The Amphibian Genomics Consortium: advancing genomic and genetic resources for amphibian research and conservation

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Abstract

Amphibians represent a diverse group of tetrapods, marked by deep divergence times between their three systematic orders and families. Studying amphibian biology through the genomics lens increases our understanding of the features of this animal class and that of other terrestrial vertebrates. The need for amphibian genomics resources is more urgent than ever due to the increasing threats to this group. Amphibians are one of the most imperiled taxonomic groups, with approximately 41% of species threatened with extinction due to habitat loss, changes in land use patterns, disease, climate change, and their synergistic effects. Amphibian genomics resources have provided a better understanding of ontogenetic diversity, tissue regeneration, diverse life history and reproductive modes, anti-predator strategies, and resilience and adaptive responses. They also serve as critical models for understanding widespread genomic characteristics, including evolutionary genome expansions and contractions given they have the largest range in genome sizes of any animal taxon and multiple mechanisms of genetic sex determination. Despite these features, genome sequencing of amphibians has significantly lagged behind that of other vertebrates, primarily due to the challenges

of assembling their large, repeat-rich genomes and the relative lack of societal support. The advent of long-read sequencing technologies, along with computational techniques that enhance scaffolding capabilities and streamline computational workload is now enabling the ability to overcome some of these challenges. To promote and accelerate the production and use of amphibian genomics research through international coordination and collaboration, we launched the Amphibian Genomics Consortium (AGC) in early 2023. This burgeoning community already has more than 282 members from 41 countries (6 in Africa, 131 in the Americas, 27 in Asia, 29 in Australasia, and 89 in Europe). The AGC aims to leverage the diverse capabilities of its members to advance genomic resources for amphibians and bridge the implementation gap between biologists, bioinformaticians, and conservation practitioners. Here we evaluate the state of the field of amphibian genomics, highlight previous studies, present challenges to overcome, and outline how the AGC can enable amphibian genomics research to “leap” to the next level.

Keywords

Amphibians, Biodiversity conservation, Comparative genomics, Genomics, Lissamphibia, Metagenomics, Phylogenomics, Population genomics, Taxonomy, Transcriptomics.

State of the field of amphibian genomics

In 2010, the genome of the Western clawed frog (*Xenopus tropicalis*) was sequenced, marking the first genome assembly for Class Amphibia [1]. This species serves as a crucial laboratory model organism for cell biology, molecular genetics, and developmental biology [2]. The first amphibian genome assembly came years after the completion of the first genomes for other vertebrate groups: fishes in 2002

(*Fugu rubripes*; [3]), mammals in 2003 (*Homo sapiens*; [4]), birds in 2004 (*Gallus gallus*; [5]), and reptiles in 2007 (*Anolis carolinensis*; Anolis Genome Project https://www.broadinstitute.org/anolis/anolis-genome-project). Since then, the generation and annotation of amphibian reference genomes has dramatically lagged behind those of other vertebrates [6], even though amphibians represent nearly 22% of all tetrapods [7]. Nearly 15 years later, amphibians are still the tetrapod class with the lowest number of sequenced genomes (111 genomes of 8648 described amphibian species being the tetrapod class with the second lowest proportion after Reptiles [database records accessed on 1 March 2024], Fig. 1A and Supplementary File 1). This is likely attributable to the size of amphibian genomes, which are generally larger than the genomes of other terrestrial vertebrates (Fig. 1B and Fig. S1; see Supplementary Material for methodological information). Indeed, among all vertebrates, only the genomes of lungfish are larger (up to 130 Gb) than the largest amphibian genomes (up to ~120 Gb in *Necturus lewisi*) [8-10].

To lower cost and enhance feasibility, early amphibian genome sequencing projects tended to select species with comparatively small genomes (Fig. 1B). This has resulted in the disproportionately fewer sequenced salamander genomes [11]. To date, the largest amphibian genome assemblies belong to three salamander species: *Ambystoma mexicanum* (27.3 Gb assembly; [12]), *Pleurodeles waltl* (20.3 Gb; [13]), and *Calotriton arnoldi* (22.8 Gb; [14]). However, these only represent the lower end of the genome size range for this group, with the genomes of *Necturus* salamanders exceeding 100 Gb (Fig. 2) [10].

In addition to their large sizes, amphibian genomes have also been challenging to assemble due to their extensive repeat content (up to 82% [15]). Amphibian transposable elements have expanded and become highly abundant in younger clades, posing challenges for the construction of contiguous genome assemblies [16]. These characteristics of amphibian genomes make sequencing and assembly both costly and technically challenging (e.g., repetitive regions can often lead to fragmented assemblies when using short-read sequencing). However, the advent of new sequencing approaches such as long-read sequencing (e.g., PacBio HiFi and Oxford Nanopore Duplex), Hi-C scaffolding, along with reduced sequencing costs have resolved many of these assembly challenges (e.g., *Nanorana parkeri*; [17]).

Thus, the number of amphibian genome assemblies has increased rapidly in recent years, reaching 111 currently listed as reference genomes at scaffold level or higher in the National Center for Biotechnology Information (NCBI) genome database (52 for Anura, 55 for Urodela, and four for Gymnophiona; NCBI genome database records accessed on 1 March 2024). Despite this rapid increase, the quality of available amphibian genomes varies significantly, only 38 are chromosome-level assemblies, and among these, only 16 are annotated. This indicates that the majority of available assemblies are incomplete or partial. For example, several recently published salamanders genomes of the genus *Desmognathus* have assembly sizes of ~1 Gb while their genome size estimates based on flow cytometry or image densitometry average 14 Gb [18, 19]. Furthermore, the gene content values for many of these incomplete genomes can be as low as 0.7% [15]. Besides the variation in quality, there are substantial taxonomic gaps in genome representation across Amphibia. Notably, 48 of the 77 amphibian families (62%) lack a

representative genome assembly in the NCBI genome database (Fig. 2B), indicating significant gaps in our understanding (see “The AGC’s genome sequencing targets” section and Table 1 for more information about these 48 families).

Due to the difficulty of assembling genomes, most previous genomic research in amphibians has relied on alternative high-throughput sequencing methodologies, including RNA sequencing (RNA-seq), reduced representation or target-capture approaches, or metagenomic methods (Fig. 3 and Supplementary File 2). For example, RNA sequencing (RNA-Seq) techniques have been used to explore gene expression across more than 300 different amphibian species. Furthermore, a substantial number of *de novo* transcriptomes are available through the NCBI Transcriptome Shotgun Assemblies (TSA) database (79 total: 59 for Anura, 15 for Urodela, and 5 for Gymnophiona). Various reduced-representation (e.g., ddRADseq) and targeted-capture sequencing approaches have also been implemented in recent years to obtain genome-wide sequence information from more than 1,400 amphibian species. All this information—from whole genomes to gene transcript features—has advanced the understanding of amphibian biology and directly contributed to conservation efforts as described below.

Advancing research and conservation through amphibian genomics

Amphibians have many unique characteristics that make them subjects of interest to a wide variety of scientific disciplines, spanning from developmental biology and medical research to ecology and evolution. The rapid development of genomic tools is galvanizing the study of amphibian biology and uncovering important facets of their biology and conservation [20-22]. We highlight some examples here.

Embryogenesis, developmental and regenerative biology

Amphibians have played a fundamental role in uncovering development principles [for a detailed review see 23]. Research on anurans has enabled the understanding of critical mechanisms underlying such as the breaking of egg asymmetry [24], axis establishment, and nerve transmission [25]. Notably, the availability of genome assemblies for *Xenopus laevis* and *X. tropicalis* has significantly advanced embryological and developmental biology. This advancement has enabled gene loss-of-function research through the combination of transgenesis with RNA interference, gene editing, and enhanced morpholino design. This has facilitated the in-depth analysis of regulatory and non-coding genomic influences in developmental processes [26, 27]. Consequently, these studies have generated thousands of genomic and transcriptomic resources for these two species [28, 29].

Yet, there is much more to uncover about amphibian development, especially given the numerous developmental modalities found across amphibian species, which likely demonstrates the highest diversity among vertebrates [30]. This includes direct development (egg to froglet; the first genome of a direct-developing amphibian, *Eleutherodactylus coqui*, was published in 2024 [31]), and phenotypic plasticity [32, 33].

Sexual development and determination are also diverse in amphibians [34], encompassing female and male heterogamety, unique microscopically recognizable sex chromosomes, and species with undifferentiated sex chromosomes. Sex-determining genes across these systems are starting to be explored through high-throughput sequencing [6, 35-38]. For instance, a Y-specific non-coding RNA

involved in male sex determination in *Bufotes viridis* was identified through the application of multiple omics techniques [38]. Strikingly, some salamanders in the genus *Ambystoma* exist as a single all-female, polyploid lineage that can incorporate new chromosome sets from up to five other sexual species [39]. Transcriptomes from these salamanders have shown that gene expression from their divergent genomes is balanced for some genes but biased for others [40]. Sexual development in amphibians can result in sexually dimorphic features such as nuptial spines, which have been explored using comparative genomics approaches in the frog *Leptobrachium leishanense* [41]. The increasing availability of amphibian genomes will enable a deeper understanding of the molecular mechanisms underlying such ontogenetic diversity.

Metamorphosis sets many amphibian species apart from amniotes. Transcriptomics has revealed a remarkable turnover in gene expression between larval and adult stages of both frogs [42-45] and salamanders [46, 47]. This represents genomic uncoupling of these life history phases with major macroevolutionary implications [44, 48]. Amphibian omics approaches are rapidly increasing our understanding of the developmental process of metamorphosis, including the role of methylation in gene regulation [49]. Amphibians have also been found to respond to environmental perturbations by altering their behavior or phenotypes in various ways. These include changing developmental rate [32], hybridization with positive fitness effects [50], producing novel trophic morphologies [51], and kin detection to avoid cannibalizing relatives [52-54].

Due to their exceptional tissue repair and regenerative capacities [55, 56], amphibians are leading models for understanding the mechanisms of regeneration. This is particularly true for salamanders, which display the most extensive adult regenerative repertoire among vertebrates, including the ability to regenerate parts of their eyes, brain, heart, jaws, lungs, spinal cord, tail, and entire limbs [56]. Due to new genome assemblies for urodele species, *Ambystoma mexicanum* and the *Pleurodeles waltl*, regeneration can now be studied with transgenesis, advanced imaging, and genome editing. Intensive transcriptomic sequencing for these two salamander species has facilitated gene expression studies, including investigations into regeneration processes and characterization of other genomic features [57]. Additionally, a novel mechanism of telomere length maintenance and elongation has recently been described in *P. waltl* [58], potentially linking regenerative capability with longevity. Other amphibian species have also contributed to genomic research on regeneration. For example, databases compiled from gene expression resources of *Notophthalmus viridescens* have enabled comparative studies [59].

Ecology and evolution

Modern amphibians are the sister lineage of all amniotes, making them a valuable resource for studying species relationships and trait evolution. This is exemplified by studies that explore the rapid diversification of frogs [60], the evolution of vision [61], hybridogenesis [62-64], and the evolution of limblessness [65]. Amphibian phylogenomics has addressed many longstanding questions in amphibian evolution [66-69]. Comparative genomic analyses including amphibian groups have also revealed important gaps in our understanding of tetrapod molecular evolution such as chromosomal rearrangements and group-specific gene families that remain

unclassified to date [65, 70, 71]. In this section, we explore how genomics is being applied to understand the diverse ecological and evolutionary features unique to amphibians.

Like mammals, birds, and reptiles [72-74], some amphibians have evolved the ability to live in high-elevation environments such as the Andes (up to 5400 m) [75, 76] and the Tibetan Plateau (4478 m) [17]. However, unlike other groups, amphibians lack fur, feathers, or scales to protect them from physiological stressors such as UV exposure. This vulnerability makes them an intriguing model for studying the effects of UV radiation, which is relevant not only to humans [17] but also to species impacted by climate change. Amphibians have evolved multiple mechanisms of resisting UV including increasing antioxidant efficiency and gene regulatory changes in defense pathways [17, 77]. There is also evidence from Tibetan anurans that genes that impact other high-elevation traits (e.g., hypoxia resistance, immunity, cold tolerance) have evolved convergently across distantly related families (e.g., Dicroglossidae, Bufonidae, Megophryidae) [78], and that intraspecific divergence in many of these genes correlates with elevation [79, 80]. Additional genomic signatures of elevation adaptations, including genes regulating resistance to cold, hypoxia, immunity, and reproduction, have been described in ranid species of western North America inhabiting elevations from the sea level to nearly 3000 m [81]. While we are beginning to understand the genetic mechanisms of high-elevation adaptation in some Asian and North American frogs, this has yet to be investigated in other high-elevation amphibians, including Andean anurans (e.g., *Telmatobius culeus* [82]) and high-elevation salamanders, such as *Pseudoeurycea gadovii* [83]).

The ability to produce toxins has evolved across all three amphibian orders, where it primarily serves as an anti-predation mechanism. The source of amphibian toxins varies: some species are capable of synthesizing poisonous compounds (e.g., bufonids, myobatrachids), whereas others sequester toxic substances from their diet (e.g., dendrobatids, mantellids) [84-87] or microbial symbionts (e.g., newts) [88]. Since dendrobatid frogs sequester their toxins from prey (e.g., mites and ants), they lack genes encoding these toxins [89, 90]. However, they require genes to facilitate the transport of these toxins to the skin. Recent genomic and proteomic research has identified candidate genes coding for proteins that may serve dual roles in toxin transport and resistance [91-93]. Comparative genomic research has identified specific substitutions that allow toxic amphibian species to effectively mitigate the effects of the sequestered toxins on their own tissues [94-96]. Skin transcriptomes have also proven to be a rich source for data mining and the identification of candidate toxins and antimicrobial peptides in various amphibians, including caecilians [97-101].

