Comparative analysis of amphibian genomes: an emerging resource for basic and applied research

Running title: Comparative analysis of amphibian genomes

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ABSTRACT

Amphibians are the most threatened group of vertebrates and are in dire need of conservation intervention to ensure their continued survival. They exhibit unique features including a high diversity of reproductive strategies, permeable and specialized skin capable of producing toxins and antimicrobial compounds, multiple genetic mechanisms of sex determination, and in some lineages, the ability to regenerate limbs and organs. Although genomics approaches would shed light on these unique traits and aid conservation, sequencing and assembly of amphibian genomes has lagged behind other taxa due to their comparatively large genome sizes. Fortunately, the development of long-read sequencing technologies and initiatives has led to a recent burst of new amphibian genome assemblies. Although growing, the field of amphibian genomics suffers from the lack of annotation resources, tools for working with challenging genomes, and lack of high-quality assemblies in multiple clades of amphibians. Here we analyze 51 publicly available amphibian genomes to evaluate their usefulness for functional genomics research. We report considerable variation in genome assembly quality and completeness, and report some of the highest transposable element and repeat contents of any vertebrate. Additionally, we detected an association between transposable element content and climatic variables. Our analysis provides evidence of conserved genome synteny despite the long divergence times of this group, but we also highlight inconsistencies in chromosome naming and orientation across genome assemblies. We discuss sequencing gaps in the phylogeny and suggest key targets for future sequencing endeavors. Finally, we propose increased investment in amphibian genomics research to promote their conservation.

KEYWORDS

Amphibian genomes; comparative genomics; transposable elements; repeat expansion; genome synteny

INTRODUCTION

Amphibians are an ancient lineage of vertebrates that predate amniotes by more than 100 million years. Despite the considerable age of this lineage, amphibians are now the most threatened group of vertebrates with more that 40% of species and are threatened by factors such as habitat change, disease, and over-exploitation (IUCN, 2022; Scheele et al., 2019). Notably, many of these threats are hard to reverse, suggesting that novel approaches that utilize genomic resources may lead to improved management decisions for some of the most endangered taxa (Kosch et al., 2022; Scheele et al., 2014).

We are only just beginning to understand the genetic basis of many of the unique features of amphibians. Amphibians exhibit a high diversity of reproductive strategies including biphasic and direct development, uniparental and biparental care, mouth and gastric brooding, and foam nesting (Brown et al., 2010; Nunes-de-Almeida et al., 2021; Schulte et al., 2020). They also have specialized skin capable of producing complex compounds of interest for drug discovery for the development of antimicrobial drugs and analgesics (Daly et al., 2000; De Angelis et al., 2021; Liu et al., 2020). Amphibians occur across habitat types from rainforests to deserts, freshwater streams to salt marshes, and tropical to arctic climates (Duellman, 1999), but it is unclear how this ecological diversity is reflected in genome composition. One potential way is the number of transposable elements (TEs) present in the genome. TEs have a huge impact on the structure and function of eukaryotic genomes, with amphibians having among the largest TE content among vertebrates. There is increasing evidence that TE activity, and thus their relative proportion in genomes, is

influenced by abiotic factors (Pimpinelli & Piacentini, 2020). This in turn highlights their potential role in the regulation of genetic mechanisms responsible for environmental adaptation (Casacuberta & González, 2013; Pappalardo et al., 2021). Salamanders are an important resource for transplant and regeneration research due to their ability to regenerate limbs and internal organs (Elewa et al., 2017; Nowoshilow et al., 2018). Amphibians also have many of the same immune components of mammals making them an important model resource for immunology (Paiola et al., 2023; Robert, 2020).

Despite the obvious value of amphibian genomes for research on ecology, evolution, medicine, and improving their conservation, until recently, the generation of amphibian reference genomes has been markedly slower than other vertebrates (Hotaling et al., 2021a; Womack et al., 2022). This lag can be attributed to high costs and the computational challenges of assembling their often large and complex genomes (Sun et al., 2020). Recent advances in sequencing technologies such as long read sequencing and assembly algorithms that incorporate hybrid approaches have circumvented many of these challenges leading to a surge of high quality, chromosome-level reference genomes. The next challenge will be developing the tools for annotation and comparative analyses of these large genomes.

In this study, we provide a synthesis of all available amphibian reference genome assemblies, 51 at the time of our analysis, with the number growing every day. We evaluate assembly quality, sequencing technology, gene completeness, transposable element and repeat content and its ecological correlates, taxonomic representation, and synteny.

98 **MATERIALS AND METHODS**

99 ***Genomes***

100 A search of the NCBI genome website using the search term “amphibians” conducted on August 25,
101 2023, revealed there were 90 amphibian genomes from 68 species. All genome files in fasta format
102 were downloaded for assessment. Sixteen salamander genomes (Pyron et al., 2024) were excluded
103 from our analyses due to their high degree of incompleteness (i.e., <10% of genome assembled). Of
104 the remaining genomes, one genome was selected for each species for subsequent analysis. If there
105 was more than one draft of a genome, the most recent draft and/or the primary haplotype was
106 selected. In cases where there were multiple versions sequenced by different groups, the best
107 genome was selected by lowest scaffold number. Entire genomes (including uncharacterized contigs
108 but excluding mitochondrial genomes) were used for assessment unless indicated otherwise.

109

110 Genome databases NCBI Genomes, NCBI RefSeq (O'Leary et al., 2016), Ensembl (Cunningham et al.,
111 2022), UCSC Genome Browser (Lee et al., 2022), and Genomes on a Tree (GoaT) (Sotero-Caio et al.,
112 2021) were searched for information on the 51 amphibian species with reference genomes including
113 chromosome number, annotation data, proteome availability, C-value, and sequencing technology.
114 Sequencing strategy was classified as “short-single” for Illumina only sequencing, “long-single” for
115 sequencing using long read technologies (e.g., PacBio and Oxford Nanopore), and “hybrid” for
116 sequencing approaches using more than one approach (e.g., PacBio and Hi-C).

117

118 A search for amphibian proteome datasets on NCBI RefSeq (O'Leary et al., 2016), Ensembl
119 (Cunningham et al., 2022), and UCSC Genome Browser (Lee et al., 2022) databases on June 24, 2022
120 revealed 11 proteomics datasets.

121

122 A search of the NCBI Organelle database on 15, February 2023 using search term "amphibian"
123 resulted in 353 mitochondrial genomes belonging to 345 species (Table S11). Seventeen
124 mitochondrial genomes overlapped with the amphibian nuclear genomes analyzed in this study.
125

126 ***Reference genome availability summary***

127 The GoaT online database (Sotero-Caio et al., 2021) was searched on August 28, 2023 to summarize
128 genomes in progress or publicly available using the search terms "tax_tree(Amphibia) AND
129 tax_rank(species) AND sequencing_status=in_progress" or "tax_tree(Amphibia) AND
130 tax_rank(species) AND sequencing_status=insdc_open". The same search terms were used to
131 summarize publicly available genomes for mammals, birds, and non-avian reptiles with the
132 "tax_tree" search term replaced by appropriate Class.
133

134 ***Genome quality analyses***

135 Genome quality assessment was performed with BBMap (v. 39.01) "statswrapper.sh" bash script
136 (https://github.com/BioInfoTools/BBMap). This tool generates metrics such as genome size, contig
137 N50, and scaffold count. Benchmarking Universal Single-Copy Orthologs (BUSCO) were summarized
138 with the BUSCO tool (v. 5.1.2) (Manni et al., 2021) using the OrthoDB Tetrapoda ortholog library (v.
139 odb10) (Kriventseva et al., 2018) (N=5310 orthologs) with the prompt "-m genome". Percentage of
140 the genome assembled to chromosomes was calculated with a custom bash script that computes the
141 genome length assigned to chromosomes and divides it by the "assembly length" value computed by
142 BBMap.
143

