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

Authors

Tiffany A. Kosch[1§]*, María Torres-Sánchez[2§]*, H. Christoph Liedtke[3], Kyle Summers[4], Maximina H. Yun[5,6], Andrew J. Crawford[7,8], Simon T. Maddock[9,10], Md. Sabbir Ahammed[11], Victor L. N. Araújo[7], Lorenzo V. Bertola[12], Gary M. Bucciarelli[13], Albert Carné[14], Céline M. Carneiro[15], Kin O. Chan[16], Ying Chen[17], Angelica Crottini[18,19,20], Jessica M. da Silva[21,22], Robert D. Denton[23], Carolin Dittrich[24], Gonçalo Espregueira Themudo[25], Katherine A. Farquharson[26,27], Natalie J. Forsdick[28], Edward Gilbert[29,30], Jing Che[31,32], Barbara A. Katzenback[33], Ramachandran Kotharambath[34], Nicholas A. Levis[35], Roberto Márquez[36], Glib Mazepa[37,38], Kevin P. Mulder[39], Hendrik Müller[40], Mary J. O'Connell[41], Pablo Orozco-terWengel[42], Gemma Palomar[43,44], Alice Petzold[45], David W. Pfennig[46], Karin S. Pfennig[46], Michael S. Reichert[47], Jacques Robert[48], Mark D. Scherz[49], Karen Siu-Ting[50,51,52], Anthony A. Snead[53], Matthias Stöck[54], Adam M. M. Stuckert[55], Jennifer L. Stynoski[56], Rebecca D. Tarvin[57], Katharina C. Wollenberg Valero[58], and The Amphibian Genomics Consortium (AGC).

§contributed equally to the manuscript

*correspondence:

Tiffany A. Kosch (tiffany.kosch@gmail.com)

María Torres-Sánchez (torressanchez.maria@gmail.com)

Affiliations

[1]One Health Research Group, Melbourne Veterinary School, Faculty of Science, University of Melbourne, Werribee, Victoria, Australia

[2]Department of Biodiversity, Ecology, and Evolution, Complutense University of Madrid, 28040 Madrid, Spain

[3]Eco-Evo-Devo Group, Estación Biológica de Doñana CSIC, Seville, Spain

[4]Biology Department, East Carolina University, Greenville, NC, USA 27858

[5]Technische Universität Dresden, CRTD/Center for Regenerative Therapies Dresden, Dresden, Germany

[6]Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany

[7]Department of Biological Sciences, Universidad de los Andes, Bogotá, 111711, Colombia

[8]Museo de Historia Natural C.J. Marinkelle, Universidad de los Andes, Bogotá, 111711, Colombia.

[9]School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne, UK

[10]Island Biodiversity and Conservation Centre, University of Seychelles, Anse Royale Seychelles

[11]Department of Zoology, Jagannath University, Dhaka 1100, Bangladesh

[12]Centre for Tropical Bioinformatics and Molecular Biology, James Cook University, Townsville, QLD 4810, Australia

[13]Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, USA

[14]Museo Nacional de Ciencias Naturales-CSIC, Madrid, Spain

[15]Department of Integrative Biology, The University of Texas at Austin, Austin, TX, USA

[16]University of Kansas Biodiversity Institute and Natural History Museum, Lawrence, Kansas 66045, USA

[17]Biology Department, Queen's University, Kingston, Ontario, Canada

[18]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão, Portugal

[19]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, rua do Campo Alegre s/n, 4169– 007 Porto, Portugal

[20]BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

[21]Evolutionary Genomics and Wildlife Management, Foundatonal Biodiversity Science, Kirstenbosch Research Centre, South African National Biodiversity Institute, Newlands 7735, Cape Town, South Africa

[22]Centre for Evolutionary Genomics and Wildlife Conservation, Department of Zoology, University of Johannesburg, Auckland Park 2006, Johannesburg, South Africa

[23]Department of Biology, Marian University, Indianapolis, IN 46222, USA

[24]Rojas Lab, Konrad-Lorenz-Institute of Ethology, Department of Life Science, University of Veterinary Medicine, Vienna, Austria

[25]CIIMAR Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de Matos, S/N, Matosinhos, Portugal