Interactions between toxic amphibians and their predators have resulted in a fascinating variety of co-evolutionary arms races. These include well-characterized systems of toxicity resistance mechanisms in amphibian predators [102-106] and aposematism and mimicry in toxic species [107, 108]. Research on aposematism and mimicry has utilized whole genome, exome capture, and transcriptome sequencing to elucidate the genes underlying the vast diversity of color patterns across populations and species in dendrobatids [109-114]. These approaches have yielded a goldmine of information that can be used to understand the genes, gene networks, and biochemical pathways that underlie variation in coloration in other

amphibian groups including highly diverged aposematic taxa such as Australian myobatrachid frogs (e.g., *Pseudophryne corroboree)*, Malagasy poison frogs (Mantellidae), caecilians (e.g., *Schistometopum thomense*), and salamanders (e.g., *Salamandra salamandra*). Indeed, these methods have already enabled the identification of genes and loci involved in coloration in the salamander *S. salamandra bernardezi* [115].

Despite the numerous advances made with amphibian omics in elucidating evolutionary and ecological mechanisms, fully unraveling their genetic basis requires generating a vast number of genomes due to the comparative nature of these fields. Some of the exciting research avenues in amphibians include parental care [116, 117], gliding ability [118], lunglessness [119, 120], unusual defense mechanisms, such as the ability of some newts to pierce their ribs through toxin glands in their skin [121, 122], milk production or skin feeding in caecilians [123, 124], and spatial navigation [125].

Conservation

Amphibians are the most endangered class of vertebrates with current estimates suggesting that more than 40% of species are threatened with extinction [126]. The threats amphibians are facing continue to increase [126], creating a clear need to develop innovative and effective methods to conserve them. Paradoxically, current rates of amphibian species description are exponential, and numerous candidate species are being flagged worldwide. This suggests that we are still far from overcoming the amphibian Linnean shortfall, especially in tropical regions [127, 128]. Hence, numbers of threatened species are likely underestimated, as undescribed

species cannot be assessed and are more likely to become extinct [129]. Further, the conservation status of many amphibians remains unknown, especially for tropical species [130] and for a number of soil-dwelling caecilians known only from a few specimens [131]. Generating genomic data is one method to address this challenge, as it can be used to estimate both evolutionary potential and extinction risk [132]. Genomes are also vital for understanding species boundaries and the geographic distribution of genetic diversity within species, and for identifying populations under higher risk due to anthropogenic pressures or climate change [20, 21, 133, 134]. These features make genomic resources invaluable for developing species conservation action plans [135].

Amphibian conservation efforts should leverage population genetic theory and the burgeoning field of conservation genomics. This approach enables the quantification of both neutral and adaptive diversity across genomes, thereby facilitating the promotion of adaptive potential or genetic rescue through translocation programs [136-139]. Typically, these studies begin with the genomic characterization of populations across various environmental conditions, assessing population genetic health and disease risk [140, 141]. Omics resources are becoming increasingly important in species detection (e.g., via eDNA [142-144]). They can also support monitoring and surveillance efforts by identifying populations most at risk of declines due to potential genetic threats such as maladaptive alleles, genetic load, inbreeding and outbreeding depression, hybridization, and/or genetic incompatibility [136, 145]. Increased monitoring and maintenance of genomic diversity are key targets of many national and international recommendations such as the US Endangered Species

Act [146], the Kunming-Montreal Global Biodiversity Monitoring Framework [147], and the Amphibian Conservation Action Plan [135].

A more specific application of amphibian genomics for conservation requires understanding the genetic basis of traits that impact fitness, such as disease resistance or climate change tolerance. This information can be used to promote adaptation using approaches like Targeted Genetic Intervention (TGI), which aims to increase the frequency of adaptive alleles with approaches such as selective breeding, genome editing, or targeted gene flow [148]. Considerable effort has been invested in understanding the genetic basis of resistance to the devastating amphibian disease chytridiomycosis, which has resulted in the identification of multiple candidate genes [149-151] that could be targeted to increase chytridiomycosis resistance with TGI. Additionally, the efficacy of TGI at increasing chytridiomycosis resistance has already been demonstrated in North American mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) where translocation of resistant individuals increased recipient population persistence [152]. Despite the obvious appeal of using genetic intervention approaches for conservation, these methods should be evaluated in contained facilities whenever possible and accompanied by long-term monitoring to ensure their efficacy and rule out any unintended impacts [148, 153-155]. Although such conservation interventions require extensive resources, this may be the only effective method for restoring some species to the wild, especially in those threatened by intractable threats such as chytridiomycosis [156].

Challenges for amphibian genomic research and ways forward

A major challenge for amphibian omics research, which will be anchored by high-quality reference genomes, is gaining access to starting material (e.g., tissue, blood). Common logistic challenges include: 1) obtaining research funding, 2) collaborating equitably with local or Indigenous communities on the development and execution of biodiversity genomics research, 3) obtaining collection and research permits from the state, 4) obtaining samples from difficult-to-access regions, and 5) obtaining high-quality samples and maintaining quality during transport.

With increasingly easy access to genomic data, researchers and industry need to be even more aware of the principles of fair and equitable access to genetic resources, as stipulated by Convention on Biological Diversity (CBD) and expanded upon by the Nagoya Protocol https://www.cbd.int/abs/default.shtml). Indigenous peoples and local communities (IPLC) are often custodians of genetic resources (physical material) sought by researchers, requiring that all parties enter into collaborative and equitable agreements on access and benefit-sharing (ABS) before embarking on a genomics project [157-161]. As a negative example from amphibians, *Phyllomedusa bicolor* skin secretions traditionally used by Amazonian indigenous peoples were patented by actors in the US, Japan, Russia and elsewhere, promoting the 'legal' but unfair appropriation of genetic resources and potentially the traditional knowledge itself from the Matses and other Indigenous tribes [162]. To promote better practices, researchers should budget the time and money required to engage in prior consultation as part of planning field work, and consult their National Focal Points on ABS. How the concept of ABS will or could be applied to the downstream use of the digital sequence information (DSI) generated has yet to be resolved (although there

are currently developments underway, https://www.cbd.int/dsi-gr) but must also be considered going forward [see for example 163]. Moreover, voucher specimens and duplicate tissue samples should be deposited in local museums or preferred partners of the local communities [164, 165].

Amphibian fieldwork often involves overcoming numerous hurdles such as navigating socio-political conflicts and accessing remote field sites. These challenges may be exacerbated in developing economies or for researchers with limited access to high volume funding streams. In addition, inadequate infrastructure for accessing field sites, and the need to time of fieldwork to coincide with the often highly seasonal and cryptic activities of amphibians [166] can further complicate the task. Attention should also be directed towards overcoming inequities that may pose additional obstacles to fieldwork for underrepresented groups [161].

Once amphibians are collected, selecting the tissue sample to obtain sufficient high-molecular-weight DNA (HMW, reaching 100 Kb or ultra HMW, reaching 1 Mb) can be challenging due to the small body sizes of most amphibians (e.g., < 30 g). For generating reference genomes, blood is one of the most recommended sample types for amphibian genomics [167]. However, the blood volume of most amphibians is too small for non-lethal collection [168]. This presents a significant challenge because obtaining the required quantity of HMW DNA often necessitates lethal sampling, which may not always be legally permitted or ethically advisable, especially for threatened species or those in captive collections [169]. Non-lethal sampling approaches, such as buccal swabs or toe or tail clips, are increasingly viable for various genomic applications, including low-coverage whole genome sequencing or

targeted sequencing approaches [170, 171]. Another alternative is to use tadpoles instead of adults as was done to generate the genome of *Taudactylus pleione* [172].

Most tissue sampling protocols for reference genomes or transcriptome sequencing recommend harvesting samples from fresh tissue, followed immediately by flash freezing in liquid nitrogen (LN2) and storing at -80°C until extraction (https://www.vertebrategenomelab.org/resources/guidelines). Maintaining ultracold storage and the cold chain during transport from remote collection sites can be challenging. After being adequately charged with LN2, dry vapor shippers can keep samples ultra-cold for a week or more in the field. While LN2 is the gold standard, other preservatives like 95% EtOH, 20-25% DMSO in EDTA may adequately preserve DNA for long-read sequencing, although efficacy may vary depending on the tissue type [167, 173]. Other options include lysis buffers or commercial products such as Zymo DNA/RNA shield (Zymo Research, USA). However, it remains essential to test the impact of these preservatives on sequencing outcomes beforehand, specifically for the given taxon and tissue type, since preservation methods may inhibit downstream approaches, such as Hi-C library construction [167, 174].

While these challenges apply to any researcher or organization, they become particularly challenging when the sequencing work occurs outside the species' country of origin. This difficulty arises not only because genetic material is prone to degradation but also because of regulations on moving biological samples across political borders [175, 176]. The global genomics community should strive to ensure that sequencing projects occur within the country of origin of the samples and

discourage ‘parachute’ or ‘helicopter science’ [177, 178]. Oxford Nanopore Technology (ONT) may be promising solution, providing comparatively affordable access to equipment and reagents for ultra-long read sequencing that can even be done directly in the field [179]. However, working with non-model organisms requires prior optimization, and the startup costs for this infrastructure remain prohibitive for many scientists from low-income countries. Moving forward, the goal should be to apply these technologies in collaboration with local researchers. For example, programs like the In Situ Laboratories Initiative (https://insitulabs.org/hubs/) aim to provide affordable access to high-tech laboratories in remote biodiverse areas). Such collaboration should proceed from finding shared interests, developing ideas, realizing the shared benefits from research outputs, and focusing on capacity-building efforts [180].

Working with museum specimens [the burgeoning field of “museomics”; 181] is another promising avenue of research allowing to access to past amphibian biodiversity. However, there are several additional challenges associated with DNA degradation, preservation methods, and contamination that need to be overcome [182-184]. This is particularly relevant for wet-preserved amphibian specimens, as retrieving DNA can be challenging due to the often unknown fixation and preservation methods that can alter nucleotide integrity. Methodological advances in laboratory protocols [e.g., 185, 186, 187] and the development of sequencing strategies, such as ‘Barcode Fishing’ [188, 189], have made significant progress in addressing these challenges, including the ability to sequence extinct species [190, 191]. In the current era, even limited sequences from taxonomic type specimens are of unparalleled importance, especially for species identification using genetic data,

by those applying methods like eDNA and metagenomics [192]. Museomics has also revolutionized amphibian taxonomy by integrating DNA from name-bearing types, overcoming impediments like uncertainty in taxonomic names, species complexes, and cryptic species [188, 189, 193, 194].

As mentioned earlier, the large genome sizes of many amphibians affect sequencing, computing, and storage costs, as well as computational requirements for data analysis [6, 195]. However, the greater challenges lie in the methodological and theoretical limitations of assembling large genomes. Additionally, access to cluster computing for genome assembly remains limited for many scientists, particularly those from low-income countries. Genome size in amphibians is correlated with increased intron lengths and repeat content [196], posing challenges for assembly, especially when using with short-read sequencing technologies or during contig joining processes. Regions misassembled due to low complexity have previously resulted in a significant loss of sequence information (e.g., by as much as 16%) through the collapsing of repetitive sequences [197]. Repetitive regions can also result in the formation of problematic chimeras during assembly, where two distant contigs are erroneously joined due to a shared repeat sequence [198]. These problems are exacerbated when repeats are longer than sequencing reads. Further challenges are posed by polyploidy [199], which has evolved repeatedly in amphibians [200, 201]. This can make developing haplotype-specific assemblies challenging and may require dramatically increased sequencing and computational efforts [199, 202]. Thus, the development of long-read and 3C technology (i.e., Hi-C scaffolding) is especially important for assembling amphibian genomes [198, 203]. Recent technological advances, including long-read techniques such as PacBio HiFi,

ONT, as well as scaffolding methods such as Hi-C and optical mapping, now make it feasible and affordable to generate reference genomes for most frogs, caecilians, and increasingly for salamanders [203].

Gene and repeat annotation resources in amphibians are also under-developed. Although this may not pose a significant issue for well-conserved genes, where researchers can retrieve annotation information from orthologous regions using existing databases like UniProt [204], it often results in missed or poorly annotated genes, particularly for highly polymorphic genes or genes lacking representation in model taxa. Hence, the ability to study features such as gene evolution [67], repeats [15, 196], immune genes [205], and genetic sex determination [206] is still limited and requires caution to prevent overinterpretation.

Although key tools for understanding functional genomics exist, tools and protocols for gene editing, transgenesis, *in vitro* fertilization, are rare or non-existent for most amphibians [148, 207], with the exception of some model species (e.g., *Xenopus laevis*, *X. tropicalis*, and *Ambystoma mexicanum*) [56, 208-211]. Immortal cell lines have been successfully generated for some amphibians [212] and protocols have been established to facilitate the initiation of spontaneously arising cell lines for a subset of anurans [213]. However, establishing cell cultures for most species requires extensive problem-solving and expertise [212].

Aims, priorities, and structure of the Amphibian Genomics Consortium (AGC)

The AGC was launched in March 2023 to address the aforementioned knowledge gaps through technological advances and international cooperation. The mission of the AGC is to increase collaboration and communication among amphibian

researchers internationally and across scientific disciplines, to increase amphibian genomics resources, and to apply genomic data and functional resources to help bridge the implementation gaps between genome biologists, other scientists and conservation practitioners. The leadership structure of the AGC consists of a director, two co-directors, and a 10-member board. The board was carefully chosen to ensure gender equality, diversity of scientific disciplines, career stages, and representation from various geographic regions.

Currently the AGC organizes monthly virtual meetings and a monthly virtual seminar series. They maintain a website (https://mvs.unimelb.edu.au/amphibian-genomics-consortium), facilitate active discussion groups, and have hosted social events at several scientific conferences. Additionally, the AGC is preparing to host a full-day symposium titled “Beyond the reference: genomics for amphibian research and conservation” at the World Congress of Herpetology in August 2024 in Kuching, Malaysia.

