DIRS1 | 42,036 | 16,579,020 | 0.76 |
| Retroviral | 11,359 | 4,052,432 | 0.19 |
| DNA Transposons | 1,733,248 | 208,812,457 | 9.57 |
| hobo-Activator | 932,141 | 100,553,364 | 4.61 |
| Tc1-IS630-Pogo | 386,030 | 59,340,280 | 2.72 |
| En-Spm | 0 | 0 | 0 |
| MuDR-IS905 | 0 | 0 | 0 |
| PiggyBac | 0 | 0 | 0 |
| Tourist/Harbinger | 228,980 | 24,599,854 | 1.13 |
| Other (Mirage, P-element, Transib) | 0 | 0 | 0 |
| Rolling circles | 0 | 0 | 0 |
| Unclassified | 146,049 | 20,051,178 | 0.92 |
| Total interspersed repeats | | 598,595,006 | 27.43 |
| Small RNA | 43,723 | 4,285,943 | 0.2 |
| Satellites | 3,958 | 1,481,294 | 0.07 |
| Simple Repeats | 563,012 | 30,756,337 | 1.41 |
| Low complexity | 80,008 | 9,305,424 | 0.43 |

**Supplementary Table 10. Node constraints and divergence time estimates used in the semiparametric penalized likelihood analysis. *Fixed node age**

| Node | Node Name | Minimum constraint (MYA) | Maximum constraint (MYA) | Estimated age (MYA) |
|---|---|---|---|---|
| 1 | Sarcopterygia | | | 415* |
| 2 | Amniota | | | 302.57 |
| 3 | Sauropsida | 255.9 | | 278.24 |
| 4 | Bifurcata | | | 174.94 |
| 5 | Toxicofera | | | 153.57 |
| 6 | Iguania | | | 136.19 |
| 7 | Ancestral Anoles | | | 45.05 |
| 8 | Acar-Aaur-Aapl | | | 34.73 |
| 9 | Aaur-Aapl | | | 21.04 |
| 10 | Snakes | | | 60.60 |
| 11 | Pbiv-Bcon | | | 44.96 |
| 12 | Colubroidia | | | 36.30 |
| 13 | Archelosauria | | | 253.98 |
| 14 | Testudines | | | 101.61 |
| 15 | Archosaurs | 247.1 | | 247.10 |
| 16 | Crocodilia | 77.8 | 83.6 | 77.8 |
| 17 | Longirostres | | | 37.34 |
| 18 | Aves | | | 111.32 |
| 19 | Neognaths | 66 | 86.8 | 86.80 |
| 20 | Galloanserae | | | 71.67 |
| 21 | Galliformes | | | 27.82 |
| 22 | Psittacopasserae | | | 71.67 |
| 23 | Thraupidae-Estrildidae | | | 24.57 |
| 24 | Mammalia | 164.9 | 201.5 | 201.50 |
| 25 | Metatheria | | | 177.22 |
| 26 | Eutheria | | | 98.72 |
| 27 | Atlantogenata | | | 91.47 |
| 28 | Boreoeutheria | | | 92.13 |
| 29 | Laurasiatheria | | | 77.13 |
| 30 | Euarchontoglires | | | 84.66 |

**Supplementary Table 11. Species and GenBank accession numbers of iguanian lizard mitochondrial genomes used to assemble the *A. frenatus* and *A. auratus* mitochondrial genomes, as well as those included in the phylogenetic analysis.**

| **Species** | **Accession No.** |
|---|---|
| *Anolis carolinensis* | NC_010972 |
| *Anolis cybotes* | AB218960 |
| *Basiliscus vittatus* | NC_012829 |
| *Iguana iguana* | AJ278511 |
| *Polychrus marmoratus* | NC_012839 |
| *Sceloporus occidentalis* | AB079242 |
| *Chamaeleo africanus* | NC_012422 |
| *Chamaeleo chamaeleon* | NC_012427 |
| *Furcifer oustaleti* | NC_008777 |
| *Leiolepis guttata* | NC_014179 |
| *Phrynocephalus guinanensis* | NC_024875 |
| *Pogona vitticeps* | NC_006922 |
| *Uromastyx benti* | NC_014182 |