[26]School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia
[27]Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, The University of Sydney, Sydney, New South Wales, Australia
[28]Manaaki Whenua – Landcare Research, Auckland, New Zealand
[29]School of Natural Sciences, The University of Hull, Hull, HU6 7RX, United Kingdom
[30]Energy and Environment Institute, The University of Hull, Hull, HU6 7RX, United Kingdom
[31] Key Laboratory of Genetic Evolution and Animal Models, and Yunnan Key Laboratory of Biodiversity and Ecological Conservation of Gaoligong Mountain, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China
[32] Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar
[33]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
[34]Herpetology Lab, Dept. of Zoology, Central University of Kerala, Tejaswini Hills, Kasaragod, Kerala, 671320, India
[35]Department of Biology, Indiana University, Bloomington, IN 47405, USA
[36]Department of Biological Sciences, Virginia Tech, Blacksburg, VA 24060, USA
[37]Department of Ecology and Evolution, University of Lausanne, Biophore, 1015, Switzerland
[38]Department of Ecology and Genetics, Evolutionary Biology, Norbyvägen 18D, 75236 Uppsala, Sweden
[39]Wildlife Health Ghent, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

[40]Central Natural Science Collections, Martin Luther University Halle-Wittenberg, D-06108 Halle (Saale), Germany

[41]School of Life Sciences, Faculty of Medicine and Health Sciences, University of Nottingham, Nottingham, UK

[42]School of Biosciences, Cardiff University, Museum Avenue, CF10 3AX Cardiff, United Kingdom

[43]Department of Genetics, Physiology, and Microbiology; Faculty of Biological Sciences; Complutense University of Madrid, Madrid, Spain

[44]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University, Kraków, Poland

[45]Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht Str.24-25, 14476 Potsdam, Germany

[46]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

[47]Department of Integrative Biology, Oklahoma State University, Stillwater OK, USA

[48]Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY, 14642, USA

[49]Natural History Museum of Denmark, University of Copenhagen, Universitetsparken 15, 2100, Copenhagen Ø, Denmark

[50]School of Biological Sciences, Queen's University Belfast, Belfast, BT7 1NN, Northern Ireland, United Kingdom

[51]Instituto Peruano de Herpetología, Ca. Augusto Salazar Bondy 136, Surco, Lima, Peru

[52]Herpetology Lab, The Natural History Museum, London, United Kingdom

[53]Department of Biology, New York University, New York, NY, USA

[54]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 301, D-12587 Berlin, Germany
[55]Department of Biology and Biochemistry, University of Houston, Houston, Texas, 77204, USA
[56]Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica
[57]Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720, USA
[58]School of Biology and Environmental Science, University College Dublin, Belfield Campus, Dublin 4, Ireland

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 genomic 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 genomic 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 essential models for studying broad genomic traits, such as evolutionary genome expansions and contractions, as they exhibit the widest range of genome sizes among all animal taxa and possess 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 emergence of long-read sequencing technologies, combined with advanced molecular and computational techniques that improve scaffolding and reduce computational workloads, is now making it possible to address 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, https://mvs.unimelb.edu.au/amphibian-genomics-consortium) in early 2023. This burgeoning community already has more than 282 members from 41 countries. 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 call on the research and conservation communities to unite as part of the AGC to 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 non-avian Reptiles, i.e. crocodilians, lepidosaurs, and testudines [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*) [8-11].

To reduce costs and enhance feasibility, early amphibian genome sequencing projects tended to select species with comparatively small genomes (Fig. 1B). This has resulted in disproportionately fewer sequenced salamander genomes, given this is the amphibian order with the largest genomes [12]. To date, the largest amphibian genome assemblies belong to three salamander species: *Ambystoma mexicanum* (27.3 Gb assembly; [13]), *Pleurodeles waltl* (20.3 Gb; [14]), and *Calotriton arnoldi*

(22.8 Gb; [15]). 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% [16]). Amphibian transposable elements have expanded and become highly abundant in younger clades, posing challenges for the construction of contiguous genome assemblies [17]. 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*; [18]).

Thus, the number of amphibian genome assemblies has increased rapidly in recent years, reaching 111 listed in early 2024 as reference genomes at the 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 salamander 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 [19, 20]. Furthermore, the gene content values for many of these incomplete genomes can be as low as 0.7% [16]. 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 that contains the information for the search term “Amphibia” of the NCBI Sequence Read Archive [SRA] accessed on 1 March 2024). For example, RNA-Seq techniques have been used to explore gene expression across more than 300 different amphibian species (see Supplementary File 2 and Supplementary Methods for detail information about how SRA records were summarized). 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 (see Supplementary File 2 and Supplementary Methods for detail information about how SRA records were summarized). 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 [21-23]. We highlight some examples here and state the imperious need to generate amphibian genomic resources to decrease further biodiversity loss as the ultimate reason.