144 ***Phylogenetic tree***

145 A species to family correspondence table was obtained from Jetz and Pyron (2018)
146 (https://vertlife.org/files_20170703/) and was filtered to include only the species with the longest

147 nucleotide sequence per family. This taxa subset was used to obtain a subset of 100 phylogenetic
148 trees from the posterior distribution of the Jetz and Pyron (2018) dataset, as available
149 from http://vertlife.org/phylosubsets. A consensus tree from these 100 trees was then obtained
150 using treeannotator (v2.7.5) (settings - target tree type: maximum clade credibility, node heights:
151 median burn-in percentage: 0, posterior probability limit: 0.0) (Drummond & Rambaut, 2007). The
152 species names of the tree tips were then substituted with the corresponding family names using the
153 "sub.taxa.label" function in the phylotools package (https://github.com/helixcn/phylotools) in R with
154 the aid of the species to family correspondence table, which was updated with the most recent
155 classification available in AmphibiaWeb (https://amphibiaweb.org) and the Amphibian Species of the
156 World database (https://amphibiansoftheworld.amnh.org/). In cases where these two references
157 disagreed, the AmphibiaWeb taxonomy was used.
158

159 ***Repeat modelling and annotation***

160 Repeats were *de novo* modelled with RepeatModeler (Apptainer v. 1.2.3) (Flynn et al., 2020).
161 Genomes were then annotated using RepeatMasker (v. 4.1.2-p1) (Smit et al., 2013) with a
162 concatenated library of genome-specific repeats generated from RepeatModeler and the Dfam
163 amphibian repeat library (v. Dfam.h5) (Storer et al., 2021). Before annotation, any previous soft
164 masking of the genomes was reversed. The results were summarized using a custom bash and R
165 scripts.
166

167 ***Ecological correlates of transposable element content***

168 Occurrence data for the 51 species were downloaded from the Global Biodiversity Information
169 Facility (GBIF) (https://www.gbif/org/; last accessed February 2024 (full DOI's for each occurrence
170 data set in Table S5). In addition, due to the putative involvement of temperature in TE activity,
171 BioClim variables associated with temperature (Bio1-Bio11) were obtained for the 51 amphibian

172 species (Table S6). As previous studies have explored the relationship between amphibian genome
173 size and environmental variables (Liedtke et al., 2022), here we focused on the relationship between
174 temperature variables, elevation, and amphibian transposable elements. Influence of these
175 bioclimatic variables (after removing highly collinear variables, see supplementary methods) on
176 transposable element content (summarized into three groups: proportion of total transposable
177 elements (TEs), proportion of retroelements, and proportion of DNA transposons) was modelled
178 using Bayesian mixed effect models (Hadfield, 2010). To correct for body size, log transformed body
179 size was included in the model structure with log transformed Bio2 (Mean diurnal range), Bio4
180 (temperature seasonality), Bio8 (mean temperature of wettest quarter), Bio10 (mean temperature of
181 warmest quarter) and elevation. Models were also corrected for phylogenetic non-independence
182 (Figure S5, see Supplementary Methods for further information) with phylogenetically independent
183 contrasts (Felsenstein, 1985; Garland Jr et al., 1992) plotted with and (Revell, 2024).
184

185 ***Synteny analysis***

186 Synteny of BUSCO genes for chromosome level assemblies was analyzed with R Package GENESPACE
187 (v. 1.1.4) (Lovell et al., 2022), which uses OrthoFinder (v. 2.5.4) (Emms & Kelly, 2019) to infer
188 orthology. Synteny was analyzed using BUSCO "full_table.tsv" results files that were reformatted for
189 GENESPACE input using a custom bash script. Synteny plots were generated for all chromosome level
190 assemblies, all anuran chromosome level assemblies, for the two salamander genomes, and for the
191 three caecilian genomes using the GENESPACE plotting tool "plot_riparian". Chromosomes with
192 reversed orientation compared to the reference genome were inverted to improve visualization.
193

194 ***Quantification and statistical analysis***

195 Regression analyses, ANOVAs, and Student's t-tests for comparing genome quality measurements
196 were conducted with the R statistics package (v. 4.1.2) (Team, 2013) in R Studio (v. 2022.02.3) (Team,

197 2022). Genome quality measures, contigN50, and scaffold count, were log transformed prior to
198 analysis. R-scripts for statistical analysis and plotting are available on GitHub at
199 https://doi.org/10.5281/zenodo.7679280.
200

201 **RESULTS**

202 ***Genome quality***

203 A total of 51 nuclear amphibian genome assemblies were available for our study and were generated
204 with a variety of sequencing technologies, including Illumina (NextSeq, HiSeq), PacBio (RS11, Sequel),
205 and Oxford Nanopore. Sequenced genomes represented 25 of 73 amphibian families with reference
206 genomes distributed unevenly across the phylogeny (Fig. 1). For example, there are only two
207 salamander genomes representing the 798 extant species, no genomes representing anuran families
208 such as Leiopelmatidae or Hyperoliidae, yet there are seven Ranidae and six Pipidae genomes (Fig. 1).
209

210 Genome assembly length ranged from 0.48 Gb in *Scaphiopus couchii* to 28.21 Gb in *Ambystoma*
211 *mexicanum* and was strongly positively associated with c-value estimates of genome size (F_{49} = 330.5,
212 $p < 1 \times 10^{-15}$) (Table 1, Fig. S1). Twenty-eight of these genomes were assembled to the chromosome
213 level of which the percentage of the genome assigned to chromosomes ranged from 63.88 to 99.99%
214 (Table 1). Percentage of the genome assigned to chromosomes was positively associated with contig
215 N50 (F_{26} = 8.6, p = 0.007) and read length ($t_{29.2}$ = 3.07, p = 0.005) and negatively associated with the
216 number of scaffolds (F_{26} = 25.2, p < 0.00001). There are additionally mitochondrial genome
217 assemblies for 345 species of which 17 had nuclear reference genomes. Eleven of the species with
218 genomes had proteomics data (Table S1).
219

220 The quality of the amphibian genomes varied considerably (Table 1). Genomes generated with short-
221 read technologies were of lower quality than long-read or hybrid genome assemblies as indicated by
222 significantly lower contig N50s ($F_{2,48}$ = 26.91, p < 10^{-6}), percentage of complete Benchmarking
223 Universal Single-Copy Ortholog (BUSCO) genes (Fig. S3; $F_{2,48}$ = 10.52, p < 0.001), and higher scaffold
224 numbers ($F_{2,48}$ = 15.8, p < 10^{-5}).
225
226 Contig N50 ranged from 362 bp in *S. couchii* to 45.59 Mb in *Pleurodeles waltl* with a median of 611.23
227 Kb. Scaffold count varied considerably from 17 in *Spea bombifrons* to more than four million in
228 *Bombina variegata* with a median of 6.66 Kb (Table 1). Benchmarking Universal Single-Copy
229 Orthologs (BUSCO) scores ranged from 0.7 to 99.5% completeness (Tables 1, S1; Fig. 2) and were
230 positively associated with contig N50 (F_{49} = 82.6, p < 10^{-10}; Fig. S2) and scaffold count (F_{49} = 66.04, p <
231 10^{-8}). Most genomes had low percentages of duplicate BUSCO genes (< 6%), suggesting they may be
232 diploid except for *Ranitomeya imitator* and the known tetraploid species, *X. laevis and X. borealis*
233 (Fig. 2) (Tymowska & Fischberg, 1973).
234
235 ***Repeat content***
236 Overall identified repeat percentage of the genomes ranged from 23% in *Platyplectrum ornatum* to
237 82% in *Oophaga sylvatica* and was positively associated with genome size (F_{49} = 13.24, p = 0.0006)
238 (Tables 1; Fig. S3). Repeat content varied across genomes with the anurans *Pseudophrne corroboree*,
239 *Bombina bombina*, and *O. sylvatica* dominated by Long Terminal Repeats (LTRs), the three caecilians
240 dominated by Long Interspersed Nuclear Elements (LINEs), and many of the ranid and bufonid
241 anurans dominated by DNA transposons (Fig. 3; Tables S2-S4). Salamander genomes *Ambystoma*
242 *mexicanum* and *Pleurodeles waltl* had fewer repeats than might be predicted given their large sizes
243 (Fig. S3).
244