The first actions of the AGC include raising funds for genome sequencing, developing technical resources and best practices guidelines, improving amphibian genome annotation, supporting travel for students and early career researchers, and conducting virtual and in-person computational workshops. The AGC plans to secure funding to sequence high-priority amphibian species (see The AGC’s genome sequencing targets section and Table 1). Additionally, the AGC aims to facilitate amphibian sample collection for broader taxonomic consortia. The AGC is already affiliated with the Earth BioGenome Project (EBP) and AmphibiaWeb

(https://amphibiaweb.org), reinforcing its commitment to advancing amphibian genomics and conservation efforts.

AGC membership

At the time of the submission of this work, the AGC had 282 members from 41 countries (Fig. 4). Although the membership is geographically diverse, there remains some disparity across regions. Efforts will be intensified to attract members from underrepresented countries, particularly regions known for high amphibian diversity and/or endemism such as Central and South America, and Southeast Asia. We promote equity between members by providing additional support and opportunities to those from developing countries and underrepresented groups. This includes eliminating membership fees, scheduling online meetings at alternating times to accommodate global time zones, facilitating discussion groups on the cloud-based collaboration platform Discord, and translating AGC correspondence into multiple languages. Furthermore, we are also committed to fostering knowledge and skills transfer to all emerging scientists worldwide, and we actively encourage early career researchers to join the initiative and participate in governance.

Current use and perception of genomics technologies by members of the AGC

The AGC leadership designed a 23-question survey to investigate consortium members' experiences in amphibian genomics (questions can be found in Supplementary Table S1). The survey was distributed using the Qualtrics XM platform and remained active from the 4th of March to the 27th of December 2023. We collected responses from a total of 133 AGC members from 32 countries with different expertise in sequencing approaches and bioinformatics techniques, who

primarily work on the ecology and evolution of anurans. Overall, respondents emphasized the urgency of filling knowledge gaps in amphibian genomics due to the current conservation crisis, pinpointing the necessity to expand the number of high-quality chromosome-level amphibian genomes. Additionally, there was strong agreement among survey respondents that the generation of new genomics resources needs to be coupled with the improvement and accessibility of annotation processes. A better development of sharing computational expertise among members and resources internationally was also underscored. More than half of the survey participants said they use sequencing technologies for their studies (70 of the 133). About half of the respondents said their main work activities were “genomics lab work” or “computational analyses” (48% and 57%, respectively).

To evaluate consortium members’ experience in amphibian genomics, we applied a principal components analysis to the quantitative responses. Bioinformatic competencies and perceived challenges of the AGC respondents were grouped in two dimensions, respectively (Fig. 5A and Fig. S2; see Supplementary Material for methodological information). To explain the variation of these two new variables, we used the scientific expertise of AGC members, the funding success, and two variables related to the country of main affiliation of the respondent: the number of amphibian species and gross domestic expenditure on R&D (GERD) per capita, as explanatory variables. Amphibian genomics expertise and identified challenges varied substantially among respondents. The number of amphibian species and GERD per capita of the respondent’s main affiliation country did not capture this variation (Fig. 5B and Fig. S3; see Supplementary Material for methodological information). Instead, genomics funding success and years of scientific expertise

were, as expected, positively correlated and both variables and were associated with a reduction in the perceived challenges associated with amphibian genomics.

The AGC's genome sequencing targets

Following the efforts of genomics consortia for other tetrapod groups [e.g., 214], and previous research efforts [21], we identified amphibian families without any representative genomes and selected one representative species per family for our sequencing priority list (Fig. 2B and Table 1). We propose 48 candidate species based on their IUCN Red List category, ecological and evolutionary distinctiveness, and the availability of other genomics records, especially transcriptomics. This list includes 38 anurans, four urodeles, and six caecilians. We aim to build upon the efforts of existing genomics consortia such as the Vertebrate Genomes Project (VGP), hence, we included in our sequencing target list species with draft genomes in the GenomeArk (https://www.genomeark.org/).

Conclusion and outlook

Amphibians are declining faster than they can be discovered [215]. Our hope is that the recent advancements in technology (e.g., long-read sequencing, computational tools) and integration of the research community to from the Amphibian Genomics Consortium (AGC) will ignite research to revolutionize amphibian conservation and our understanding of their fascinating biology, ecology and evolution. By supporting amphibian genomics research and uniting amphibian researchers worldwide, the AGC aims to propel amphibian genomics research into the future.

Moving forward, the AGC is committed to supporting amphibian sequencing initiatives worldwide, with a particular emphasis on families lacking representation

and species from biodiverse countries (Table 1). Local sequencing initiatives will be given priority whenever feasible to promote the development of *in situ* research efforts and facilities. Additionally, we aim to provide funding and training opportunities to facilitate collaboration among underrepresented groups, molecular and organismal biologists, bioinformaticians, and conservation practitioners. We aspire to stimulate public and scientific interest in amphibian research and enhance conservation efforts for this intriguing and highly endangered group of vertebrates.

List of Abbreviations

ABS: access and benefit-sharing

AGC: Amphibian Genomics Consortium

CBD: Convention on Biological Diversity

DSI: digital sequence information

EBP: Earth BioGenome Project

GERD: gross domestic expenditure on research and development

GoaT: Genomes on a Tree

HMW: High molecular weight DNA

IPLC: Indigenous peoples and local communities

IUCN: International Union for Conservation of Nature

ONT: Oxford Nanopore Technology

VGP: Vertebrate Genomes Project

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare no competing interests.

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Authors' contributions

T.A.K. and M.T.-S. drafted the manuscript. T.A.K., M.T.-S., H.C.L., K.S., M.H.Y., S.T.M., A.J.C. contributed text to the first draft, M.T.-S. and T.A.K. analyzed the data and created the figures, members of the Amphibian Genomics Consortium (AGC) reviewed later drafts. All authors reviewed the manuscript.

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Table 1. Amphibian Genomics Consortium (AGC) sequencing priority list. Table of amphibian families without any sequenced genomes. For each family, AGC proposed a candidate species based on its IUCN Red List category (LC: Least Concern, NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically Endangered, and NA: Not evaluated), ecological and evolutionary distinctiveness, and availability of other genomic records. This table shows the amphibian order to which each family belongs and its number of genera (#G) and described extant species (#S) as well as distribution region. *Species with available draft genome assemblies in the GenomeArk (https://www.genomeark.org/).

Family	Region	#G	#S	Candidate species	IUCN	Other motives
Anura: Allophrynidae	South America	1	3	*Allophryne relicta*	EN	
Anura: Alsodidae	South America	3	26	*Alsodes gargola*	LC	High altitude adaptation
Anura: Arthroleptidae	Africa	8	151	*Leptopelis vermiculatus*	EN	
Anura: Ascaphidae	North America	1	2	*Ascaphus montanus**	LC	High altitude adaptation
Anura: Batrachylidae	South America	4	13	*Batrachyla leptopus*	LC	
Anura: Brachycephalidae	South America	2	79	*Brachycephalus pitanga*	LC	Transcriptomic resources
Anura: Brevicipitidae	Africa	5	36	*Breviceps fuscus*	LC	Burrowing adaptation
Anura: Caligophrynidae	South America	1	1	*Caligophryne doylei*	NA	Pantepui endemism
Anura: Calyptocephalellidae	South America	2	5	*Telmatobufo bullocki*	EN	
Anura: Centrolenidae	Central & South America	12	166	*Centrolene pipilata*	CR	Gigantism
Anura: Ceratobatrachidae	Southeast Asia	4	103	*Platymantis spelaeus*	EN	Cave-dweller

Family	Region	#G	#S	Candidate species	IUCN	Other motives
Anura: Ceratophryidae	South America	3	12	*Lepidobatrachus laevis*	LC	Transcriptomic resources
Anura: Ceuthomantidae	South America	2	6	*Ceuthomantis cavernibardus*	LC	Cave-dweller
Anura: Conrauidae	Africa	1	8	*Conraua goliath*	EN	Giantism
Anura: Craugastoridae	Central America	3	136	*Craugastor fitzingeri*	LC	Transcriptomic resources
Anura: Cycloramphidae	South America	3	37	*Cycloramphus granulosus*	CR	
Anura: Heleophrynidae	South Africa	2	6	*Heleophryne rosei*	CR	
Anura: Hemiphractidae	Central & South America	6	123	*Gastrotheca cornuta*	CR	
Anura: Hemisotidae	Sub-Saharan Africa	1	9	*Hemisus marmoratus*	LC	Transcriptomic resources
Anura: Hylodidae	South America	4	49	*Phantasmarana massarti*	EN	
Anura: Hyperoliidae	Sub-Saharan Africa & Madagascar	17	236	*Hyperolius thomensis*	EN	Population genomic resources
Anura: Leiopelmatidae	New Zealand	1	3	*Leiopelma archeyi*	CR	
Anura: Mantellidae	Madagascar	12	272	*Mantidactylus betsileanus*	LC	Transcriptomic resources
Anura: Micrixalidae	India	1	24	*Micrixalus mallani*	EN	Transcriptomic resources
Anura: Nasikabatrachidae	India	1	2	*Nasikabatrachus sahyadrensis*	NT	EDGE target species
Anura: Neblinaphrynidae	South America	1	1	*Neblinaphryne mayeri*	NA	Pantepui endemism
Anura: Nyctibatrachidae	India & Sri Lanka	3	37	*Nyctibatrachus grandis*	EN	Transcriptomic resources
Anura: Odontobatrachidae	Tropical West Africa	1	5	*Odontobatrachus fouta*	EN	
Anura: Odontophrynidae	South America	3	54	*Proceratophrys redacta*	EN	
Anura: Petropedetidae	Sub-Saharan tropical Africa	3	13	*Petropedetes perreti*	CR	
Anura: Phrynobatrachidae	Africa	1	99	*Phrynobatrachus guineensis*	LC	Tree-hole breeder
Anura: Ranixalidae	India	2	19	*Indirana chiravasi*	LC	Transcriptomic

Family	Region	#G	#S	Candidate species	IUCN	Other motives
						resources
Anura: Rhacophoridae	Eastern Asia	22	444	*Buergeria otai*	LC	Transcriptomic resources
Anura: Rhinodermatidae	South America	1	3	*Rhinoderma darwinii*	EN	Targeted sequencing resources
Anura: Rhinophrynidae	Central America	1	1	*Rhinophrynus dorsalis**	LC	Targeted sequencing resources
Anura: Sooglossidae	Seychelles Islands	2	4	*Sooglossus sechellensis*	EN	EDGE target species
Anura: Strabomantidae	South America	19	792	*Oreobates cruralis*	LC	Transcriptomic resources
Anura: Telmatobiidae	South America	1	63	*Telmatobius simonsi*	CR	
Gymnophiona: Caeciliidae	Central & South America	2	49	*Caecilia tentaculata*	LC	Transcriptomic resources
Gymnophiona: Chikilidae	India	1	4	*Chikila gaiduwani*	LC	
Gymnophiona: Grandisoniidae	Africa, Seychelles & India	7	24	*Hypogeophis montanus*	NA	
Gymnophiona: Herpelidae	Sub-Saharan Africa	2	11	*Boulengerula niedeni*	EN	EDGE target species
Gymnophiona: Scolecomorphidae	Africa	2	6	*Crotaphatrema lamottei*	CR	
Gymnophiona: Typhlonectidae	South America	5	14	*Typhlonectes compressicauda*	LC	Transcriptomic resources
Urodela: Cryptobranchidae	Asia & North America	2	6	*Cryptobranchus alleganiensis*	VU	Transcriptomic resources
Urodela: Dicamptodontidae	North America	1	4	*Dicamptodon tenebrosus*	LC	Giantism
Urodela: Hynobiidae	Eastern Asia	9	98	*Hynobius vandenburghi*	VU	Transcriptomic resources
Urodela: Rhyacotritonidae	North America	1	4	*Rhyacotriton olympicus*	NT	

Figures

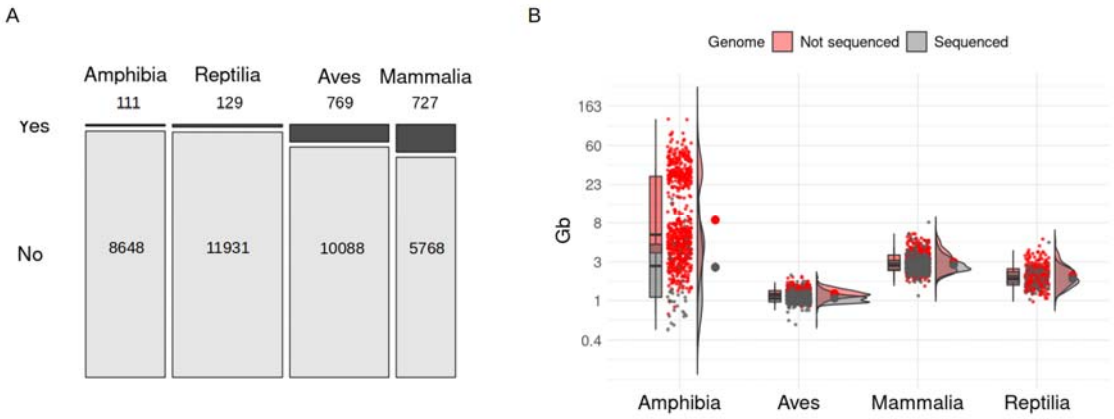

Figure 1. **Estimated genome size across tetrapod classes in relation to sequenced genomes.** (A) Mosaic plot representing the percentage of species with sequenced genomes as a proportion of the number of described species for each tetrapod class (Yes: % species with sequenced genome; No: % species without sequenced genome). (B) Combined box and density plot with raw data as points comparing genome size of species with sequenced genome (gray; genome sizes from NCBI genome assemblies) versus a subset of species without a sequenced genome (red; genome sizes from the Animal Genome Size Database) for each tetrapod class. The y-axis is log-transformed to facilitate visualization. Information about sequenced genomes and genome sizes was obtained from the NCBI Genome Browser, the Animal Genome Size database, and amphibian records from [11, 19].