**Supplementary Table 12. Mean pairwise evolutionary rates.** Mean pairwise nonsynonymous ($d_N$), synonymous ($d_S$), and the ratio of non-synonymous/synonymous ($d_N/d_S$) substitution rates for genes located on *A. carolinensis* autosomes 1-6 and Linkage Group b (LGb), which has been identified as X-linked. All comparisons are pairwise between *A. carolinensis* and the indicated comparisons species. We also analyzed genes on unplaced contigs and scaffolds, which are not included here (see Supplementary Data 2, Supplementary Data 3, and Supplementary Data 4).

| Comparison | Region | $d_N$ (95% CI) | $d_S$ (95% CI) | $d_N/d_S$ (95% CI) | Observed Genes |
|---|---|---|---|---|---|
| *A. apletophallus* | Autosomes | 0.0311 (0.0236, 0.0339) | 0.1470 (0.1180, 0.1377) | 0.2161 (0.2081, 0.2651) | 5571 |
|  | LGb | 0.0210 (0.0184, 0.0218) | 0.1751 (0.1610, 0.1857) | 0.2847 (0.2979, 0.4185) | 32 |
| *A. auratus* | Autosomes | 0.0279 (0.0321, 0.0400) | 0.1536 (0.1546, 0.1706) | 0.1833 (0.1438, 0.1915) | 6120 |
|  | LGb | 0.0154 (0.0130, 0.0157) | 0.1865 (0.1647,0.1809) | 0.0912 (0.0890, 0.1057) | 39 |
| *A. frenatus* | Autosomes | 0.0330 (0.0299, 0.0395) | 0.1628 (0.1316, 0.1557) | 0.2105 (0.1559, 0.2190) | 5483 |
|  | LGb | 0.0247 (0.0206, 0.0283) | 0.1900 (0.1675, 0.1856) | 0.2352 (0.2434, 0.3549) | 38 |

**Supplementary Table 13. Median pairwise evolutionary rates.** Median pairwise nonsynonymous ($d_N$), synonymous ($d_S$), and the ratio of non-synonymous/synonymous ($d_N/d_S$) substitution rates for genes located on *A. carolinensis* autosomes 1-6 and Linkage Group b (LGb), which has been identified as X-linked. All comparisons are pairwise between *A. carolinensis* and the indicated comparisons species.

| Comparison | Region | $d_N$ (95% CI) | $d_S$ (95% CI) | $d_N/d_S$ (95% CI) | Observed Genes |
|---|---|---|---|---|---|
| *A. apletophallus* | Autosomes | 0.0201 (0.0197, 0.0255) | 0.1352 (0.1227, 0.1332) | 0.1497 (0.1114, 0.1506) | 5571 |
| | LGb | 0.0154 (0.0150, 0.0219) | 0.1371 (0.1353, 0.1482) | 0.0971 (0.0869, 0.1103) | 32 |
| *A. auratus* | Autosomes | 0.0185 (0.0103, 0.0159) | 0.1456 (0.1435, 0.1545) | 0.1268 (0.0926, 0.1262) | 6120 |
| | LGb | 0.0114 (0.0108, 0.0120) | 0.1609 (0.1528, 0.1673) | 0.0683 (0.0727, 0.0952) | 39 |
| *A. frenatus* | Autosomes | 0.0220 (0.0087, 0.1479) | 0.1479 (0.1447, 0.1572) | 0.1471 (0.1206, 0.1565) | 5483 |
| | LGb | 0.0148 (0.0104, 0.0171) | 0.1811 (0.1707, 0.1917) | 0.0814 (0.0716, 0.0961) | 38 |

**Supplementary Table 14. The proportion of genes in each pairwise comparison with $d_N/d_S$ less than or equal to 0.5 and 1.**

| **Alignment** | **$d_N/d_S \leq 0.5$** | **$d_N/d_S \leq 1$** |
|---|---|---|
| Acar to Aapl | 0.8852 | 0.9739 |
| Acar to Aaur | 0.9339 | 0.9876 |
| Acar to Afre | 0.8915 | 0.9748 |