245 The proportion of repeats that could be classified by RepeatMasker ranged from 7.4 % in *P. ornatum*
246 to 47.8 % in *P. corroboree* (Table S1) and was positively associated with genome quality measures
247 contigN50 (F_{49} = 23.49, p = 0.001), scaffold count (F_{49} = 8.71, p = 0.005), and percent BUSCO complete
248 (F_{49} = 10.27, p = 0.002). The ability to classify repeats was also positively associated with read length,
249 with longer reads resulting in better classification ($t_{35.622}$ = 4.73, p < 0.001).
250

251 ***Ecological correlates of transposable element content***

252 A Bayesian mixed effect modelling approach was employed to examine the relationships between
253 proportion of transposable elements and environmental variables. Controlling for phylogenetic
254 relationships (by estimating Pagel's lambda, λ; de Villemereuil & Nakagawa, 2014), including body
255 size as a covariate (Spearman correlation with transposable element content = -0.772, p<0.001) and
256 excluding the three globally invasive species (*Rhinella marina, X. laevis*, and *Lithobates catesbeianus*)
257 our analysis revealed a significant influence (pMCMC = 0.014) of Bio8 (mean temperature of the
258 wettest quarter) on the proportion of total transposable elements (Figs 4, S5; Table S8). Inclusion of
259 these three invasive species did not change this relationship (Table S7). Further analysis indicated
260 that the relationship with Bio8 was not specific to a particular class of transposable elements, such as
261 retroelements or DNA transposons (Tables S9 and S10). Phylogenetic signal (Pagel's lambda, λ) was
262 moderate when considering total transposable elements and retroelements (0.555; Table S7) and
263 increased when we considered retroelements and DNA transposons alone (0.616 and 0.649; Table
264 S9).
265

266 ***Genome synteny***

267 Genome synteny of BUSCO genes was highly conserved within amphibian orders (caecilians (Fig. S7),
268 caudates (Fig. S8), and anurans (Fig. S9); but was less conserved across the amphibian orders (Fig. 5,
269 S6). However, chromosome naming was inconsistent across all taxa (Figs 5, S6-S9). For example, *X.*

270 *tropicalis* chr1 is chr12 in *Leptobrachium ailaonicum* (but not *L. leishanense*), chr2 in *Bufo bufo* (but
271 not *Bufo gargarizans*) (Fig. S9), and most of the chromosomes for the two salamander genomes (Fig.
272 S8). Orientation of chromosomes was also inconsistent, including between species of the same genus
273 (e.g., *Bufo*, *Leptobrachium*) (Fig. S59) and among the three caecilians (Fig. S7). Multiple inversions
274 were evident including between chr3 of pipids *(Xenopus tropicalis* and *Hymenochirus boettgeri*) and
275 other anurans (chromosomes 1, 2, 3, 4, or 10), caecilians (chr3 and chr4/5/6), and even within
276 species of the same genus (chr7 *Bufo gargarizans*, chr 9 *B. bufo*; Figs 5, S7, S9). There was also
277 evidence of several chromosomal fissions including the separation of chr1 of *Leptobrachium*
278 *leishanense* into chr3 and chr6 in *Pyxicephalus adspersus* and into chr3 and chr7 in *Engystomops*
279 *pustulosus;* however, this chromosome remained mostly intact in the other anuran genomes (Fig.
280 S9).

281 **DISCUSSION**

282 In this study we analyzed 51 amphibian reference genomes from the public domain to evaluate their
283 content and usefulness for functional genetics research (Fig. 1, Table 1). There are considerably
284 fewer reference genomes for amphibians than exist for birds (N=754), mammals (N=406), and non-
285 avian reptiles (N=108). This scarcity of reference genomes results in many gaps in genome
286 representation across the amphibian tree of life including many entirely unrepresented groups and
287 with only two genomes representing the entire order Caudata (but see Myers & Pyron, 2024). The
288 unrepresented families include many of interest from a conservation perspective due to their high
289 number of IUCN RedList Critically Endangered species (e.g., Cryptobranchidae, Plethodontidae,
290 Strabomantidae, and Craugastoridae) (IUCN, 2022). However, our search of the Genomes on a Tree
291 (GoaT) database (Sotero-Caio et al., 2021) indicated that there are a further 20 amphibian genome
292 assemblies in progress (15 anurans, 5 caudates; Table S10) indicating that this resource will be
293 increasing by more than 40% in the next few years.
294

295 The quality and completeness of the genomes in our dataset varied considerably (e.g., Fig. 2). Much
296 of this variation can be attributed to the sequencing technology used to generate them, with short-
297 read sequencing approaches resulting in lower completeness and continuity (Fig. S2). These impacts
298 are a recognized limitation of short-read sequencing and have been reported to impact genome
299 quality in taxa from insects (Hotaling et al., 2021b) to other vertebrates (Rhie et al., 2021), but have
300 likely had a disproportionate impact in amphibian genomes due to the difficulty of assembling
301 genomes with high repeat content (Sun et al., 2020). Fortunately, most ongoing sequencing efforts
302 now use long-read or hybrid sequencing approaches (i.e., that incorporate scaffolding technologies
303 such as Hi-C sequencing), which along with improved sequencing algorithms, should result in higher
304 quality amphibian genomes (Hotaling et al., 2021a; Lawniczak et al., 2022; Rhie et al., 2021).
305
306 The variation we report here in genome quality, contiguity, and completeness may impact the value
307 of the genomes for functional genomics research. However, the improvements in all these measures
308 seen with the utilization of long read technologies or hybrid assemblies suggests that genome quality
309 will continue to improve as these approaches are used more frequently. Genome quality (i.e., high
310 continuity, contiguity, accuracy, completeness (Rhie et al., 2021)) are critical for applications such as
311 quantitative genetics where assembly errors can lead to incorrect inferences in genetic association or
312 genetic prediction. Quality also enhances the usefulness of genomes. For example, highly contiguous
313 chromosome-level assemblies decrease computational requirements for downstream analyses such
314 as mapping, variant calling, and alignment (Aganezov et al., 2022).
315
316 One of the most intriguing features of amphibian genomes is the huge range they exhibit in size
317 (Biscotti et al., 2019). This was exemplified in our dataset where assembly length ranged from 0.48
318 Gb in *Scaphiopus couchii* to 28 Gb in *Ambystoma mexicanum*. Why gigantic genomes exist in some
319 species, but not others, remains a key evolutionary question (Kapusta et al., 2017; Wang et al., 2021).