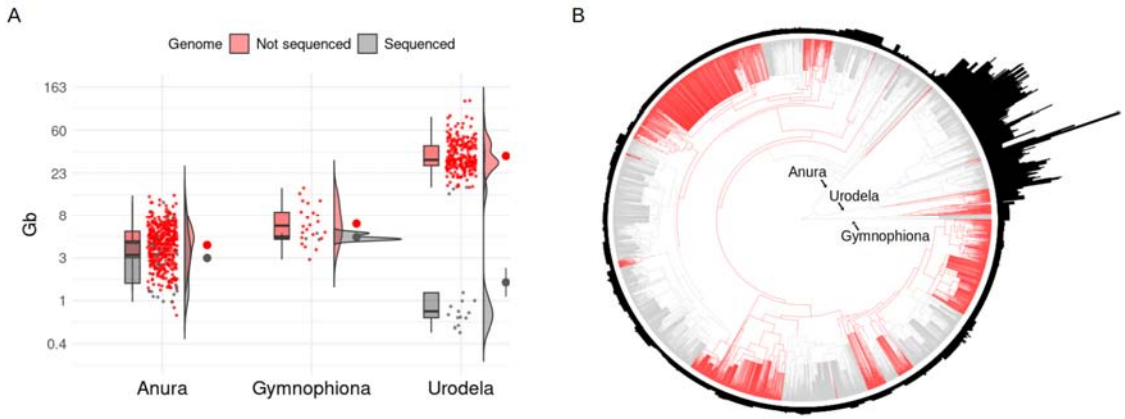

Figure 2. Estimated genome size across amphibian orders in relation to sequenced genomes. (A) Combined box and density plot with raw data as points showing genome size of species with sequenced genome (gray color; genome sizes from NCBI genome assemblies) versus a subset of species without available genome assembly (red color; genome sizes from the Animal Genome Size Database) for each amphibian order. The y-axis is logarithmic transformed to facilitate visualization. Information about sequenced genomes and genome sizes was obtained from the NCBI Genome Browser, the Animal Genome Size database [19], and amphibian records from [11]. (B) Amphibian phylogenetic tree was adapted from [66], which includes species with genome size estimates from Genomes on a Tree (GoaT) [18]. Branches are color coded to represent families without any genomic record (in red) and families with at least a representative genome sequenced (in gray). Bar plots around the phylogeny indicate relative genome sizes.

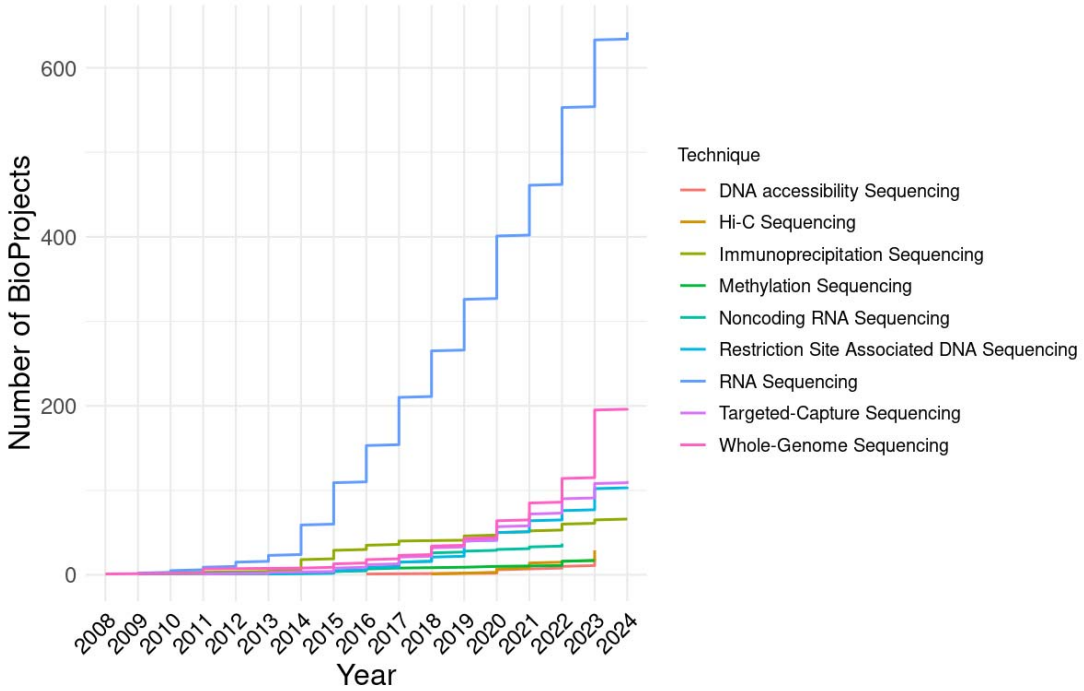

Figure 3. Main sequencing techniques applied to amphibian genomics studies. Yearly cumulative number of amphibian BioProjects split and color-coded by sequencing technique (DNA accessibility Sequencing includes ATAC-Seq and Mnase-Seq; Immunoprecipitation Sequencing includes: ChIP-Seq and RIP-Seq; Amplicon sequencing was included with Targeted-Capture Sequencing; Noncoding RNA Sequencing includes: miRNA-Seq and ncRNA-Seq). BioProject information was obtained from the NCBI Sequence Read Archive (SRA, accessed 1 March 2024).

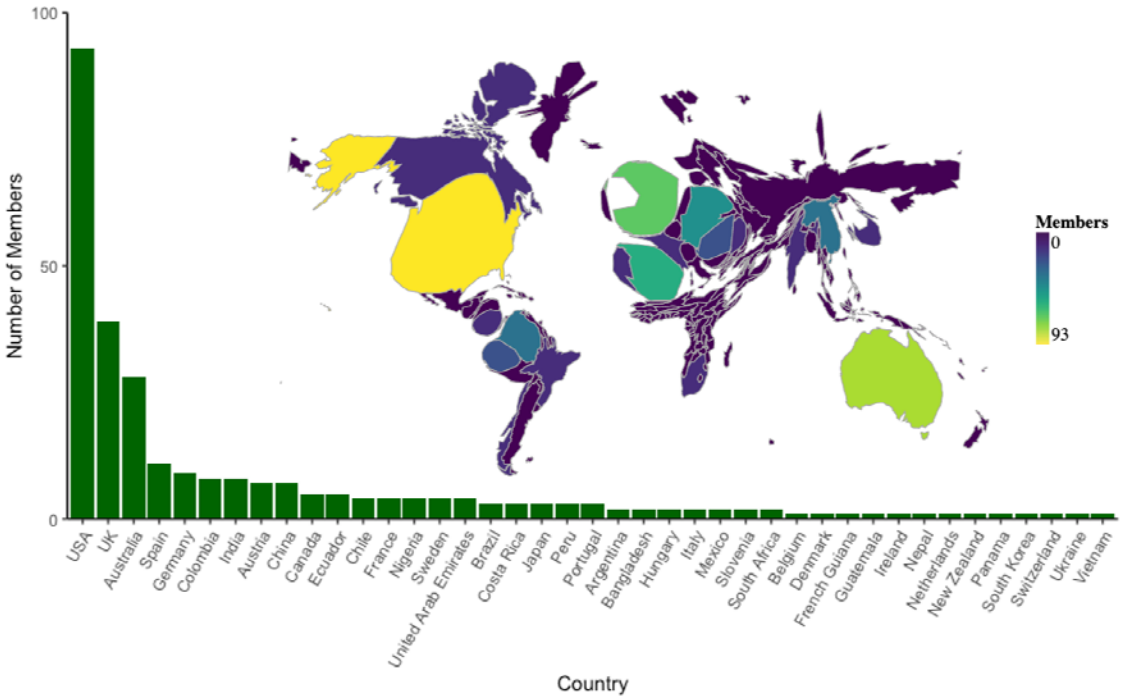

Figure 4. Amphibian Genomics Consortium (AGC) membership by country. Inset map showing the size of each country scaled by number of members in the AGC.

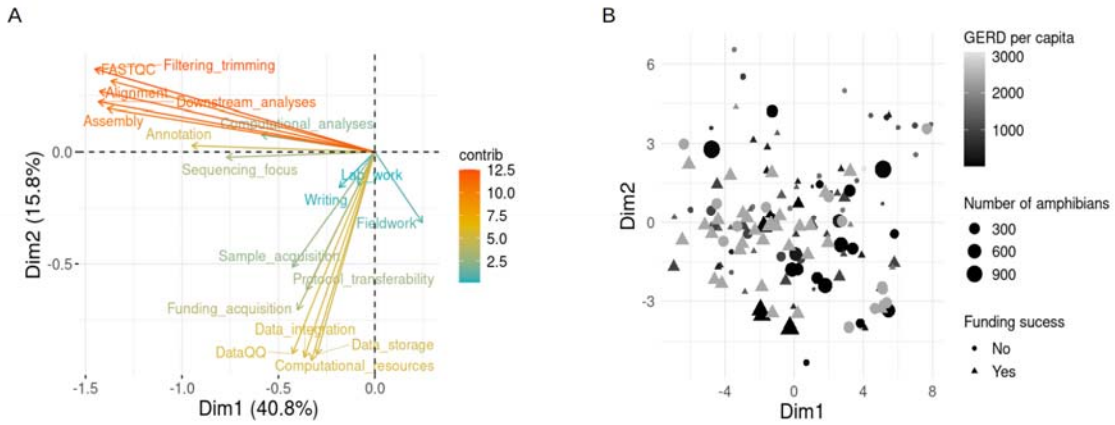

Figure 5. Sequencing competencies and identified challenges of the members of the Amphibian Genomics Consortium (AGC). (A) Representation of the contribution of the AGC survey quantitative questions to the first dimensions after computing a principal component analysis (PCA). Bioinformatic competencies and perceived challenges were grouped into dimensions one and two, respectively. (B) Scatter plot showing PCA scores for each AGC survey respondent. Respondent answers are coded by the qualitative question about funding success for amphibian genomics projects using shape; number of amphibian species of the respondent main affiliation country by size, and gross domestic expenditure on R&D (GERD) per capita of the respondent main affiliation country by gray-scale color coded. Information about the number of amphibian species per country was obtained from AmphibiaWeb. GERD per capita was calculated using information from the UNESCO and World Bank websites from the information about the most recent year for each country.

References

1. Hellsten U, Harland RM, Gilchrist MJ, Hendrix D, Jurka J, Kapitonov V, Ovcharenko I, Putnam NH, Shu S, Taher L *et al*: **The Genome of the Western Clawed Frog *Xenopus tropicalis***. *Science* 2010, **328**(5978):633-636.
2. Band MR, Larson JH, Rebeiz M, Green CA, Heyen DW, Donovan J, Windish R, Steining C, Mahyuddin P, Womack JE *et al*: **An ordered comparative map of the cattle and human genomes**. *Genome Res* 2000, **10**(9):1359-1368.
3. Aparicio S, Chapman J, Stupka E, Putnam N, Chia JM, Dehal P, Christoffels A, Rash S, Hoon S, Smit A *et al*: **Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes***. *Science* 2002, **297**(5585):1301-1310.
4. Collins FS, Green ED, Guttmacher AE, Guyer MS, on behalf of the USNHGRI: **A vision for the future of genomics research**. *Nature* 2003, **422**(6934):835-847.
5. Hillier LW, Miller W, Birney E, Warren W, Hardison RC, Ponting CP, Bork P, Burt DW, Groenen MAM, Delany ME *et al*: **Sequence and comparative analysis of the chicken genome provide unique perspectives on vertebrate evolution**. *Nature* 2004, **432**(7018):695-716.
6. Stöck M, Kratochvíl L, Kuhl H, Rovatsos M, Evans BJ, Suh A, Valenzuela N, Veyrunes F, Zhou Q, Gamble T *et al*: **A brief review of vertebrate sex evolution with a pledge for integrative research: towards 'sexomics'**. *Philosophical Transactions of the Royal Society B: Biological Sciences* 2021, **376**(1832):20200426.
7. Pillay R, Venter M, Aragon-Osejo J, González-del-Pliego P, Hansen AJ, Watson JE, Venter O: **Tropical forests are home to over half of the world's vertebrate species**. *Front Ecol Environ* 2022, **20**(1):10-15.
8. Gregory TR: **The evolution of the genome**: Elsevier; 2011.
9. Biscotti MA, Carducci F, Olmo E, Canapa A: **Vertebrate Genome Size and the Impact of Transposable Elements in Genome Evolution**. In: *Evolution, Origin of Life, Concepts and Methods.* Edited by Pontarotti P. Cham: Springer International Publishing; 2019: 233-251.
10. Weisrock DW, Hime PM, Nunziata SO, Jones KS, Murphy MO, Hotaling S, Kratovil JD: **Surmounting the large-genome "problem" for genomic data generation in salamanders**. *Population genomics: wildlife* 2021:115-142.
11. Liedtke HC, Gower DJ, Wilkinson M, Gomez-Mestre I: **Macroevolutionary shift in the size of amphibian genomes and the role of life history and climate**. *Nature Ecology & Evolution* 2018, **2**(11):1792-1799.
12. Nowoshilow S, Schloissnig S, Fei J-F, Dahl A, Pang AWC, Pippel M, Winkler S, Hastie AR, Young G, Roscito JG *et al*: **The axolotl genome and the evolution of key tissue formation regulators**. *Nature* 2018.
13. Brown T, Elewa A, Iarovenko S, Subramanian E, Araus AJ, Petzold A, Susuki M, Suzuki K-iT, Hayashi T, Toyoda A *et al*: **Sequencing and chromosome-scale assembly of the giant *Pleurodeles waltl* genome**. *bioRxiv* 2022:2022.2010.2019.512763.
14. Talavera A, Palmada-Flores M, Burriel-Carranza B, Valbuena-Ureña E, Mochales-Riaño G, Adams DC, Tejero-Cicuéndez H, Soler-Membrives A, Amat F, Guinart D *et al*: **Genomic insights into the Montseny brook newt (*Calotriton arnoldi*), a Critically Endangered glacial relict**. *iScience* 2024, **27**(1):108665.
15. Kosch TA, Crawford AJ, Mueller RL, Wollenberg Valero KC, Rodríguez A, O'Connell LA, Young ND, Skerratt LF: **Comparative analysis of amphibian genomes: an emerging resource for basic and applied research**. *bioRxiv* 2023:2023.2002.2027.530355.