**Supplementary Table 15. Genes with significantly increased rate of evolution.** Genes with a pairwise $d_N/d_S$ that is significantly greater than 1, computed from a Fisher Exact Test.

| ***A. carolinensis* to:** | **Ensembl Gene ID** | **Gene Symbol** | **$d_N/d_S$** | **Bonferroni Corrected P -Value** | **Function** |
|---|---|---|---|---|---|
| *A. apletophallus* | ENSACAG00000006068 | *dgkk* | 2.69751 | $2.0859 \times 10^{-6}$ | phosphorylates diacylglycerol, converting it to phosphatidic acid |
| | ENSACAG00000007232 | *abca2* | 2.12148 | $4.4612 \times 10^{-4}$ | probable transporter |
| | ENSACAG00000003147 | *sptbn4* | 1.9121 | $6.9351 \times 10^{-4}$ | Spectrin- links the plasma membrane to the actin cytoskeleton |
| | ENSACAG00000001632 | *tet3* | 1.97406 | $2.046 \times 10^{-3}$ | plays a role in the DNA methylation process |
| | ENSACAG00000016669 | *rhobtb2* | 20.6284 | $8.373 \times 10^{-3}$ | small Rho GTPase and candidate tumor suppressor |
| | ENSACAG00000010939 | *tln1* | 1.75569 | 0.01817 | plays a significant role in the assembly of actin filaments |
| *A. auratus* | ENSACAG00000012940 | *myo18a* | 2.59759 | $1.4407 \times 10^{-8}$ | potential Golgi membrane trafficking |
| *A. frenatus* | ENSACAG00000017766 | *N/A* | 3.30476 | $1.22 \times 10^{-7}$ | N/A |
| | ENSACAG00000011444 | *N/A* | 1.75701 | $2.6656 \times 10^{-4}$ | N/A |
| | ENSACAG00000001978 | *cog4* | 5.40433 | $8.2693 \times 10^{-4}$ | Plays a role in SNARE-pin assembly and Golgi-to-ER retrograde transport |
| | ENSACAG00000002647 | *depdc5* | 2.5966 | $2.3950 \times 10^{-3}$ | GATOR1 complex component, inhibits amino acid-sensing branch of the mTORC1 pathway |
| | ENSACAG00000012644 | *slc9a6* | 4.55244 | 0.01916 | Contributes to calcium homeostasis |

**Supplementary Table 16. Codeml results for the null model (M0) of a single $d_N/d_S$ value for the entire phylogeny. np=Number of parameters in the model.**

| Gene | Sequences | Alignment Length | Codons | np | Lnl | Omega ($d_N/d_S$) |
|---|---|---|---|---|---|---|
| *alx4* | 17 | 774 | 258 | 34 | -4342.68453 | 0.03698 |
| *bmp2* | 18 | 843 | 281 | 36 | -5079.851869 | 0.0966 |
| *bmp4* | 18 | 1,125 | 375 | 36 | -6977.761119 | 0.06175 |
| *en1* | 17 | 318 | 106 | 34 | -1454.791545 | 0.01004 |
| *fgf4* | 17 | 411 | 137 | 34 | -2274.070988 | 0.05384 |
| *fgf8* | 18 | 501 | 167 | 36 | -2349.538271 | 0.0066 |
| *grem1* | 16 | 558 | 186 | 32 | -3518.54477 | 0.06818 |
| *hand2* | 17 | 312 | 104 | 34 | -1187.12826 | 0.0144 |
| *hoxa10* | 18 | 246 | 82 | 36 | -1096.138054 | 0.02562 |
| *hoxa11* | 18 | 798 | 266 | 36 | -3867.796332 | 0.05536 |
| *hoxc10* | 15 | 354 | 118 | 30 | -1496.020517 | 0.063 |
| *hoxd10* | 18 | 1,026 | 342 | 36 | -4234.968702 | 0.07734 |
| *hoxd11* | 18 | 984 | 328 | 36 | -5417.046313 | 0.10432 |
| *hoxd12* | 16 | 240 | 80 | 32 | -1297.098741 | 0.01916 |
| *hoxd13* | 18 | 384 | 128 | 36 | -1893.218381 | 0.01303 |
| *pitx1* | 16 | 336 | 112 | 32 | -1847.600999 | 0.03404 |
| *ptch1* | 18 | 3,168 | 1,056 | 36 | -19781.70512 | 0.08142 |
| *sall4* | 18 | 3,405 | 1,135 | 36 | -28910.15541 | 0.14694 |
| *shh* | 15 | 678 | 226 | 30 | -4221.417611 | 0.05151 |
| *shox2* | 17 | 216 | 72 | 34 | -1037.966843 | 0.01174 |
| *tbx3* | 18 | 420 | 140 | 36 | -2021.42278 | 0.00497 |
| *tbx4* | 17 | 915 | 305 | 34 | -5641.334915 | 0.04314 |
| *tbx5* | 18 | 1,323 | 441 | 36 | -8150.871661 | 0.02938 |
| *twist1* | 15 | 297 | 99 | 30 | -1045.685444 | 0.00298 |
| *wnt7a* | 18 | 978 | 326 | 36 | -4689.970265 | 0.00653 |