320 Explanations include differences in genome-level processes (e.g., insertion and deletion rates)
321 (Frahry et al., 2015; Sun et al., 2012b), development (e.g., developmental rate and complexity)
322 (Gregory, 2002; Liedtke et al., 2018), physiology (e.g., water loss) (Johnson et al., 2021), body size
323 (e.g. miniaturization) (Decena-Segarra et al., 2020), and demography (e.g., effective population size)
324 (Liedtke et al., 2018; Lynch & Walsh, 2007) (but see Mohlhenrich & Mueller, 2016). As more
325 amphibian genomes become available, these hypotheses can be more rigorously evaluated.
326
327 We report some of the largest estimates of repeat content of any vertebrate (82% in *Oophaga*
328 *sylvatica* and 77% in *Rana muscosa*), exceeded only by the Australian lungfish at 90% (Meyer et al.,
329 2021). As expected, genome size was correlated with repeat content affirming that much of the
330 variation in amphibian genome size is due to an excess of repeats and transposable elements rather
331 than coding regionds (Biscotti et al., 2019; Lamichhaney et al., 2021; Zuo et al., 2023).
332
333 In contrast to mammals, whose repeat landscape is mainly dominated by LTR retrotransposons (Platt
334 et al., 2018), amphibian repeat content varied considerably with some species dominated by DNA
335 transposons (as previously reported (Suda et al., 2022; Zuo et al., 2023), and others by non-LTR
336 retrotransposons including the three caecilian genomes which were dominated by LINEs. This agrees
337 with genomic data and transcriptomic data from the caecilian *Ichthyophis bannanicus*, where LINEs
338 were the second most abundant type of repeat (26% of the genome) behind Dictyostelium
339 intermediate repeat sequences (DIRS) (30%) (Wang et al., 2021); this is a similar percentage of LINES
340 to what we report in the three caecilian genomes in this study (19 to 26%) (Table S4).
341
342 These disparities in repeat percentage and content likely reflect differing evolutionary histories
343 among species, as indicated by three of the four congeneric species pairs in our dataset having
344 similar values (i.e., *Bufo*, *Leptobrachium,* and *Xenopus*; but not *Oophaga*). The differences we

345 observed in *Oophaga pumilio* and *O. sylvatica* are likely due to assembly quality rather than genome
346 content given that these two genomes were sequenced with different technologies and have
347 dramatically different genome qualities (e.g., contig N50s of 5.8 vs. 97.8 Kbp respectively).
348
349 A considerable proportion of the repeats could not be classified. This was likely due to incorrect
350 classification (e.g., genes categorized as repeats) and the lack of good amphibian-specific repeat
351 resources (Ou et al., 2019) for classification via nucleotide sequence homology. The majority of
352 amphibian curated repeat libraries are generated in reference to *Xenopus* species (e.g., Dfam); the
353 large divergence times of this genus from the other amphibian species suggests that it may be a
354 contributing factor to the lack of classification. However, we also report many unclassified repeats in
355 the two *Xenopus* genomes.
356
357 The largest genomes in our dataset from caudates, *A. mexicanum and P. waltl*, had fewer repeats
358 than predicted given their size (Fig S4) (Nowoshilow et al., 2018). This may be due, in part, to the
359 Dfam (Storer et al., 2021) library used for repeat annotation being anuran-based; however, we did
360 not observe this trend in the three caecilian genomes in our dataset. Also, we performed *de novo*
361 annotation of these genomes, which should have captured repetitive elements missing from Dfam.
362 More likely, this low number of repeats reflects low deletion rates and, thus, persistence of repeats
363 in the genome for extremely long periods of time, leading to their mutational decay into unique
364 sequences whose repetitive origin is obscured (Frahry et al., 2015; Keinath et al., 2015; Novák et al.,
365 2020; Sun et al., 2012a).
366
367 We also show that amphibian species that inhabit warm climates particularly during months with
368 high precipitation have a greater proportion of transposable elements. This observed trend does not
369 appear to be driven by a specific group of transposons suggesting it may be caused by climatic

370 factors. Recent studies indicate that transposable elements exhibit greater activity in hotter climates
371 (Baduel et al., 2021) with an increasing number of studies suggesting increased transposable element
372 activity contributes to genetic diversification and facilitates species adaptation (Li et al., 2018;
373 Schrader & Schmitz, 2019; Stapley et al., 2015). The pattern observed here likewise suggests the
374 potential for heightened transposable element activity and may help explain transposable element
375 accumulation and potentially the higher evolutionary rates observed in the genomes of tropical
376 amphibians (Pyron & Wiens, 2013).
377
378 Our study is the first to examine chromosomal synteny across all amphibian orders. We show that
379 overall synteny of amphibian genomes is relatively conserved, particularly within orders (Figs 5 and
380 S7). This aligns with previous results from anurans that reported conserved genome organization in
381 this group (Bredeson et al., 2021; Wu et al., 2022). However, chromosome content and number
382 varied across species, which seems to have been driven by multiple occurrences of chromosomal
383 fusions and fissions (e.g., Fig. 5). Chromosomal rearrangements have occurred throughout vertebrate
384 evolution, including the hypothesized fusion of microchromosomes in the ancestor of tetrapods to
385 create the larger macrochromosomes seen in amphibians and mammals and their subsequent fission
386 to create the microchromosomes of modern birds and non-avian reptiles (Waters et al., 2021).
387
388 Some of the structural rearrangements we detected may be due to assembly errors and should be
389 evaluated in future assemblies using long-read scaffolding approaches (e.g., Oxford nanopore
390 sequencing), chromosome conformation capture technologies (e.g., Hi-C), or chromosome mapping
391 approaches (e.g., FISH). We also identified incongruities with chromosome naming and orientation
392 caused by differences in assembly methods. These were apparent even within species of the same
393 genus (e.g., *Bufo*). We suggest potential revisions of existing genome annotations to improve

394 congruity and that future assemblies are curated consistently against high-quality reference genomes
395 (e.g., *Xenopus laevis*).
396

397 **Conclusions**

398 New sequencing technologies and assembly algorithms have resulted in a good number of genomes
399 for comparative analyses spanning the amphibian phylogeny. This has already begun to yield
400 important insights on the evolution (Lamichhaney et al., 2021; Wu et al., 2022), development
401 (Schloissnig et al., 2021; Stuckert et al., 2021), sex determination (Hime et al., 2019; Ma & Veltsos,
402 2021), and unique features (Fischer et al., 2019; Nowoshilow et al., 2018; Seidl et al., 2019) of this
403 interesting group of animals.
404

405 The increased availability of amphibian genomes can also aid conservation efforts in this highly
406 threatened group by facilitating research on genome-wide functional diversity, which can be used to
407 inform management decisions such as genetic rescue or targeted genetic intervention for species
408 threatened by habitat loss or chytridiomycosis (Chestnut et al., 2014; Kosch et al., 2022).
409 Additionally, well-annotated genomes can be used to create eDNA assays for population monitoring
410 (Breton et al., 2022; Saeed et al., 2022).
411

412 Future research efforts should focus on generating more reference genomes to fill the gaps in the
413 amphibian phylogeny and the identification of advantageous genetic traits against threats. Efforts
414 should also be made to increase the quality of genomes and expand transcriptome and annotation
415 databases. We suggest that these efforts strive to follow the recommendations of initiatives such as
416 the Earth BioGenome Project (Lawniczak et al., 2022), the Darwin Tree of Life Project (Blaxter et al.,
417 2022), and the Threatened Species Initiative (Hogg et al., 2022) to sequence at least one
418 representative from each family to ensure taxonomic coverage. Species selection should prioritize

species of interest for understanding valuable functional genetic traits; for example, for the purpose of immunological research to understand disease resistance, or for conservation purposes to enhance fitness.

ACKNOWLEDGEMENTS

T.A.K, N.D.Y, and L.F.S research were supported by The University of Melbourne's Research Computing Services and the Petascale Campus Initiative. T.A.K. and L.F.S were supported by Australian Research Council Grants (FT190100462, LP200301370). K.W.V and M.L.P were funded by the European Union (ERC, MolStressH2O, 101044202). Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Research Council Executive Agency. Neither the European Union nor the granting authority can be held responsible for them. We are grateful to A. Stuckert, N. Brajuka, J. Sproul, and E. Tescari for their advice on repeat modelling, and J.T. Li for providing suggestions on synteny analyses. We thank M. Moore for exploratory repeat analyses.