Embryogenesis, developmental and regenerative biology

Amphibians have played a fundamental role in uncovering developmental principles [for a detailed review see 24]. Research on anurans has enabled the understanding of critical developmental mechanisms such as the breaking of egg asymmetry [25], axis establishment, and nerve transmission [26]. 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 [27, 28]. Consequently, these studies have generated thousands of genomic and transcriptomic resources for these two species [29, 30].

Yet, there is much more to uncover about amphibian development, especially given the numerous developmental modalities found across amphibians, which likely demonstrates the highest diversity among vertebrates [31]. This includes direct development (egg to froglet; the first genome of a direct-developing amphibian, *Eleutherodactylus coqui*, was published in 2024 [32]), and phenotypic plasticity [33, 34].

Sexual development and determination are also diverse and unique in amphibians [35]. Unlike most mammals and birds who have degenerate Y and W chromosomes, most amphibians have undifferentiated sex chromosomes, making it extremely difficult to study sex evolution through traditional cytogenetic techniques [36, 37]. However, sex-determining systems are starting to be explored through high-throughput sequencing [6, 38-42]. For example, the application of multiple omics techniques led to the identification of a Y-specific non-coding RNA in the 5’-region of the *bod1l* gene, which is involved in male sex determination in *Bufotes viridis* [41].

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 [43]. Transcriptomes from these salamanders have shown that gene expression from their divergent genomes is balanced for some genes but biased for others [44]. Sexual development in amphibians can result in sexually dimorphic features such as nuptial spines, which have been explored using comparative genomics approaches such as in the frog *Leptobrachium leishanense* [45].

The increasing availability of amphibian genomes will enable a deeper understanding of the molecular mechanisms underlying such ontogenetic diversity. Chromosome-level reference genomes provide high-resolution data crucial for identifying sex-determining regions, revealing new insights about these processes and, helping to address challenges of sex reversal due to temperature fluctuations and the increasing presence of endocrine disruptors [46].

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 [47-50] and salamanders [51, 52]. This represents genomic uncoupling of these life history phases with major macroevolutionary implications [49, 53]. Amphibian omics approaches are rapidly increasing our understanding of the developmental process of metamorphosis, including the role of methylation in gene regulation and other epigenetic markers [54]. Amphibians have also been found to respond to environmental perturbations by altering their behavior or phenotypes in various ways. These mechanisms, including change developmental rate [33], hybridization with positive fitness effects [55], production of novel trophic morphologies [56], and kin recognition to avoid cannibalizing relatives [57-59], remain poorly understood, and would benefit from further genomic research.

Due to their exceptional tissue repair and regenerative capacities [60, 61], 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 [61]. Due to new genome assemblies for urodele species, *Ambystoma mexicanum* and *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 [62]. Additionally, a novel mechanism of telomere length maintenance and elongation has recently been described in *P. waltl* [63] and, potentially linking regenerative capability with longevity. Other amphibian species have also contributed to genomic research on regeneration, for example, an early database compiled from gene expression resources of *Notophthalmus viridescens* [64].

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 [65], the evolution of vision [66], hybridogenesis [67-69], and the evolution of limblessness [70]. Amphibian phylogenomics has addressed many longstanding questions in amphibian evolution [71-74]. 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 [70, 75, 76]. Nevertheless, there are numerous open questions and unresolved evolutionary relationships that could benefit from high-quality genomes, which are especially powerful in revealing the role of transposable elements in adaptation and evolution [77]. 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 [78-80], some amphibians have evolved the ability to live in high-elevation environments such as the Andes (up to 5400 m) [81, 82] and the Tibetan Plateau (4478 m) [18]. 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 [18] 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 [18, 83]. There is evidence 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, Ranidae) [84, 85], and that intraspecific divergence in many of these genes correlates with elevation deepening our understanding of evolutionary processes shaped by environmental conditions [86, 87]. 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 where genomic data is still missing, including Andean anurans (e.g., *Telmatobius culeus* [88]) and high-elevation salamanders, such as *Pseudoeurycea gadovii* [89]).