**Supplementary Table 17. Codeml results for the first alternative model (M2) of two dn/ds values for *Anolis* (foreground branch) and the remainder of the phylogeny (background branches).** The same numbers of sequences, alignment lengths, and codons as Supplementary Table 13 apply. *=p<0.1, **=p<0.01, ***=p<0.001; Likelihood Ratio Test.

| Gene | np | Lnl | Background Omega | Foreground Omega |
|---|---|---|---|---|
| *alx4* | 35 | -4342.635348 | 0.03665 | 0.04237 |
| *bmp2* | 37 | -5078.998785 | 0.09298 | 0.12883 |
| *bmp4* | 37 | -6977.634304 | 0.06277 | 0.05698 |
| *en1* | 35 | -1447.751817*** | 0.00605 | 0.02878 |
| *fgf4* | 35 | -2272.634276* | 0.05741 | 0.02541 |
| *fgf8* | 37 | -2348.783806 | 0.00739 | 0.0001 |
| *grem1* | 33 | -3500.08397*** | 0.04601 | 0.19795 |
| *hand2* | 35 | -1180.944534*** | 0.00911 | 0.06721 |
| *hoxa10* | 37 | -1092.703716** | 0.01749 | 0.08284 |
| *hoxa11* | 37 | -3867.135891 | 0.05816 | 0.04207 |
| *hoxc10* | 31 | -1496.019558 | 0.06313 | 0.06126 |
| *hoxd10* | 37 | -4234.410559** | 0.08013 | 0.05518 |
| *hoxd11* | 37 | -5412.905793*** | 0.09477 | 0.18627 |
| *hoxd12* | 33 | -1289.959188*** | 0.01129 | 0.07234 |
| *hoxd13* | 37 | -1893.133809 | 0.01241 | 0.01682 |
| *pitx1* | 33 | -1845.151291* | 0.0283 | 0.06094 |
| *ptch1* | 37 | -19781.57341 | 0.08085 | 0.08756 |
| *sall4* | 37 | -28883.4628*** | 0.13551 | 0.29613 |
| *shh* | 31 | -4221.176226 | 0.05309 | 0.04352 |
| *shox2* | 35 | -1037.598664 | 0.01 | 0.01522 |
| *tbx3* | 37 | -2019.968118 | 0.00323 | 0.00856 |
| *tbx4* | 35 | -5639.62159* | 0.04617 | 0.02977 |
| *tbx5* | 37 | -8150.849303 | 0.02959 | 0.02804 |
| *twist1* | 31 | -1043.430261* | 0.00286 | 0.02772 |
| *wnt7a* | 37 | -4689.959314 | 0.00649 | 0.00726 |