16. Sun C, Shepard DB, Chong RA, López Arriaza J, Hall K, Castoe TA, Feschotte C, Pollock DD, Mueller RL: **LTR retrotransposons contribute to genomic gigantism in plethodontid salamanders**. *Genome Biol Evol* 2012, **4**(2):168-183.
17. Fu T-T, Sun Y-B, Gao W, Long C-B, Yang C-H, Yang X-W, Zhang Y, Lan X-Q, Huang S, Jin J-Q *et al*: **The highest-elevation frog provides insights into mechanisms and evolution of defenses against high UV radiation**. *Proceedings of the National Academy of Sciences* 2022, **119**(46):e2212406119.
18. Challis R, Kumar S, Sotero-Caio C, Brown M, Blaxter M: **Genomes on a Tree (GoaT): A versatile, scalable search engine for genomic and sequencing project metadata across the eukaryotic tree of life [version 1; peer review: 2 approved]**. *Wellcome Open Research* 2023, **8**(24).
19. Gregory TR: **Animal genome size database**. 2024.
20. Calboli FCF, Fisher MC, Garner TWJ, Jehle R: **The need for jumpstarting amphibian genome projects**. *Trends Ecol Evol* 2011, **26**(8):378-379.
21. Funk WC, Zamudio KR, Crawford AJ: **Advancing Understanding of Amphibian Evolution, Ecology, Behavior, and Conservation with Massively Parallel Sequencing**. In: *Population Genomics: Wildlife.* Edited by Hohenlohe PA, Rajora OP. Cham: Springer International Publishing; 2018: 211-254.
22. Sun Y-B, Zhang Y, Wang K: **Perspectives on studying molecular adaptations of amphibians in the genomic era**. *Zool Res* 2020, **41**(4):351.
23. Callery EM: **There's more than one frog in the pond: A survey of the Amphibia and their contributions to developmental biology**. In: *Semin Cell Dev Biol: 2006*: Elsevier; 2006: 80-92.
24. Weaver C, Kimelman D: **Move it or lose it: axis specification in *Xenopus***. *Development* 2004, **131**(15):3491-3499.
25. Burggren WW, Warburton S: **Amphibians as animal models for laboratory research in physiology**. *ILAR journal* 2007, **48**(3):260-269.
26. Naert T, Van Nieuwenhuysen T, Vleminckx K: **TALENs and CRISPR/Cas9 fuel genetically engineered clinically relevant *Xenopus tropicalis* tumor models**. *Genesis* 2017, **55**(1-2):e23005.
27. Guille M, Grainger R: **Genetics and Gene Editing Methods in *Xenopus laevis* and *Xenopus tropicalis***. *Cold Spring Harbor Protocols* 2023, **2023**(6):pdb. top107045.
28. Horb M, Wlizla M, Abu-Daya A, McNamara S, Gajdasik D, Igawa T, Suzuki A, Ogino H, Noble A, France CdRBXti: ***Xenopus* resources: transgenic, inbred and mutant animals, training opportunities, and web-based support**. *Frontiers in physiology* 2019, **10**:387.
29. Fisher M, James-Zorn C, Ponferrada V, Bell AJ, Sundararaj N, Segerdell E, Chaturvedi P, Bayyari N, Chu S, Pells T: **Xenbase: key features and resources of the *Xenopus* model organism knowledgebase**. *Genetics* 2023, **224**(1):iyad018.
30. Liedtke HC, Wiens JJ, Gomez-Mestre I: **The evolution of reproductive modes and life cycles in amphibians**. *Nat Commun* 2022, **13**(1):7039.
31. Bredeson JV, Mudd AB, Medina-Ruiz S, Mitros T, Smith OK, Miller KE, Lyons JB, Batra SS, Park J, Berkoff KC *et al*: **Conserved chromatin and repetitive patterns reveal slow genome evolution in frogs**. *Nat Commun* 2024, **15**(1):579.
32. Liedtke HC, Harney E, Gomez-Mestre I: **Cross-species transcriptomics uncovers genes underlying genetic accommodation of developmental plasticity in spadefoot toads**. *Mol Ecol* 2021, **30**(10):2220-2234.

928 33. Isdaner AJ, Levis NA, Pfennig DW: **Comparative transcriptomics reveals that a novel**
929 **form of phenotypic plasticity evolved via lineage-specific changes in gene**
930 **expression**. *Ecol Evol* 2023, **13**(10):e10646.
931 34. Nemesházi E, Bókony V: **HerpSexDet: the herpetological database of sex**
932 **determination and sex reversal**. *Scientific Data* 2023, **10**(1):377.
933 35. Roco ÁS, Olmstead AW, Degitz SJ, Amano T, Zimmerman LB, Bullejos M: **Coexistence**
934 **of Y, W, and Z sex chromosomes in *Xenopus tropicalis***. *Proceedings of the National*
935 *Academy of Sciences* 2015, **112**(34):E4752-E4761.
936 36. Jeffries DL, Lavanchy G, Sermier R, Sredl MJ, Miura I, Borzée A, Barrow LN, Canestrelli
937 D, Crochet P-A, Dufresnes C *et al*: **A rapid rate of sex-chromosome turnover and**
938 **non-random transitions in true frogs**. *Nat Commun* 2018, **9**(1):4088.
939 37. Cauret CM, Jordan DC, Kukoly LM, Burton SR, Anele EU, Kwiecien JM, Gansauge M-T,
940 Senthillmohan S, Greenbaum E, Meyer M: **Functional dissection and assembly of a**
941 **small, newly evolved, W chromosome-specific genomic region of the African**
942 **clawed frog *Xenopus laevis***. *PLoS Genet* 2023, **19**(10):e1010990.
943 38. Kuhl H, Tan WH, Klopp C, Kleiner W, Koyun B, Ciorpac M, Feron R, Knytl M, Kloas W,
944 Schartl M *et al*: **A candidate sex determination locus in amphibians which evolved**
945 **by structural variation between X- and Y-chromosomes**. *Nat Commun* 2024,
946 **15**(1):4781.
947 39. Bogart JP, Bi K, Fu J, Noble DW, Niedzwiecki J: **Unisexual salamanders (genus**
948 ***Ambystoma*) present a new reproductive mode for eukaryotes**. *Genome* 2007,
949 **50**(2):119-136.
950 40. McElroy KE, Denton RD, Sharbrough J, Bankers L, Neiman M, Gibbs HL: **Genome**
951 **expression balance in a triploid trihybrid vertebrate**. *Genome Biol Evol* 2017,
952 **9**(4):968-980.
953 41. Li Y, Ren Y, Zhang D, Jiang H, Wang Z, Li X, Rao D: **Chromosome-level assembly of the**
954 **mustache toad genome using third-generation DNA sequencing and Hi-C analysis**.
955 *GigaScience* 2019, **8**(9):giz114.
956 42. Das B, Cai L, Carter MG, Piao Y-L, Sharov AA, Ko MS, Brown DD: **Gene expression**
957 **changes at metamorphosis induced by thyroid hormone in *Xenopus laevis* tadpoles**.
958 *Dev Biol* 2006, **291**(2):342-355.
959 43. Schott RK, Bell RC, Loew ER, Thomas KN, Gower DJ, Streicher JW, Fujita MK:
960 **Transcriptomic evidence for visual adaptation during the aquatic to terrestrial**
961 **metamorphosis in leopard frogs**. *BMC Biol* 2022, **20**(1):138.
962 44. Wollenberg Valero KC, Garcia-Porta J, Rodríguez A, Arias M, Shah A, Randrianiaina
963 RD, Brown JL, Glaw F, Amat F, Künzel S *et al*: **Transcriptomic and macroevolutionary**
964 **evidence for phenotypic uncoupling between frog life history phases**. *Nat Commun*
965 2017, **8**(1):15213.
966 45. Zhao L, Liu L, Wang S, Wang H, Jiang J: **Transcriptome profiles of metamorphosis in**
967 **the ornamented pygmy frog *Microhyla fissipes* clarify the functions of thyroid**
968 **hormone receptors in metamorphosis**. *Sci Rep* 2016, **6**(1):27310.
969 46. Palacios-Martinez J, Caballero-Perez J, Espinal-Centeno A, Marquez-Chavoya G,
970 Lomeli H, Salas-Vidal E, Schnabel D, Chimal-Monroy J, Cruz-Ramirez A: **Multi-organ**
971 **transcriptomic landscape of *Ambystoma velasci* metamorphosis**. *Dev Biol* 2020,
972 **466**(1-2):22-35.
973 47. Sanchez E, Küpfer E, Goedbloed DJ, Nolte AW, Lüddecke T, Schulz S, Vences M,
974 Steinfartz S: **Morphological and transcriptomic analyses reveal three discrete**

975 **primary stages of postembryonic development in the common fire salamander,**
976 ***Salamandra salamandra***. *Journal of Experimental Zoology Part B: Molecular and*
977 *Developmental Evolution* 2018, **330**(2):96-108.
978 48. Goedert D, Calsbeek R: **Experimental evidence that metamorphosis alleviates**
979 **genomic conflict**. *The American Naturalist* 2019, **194**(3):356-366.
980 49. Kyono Y, Raj S, Sifuentes CJ, Buisine N, Sachs L, Denver RJ: **DNA methylation**
981 **dynamics underlie metamorphic gene regulation programs in *Xenopus* tadpole**
982 **brain**. *Dev Biol* 2020, **462**(2):180-196.
983 50. Pfennig KS: **Facultative mate choice drives adaptive hybridization**. *Science* 2007,
984 **318**(5852):965-967.
985 51. Levis NA, Pfennig DW: **Innovation and diversification via plasticity-led evolution**. In:
986 *Phenotypic plasticity & evolution*. CRC Press; 2021: 211-240.
987 52. Pfennig DW, Collins JP: **Kinship affects morphogenesis in cannibalistic salamanders**.
988 *Nature* 1993, **362**(6423):836-838.
989 53. Pfennig DW, Reeve HK, Sherman PW: **Kin recognition and cannibalism in spadefoot**
990 **toad tadpoles**. *Anim Behav* 1993, **46**(1):87-94.
991 54. DeVore JL, Crossland MR, Shine R, Ducatez S: **The evolution of targeted cannibalism**
992 **and cannibal-induced defenses in invasive populations of cane toads**. *Proceedings*
993 *of the National Academy of Sciences* 2021, **118**(35):e2100765118.
994 55. Brockes JP, Kumar A: **Comparative aspects of animal regeneration**. *Annu Rev Cell Dev*
995 *Biol* 2008, **24**:525-549.
996 56. Cox BD, Yun MH, Poss KD: **Can laboratory model systems instruct human limb**
997 **regeneration?** *Development* 2019, **146**(20):dev181016.
998 57. Torres-Sánchez M: **Variation under domestication in animal models: the case of the**
999 **Mexican axolotl**. *BMC Genomics* 2020, **21**(1):827.
1000 58. Yu Q, Gates PB, Rogers S, Mikicic I, Elewa A, Salomon F, Lachnit M, Caldarelli A,
1001 Flores-Rodriguez N, Cesare AJ *et al*: **Telomerase-independent maintenance of**
1002 **telomere length in a vertebrate**. *bioRxiv* 2022:2022.2003.2025.485759.
1003 59. Bruckskotten M, Looso M, Reinhardt R, Braun T, Borchardt T: **Newt-omics: a**
1004 **comprehensive repository for omics data from the newt *Notophthalmus***
1005 ***viridescens***. *Nucleic Acids Res* 2012, **40**(Database issue):D895-900.
1006 60. Feng Y-J, Blackburn DC, Liang D, Hillis DM, Wake DB, Cannatella DC, Zhang P:
1007 **Phylogenomics reveals rapid, simultaneous diversification of three major clades of**
1008 **Gondwanan frogs at the Cretaceous–Paleogene boundary**. *Proceedings of the*
1009 *National Academy of Sciences* 2017, **114**(29):E5864-E5870.
1010 61. Schott RK, Fujita MK, Streicher JW, Gower DJ, Thomas KN, Loew ER, Bamba Kaya AG,
1011 Bittencourt-Silva GB, Guillherme Becker C, Cisneros-Heredia D: **Diversity and**
1012 **Evolution of Frog Visual Opsins: Spectral Tuning and Adaptation to Distinct Light**
1013 **Environments**. *Mol Biol Evol* 2024, **41**(4):msae049.
1014 62. Rancilhac L, Irisarri I, Angelini C, Arntzen JW, Babik W, Bossuyt F, Künzel S, Lüddecke T,
1015 Pasmans F, Sanchez E *et al*: **Phylotranscriptomic evidence for pervasive ancient**
1016 **hybridization among Old World salamanders**. *Mol Phylogen Evol* 2021, **155**:106967.
1017 63. Dubey S, Maddalena T, Bonny L, Jeffries DL, Dufresnes C: **Population genomics of an**
1018 **exceptional hybridogenetic system of *Pelophylax* water frogs**. *BMC Evol Biol* 2019,
1019 **19**(1):164.