The ability to produce or sequester 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) [90-93] or microbial symbionts (e.g., newts) [94]. Since dendrobatid frogs sequester their toxins from prey (e.g., mites and ants), they lack genes encoding these toxins [95, 96]. 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 [97-99]. 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 [100-102]. 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 [103-107], which could potentially be used for future human medical treatments.

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 [108-112] and aposematism and mimicry in toxic species [113, 114]. 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 [115-120]. 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* [121].

Despite the numerous advances made with amphibian omics in elucidating evolutionary and ecological mechanisms, fully unraveling their genetic basis requires the generation of a vast number of genomes, given the comparative nature of these fields and the evolutionary uniqueness of each lineage. Some of the exciting research avenues in amphibians include behavioral adaptations like parental care [122, 123], milk production or skin feeding in caecilians [124, 125], spatial navigation [126]; adaptations to environmental conditions, like niche expansion due to the evolution of gliding ability [127], the evolution of lunglessness [128, 129] or predator-prey interactions like unusual defense mechanisms, such as the ability of some newts to pierce their ribs through toxin glands in their skin [130, 131].

Conservation

Amphibians are the most endangered class of vertebrates with current estimates suggesting that more than 40% of species are threatened with extinction [132]. The threats amphibians face continue to increase [132], 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 [133, 134]. Hence, numbers of threatened species are likely underestimated, as undescribed species cannot be assessed and are more likely to become extinct [135]. Further, the conservation status of many amphibians remains unknown, especially for tropical species [136] and for a number of soil-dwelling caecilians for which only a limited number of specimens are available [137]. Generating genomic data is one method to address this challenge, as it can be used to estimate both evolutionary potential and extinction risk [138, 139]. 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 [21, 22, 140, 141]. These features make genomic resources invaluable for developing species conservation action plans [142].

Amphibian conservation efforts should leverage population genetic theory and the burgeoning field of conservation genomics. These approaches enable the quantification of both neutral and adaptive diversity across genomes, thereby facilitating the promotion of adaptive potential or genetic rescue through translocation programs [143-146]. High quality genomes can also facilitate more comprehensive genomic diversity analyses, enabling the analyses of structural variants in addition to single nucleotide polymorphisms (SNPs), which are often overlooked, and an improve of the runs of homozygosity (ROH) analyses.

Typically, these studies begin with the genomic characterization of populations across various environmental conditions, assessing population genetic health and disease risk [147, 148]. They can also support monitoring and surveillance efforts by identifying populations most at risk of declines due to potential genetic threats like maladaptive alleles, genetic load, inbreeding and outbreeding depression, hybridization, and/or genetic incompatibility [143, 149]. Increased monitoring and maintenance of genomic diversity are key targets of many national and international recommendations such as the US Endangered Species Act [150], the Kunming-Montreal Global Biodiversity Monitoring Framework [151], and the Amphibian Conservation Action Plan[142] .

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. The increased availability of long-read sequencing technology is particularly valuable in addressing the challenges of identifying highly variable gene regions accountable for immunological processes such as the major histocompatibility complex (MHC) [152]. 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 [153]. Considerable effort has been invested in understanding the genetic basis of resistance to the devastating amphibian disease chytridiomycosis. This has resulted in the identification of multiple candidate genes [154-156] 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 [157]. 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 [153, 158-160]. 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 [161].

Challenges for amphibian genomic research and ways forward

The future of amphibian omics research will rely on high-quality reference genomes, which necessitates overcoming unique bioinformatic challenges in genome assembly and securing high-quality starting materials (e.g., tissue, blood). Additionally, challenges in obtaining funding, particularly in low-income countries, exacerbate these issues. Here, we outline these challenges in amphibian omics and highlight emerging developments aimed at addressing them.

The large genomes of amphibians increase requirements and costs for sequencing, computing, and data storage [6, 162]. Despite technological advancements and decreasing service costs, assembling these genomes remains methodologically challenging due to the notable intron lengths and repetitive content of amphibian genomes [163], especially when repeat lengths exceed sequencing read lengths. Regions of low complexity can result in erroneously joined contigs [164] or a significant loss of sequence information (by as much as 16%) through the collapsing of repetitive sequences [165]. Polyploidy has also evolved repeatedly in amphibians [166, 167], making haplotype-specific assemblies challenging and may require dramatically increased sequencing and computational efforts [168, 169]. The development of long-read sequencing (e.g. PacBio HiFi, ONT), optical mapping and 3C technology (i.e., Hi-C scaffolding) is therefore especially important for assembling amphibian genomes [164, 170].