**Supplementary Table 18. Codeml results for the second alternative model (M2b), with up to 7 dn/ds values across the phylogeny.** The same numbers of sequences, alignment lengths, and codons as Supplementary Table 13 apply. This model was compared to the M2 model using the Likelihood Ratio Test. *=p<0.1, **=p<0.01, ***=p<0.001; Likelihood Ratio Test.

| Gene | np | Lnl | Background Omega | Omega Eutheria | Omega Aves | Omega Anolis | Omega Python | Omega Turtles | Omega Alligator |
|---|---|---|---|---|---|---|---|---|---|
| *alx4* | 39 | -4336.43469* | 0.05319 | 0.02841 | 0.03697 | 0.04095 | 0.01019 | 0.01215 | 0.02052 |
| *bmp2* | 42 | -5073.081974* | 0.09845 | 0.06913 | 0.0931 | 0.12519 | 0.16449 | 0.1426 | 0.06201 |
| *bmp4* | 42 | -6967.184679*** | 0.06444 | 0.03147 | 0.05161 | 0.05592 | 0.08008 | 0.12469 | 0.0949 |
| *en1* | 39 | -1444.393008*** | 0.00481 | 0.00168 | 0.00614 | 0.02871 | NA | 0.00997 | 0.03723 |
| *fgf4* | 39 | -2267.887311* | 0.03182 | 0.07119 | 0.07056 | 0.02519 | NA | 0.03698 | 0.11104 |
| *fgf8* | 42 | -2336.890626*** | 0.02393 | 0.00307 | 0.00264 | 0.0001 | 0.0001 | 0.01373 | 0.01489 |
| *grem1* | 37 | -3486.975608*** | 0.04247 | 0.01735 | 0.0533 | 0.19912 | NA | 0.13385 | 0.13448 |
| *hand2* | 39 | -1178.478673** | 0.01496 | 0.00281 | 0.01433 | 0.06722 | 0.00791 | 0.0001 | 0.00691 |
| *hoxa10* | 42 | -1089.309432* | 0.02431 | 0.00788 | 0.0001 | 0.07486 | 0.04055 | 0.0001 | 0.04828 |
| *hoxa11* | 42 | -3860.293118** | 0.05241 | 0.0464 | 0.05942 | 0.04071 | 0.17157 | 0.04561 | 0.03296 |
| *hoxc10* | 35 | -1494.629652 | 0.07424 | 0.06657 | 0.04969 | 0.06106 | NA | 0.07647 | 0.17935 |
| *hoxd10* | 42 | -4231.197029* | 0.09346 | 0.05926 | 0.06831 | 0.05294 | 0.12678 | 0.06427 | 0.04469 |
| *hoxd11* | 42 | -5403.492265*** | 0.11446 | 0.0589 | 0.10227 | 0.16324 | 0.28514 | 0.11168 | 0.08595 |
| *hoxd12* | 37 | -1286.896109*** | 0.00807 | 0.01893 | 0.01325 | 0.07335 | NA | 0.07641 | 0.00459 |
| *hoxd13* | 42 | -1884.497035 | 0.01351 | 0.00693 | 0.01698 | 0.01012 | 999 | 0.01649 | 0.0001 |
| *pitx1* | 37 | -1840.017926** | 0.04957 | 0.02113 | 0.00774 | 0.05985 | 0.04871 | 0.02067 | NA |
| *ptch1* | 42 | -19751.96054*** | 0.08306 | 0.04653 | 0.11208 | 0.08576 | 0.11617 | 0.09496 | 0.13345 |
| *sall4* | 42 | -28861.65703*** | 0.11976 | 0.17435 | 0.10239 | 0.29127 | 0.16846 | 0.14481 | 0.12623 |
| *shh* | 36 | -4219.334731 | 0.04281 | 0.05443 | 0.06716 | 0.0409 | 0.08909 | 0.0782 | 0.04074 |
| *shox2* | 40 | -1034.350218* | 0.01211 | 0.00581 | 0.01172 | 0.02303 | 0.0001 | 0.01948 | 0.0001 |
| *tbx3* | 42 | -2016.375476* | 0.00153 | 0.00535 | 0.0001 | 0.00855 | 0.0001 | 0.01572 | 0.0001 |
| *tbx4* | 39 | -5638.22143* | 0.04135 | 0.04189 | 0.05634 | 0.02999 | NA | 0.05363 | 0.03469 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *tbx5* | 42 | 8136.540014*** | 0.03072 | 0.02497 | 0.01929 | 0.02562 | 0.08473 | 0.06408 | 0.0167 |
| *twist1* | 36 | -1038.768748* | 0.00188 | 0.0001 | 0.04271 | 0.02772 | 0.00607 | 0.0001 | 0.0001 |
| *wnt7a* | 42 | 4682.861863* | 0.01139 | 0.00769 | 0.00172 | 0.00721 | 0.01431 | 0.00648 | 0.0001 |