1020 64. Dufresnes C, Ambu J, Galán P, Sequeira F, Viesca L, Choda M, Álvarez D, Alard B,
1021 Suchan T, Künzel S: **Delimiting phylogeographic diversity in the genomic era:**
1022 **application to an Iberian endemic frog**. *Zool J Linn Soc* 2023:zlad170.
1023 65. Ovchinnikov V, Uliano-Silva M, Wilkinson M, Wood J, Smith M, Oliver K, Sims Y,
1024 Torrance J, Suh A, McCarthy SA *et al*: **Caecilian Genomes Reveal the Molecular Basis**
1025 **of Adaptation and Convergent Evolution of Limblessness in Snakes and Caecilians**.
1026 *Mol Biol Evol* 2023, **40**(5).
1027 66. Jetz W, Pyron RA: **The interplay of past diversification and evolutionary isolation**
1028 **with present imperilment across the amphibian tree of life**. *Nature Ecology &*
1029 *Evolution* 2018, **2**(5):850-858.
1030 67. Siu-Ting K, Torres-Sánchez M, San Mauro D, Wilcockson D, Wilkinson M, Pisani D,
1031 O'Connell MJ, Creevey CJ: **Inadvertent Paralog Inclusion Drives Artifactual**
1032 **Topologies and Timetree Estimates in Phylogenomics**. *Mol Biol Evol* 2019,
1033 **36**(6):1344-1356.
1034 68. Hime PM, Lemmon AR, Lemmon ECM, Prendini E, Brown JM, Thomson RC, Kratovil
1035 JD, Noonan BP, Pyron RA, Peloso PL: **Phylogenomics reveals ancient gene tree**
1036 **discordance in the amphibian tree of life**. *Syst Biol* 2021, **70**(1):49-66.
1037 69. Portik DM, Streicher JW, Blackburn DC, Moen DS, Hutter CR, Wiens JJ: **Redefining**
1038 **Possible: Combining Phylogenomic and Supersparse Data in Frogs**. *Mol Biol Evol*
1039 2023, **40**(5).
1040 70. Sun Y-B, Xiong Z-J, Xiang X-Y, Liu S-P, Zhou W-W, Tu X-L, Zhong L, Wang L, Wu D-D,
1041 Zhang B-L *et al*: **Whole-genome sequence of the Tibetan frog *Nanorana parkeri* and**
1042 **the comparative evolution of tetrapod genomes**. *Proceedings of the National*
1043 *Academy of Sciences* 2015, **112**(11):E1257-E1262.
1044 71. Torres-Sánchez M, Gower DJ, Alvarez-Ponce D, Creevey CJ, Wilkinson M, San Mauro
1045 D: **What lies beneath? Molecular evolution during the radiation of caecilian**
1046 **amphibians**. *BMC Genomics* 2019, **20**(1):354.
1047 72. Li J-T, Gao Y-D, Xie L, Deng C, Shi P, Guan M-L, Huang S, Ren J-L, Wu D-D, Ding L:
1048 **Comparative genomic investigation of high-elevation adaptation in ectothermic**
1049 **snakes**. *Proceedings of the National Academy of Sciences* 2018, **115**(33):8406-8411.
1050 73. Storz JF: **High-Altitude Adaptation: Mechanistic Insights from Integrated Genomics**
1051 **and Physiology**. *Mol Biol Evol* 2021, **38**(7):2677-2691.
1052 74. Yu L, Wang G-D, Ruan J, Chen Y-B, Yang C-P, Cao X, Wu H, Liu Y-H, Du Z-L, Wang X-P:
1053 **Genomic analysis of snub-nosed monkeys (*Rhinopithecus*) identifies genes and**
1054 **processes related to high-altitude adaptation**. *Nat Genet* 2016, **48**(8):947-952.
1055 75. Seimon TA, Seimon A, Daszak P, Halloy SR, Schloegel LM, Aguilar CA, Sowell P, Hyatt
1056 AD, Konecky B, E Simmons J: **Upward range extension of Andean anurans and**
1057 **chytridiomycosis to extreme elevations in response to tropical deglaciation**. *Global*
1058 *Change Biol* 2007, **13**(1):288-299.
1059 76. Acosta-Galvis AR: **Ranas, salamandras y caecilias (Tetrapoda: Amphibia) de**
1060 **Colombia**. *Biota colombiana* 2000, **1**(3).
1061 77. Yang W, Qi Y, Fu J: **Genetic signals of high-altitude adaptation in amphibians: a**
1062 **comparative transcriptome analysis**. *BMC Genet* 2016, **17**(1):134.
1063 78. Lu B, Jin H, Fu J: **Molecular convergent and parallel evolution among four high-**
1064 **elevation anuran species from the Tibetan region**. *BMC Genomics* 2020, **21**:1-14.

1065 79. Wang G-D, Zhang B-L, Zhou W-W, Li Y-X, Jin J-Q, Shao Y, Yang H-c, Liu Y-H, Yan F, Chen
1066 H-M: **Selection and environmental adaptation along a path to speciation in the**
1067 **Tibetan frog *Nanorana parkeri***. *Proc Natl Acad Sci USA* 2018, **115**(22):E5056-E5065.
1068 80. Sun Y-B, Fu T-T, Jin J-Q, Murphy RW, Hillis DM, Zhang Y-P, Che J: **Species groups**
1069 **distributed across elevational gradients reveal convergent and continuous genetic**
1070 **adaptation to high elevations**. *Proceedings of the National Academy of Sciences*
1071 2018, **115**(45):E10634-E10641.
1072 81. Cayuela H, Dorant Y, Forester BR, Jeffries DL, Mccaffery RM, Eby LA, Hossack BR,
1073 Gippet JM, Pilliod DS, Chris Funk W: **Genomic signatures of thermal adaptation are**
1074 **associated with clinal shifts of life history in a broadly distributed frog**. *J Anim Ecol*
1075 2022, **91**(6):1222-1238.
1076 82. Hutchison VH, Haines HB, Engbretson G: **Aquatic life at high altitude: Respiratory**
1077 **adaptations in the lake titicaca frog, *Telmatobius culeus***. *Respiration Physiology*
1078 1976, **27**(1):115-129.
1079 83. Dunn ER: **The salamanders of the family Plethodontidae**, vol. 7: Smith College; 1926.
1080 84. Daly J, Garraffo H, Pannell L, Spande T, Severini C, Erspamer V: **Alkaloids from**
1081 **Australian frogs (Myobatrachidae): pseudophrynamines and pumiliotoxins**. *J Nat*
1082 *Prod* 1990, **53**(2):407-421.
1083 85. Darst CR, Cummings ME: **Predator learning favours mimicry of a less-toxic model in**
1084 **poison frogs**. *Nature* 2006, **440**(7081):208-211.
1085 86. Hayes RA, Piggott AM, Dalle K, Capon RJ: **Microbial biotransformation as a source of**
1086 **chemical diversity in cane toad steroid toxins**. *Bioorganic & Medicinal Chemistry*
1087 *Letters* 2009, **19**(6):1790-1792.
1088 87. Tóth Z, Kurali A, Móricz ÁM, Hettyey A: **Changes in Toxin Quantities Following**
1089 **Experimental Manipulation of Toxin Reserves in *Bufo bufo* Tadpoles**. *J Chem Ecol*
1090 2019, **45**(3):253-263.
1091 88. Vaelli PM, Theis KR, Williams JE, O'Connell LA, Foster JA, Eisthen HL: **The skin**
1092 **microbiome facilitates adaptive tetrodotoxin production in poisonous newts**. *eLife*
1093 2020, **9**:e53898.
1094 89. Daly JW, Martin Garraffo H, Spande TF, Jaramillo C, Stanley Rand A: **Dietary source**
1095 **for skin alkaloids of poison frogs (Dendrobatidae)?** *J Chem Ecol* 1994, **20**:943-955.
1096 90. Darst CR, Menéndez-Guerrero PA, Coloma LA, Cannatella DC: **Evolution of dietary**
1097 **specialization and chemical defense in poison frogs (Dendrobatidae): a**
1098 **comparative analysis**. *The American Naturalist* 2005, **165**(1):56-69.
1099 91. Caty SN, Alvarez-Buylla A, Byrd GD, Vidoudez C, Roland AB, Tapia EE, Budnik B,
1100 Trauger SA, Coloma LA, O'Connell LA: **Molecular physiology of chemical defenses in**
1101 **a poison frog**. *J Exp Biol* 2019, **222**(12):jeb204149.
1102 92. Alvarez-Buylla A, Fischer M-T, Moya Garzon MD, Rangel AE, Tapia EE, Tanzo JT, Soh
1103 HT, Coloma LA, Long JZ, O'Connell LA: **Binding and sequestration of poison frog**
1104 **alkaloids by a plasma globulin**. *eLife* 2023, **12**:e85096.
1105 93. Abderemane-Ali F, Rossen ND, Kobiela ME, Craig RA, Garrison CE, Chen Z, Colleran
1106 CM, O'Connell LA, Du Bois J, Dumbacher JP: **Evidence that toxin resistance in poison**
1107 **birds and frogs is not rooted in sodium channel mutations and may rely on "toxin**
1108 **sponge" proteins**. *J Gen Physiol* 2021, **153**(9):e202112872.
1109 94. Márquez R, Ramírez-Castañeda V, Amézquita A: **Does batrachotoxin autoresistance**
1110 **coevolve with toxicity in *Phyllobates* poison-dart frogs?** *Evolution* 2019, **73**(2):390-
1111 400.

1112 95. Tarvin RD, Borghese CM, Sachs W, Santos JC, Lu Y, O'Connell LA, Cannatella DC, Harris
1113 RA, Zakon HH: **Interacting amino acid replacements allow poison frogs to evolve**
1114 **epibatidine resistance**. *Science* 2017, **357**(6357):1261-1266.
1115 96. Tarvin RD, Santos JC, O'Connell LA, Zakon HH, Cannatella DC: **Convergent**
1116 **substitutions in a sodium channel suggest multiple origins of toxin resistance in**
1117 **poison frogs**. *Mol Biol Evol* 2016, **33**(4):1068-1080.
1118 97. Shibao PYT, Cologna CT, Morandi-Filho R, Wiezel GA, Fujimura PT, Ueira-Vieira C,
1119 Arantes EC: **Deep sequencing analysis of toad *Rhinella schneideri* skin glands and**
1120 **partial biochemical characterization of its cutaneous secretion**. *J Venom Anim Tox*
1121 *incl Trop Dis* 2018, **24**(1):36.
1122 98. Torres-Sánchez M, Wilkinson M, Gower DJ, Creevey CJ, San Mauro D: **Insights into**
1123 **the skin of caecilian amphibians from gene expression profiles**. *BMC Genomics*
1124 2020, **21**(1):515.
1125 99. Liscano Martinez Y, Arenas Gómez CM, Smith J, Delgado JP: **A tree frog (*Boana***
1126 ***pugnax*) dataset of skin transcriptome for the identification of biomolecules with**
1127 **potential antimicrobial activities**. *Data in Brief* 2020, **32**:106084.
1128 100. Lan Y, He L, Dong X, Tang R, Li W, Wang J, Wang L, Yue B, Price M, Guo T *et al*:
1129 **Comparative transcriptomes of three different skin sites for the Asiatic toad (*Bufo***
1130 ***gargarizans*)**. *PeerJ* 2022, **10**:e12993.
1131 101. Lv Y, Li Y, Wen Z, Shi Q: **Transcriptomic and gene-family dynamic analyses reveal**
1132 **gene expression pattern and evolution in toxin-producing tissues of Asiatic toad**
1133 **(*Bufo gargarizans*)**. *Frontiers in Ecology and Evolution* 2022, **10**:924248.
1134 102. Mohammadi S, Herrera-Álvarez S, Yang L, Rodriguez-Ordonez MdP, Zhang K, Storz JF,
1135 Dobler S, Crawford AJ, Andolfatto P: **Constraints on the evolution of toxin-resistant**
1136 **Na, K-ATPases have limited dependence on sequence divergence**. *PLoS Genet* 2022,
1137 **18**(8):e1010323.
1138 103. Mohammadi S, Yang L, Harpak A, Herrera-Álvarez S, del Pilar Rodríguez-Ordoñez M,
1139 Peng J, Zhang K, Storz JF, Dobler S, Crawford AJ: **Concerted evolution reveals co-**
1140 **adapted amino acid substitutions in Na+ K+-ATPase of frogs that prey on toxic**
1141 **toads**. *Curr Biol* 2021, **31**(12):2530-2538. e2510.
1142 104. Hutchinson DA, Mori A, Savitzky AH, Burghardt GM, Wu X, Meinwald J, Schroeder FC:
1143 **Dietary sequestration of defensive steroids in nuchal glands of the Asian snake**
1144 ***Rhabdophis tigrinus***. *Proceedings of the National Academy of Sciences* 2007,
1145 **104**(7):2265-2270.
1146 105. Brodie III ED, Brodie Jr ED: **Tetrodotoxin resistance in garter snakes: an evolutionary**
1147 **response of predators to dangerous prey**. *Evolution* 1990, **44**(3):651-659.
1148 106. Mancuso M, Zaman S, Maddock ST, Kamei RG, Salazar-Valenzuela D, Wilkinson M,
1149 Roelants K, Fry BG: **Resistance Is Not Futile: Widespread Convergent Evolution of**
1150 **Resistance to Alpha-Neurotoxic Snake Venoms in Caecilians (Amphibia:**
1151 **Gymnophiona)**. *International Journal of Molecular Sciences* 2023, **24**(14):11353.
1152 107. Symula R, Schulte R, Summers K: **Molecular phylogenetic evidence for a mimetic**
1153 **radiation in Peruvian poison frogs supports a Müllerian mimicry hypothesis**.
1154 *Proceedings of the Royal Society of London Series B: Biological Sciences* 2001,
1155 **268**(1484):2415-2421.
1156 108. Daly JW, Brown GB, Mensah-Dwumah M, Myers CW: **Classification of skin alkaloids**
1157 **from neotropical poison-dart frogs (Dendrobatidae)**. *Toxicon* 1978, **16**(2):163-188.