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779

780 **DATA ACCESSIBILITY AND BENEFIT-SHARING**

781 ***Data Accessibility Statement***

782 **Genetic data:**

783 All the genomes used in this study are available on the NCBI Genomes database

784 (https://www.ncbi.nlm.nih.gov/genome/).

785 **Code:**

786 All original code has been deposited on GitHub and is publicly available at

787 (https://doi.org/10.5281/zenodo.7679280).

788

789 **AUTHOR CONTRIBUTIONS**

790 Conceptualization, T.A.K., A.J.C., L.A.O., A.R., and K.C.W.V; methodology, T.A.K, N.D.Y, R.L.M, and

791 A.R.; formal analysis, T.A.K., M.L.P.; investigation, T.A.K., N.D.Y, R.L.M., K.C.W.V, M.L.P.; resources,

792 L.A.0. and A.R.; writing – original draft, T.A.K.; writing – review & editing, all authors; project

793 administration, T.A.K; funding acquisition, T.A.K. and L.F.S.

794

TABLES AND FIGURES

Table 1. Genome quality measures.

	Order	Family	N chrs	Assembly ID	N scaf	Genome size (Gb)	Scaf N50 (kb)	Cont N50 (kb)	% chrs	% assembled
	Anura	Aromobatidae	12	in progress	6,658	5.32	625,512	879	0.00	76.60
	Anura	Bombinatoridae	12	GCA_027579735.1	2,468	10.02	1,241,341	3,760	97.73	90.00
	Anura	Bombinatoridae	12	GCA905336975.1	4,065,339	4.68	2	2	0.00	52.20
	Anura	Bufonidae	11	GCA905171765.1	1,307	5.04	635,713	3,960	98.92	78.20
	Anura	Bufonidae	11	GCA014858855.1	747	4.55	539,822	1,738	96.21	83.00
s	Anura	Hylidae	15	GCA_027789765.1	2,200	2.21	153,439	8,808	94.14	95.60
	Anura	Alytidae	14	GCA_027410445.1	1,318	3.87	453,718	3,044	95.72	75.40
	Anura	Eleutherodactylidae	13	GCA019857665.1	105,233	2.79	109,467	11	63.88	73.50
	Anura	Leptodactylidae	11	GCA019512145.1	108,304	2.56	172,109	305	75.27	108.00
	Anura	Microhylidae	11	GCA_027917425.1	1,002	4.34	489,283	4,963	98.02	91.00
	Anura	Ranidae	13	GCA018402905.1	561,726	7.63	748	11	0.00	95.70
manni	Anura	Centrolenidae	10	in progress	227,850	4.04	295,404	10	0.00	126.20
	Anura	Hylidae	12	GCA_029499605.1	3,474	4.14	384,967	3,809	90.48	96.00
	Anura	Pipidae	12	GCA019447015.1	25,835	3.21	293,321	784	82.53	135.10
m	Anura	Megophryidae	13	GCA018994145.1	5,370	3.54	412,425	821	98.22	129.60
e	Anura	Megophryidae	13	GCA009667805.1	5,302	3.55	394,693	1,946	93.34	130.10
	Anura	Leptodactylidae	11	GCA_947044405.1	3,509	2.51	4,630	4,630	0.00	79.90
	Anura	Limnodynastidae	11	GCA011038615.1	520,896	2.38	286	11	0.00	74.80
	Anura	Ranidae	13	GCA002284835.2	1,544,635	6.25	39	6	0.00	85.20
	Anura	Ranidae	13	GCA_028564925.1	4,861	5.15	611,110	2,499	98.80	90.30
	Anura	Mantellidae	13	in progress	135,214	3.41	324,859	14	0.00	72.60
	Anura	Dicroglossidae	13	GCA000935625.1	25,187	2.05	1,069	33	0.00	50.60
	Anura	Dendrobatidae	10	GCA009801035.1	125,383	3.49	78	6	0.00	39.90
	Anura	Dendrobatidae	10	in progress	15,287	5.19	571,703	98	0.00	59.30
	Anura	Pelobatidae	13	GCA_933207985.1	3,574	3.09	330,124	130	98.65	75.10
i	Anura	Dicroglossidae	13	GCA_022657655.1	1,446,664	1.83	1	1	0.00	45.10
	Anura	Hylidae	26	GCA_025379985.1	109,372	4.74	78	78	0.00	72.70
	Anura	Pipidae	10	GCA_021901965.1	830,118	1.19	2	2	0.00	50.20
	Anura	Pipidae	15	GCA019650415.1	283,592	1.37	7	6	0.00	57.70
	Anura	Limnodynastidae	11	GCA016617825.1	148,035	1.07	27	5	0.00	46.90
	Anura	Myobatrachidae	12	GCA_028390025.1	3,127	8.87	846,895	6,819	92.31	103.70
	Anura	Pyxicephalidae	13	GCA004786255.1	5,411	1.56	157,522	30	98.54	114.20
	Anura	Ranidae	12	GCA_029574335.1	2,983	4.83	547,819	1,704	92.76	92.50
	Anura	Ranidae	13	GCA_029206835.1	5,628	10.16	938,497	4,644	85.63	194.50
	Anura	Ranidae	13	GCA905171775.1	555	4.11	481,763	6,265	98.26	93.60
	Anura	Dendrobatidae	9	GCA_958301615.1	79,800	6.02	390	309	0.00	68.80
	Anura	Bufonidae	11	GCA900303285.1	31,391	2.55	167	167	0.00	54.80
	Anura	Scaphiopodidae	13	GCA009364435.1	1,378,905	0.48	0	0	0.00	46.30
	Anura	Scaphiopodidae	13	GCA009364455.1	1,509,266	0.71	1	1	0.00	51.90
	Anura	Pelobatidae	13	GCA_027358695.2	17	0.99	114,150	21,170	99.99	81.80
	Anura	Pelobatidae	13	GCA_029215755.1	351	1.16	120,843	14,336	0.00	81.80
	Anura	Pelobatidae	13	GCA009364415.1	49,736	1.08	72	31	0.00	82.10
	Anura	Ranidae	13	GCA_951230385.1	22,068	3.98	628	611	0.00	63.80
	Anura	Pipidae	18	GCA_024363595.1	23,435	2.75	143,989	21	96.13	78.90
	Anura	Pipidae	18	GCA017654675.1	54	2.74	155,251	22,452	99.97	89.00
	Anura	Pipidae	10	GCA000004195.4	166	1.45	153,961	14,634	99.86	85.30
	Caudata	Ambystomatidae	14	GCA002915635.3	27,157	28.21	1,205,707	218	94.77	81.20
	Caudata	Salamandridae	12	GCA_026652325.1	267	20.30	1,237,755	45,590	99.61	97.00
	Gymnophiona	Dermophiidae	19	GCA902459505.2	164	3.78	272,612	20,657	99.85	82.40
	Gymnophiona	Siphonopidae	12	GCA901765095.2	1,081	4.69	376,147	3,662	97.06	34.30
	Gymnophiona	Rhinatrematidae	14	GCA901001135 2	1 330	5 32	486 875	3 216	97 36	59 90