**Supplementary Table 19. Numbers and percentages of embryonic mouse enhancers identified by kmeans clustering of mouse ENCODE data that are detectable in the *A. carolinensis* genome, and alignable to the *A. auratus*, *A. apletophallus*, and *A. frenatus* genomes based on coordinates from an *Anolis* 4-way whole genome alignment.**

| Mouse Embryonic Tissue | Total Enhancers | Detected in anoCar2 | | Alignable in Aaur1 | | Alignable in Aapl1 | | Alignable in Afre1 | |
|---|---|---|---|---|---|---|---|---|---|
| Brain | 4,252 | 756 | 17.8% | 750 | 99.2% | 739 | 97.8% | 732 | 96.8% |
| Liver | 4,062 | 112 | 2.8% | 99 | 88.4% | 105 | 93.8% | 106 | 94.6% |
| Heart | 3,318 | 109 | 3.3% | 107 | 98.2% | 103 | 94.5% | 107 | 98.2% |
| Limb | 3,596 | 289 | 8.0% | 286 | 99.0% | 282 | 97.6% | 279 | 96.5% |

## SUPPLEMENTARY REFERENCES

Conrad JL, Norell MA. 2007. A complete Late Cretaceous iguanian (Squamata, Reptilia) from the Gobi and identification of a new iguanian clade. Am. Mus. Novit. 3584:1–47. doi: 10.1206/0003-0082(2007)3584[1:ACLCIS]2.0.CO;2.

Diaz A, Nellore A, Song JS. 2012. CHANCE: comprehensive software for quality control and validation of ChIP-seq data. Genome Biol. 13:R98. doi: 10.1186/gb-2012-13-10-r98.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol. 7:214. doi: 10.1186/1471-2148-7-214.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29:1969–1973. doi: 10.1093/molbev/mss075.

Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics. 5:113. doi: 10.1186/1471-2105-5-113.

Hanken J. & Wassersug R. 1981. The visible skeleton. Funct. Photogr.16: 22-26.

Kearse M et al. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28:1647–1649. doi: 10.1093/bioinformatics/bts199.

Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods. 9:357–359. doi: 10.1038/nmeth.1923.

Li H et al. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 25:2078–2079. doi: 10.1093/bioinformatics/btp352.

Liao Y, Smyth GK, Shi W. 2013. The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. Nucleic Acids Res. 41:e108–e108. doi: 10.1093/nar/gkt214.

Miller W et al. 2007. 28-way vertebrate alignment and conservation track in the UCSC Genome Browser. Genome Res. 17:1797–1808. doi: 10.1101/gr.6761107.

Quinlan AR, Hall IM. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics. 26:841–842. doi: 10.1093/bioinformatics/btq033.

R Core Team. 2016. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.* URL http://www. R-project. org.

Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 26:139–140. doi: 10.1093/bioinformatics/btp616.

Shen Y et al. 2012. A map of the cis-regulatory sequences in the mouse genome. Nature. 488:116–120. doi: 10.1038/nature11243.

Speir ML et al. 2016. The UCSC Genome Browser database: 2016 update. Nucleic Acids Res. 44:D717–25. doi: 10.1093/nar/gkv1275.

Zhang Y et al. 2008. Model-based analysis of ChIP-Seq (MACS). Genome Biol. 9:R137. doi: 10.1186/gb-2008-9-9-r137.