109. Stuckert AMM, Moore E, Coyle KP, Davison I, MacManes MD, Roberts R, Summers K: **Variation in pigmentation gene expression is associated with distinct aposematic color morphs in the poison frog *Dendrobates auratus***. *BMC Evol Biol* 2019, **19**(1):85.
110. Stuckert AM, Chouteau M, McClure M, LaPolice TM, Linderoth T, Nielsen R, Summers K, MacManes MD: **The genomics of mimicry: gene expression throughout development provides insights into convergent and divergent phenotypes in a Müllerian mimicry system**. *Mol Ecol* 2021, **30**(16):4039-4061.
111. Twomey E, Johnson JD, Castroviejo-Fisher S, Van Bocxlaer I: **A ketocarotenoid-based colour polymorphism in the Sira poison frog *Ranitomeya sirensis* indicates novel gene interactions underlying aposematic signal variation**. *Mol Ecol* 2020, **29**(11):2004-2015.
112. Twomey E, Kain M, Claeys M, Summers K, Castroviejo-Fisher S, Van Bocxlaer I: **Mechanisms for color convergence in a mimetic radiation of poison frogs**. *The American Naturalist* 2020, **195**(5):E132-E149.
113. Linderoth T, Aguilar-Gómez D, White E, Twomey E, Stuckert A, Bi K, Ko A, Graham N, Rocha JL, Chang J *et al*: **Genetic basis of aposematic coloration in a mimetic radiation of poison frogs**. *bioRxiv* 2023:2023.2004.2020.537757.
114. Stuckert AM, Freeborn L, Howell KA, Yang Y, Nielsen R, Richards-Zawacki C, MacManes MD: **Transcriptomic analyses during development reveal mechanisms of integument structuring and color production**. *Evol Ecol* 2023:1-22.
115. Burgon JD, Vieites DR, Jacobs A, Weidt SK, Gunter HM, Steinfartz S, Burgess K, Mable BK, Elmer KR: **Functional colour genes and signals of selection in colour-polymorphic salamanders**. *Mol Ecol* 2020, **29**(7):1284-1299.
116. Fischer EK, Roland AB, Moskowitz NA, Tapia EE, Summers K, Coloma LA, O'Connell LA: **The neural basis of tadpole transport in poison frogs**. *Proceedings of the Royal Society B: Biological Sciences* 2019, **286**(1907):20191084.
117. Crump ML: **Parental Care among the Amphibia**. In: *Advances in the Study of Behavior.* Edited by Rosenblatt JS, Snowdon CT, vol. 25: Academic Press; 1996: 109-144.
118. Wu W, Gao YD, Jiang DC, Lei J, Ren JL, Liao WB, Deng C, Wang Z, Hillis DM, Zhang YP *et al*: **Genomic adaptations for arboreal locomotion in Asian flying treefrogs**. *Proc Natl Acad Sci U S A* 2022, **119**(13):e2116342119.
119. Blackburn DC, Gray JA, Stanley EL: **The only "lungless" frog has a glottis and lungs**. *Curr Biol* 2024, **34**(10):R492-R493.
120. Lewis ZR, Kerney R, Hanken J: **Developmental basis of evolutionary lung loss in plethodontid salamanders**. *Sci Adv* 2022, **8**(33):eabo6108.
121. Heiss E, Natchev N, Salaberger D, Gumpenberger M, Rabanser A, Weisgram J: **Hurt yourself to hurt your enemy: new insights on the function of the bizarre antipredator mechanism in the salamandrid *Pleurodeles waltl***. *J Zool* 2010, **280**(2):156-162.
122. Brodie Jr ED, Nussbaum RA, DiGiovanni M: **Antipredator adaptations of Asian salamanders (Salamandridae)**. *Herpetologica* 1984:56-68.
123. Mailho-Fontana PL, Antoniazzi MM, Coelho GR, Pimenta DC, Fernandes LP, Kupfer A, Brodie ED, Jared C: **Milk provisioning in oviparous caecilian amphibians**. *Science* 2024, **383**(6687):1092-1095.

124. Kupfer A, Müller H, Antoniazzi MM, Jared C, Greven H, Nussbaum RA, Wilkinson M: **Parental investment by skin feeding in a caecilian amphibian**. *Nature* 2006, **440**(7086):926-929.
125. Liu Y, Jones CD, Day LB, Summers K, Burmeister SS: **Cognitive phenotype and differential gene expression in a hippocampal homologue in two species of frog**. *Integr Comp Biol* 2020, **60**(4):1007-1023.
126. Luedtke JA, Chanson J, Neam K, Hobin L, Maciel AO, Catenazzi A, Borzée A, Hamidy A, Aowphol A, Jean A *et al*: **Ongoing declines for the world's amphibians in the face of emerging threats**. *Nature* 2023, **622**(7982):308-314.
127. Vacher JP, Chave J, Ficetola FG, Sommeria-Klein G, Tao S, Thébaud C, Blanc M, Camacho A, Cassimiro J, Colston TJ: **Large-scale DNA-based survey of frogs in Amazonia suggests a vast underestimation of species richness and endemism**. *J Biogeogr* 2020, **47**(8):1781-1791.
128. Oliver PM, Bower DS, McDonald PJ, Kraus F, Luedtke J, Neam K, Hobin L, Chauvenet AL, Allison A, Arida E: **Melanesia holds the world's most diverse and intact insular amphibian fauna**. *Communications biology* 2022, **5**(1):1182.
129. Liu J, Slik F, Zheng S, Lindenmayer DB: **Undescribed species have higher extinction risk than known species**. *Conservation Letters* 2022, **15**(3):e12876.
130. Re:wild, Earth S, Group ISAS: **State of the World's Amphibians: The Second Global Amphibian Assessment**. In. Texas, USA: Re:wild; 2023.
131. Gower DJ, San Mauro D, Giri V, Bhatta G, Govindappa V, Kotharambath R, Oommen OV, Fatih FA, Mackenzie-Dodds JA, Nussbaum RA: **Molecular systematics of caeciliid caecilians (Amphibia: Gymnophiona) of the Western Ghats, India**. *Mol Phylogen Evol* 2011, **59**(3):698-707.
132. Forester BR, Beever EA, Darst C, Szymanski J, Funk WC: **Linking evolutionary potential to extinction risk: applications and future directions**. *Front Ecol Environ* 2022, **20**(9):507-515.
133. Nemesházi E, Bókony V: **Interplay of genotypic and thermal sex determination shapes climatic distribution in herpetofauna**. *bioRxiv* 2024:2024.2004.2021.589911.
134. Wollenberg Valero KC, Marshall JC, Bastiaans E, Caccone A, Camargo A, Morando M, Niemiller ML, Pabijan M, Russello MA, Sinervo B *et al*: **Patterns, Mechanisms and Genetics of Speciation in Reptiles and Amphibians**. *Genes (Basel)* 2019, **10**(9):646.
135. IUCN SSC ASG: **Draft for Open Consultation. The Amphibian Conservation Action Plan (ACAP): A status review and roadmap for global amphibian conservation**. *EcoEvoRxiv* 2022.
136. Hogg CJ: **Translating genomic advances into biodiversity conservation**. *Nat Rev Genet* 2023, **25**(5):362-373.
137. Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA: **Genetic rescue to the rescue**. *Trends Ecol Evol* 2015, **30**(1):42-49.
138. Pabijan M, Palomar G, Antunes B, Antoł W, Zieliński P, Babik W: **Evolutionary principles guiding amphibian conservation**. *Evol Appl* 2020, **13**(5):857-878.
139. Theissinger K, Fernandes C, Formenti G, Bista I, Berg PR, Bleidorn C, Bombarely A, Crottini A, Gallo GR, Godoy JA *et al*: **How genomics can help biodiversity conservation**. *Trends Genet* 2023, **39**(7):545-559.
140. Trumbo D, Hardy B, Crockett H, Muths E, Forester B, Cheek R, Zimmerman S, Corey-Rivas S, Bailey L, Funk WC: **Conservation genomics of an endangered montane**

1249 **amphibian reveals low population structure, low genomic diversity, and selection**
1250 **pressure from disease**. *Mol Ecol* 2023, **32**(24):6777-6795.
1251 141. Torres-Sánchez M, Longo AV: **Linking pathogen-microbiome-host interactions to**
1252 **explain amphibian population dynamics**. *Mol Ecol* 2022, **31**(22):5784-5794.
1253 142. Lopes CM, Baêta D, Valentini A, Lyra ML, Sabbag AF, Gasparini JL, Dejean T, Haddad
1254 CFB, Zamudio KR: **Lost and found: Frogs in a biodiversity hotspot rediscovered with**
1255 **environmental DNA**. *Mol Ecol* 2021, **30**(13):3289-3298.
1256 143. Cutajar TP, Rowley JJL: **Surveying frogs from the bellies of their parasites:**
1257 **Invertebrate-derived DNA as a novel survey method for frogs**. *Global Ecology and*
1258 *Conservation* 2020, **22**:e00978.
1259 144. Villacorta-Rath C, Hoskin CJ, Strugnell JM, Burrows D: **Long distance (> 20 km)**
1260 **downstream detection of endangered stream frogs suggests an important role for**
1261 **eDNA in surveying for remnant amphibian populations**. *PeerJ* 2021, **9**:e12013.
1262 145. Allendorf FW, Funk WC, Aitken SN, Byrne M, Luikart G: **Conservation and the**
1263 **genomics of populations**: Oxford University Press; 2022.
1264 146. Fischman RL, Ruhl JB, Forester BR, Lama TM, Kardos M, Rojas GA, Robinson NA,
1265 Shirey PD, Lamberti GA, Ando AW *et al*: **A landmark environmental law looks ahead**.
1266 *Science* 2023, **382**(6677):1348-1355.
1267 147. Programme UE: **Kunming-Montreal Global Biodiversity Framework —**
1268 **CBD/COP/15/L25**. In. Edited by Diversity UCoB. Montreal Canada; 2022.
1269 148. Kosch TA, Waddle AW, Cooper CA, Zenger KR, Garrick DJ, Berger L, Skerratt LF:
1270 **Genetic approaches for increasing fitness in endangered species**. *Trends Ecol Evol*
1271 2022, **37**(4):332-345.
1272 149. Kosch T, Silva C, Brannelly L, Roberts A, Lau Q, Marantelli G, Berger L, Skerratt L:
1273 **Genetic potential for disease resistance in critically endangered amphibians**
1274 **decimated by chytridiomycosis**. *Anim Conserv* 2019, **22**(3):238-250.
1275 150. Savage AE, Gratwicke B, Hope K, Bronikowski E, Fleischer RC: **Sustained immune**
1276 **activation is associated with susceptibility to the amphibian chytrid fungus**. *Mol*
1277 *Ecol* 2020, **29**(15):2889-2903.
1278 151. Bataille A, Cashins SD, Grogan L, Skerratt LF, Hunter D, McFadden M, Scheele B,
1279 Brannelly LA, Macris A, Harlow PS: **Susceptibility of amphibians to chytridiomycosis**
1280 **is associated with MHC class II conformation**. *Proc R Soc Lond, Ser B: Biol Sci* 2015,
1281 **282**(1805):20143127.
1282 152. Knapp RA, Wilber MQ, Byrne AQ, Joseph MB, Smith TC, Rothstein AP, Grasso RL,
1283 Rosenblum EB: **Reintroduction of resistant frogs facilitates landscape-scale recovery**
1284 **in the presence of a lethal fungal disease**. *bioRxiv* 2023:2023.2005.2022.541534.
1285 153. Byrne PG, Silla AJ: **An experimental test of the genetic consequences of population**
1286 **augmentation in an amphibian**. *Conserv Sci Pract* 2020, **2**(6):e194.
1287 154. Liddell E, Sunnucks P, Cook CN: **To mix or not to mix gene pools for threatened**
1288 **species management? Few studies use genetic data to examine the risks of both**
1289 **actions, but failing to do so leads disproportionately to recommendations for**
1290 **separate management**. *Biol Conserv* 2021, **256**:109072.
1291 155. Kyriazis CC, Wayne RK, Lohmueller KE: **Strongly deleterious mutations are a primary**
1292 **determinant of extinction risk due to inbreeding depression**. *Evolution Letters* 2021,
1293 **5**:33-47.