Annotations are as crucial as genome assemblies, but current homology-based approaches using ortholog databases like UniProt [171] often miss or poorly annotate genes, especially polymorphic genes or those lacking representation in model taxa. This limits amphibian studies on gene evolution [72], repeats [16, 163], or immune genes [172].

Additionally, functional genomics tools like gene editing, *in vitro* fertilization and transgenesis are rare for most amphibians [153, 173], developed primarily in model species (e.g., *Xenopus* spp., *Ambystoma mexicanum*) [61, 174-177]. Immortal cell lines have been successfully generated for some amphibians [178] and protocols have been established to facilitate the initiation of spontaneously arising cell lines for a subset of anurans [179]. However, establishing cell cultures for most species requires extensive problem-solving and expertise [178].

Most tissue sampling protocols for sequencing reference genomes 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). This often requires fieldwork with many logistical challenges.

The small body sizes and blood volumes of most amphibians (e.g., < 30 g) may necessitate lethal sampling to obtain sufficient high-molecular-weight DNA for generating reference genomes (HMW, reaching 100 Kb or ultra HMW, reaching 1 Mb) [180, 181]. While this characteristic is shared with other taxonomic groups (e.g., invertebrates), lethal sampling may not always be legally permitted or ethically advisable in amphibians, especially for threatened species or those in captive collections [182]. 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 [183, 184]. Until these become suitable for reference-grade genome sequencing, an alternative to minimize sampling impacts may be to use tadpoles instead of adults (e.g., to generate the genome of *Taudactylus pleione* [185]).

Working with museum or natural history collections [the burgeoning field of “museomics”; 186] is a promising avenue of research for circumventing the intrinsic problems of sample collection. Moreover, it allows access to past amphibian biodiversity and is revolutionizing amphibian taxonomy by integrating DNA from name-bearing type specimens, overcoming impediments like uncertainty in nomenclature, species complexes, and cryptic species [187-190]. Key challenges of such research include issues with DNA degradation, preservation methods, and contamination that need to be overcome [191-193]. This is particularly relevant for wet-preserved amphibian specimens, as retrieving DNA can be challenging due undocumented fixation and preservation methods that may alter nucleotide integrity. Methodological advances in laboratory protocols [e.g., 194, 195, 196] and the development of sequencing strategies, such as ‘Barcode Fishing’, have made significant progress in addressing these challenges, including the ability to sequence extinct species [187, 188, 197-199]. 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 [200].

Other noteworthy challenges, that are not necessarily unique to amphibians, include securing collection and research permits, maintaining ultracold storage and an uninterrupted cold chain during transport, and adhering to regulations for the international movement of biological samples across political borders. [201, 202]. Amphibian-specific challenges, however, can arise due to their biological, ecological, and conservation characteristics. Centers of amphibian diversity and endemism include remote, highly specialized habitats, such as tropical montane forests, cave systems or isolated wetlands. Moreover, many amphibians have specialized aquatic, subterranean or arboreal ecologies, are mostly nocturnal and highly seasonal. These factors make fieldwork and sample transportation challenging, especially in regions with poor infrastructure, inadequate storage facilities, socio-political conflicts, and limited funding for research, conservation, and public awareness.

While eliminating some of these practical and political challenges in amphibian fieldwork is beyond the scope of individual researchers, the growing accessibility of genomic data calls for increased awareness of the principles of fair and equitable access to genetic resources, as outlined by the Convention on Biological Diversity (CBD) and further elaborated 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 [203-207].

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

The AGC (https://mvs.unimelb.edu.au/amphibian-genomics-consortium) was launched in March 2023 to address the aforementioned knowledge gaps through technological advances and international cooperation. The mission of the AGC is to enhance international and interdisciplinary collaboration among amphibian researchers, expand amphibian genomic resources, and effectively utilize genomic data and functional resources to close the gap between genome biologists, 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.