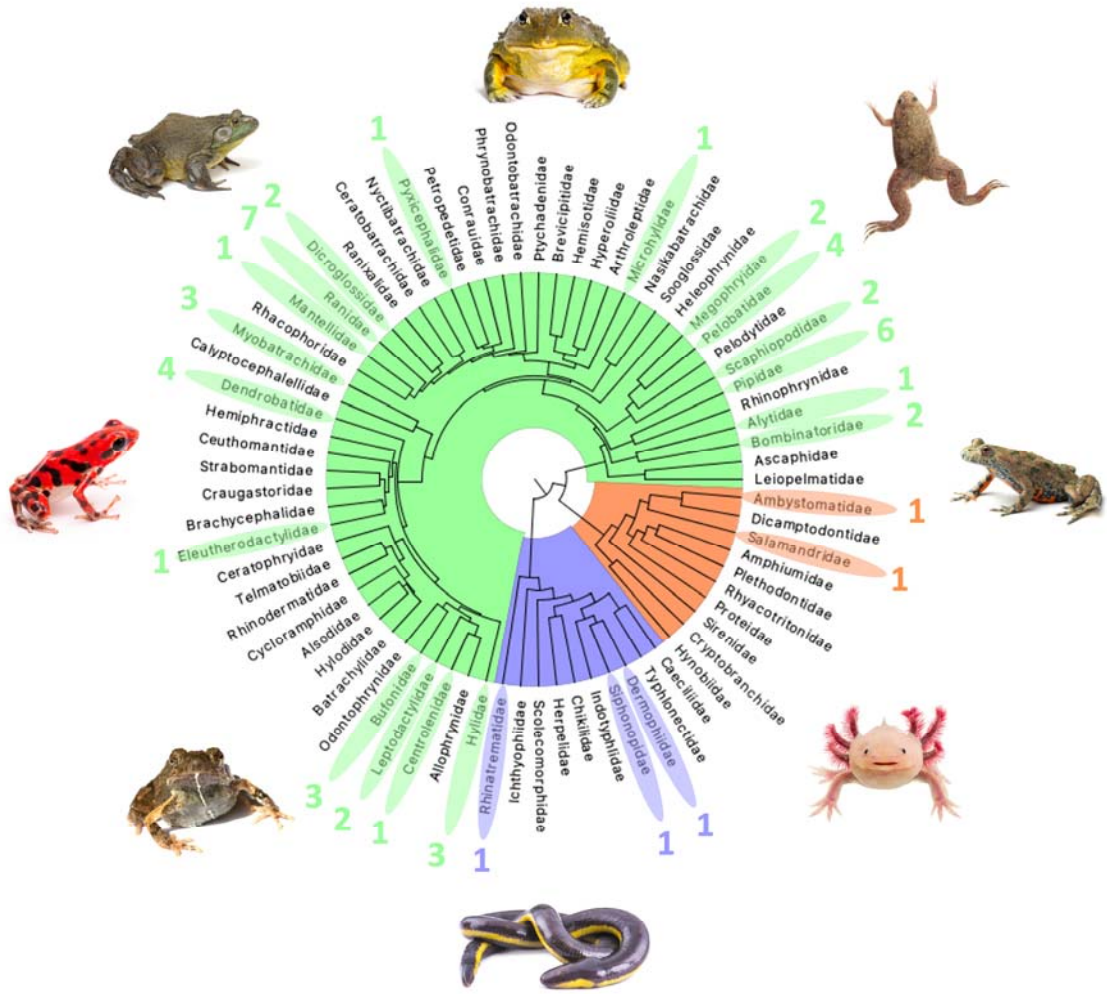

Figure 1. Phylogenetic tree of amphibian families. Amphibian families with representative genomes are highlighted and numbers indicate genome counts per family. (Green) anurans, (blue) caecilians, and (orange) salamanders. *Engystomops pustulosus* (Family) image was taken by B. Gratwicke, other amphibian images were licensed to T. Kosch by Adobe Stock and Shutterstock.

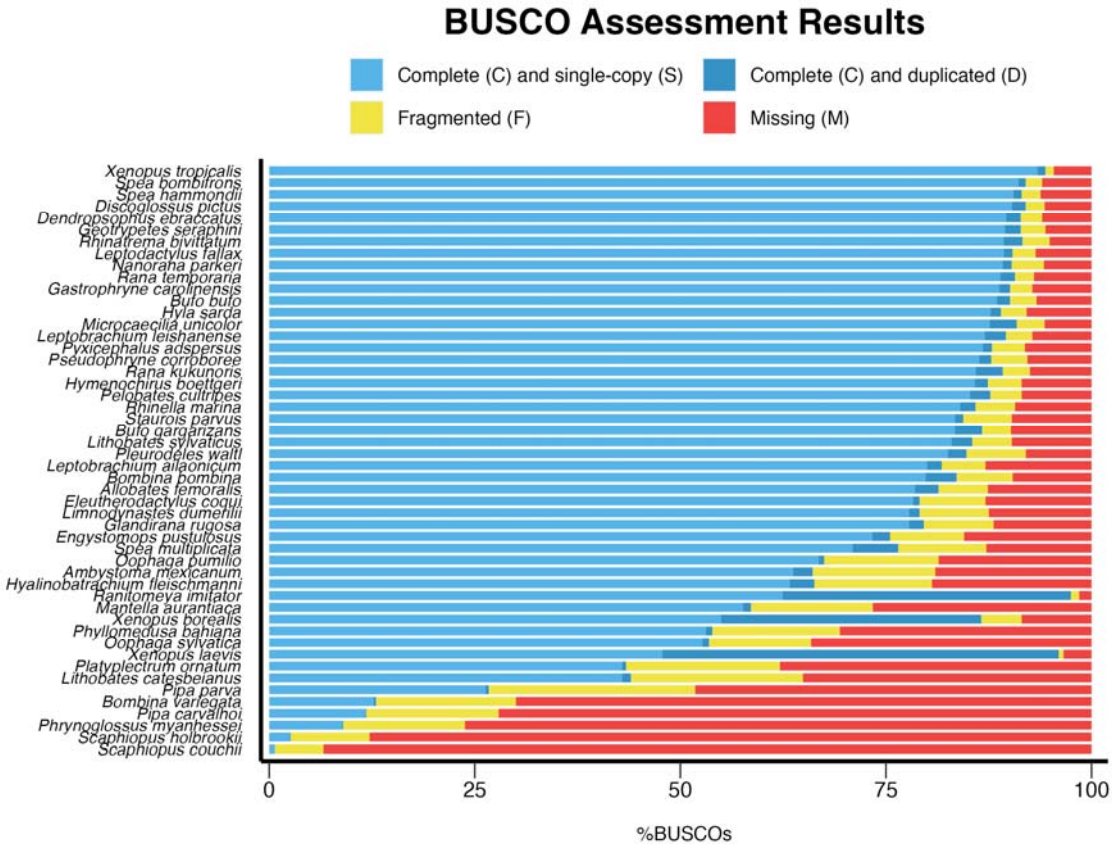

Figure 2. BUSCO (Benchmarking Universal Single-Copy Orthologs) assessment results for amphibian genomes.

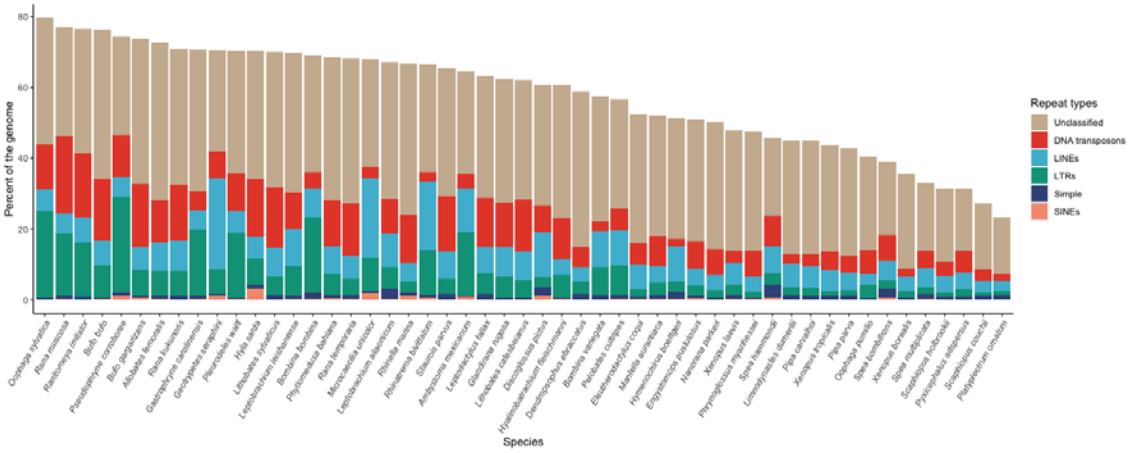

Figure 3. Repeat content across the amphibian genomes. (LINEs) long interspersed nuclear elements, (LTRs) long terminal repeats, and (SINEs) short interspersed nuclear elements.