1294 156. Berger L, Skerratt LF, Kosch TA, Brannelly LA, Webb RJ, Waddle AW: **Advances in**
1295 **Managing Chytridiomycosis for Australian Frogs: *Gradarius Firmus* Victoria**. *Annu*
1296 *Rev Anim Biosci* 2024, **12**(1):113-133.
1297 157. Collier-Robinson L, Rayne A, Rupene M, Thoms C, Steeves T: **Embedding indigenous**
1298 **principles in genomic research of culturally significant species**. *N Z J Ecol* 2019,
1299 **43**(3):1-9.
1300 158. Rayne A, Blair S, Dale M, Flack B, Hollows J, Moraga R, Parata RN, Rupene M, Tamati-
1301 Elliffe P, Wehi PM *et al*: **Weaving place-based knowledge for culturally significant**
1302 **species in the age of genomics: Looking to the past to navigate the future**. *Evol Appl*
1303 2022, **15**(5):751-772.
1304 159. Mc Cartney AM, Head MA, Tsosie KS, Sterner B, Glass JR, Paez S, Geary J, Hudson M:
1305 **Indigenous peoples and local communities as partners in the sequencing of global**
1306 **eukaryotic biodiversity**. *npj Biodiversity* 2023, **2**(1):8.
1307 160. Carroll S, Garba I, Figueroa-Rodríguez O, Holbrook J, Lovett R, Materechera S, Parsons
1308 M, Raseroka K, Rodriguez-Lonebear D, Rowe R: **The CARE principles for indigenous**
1309 **data governance**. *Data Science Journal* 2020, **19**(43):1-21.
1310 161. Ramírez-Castañeda V, Westeen EP, Frederick J, Amini S, Wait DR, Achmadi AS,
1311 Andayani N, Arida E, Arifin U, Bernal MA *et al*: **A set of principles and practical**
1312 **suggestions for equitable fieldwork in biology**. *Proceedings of the National Academy*
1313 *of Sciences* 2022, **119**(34):e2122667119.
1314 162. Feres MVC: **Biodiversity, traditional knowledge and patent rights: The case study of**
1315 ***Phyllomedusa bicolor***. *Revista Direito GV* 2022, **18**:e2205.
1316 163. Te Aika B, Liggins L, Rye C, Perkins EO, Huh J, Brauning R, Godfery T, Black MA:
1317 **Aotearoa genomic data repository: An āhuru mōwai for taonga species sequencing**
1318 **data**. *Mol Ecol Resour* 2023, **00**:1-14.
1319 164. Buckner JC, Sanders RC, Faircloth BC, Chakrabarty P: **The critical importance of**
1320 **vouchers in genomics**. *eLife* 2021, **10**:e68264.
1321 165. Golan J, Riddle K, Hudson M, Anderson J, Kusabs N, Coltman T: **Benefit sharing: Why**
1322 **inclusive provenance metadata matter**. *Front Genet* 2022, **13**:1014044.
1323 166. Duellman WE, Trueb L: **Biology of Amphibians**: JHU press; 1994.
1324 167. Dahn HA, Mountcastle J, Balacco J, Winkler S, Bista I, Schmitt AD, Pettersson OV,
1325 Formenti G, Oliver K, Smith M *et al*: **Benchmarking ultra-high molecular weight DNA**
1326 **preservation methods for long-read and long-range sequencing**. *GigaScience* 2022,
1327 **11**.
1328 168. Forzán MJ, Heatley J, Russell KE, Horney B: **Clinical pathology of amphibians: a**
1329 **review**. *Veterinary Clinical Pathology* 2017, **46**(1):11-33.
1330 169. IUCN: **IUCN policy statement on research involving species at risk of extinction**. In.
1331 Gland, Switzerland: International Union for the Conservation of Nature.; 1989.
1332 170. Ambu J, Dufresnes C: **Buccal swabs for amphibian genomics**. *Amphibia-Reptilia*
1333 2023, **44**(2):249-255.
1334 171. Oyler-McCance SJ, Ryan MJ, Sullivan BK, Fike JA, Cornman RS, Giermakowski JT,
1335 Zimmerman SJ, Harrow RL, Hedwall SJ, Hossack BR *et al*: **Genetic connectivity in the**
1336 **Arizona toad (*Anaxyrus microscaphus*): implications for conservation of a stream**
1337 **dwelling amphibian in the arid Southwestern United States**. *Conserv Genet* 2024,
1338 **25**(3):835-848.

172. Farquharson KA, McLennan EA, Belov K, Hogg CJ: **The genome sequence of the critically endangered Kroombit tinkerfrog (*Taudactylus pleione*)**. *F1000Research* 2023, **12**:845.
173. Minich JJ, Moore ML, Allsing NA, Aylward A, Murray ER, Tran L, Michael TP: **Generating high-quality plant and fish reference genomes from field-collected specimens by optimizing preservation**. *Communications Biology* 2023, **6**(1):1246.
174. Blom MPK: **Opportunities and challenges for high-quality biodiversity tissue archives in the age of long-read sequencing**. *Mol Ecol* 2021, **30**(23):5935-5948.
175. Alves RJV, Weksler M, Oliveira JA, Buckup PA, SANTANA HR, PERACCHI AL, KELLNER AW, ALEIXO A, LANGGUTH A, ALMEIDA A: **Brazilian legislation on genetic heritage harms Biodiversity Convention goals and threatens basic biology research and education**. In., vol. 90: SciELO Brasil; 2018: 1279-1284.
176. Alexander GJ, Tollev KA, Maritz B, McKechnie A, Manger P, Thomson RL, Schradin C, Fuller A, Meyer L, Hetem RS: **Excessive red tape is strangling biodiversity research in South Africa**. *S Afr J Sci* 2021, **117**(9-10):1-4.
177. Haelewaters D, Hofmann TA, Romero-Olivares AL: **Ten simple rules for Global North researchers to stop perpetuating helicopter research in the Global South**. *PLoS Comp Biol* 2021, **17**(8):e1009277.
178. de Vos A, Schwartz MW: **Confronting parachute science in conservation**. *Conserv Sci Pract* 2022, **4**(5):e12681.
179. Watsa M, Erkenswick GA, Pomerantz A, Prost S: **Portable sequencing as a teaching tool in conservation and biodiversity research**. *PLoS Biol* 2020, **18**(4):e3000667.
180. Armenteras D: **Guidelines for healthy global scientific collaborations**. *Nature Ecology & Evolution* 2021, **5**(9):1193-1194.
181. Fong JJ, Blom MP, Aowphol A, McGuire JA, Sutcharit C, Soltis PS: **Recent advances in museomics: revolutionizing biodiversity research**. *Frontiers in Ecology and Evolution* 2023, **11**:1188172.
182. Raxworthy CJ, Smith BT: **Mining museums for historical DNA: advances and challenges in museomics**. *Trends Ecol Evol* 2021, **36**(11):1049-1060.
183. Roycroft E, Moritz C, Rowe KC, Moussalli A, Eldridge MDB, Portela Miguez R, Piggott MP, Potter S: **Sequence Capture From Historical Museum Specimens: Maximizing Value for Population and Phylogenomic Studies**. *Frontiers in Ecology and Evolution* 2022, **10**:931644.
184. Speer KA, Hawkins MTR, Flores MFC, McGowen MR, Fleischer RC, Maldonado JE, Campana MG, Muletz-Wolz CR: **A comparative study of RNA yields from museum specimens, including an optimized protocol for extracting RNA from formalin-fixed specimens**. *Frontiers in Ecology and Evolution* 2022, **10**:953131.
185. Dabney J, Knapp M, Glocke I, Gansauge M-T, Weihmann A, Nickel B, Valdiosera C, García N, Pääbo S, Arsuaga J-L: **Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments**. *Proceedings of the National Academy of Sciences* 2013, **110**(39):15758-15763.
186. Gansauge M-T, Gerber T, Glocke I, Korlević P, Lippik L, Nagel S, Riehl LM, Schmidt A, Meyer M: **Single-stranded DNA library preparation from highly degraded DNA using T4 DNA ligase**. *Nucleic Acids Res* 2017, **45**(10):e79-e79.
187. Straube N, Lyra ML, Paijmans JL, Preick M, Basler N, Penner J, Rödel MO, Westbury MV, Haddad CF, Barlow A: **Successful application of ancient DNA extraction and**

1385 **library construction protocols to museum wet collection specimens**. *Mol Ecol*
1386 *Resour* 2021, **21**(7):2299-2315.
1387 188. Rancilhac L, Bruy T, Scherz MD, Pereira EA, Preick M, Straube N, Lyra ML, Ohler A,
1388 Streicher JW, Andreone F: **Target-enriched DNA sequencing from historical type**
1389 **material enables a partial revision of the Madagascar giant stream frogs (genus**
1390 ***Mantidactylus*)**. *J Nat Hist* 2020, **54**(1-4):87-118.
1391 189. Scherz MD, Rasolonjatovo SM, Köhler J, Rancilhac L, Rakotoarison A, Raselimanana
1392 AP, Ohler A, Preick M, Hofreiter M, Glaw F: **'Barcode fishing' for archival DNA from**
1393 **historical type material overcomes taxonomic hurdles, enabling the description of a**
1394 **new frog species**. *Sci Rep* 2020, **10**(1):19109.
1395 190. Dalén L, Heintzman PD, Kapp JD, Shapiro B: **Deep-time paleogenomics and the limits**
1396 **of DNA survival**. *Science* 2023, **382**(6666):48-53.
1397 191. Ramírez JP, Jaramillo CA, Lindquist ED, Crawford AJ, Ibáñez R: **Recent and Rapid**
1398 **Radiation of the Highly Endangered Harlequin Frogs (*Atelopus*) into Central**
1399 **America Inferred from Mitochondrial DNA Sequences**. *Diversity* 2020, **12**(9):360.
1400 192. Renner SS, Scherz MD, Schoch CL, Gottschling M, Vences M: **DNA sequences from**
1401 **type specimens and type strains–how to increase their number and improve their**
1402 **annotation in NCBI GenBank and related databases**. *Syst Biol* 2023:syad068.
1403 193. Rakotoarison A, Scherz MD, Mullin KE, Crottini A, Petzold A, Ranjanaharisoa FA,
1404 Maheritafika HMR, Rafanoharana JM, Raherinjatovo H, Andreone F: **Gray versus**
1405 **yellow ventral coloration: Identity, distribution, color polymorphism and molecular**
1406 **relationships of the microhylid frog *Platypelis mavomavo* Andreone, Fenolio &**
1407 **Walvoord, 2003**. *Zootaxa* 2023, **5352**(2):221-234.
1408 194. Evans BJ, Gansauge M-T, Stanley EL, Furman BLS, Cauret CMS, Ofori-Boateng C,
1409 Gvoždík V, Streicher JW, Greenbaum E, Tinsley RC *et al*: ***Xenopus fraseri*: Mr. Fraser,**
1410 **where did your frog come from?** *PLOS ONE* 2019, **14**(9):e0220892.
1411 195. Wong K-C: **Big data challenges in genome informatics**. *Biophysical Reviews* 2019,
1412 **11**(1):51-54.
1413 196. Zuo B, Nneji LM, Sun Y-B: **Comparative genomics reveals insights into anuran**
1414 **genome size evolution**. *BMC Genomics* 2023, **24**(1):379.
1415 197. Alkan C, Sajjadian S, Eichler EE: **Limitations of next-generation genome sequence**
1416 **assembly**. *Nat Methods* 2011, **8**(1):61-65.
1417 198. Treangen TJ, Salzberg SL: **Repetitive DNA and next-generation sequencing:**
1418 **computational challenges and solutions**. *Nat Rev Genet* 2012, **13**(1):36-46.
1419 199. Sun Y, Shang L, Zhu Q-H, Fan L, Guo L: **Twenty years of plant genome sequencing:**
1420 **achievements and challenges**. *Trends Plant Sci* 2022, **27**(4):391-401.
1421 200. Mable B, Alexandrou M, Taylor M: **Genome duplication in amphibians and fish: an**
1422 **extended synthesis**. *J Zool* 2011, **284**(3):151-182.
1423 201. Schmid M, Evans BJ, Bogart JP: **Polyploidy in Amphibia**. *Cytogenet Genome Res* 2015,
1424 **145**(3-4):315-330.
1425 202. Session AM, Uno Y, Kwon T, Chapman JA, Toyoda A, Takahashi S, Fukui A, Hikosaka A,
1426 Suzuki A, Kondo M *et al*: **Genome evolution in the allotetraploid frog *Xenopus***
1427 ***laevis***. *Nature* 2016, **538**(7625):336-343.
1428 203. Rhie A, McCarthy SA, Fedrigo O, Damas J, Formenti G, Koren S, Uliano-Silva M, Chow
1429 W, Fungtammasan A, Kim J *et al*: **Towards complete and error-free genome**
1430 **assemblies of all vertebrate species**. *Nature* 2021, **592**(7856):737-746.

1431 204. Consortium TU: **UniProt: the Universal Protein Knowledgebase in 2023**. *Nucleic*
1432 *Acids Res* 2022, **51**(D1):D523-D531.
1433 205. Dimitrakopoulou D, Khwatenge CN, James-Zorn C, Paiola M, Bellin EW, Tian Y,
1434 Sundararaj N, Polak EJ, Grayfer L, Barnard D *et al*: **Advances in the *Xenopus***
1435 **immunome: Diversification, expansion, and contraction**. *Dev Comp Immunol* 2023,
1436 **145**:104734.
1437 206. Bertola LV, Hoskin CJ, Jones DB, Zenger KR, McKnight DT, Higgie M: **The first linkage**
1438 **map for Australo-Papuan Treefrogs (family: Pelodryadidae) reveals the sex-**
1439 **determination system of the Green-eyed Treefrog (*Litoria serrata*)**. *Heredity* 2023,
1440 **131**(4):263-272.
1441 207. Silla AJ, Byrne PG: **The Role of Reproductive Technologies in Amphibian**
1442 **Conservation Breeding Programs**. *Annu Rev Anim Biosci* 2019, **7**(1):499-519.
1443 208. Banach M, Edholm E-S, Robert J: **Exploring the functions of nonclassical MHC class**
1444 **Ib genes in *Xenopus laevis* by the CRISPR/Cas9 system**. *Dev Biol* 2016, **426**(2):261-
1445 269.
1446 209. Grainger RM: ***Xenopus tropicalis* as a model organism for genetics and genomics:**
1447 **past, present, and future**. *Xenopus Protocols: Post-Genomic Approaches* 2012:3-15.
1448 210. Sousounis K, Courtemanche K, Whited JL: **A Practical Guide for CRISPR-Cas9-Induced**
1449 **Mutations in Axolotls**. In: *Salamanders: Methods and Protocols.* Springer; 2022: 335-
1450 349.
1451 211. Wang F, Shi Z, Cui Y, Guo X, Shi Y-B, Chen Y: **Targeted gene disruption in *Xenopus***
1452 ***laevis* using CRISPR/Cas9**. *Cell & bioscience* 2015, **5**:1-5.
1453 212. Douglas AJ, Todd LA, Katzenback BA: **The amphibian invitrome: Past, present, and**
1454 **future contributions to our understanding of amphibian immunity**. *Dev Comp*
1455 *Immunol* 2023, **142**:104644.
1456 213. Bui-Marinos MP, Todd LA, Douglas AJ, Katzenback BA: **So, you want to create a frog**
1457 **cell line? A guide to establishing frog skin cell lines from tissue explants**. *MethodsX*
1458 2022, **9**:101693.
1459 214. Stiller J, Feng S, Chowdhury A-A, Rivas-González I, Duchêne DA, Fang Q, Deng Y,
1460 Kozlov A, Stamatakis A, Claramunt S *et al*: **Complexity of avian evolution revealed by**
1461 **family-level genomes**. *Nature* 2024, **629**(818):851-860.
1462 215. Lees AC, Pimm SL: **Species, extinct before we know them?** *Curr Biol* 2015,
1463 **25**(5):R177-R180.
1464