The first actions of the AGC include hosting monthly regular meetings that showcase advances in amphibian genomics research, developing technical resources and best practices guidelines (through discussions facilitated in a Discord channel), improving amphibian genome annotation, supporting travel for students and early career researchers, hosting networking events at conferences, and conducting virtual and in-person computational workshops. Details of these activities can be found in the AGC website. 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; [208]) 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 (6 in Africa, 131 in the Americas, 27 in Asia, 29 in Australasia, and 89 in Europe), with membership continuing to increase (Fig. 4). Although the membership is geographically diverse, disparity persists across regions. The recruitment of members from underrepresented countries will be a key focus of the AGC, with a particular emphasis on 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 genomic 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 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., 209], and previous research [22], we identified 48 amphibian families for which no representative genomes had been sequenced and selected one representative species from each 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 recommend this priority list as a starting point. If suitable sample material from other species within the targeted families becomes available, those species could replace the ones currently proposed. fAdditionally, we aim to build upon the efforts of existing genomics consortia such as the Vertebrate Genomes Project (VGP), hence, we included two species with draft genomes in the GenomeArk (https://www.genomeark.org/) in our sequencing target list.

The AGC's stance on resource and benefits sharing

With increasingly easy access to genomic data, researchers and industry need to be 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). 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 [210].

To promote better practices, researchers should allocate the necessary time and funds for prior consultation during fieldwork planning and seek guidance from their National Focal Points on ABS. How the concept of ABS may be applied to the downstream use of the digital sequence information (DSI) generated has yet to be resolved. However, there are currently developments underway that may provide a solution (https://www.cbd.int/dsi-gr). It is imperative that this issue be considered going forward [see for example 211]. Moreover, voucher specimens and duplicate tissue samples should be deposited in local natural history collections or preferred partners of the local communities [212, 213].

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' [214, 215]. 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

[216]. However, optimization for non-model organisms, along with 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. Programs like the In Situ Laboratories Initiative (https://insitulabs.org/hubs/) aim to overcome these challenges by providing affordable access to high-tech laboratories in remote biodiverse areas. Such collaborative projects should proceed from finding shared interests, developing ideas, realizing the shared benefits from research outputs, and focusing on capacity-building efforts [217].

Conclusion and call to action

Moving forward, the AGC is committed to supporting amphibian sequencing initiatives worldwide, with a particular emphasis on taxonomic groups lacking representation, and species from biodiverse countries within a conservation framework (Table 1). Local sequencing initiatives will be given priority whenever feasible to promote the development of *in situ* research efforts and facilities. We will achieve this goal building strong networks between researchers and conservation practitioners and by providing an open list of members and their expertise. Additionally, we aim to provide funding and training opportunities to facilitate collaboration among underrepresented groups, molecular and organismal biologists, bioinformaticians, and conservation practitioners. We will also support the development of a concept of Access and Benefit Sharing policies that can be applied to the downstream use of the digital sequence information (DSI), including long-term storage and access. Further, the AGC aspires to stimulate public and scientific interest in amphibian research and, ultimately, to enhance conservation outcomes for this intriguing and highly endangered group of vertebrates.

We hope that the recent advancements in technology, a focus on equitable research, and the integration of the research community to form the AGC will ignite research to revolutionize amphibian conservation and our understanding of their fascinating biology, ecology and evolution. By addressing the challenges outlined, supporting and promoting amphibian genomics research and uniting amphibian researchers worldwide, the AGC aims to fill the huge gap in genomic data for this diverse group of tetrapods and in doing so, propel amphibian genomics research into the future.

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. T.A.K., M.T.-S, C.D., N.J.F, Y.C., R.D.T., H.C.L, V.L.N.A., R.M., K.S.-S., H.M., K.C.W.V, M.H.Y., and M.S. contributed to revising the manuscript for resubmission. 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	Motives
Anura: Allophrynidae	South America	1	3	*Allophryne relicta*	EN	Endangered
Anura: Alsodidae	South America	3	26	*Alsodes gargola*	LC	High altitude adaptation
Anura: Arthroleptidae	Africa	8	151	*Leptopelis vermiculatus*	EN	Endangered
Anura: Ascaphidae	North America	1	2	*Ascaphus montanus**	LC	High altitude adaptation
Anura: Batrachylidae	South America	4	13	*Batrachyla leptopus*	LC	High altitude adaptation
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	Endangered
Anura: Centrolenidae	Central & South America	12	166	*Centrolene pipilata*	CR	Endangered, Gigantism
Anura: Ceratobatrachidae	Southeast Asia	4	103	*Platymantis spelaeus*	EN	Cave-dweller, Endangered
Anura: Ceratophryidae	South America	3	12	*Lepidobatrachus laevis*	LC	Transcriptomic resources