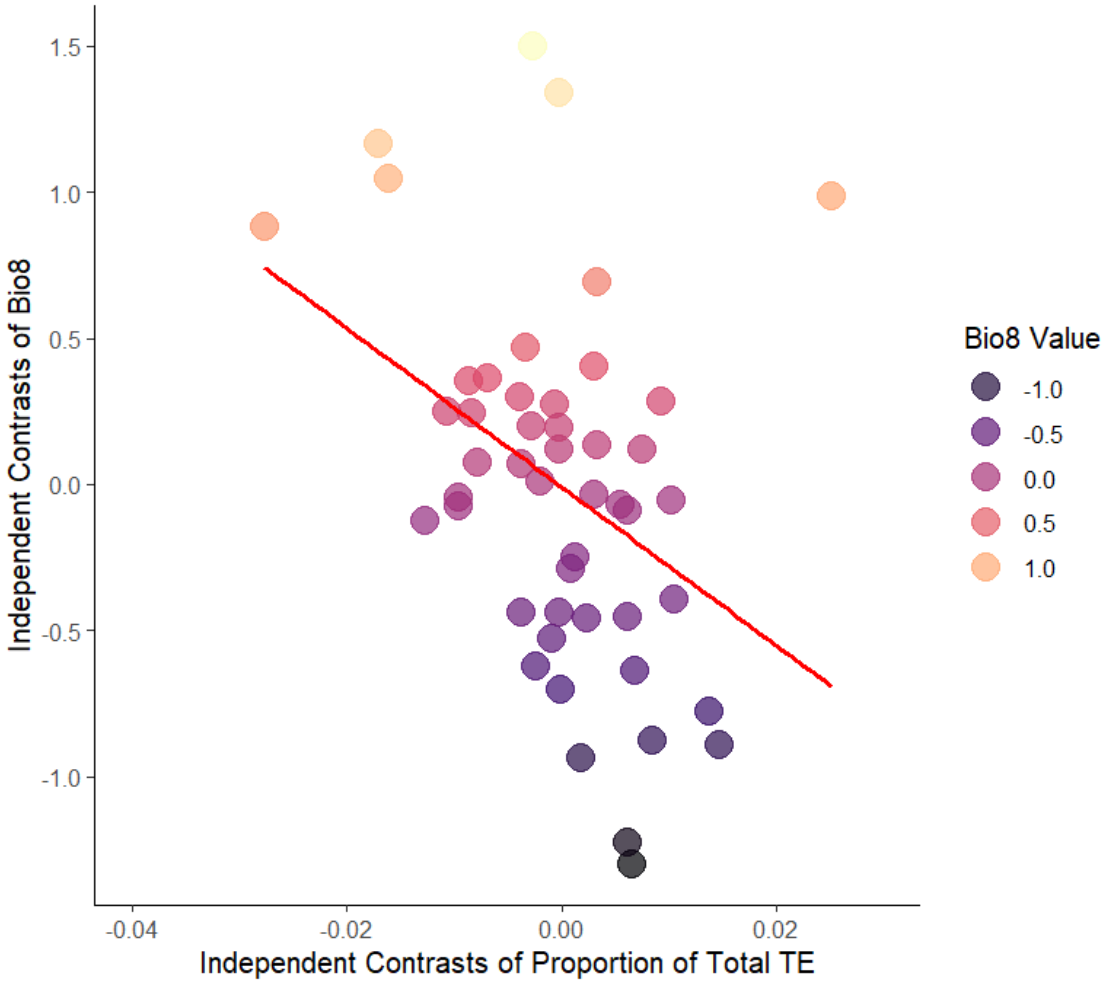

Figure 4. Phylogenetic independent contrasts (PICs) between the proportion of transposable element content relative to genome size and Bio8 (representing mean temperature of wettest quarter).

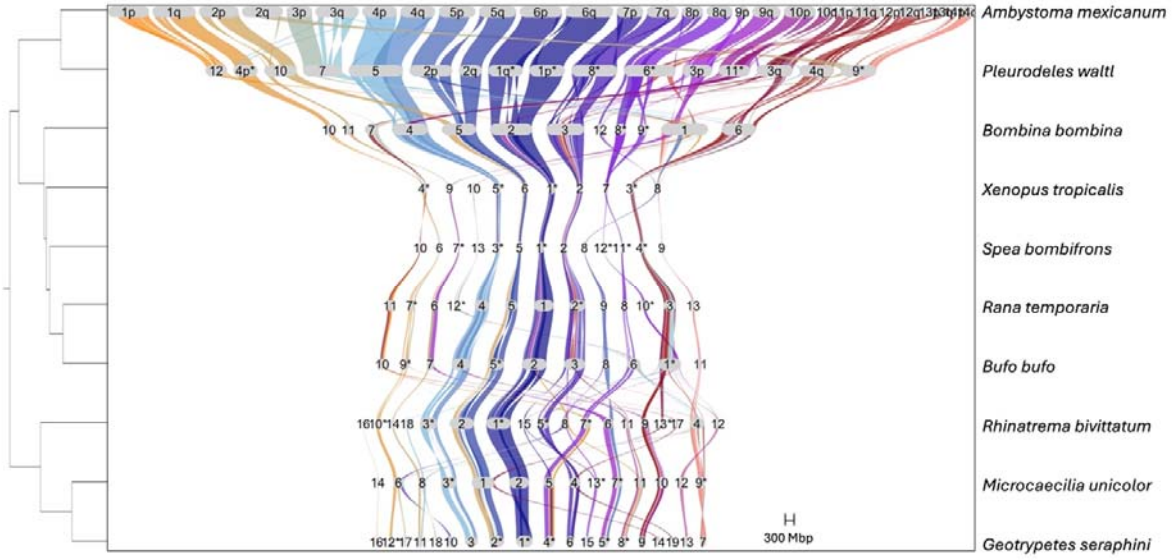

Figure 5. Synteny plot of BUSCOs (Benchmarking Universal Single-Copy Orthologs) for representative amphibian chromosome-level genomes. The phylogenetic tree was created with Timetree.org. The reference genome is *Ambystoma mexicanum*. *Indicate inverted chromosomes. Chromosomes without BUSCOs were excluded from the plot.

Species	Order	Family	N chrs	Assembly ID	N scaf	Genome size (Gb)	Scaf N50 (kb)	Cont N50 (kb)	% chrs	% assembled	% repeats	BUSCO % complete
Allobates femoralis	Anura	Aromobatidae	12	in progress	6,658	5.32	625,512	879	0.00	76.60	73.52	81.37
Bombina bombina	Anura	Bombinatoridae	12	GCA_027579735.1	2,468	10.02	1,241,341	3,760	97.73	90.00	69.56	83.62
Bombina variegata	Anura	Bombinatoridae	12	GCA905336975.1	4,065,339	4.68	2	2	0.00	52.20	57.73	13.03
Bufo bufo	Anura	Bufonidae	11	GCA905171765.1	1,307	5.04	635,713	3,960	98.92	78.20	76.92	90.11
Bufo gargarizans	Anura	Bufonidae	11	GCA014858855.1	747	4.55	539,822	1,738	96.21	83.00	73.92	86.74
Dendropsophus ebraccatus	Anura	Hylidae	15	GCA_027789765.1	2,200	2.21	153,439	8,808	94.14	95.60	59.10	91.37
Discoglossus pictus	Anura	Alytidae	14	GCA_027410445.1	1,318	3.87	453,718	3,044	95.72	75.40	64.10	92.05
Eleutherodactylus coqui	Anura	Eleutherodactylidae	13	GCA019857665.1	105,233	2.79	109,467	11	63.88	73.50	52.96	79.11
Engystomops pustulosus	Anura	Leptodactylidae	11	GCA019512145.1	108,304	2.56	172,109	305	75.27	108.00	51.56	75.48
Gastrophryne carolinensis	Anura	Microhylidae	11	GCA_027917425.1	1,002	4.34	489,283	4,963	98.02	91.00	71.17	90.06
Glandirana rugosa	Anura	Ranidae	13	GCA018402905.1	561,726	7.63	748	11	0.00	95.70	62.54	79.62
Hyalinobatrachium fleischmanni	Anura	Centrolenidae	10	in progress	227,850	4.04	295,404	10	0.00	126.20	61.12	66.27
Hyla sarda	Anura	Hylidae	12	GCA_029499605.1	3,474	4.14	384,967	3,809	90.48	96.00	70.86	88.96
Hymenochirus boettgeri	Anura	Pipidae	12	GCA019447015.1	25,835	3.21	293,321	784	82.53	135.10	51.76	87.38
Leptobrachium ailaonicum	Anura	Megophryidae	13	GCA018994145.1	5,370	3.54	412,425	821	98.22	129.60	67.52	81.79
Leptobrachium leishanense	Anura	Megophryidae	13	GCA009667805.1	5,302	3.55	394,693	1,946	93.34	130.10	70.10	89.55
Leptodactylus fallax	Anura	Leptodactylidae	11	GCA_947044405.1	3,509	2.51	4,630	4,630	0.00	79.90	63.55	90.45
Limnodynastes dumerilii	Anura	Limnodynastidae	11	GCA011038615.1	520,896	2.38	286	11	0.00	74.80	46.17	79.10
Lithobates catesbeianus	Anura	Ranidae	13	GCA002284835.2	1,544,635	6.25	39	6	0.00	85.20	62.41	44.05
Lithobates sylvaticus	Anura	Ranidae	13	GCA_028564925.1	4,861	5.15	611,110	2,499	98.80	90.30	70.19	85.44
Mantella aurantiaca	Anura	Mantellidae	13	in progress	135,214	3.41	324,859	14	0.00	72.60	52.28	58.57
Nanorana parkeri	Anura	Dicroglossidae	13	GCA000935625.1	25,187	2.05	1,069	33	0.00	50.60	50.45	90.40
Oophaga pumilio	Anura	Dendrobatidae	10	GCA009801035.1	125,383	3.49	78	6	0.00	39.90	40.55	67.57
Oophaga sylvatica	Anura	Dendrobatidae	10	in progress	15,287	5.19	571,703	98	0.00	59.30	81.63	53.47
Pelobates cultripes	Anura	Pelobatidae	13	GCA_933207985.1	3,574	3.09	330,124	130	98.65	75.10	57.39	87.74
Phrynoglossus myanhessei	Anura	Dicroglossidae	13	GCA_022657655.1	1,446,664	1.83	1	1	0.00	45.10	47.65	8.96
Phyllomedusa bahiana	Anura	Hylidae	26	GCA_025379985.1	109,372	4.74	78	78	0.00	72.70	69.02	53.94
Pipa carvalhoi	Anura	Pipidae	10	GCA_021901965.1	830,118	1.19	2	2	0.00	50.20	45.27	11.81
Pipa parva	Anura	Pipidae	15	GCA019650415.1	283,592	1.37	7	6	0.00	57.70	42.92	26.72
Platyplectrum ornatum	Anura	Limnodynastidae	11	GCA016617825.1	148,035	1.07	27	5	0.00	46.90	23.42	43.45
Pseudophryne corroboree	Anura	Myobatrachidae	12	GCA_028390025.1	3,127	8.87	846,895	6,819	92.31	103.70	75.86	87.85
Pyxicephalus adspersus	Anura	Pyxicephalidae	13	GCA004786255.1	5,411	1.56	157,522	30	98.54	114.20	31.64	87.93
Rana kukunoris	Anura	Ranidae	12	GCA_029574335.1	2,983	4.83	547,819	1,704	92.76	92.50	71.31	89.15
Rana muscosa	Anura	Ranidae	13	GCA_029206835.1	5,628	10.16	938,497	4,644	85.63	194.50	77.28	89.19
Rana temporaria	Anura	Ranidae	13	GCA905171775.1	555	4.11	481,763	6,265	98.26	93.60	68.52	90.72
Ranitomeya imitator	Anura	Dendrobatidae	9	GCA_958301615.1	79,800	6.02	390	309	0.00	68.80	76.94	97.50
Rhinella marina	Anura	Bufonidae	11	GCA900303285.1	31,391	2.55	167	167	0.00	54.80	67.02	85.89
Scaphiopus couchii	Anura	Scaphiopodidae	13	GCA009364435.1	1,378,905	0.48	0	0	0.00	46.30	27.34	0.70
Scaphiopus holbrookii	Anura	Scaphiopodidae	13	GCA009364455.1	1,509,266	0.71	1	1	0.00	51.90	31.65	2.60
Spea bombifrons	Anura	Pelobatidae	13	GCA_027358695.2	17	0.99	114,150	21,170	99.99	81.80	39.55	91.96
Spea hammondii	Anura	Pelobatidae	13	GCA_029215755.1	351	1.16	120,843	14,336	0.00	81.80	46.92	91.51
Spea multiplicata	Anura	Pelobatidae	13	GCA009364415.1	49,736	1.08	72	31	0.00	82.10	33.15	76.59
Staurois parvus	Anura	Ranidae	13	GCA_951230385.1	22,068	3.98	628	611	0.00	63.80	65.85	84.39
Xenopus borealis	Anura	Pipidae	18	GCA_024363595.1	23,435	2.75	143,989	21	96.13	78.90	35.67	86.61
Xenopus laevis	Anura	Pipidae	18	GCA017654675.1	54	2.74	155,251	22,452	99.97	89.00	48.23	95.95
Xenopus tropicalis	Anura	Pipidae	10	GCA000004195.4	166	1.45	153,961	14,634	99.86	85.30	44.21	94.44
Ambystoma mexicanum	Caudata	Ambystomatidae	14	GCA002915635.3	27,157	28.21	1,205,707	218	94.77	81.20	65.91	66.06
Pleurodeles waltl	Caudata	Salamandridae	12	GCA_026652325.1	267	20.30	1,237,755	45,590	99.61	97.00	71.01	84.82
Geotrypetes seraphini	Gymnophiona	Dermophiidae	19	GCA902459505.2	164	3.78	272,612	20,657	99.85	82.40	71.22	91.39
Microcaecilia unicolor	Gymnophiona	Siphonopidae	12	GCA901765095.2	1,081	4.69	376,147	3,662	97.06	34.30	68.35	90.89
Rhinatrema bivittatum	Gymnophiona	Rhinatrematidae	14	GCA901001135.2	1,330	5.32	486,875	3,216	97.36	59.90	66.99	91.58