Family	Region	#G	#S	Candidate species	IUCN	Motives
Anura: Ceuthomantidae	South America	2	6	*Ceuthomantis cavernibardus*	LC	Cave-dweller
Anura: Conrauidae	Africa	1	8	*Conraua goliath*	EN	Gigantism
Anura: Craugastoridae	Central America	3	136	*Craugastor fitzingeri*	LC	Transcriptomic resources
Anura: Cycloramphidae	South America	3	37	*Cycloramphus granulosus*	CR	Critically endangered
Anura: Heleophrynidae	South Africa	2	6	*Heleophryne rosei*	CR	Critically endangered
Anura: Hemiphractidae	Central & South America	6	123	*Gastrotheca cornuta*	CR	Critically endangered
Anura: Hemisotidae	Sub-Saharan Africa	1	9	*Hemisus marmoratus*	LC	Transcriptomic resources
Anura: Hylodidae	South America	4	49	*Phantasmarana massarti*	EN	Endangered
Anura: Hyperoliidae	Sub-Saharan Africa & Madagascar	17	236	*Hyperolius thomensis*	EN	Endangered
Anura: Leiopelmatidae	New Zealand	1	3	*Leiopelma archeyi*	CR	Critically endangered
Anura: Mantellidae	Madagascar	12	272	*Mantidactylus betsileanus*	LC	Transcriptomic resources
Anura: Micrixalidae	India	1	24	*Micrixalus mallani*	EN	Endangered
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	Endangered
Anura: Odontobatrachidae	Tropical West Africa	1	5	*Odontobatrachus fouta*	EN	Endangered
Anura: Odontophrynidae	South America	3	54	*Proceratophrys redacta*	EN	Endangered
Anura: Petropedetidae	Sub-Saharan tropical Africa	3	13	*Petropedetes perreti*	CR	Critically endangered
Anura: Phrynobatrachidae	Africa	1	99	*Phrynobatrachus guineensis*	LC	Tree-hole breeder
Anura: Ranixalidae	India	2	19	*Indirana chiravasi*	LC	Transcriptomic resources
Anura: Rhacophoridae	Eastern Asia	22	444	*Buergeria otai*	LC	Transcriptomic resources

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

Figures

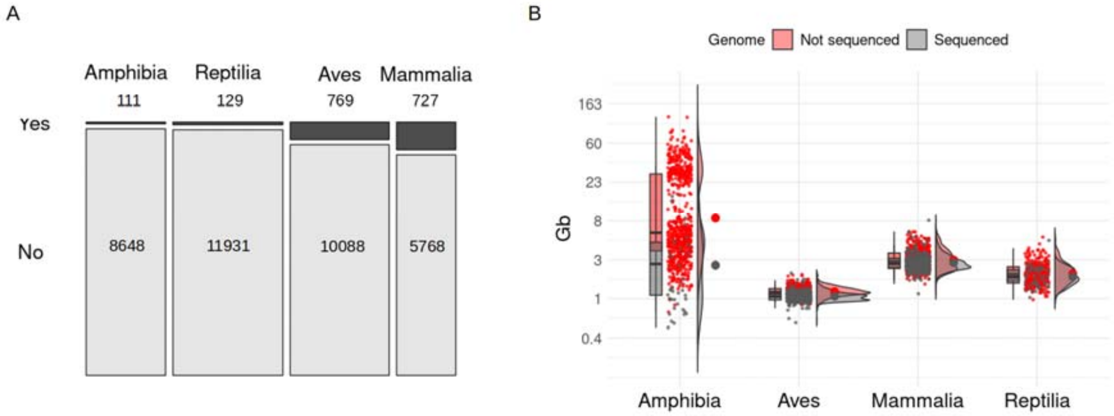

Figure 1. Estimated genome size across tetrapod classes in relation to sequenced genomes. (A) Mosaic plot representing the percentage of species with sequenced genomes as a proportion of the number of described species for each tetrapod class (Yes: % species with sequenced genome; No: % species without 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 [12, 20].

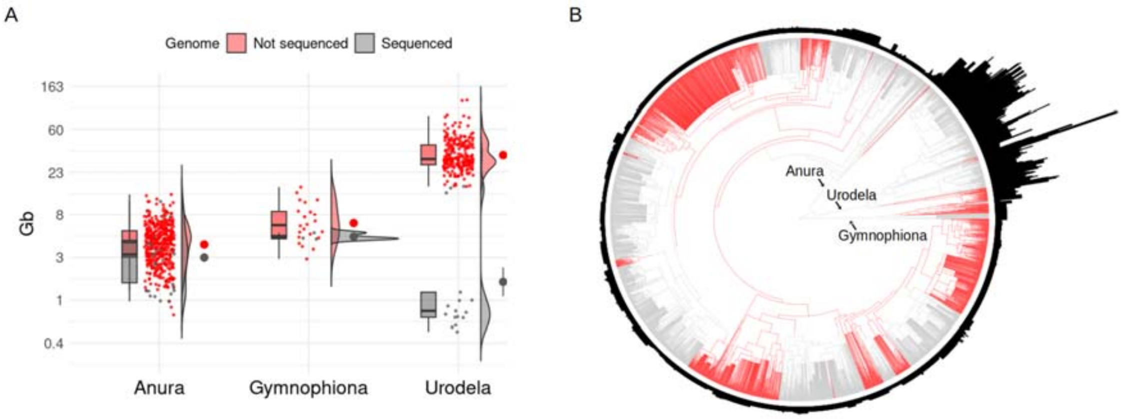

Figure 2. Estimated genome size across amphibian orders in relation to sequenced genomes. (A) Combined box and density plot with raw data as points showing genome size of species with sequenced genome (gray color; genome sizes from NCBI genome assemblies) versus a subset of species without available 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 [20], and amphibian records from [12]. (B) Amphibian phylogenetic tree was adapted from [71], which includes species with genome size estimates from Genomes on a Tree (GoaT) [19]. 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.

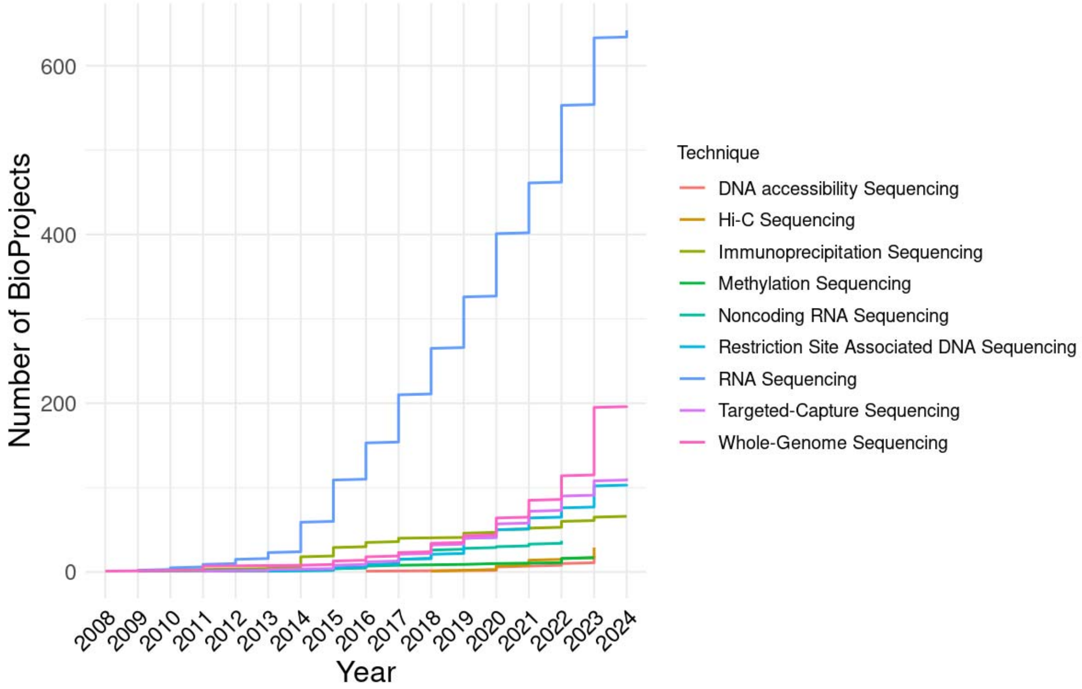

Figure 3. Main sequencing techniques applied to amphibian genomics studies. Yearly cumulative number of amphibian BioProjects split and color-coded by sequencing technique (DNA accessibility Sequencing includes ATAC-Seq and Mnase-Seq; Immunoprecipitation Sequencing includes: ChIP-Seq and RIP-Seq; Amplicon 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).

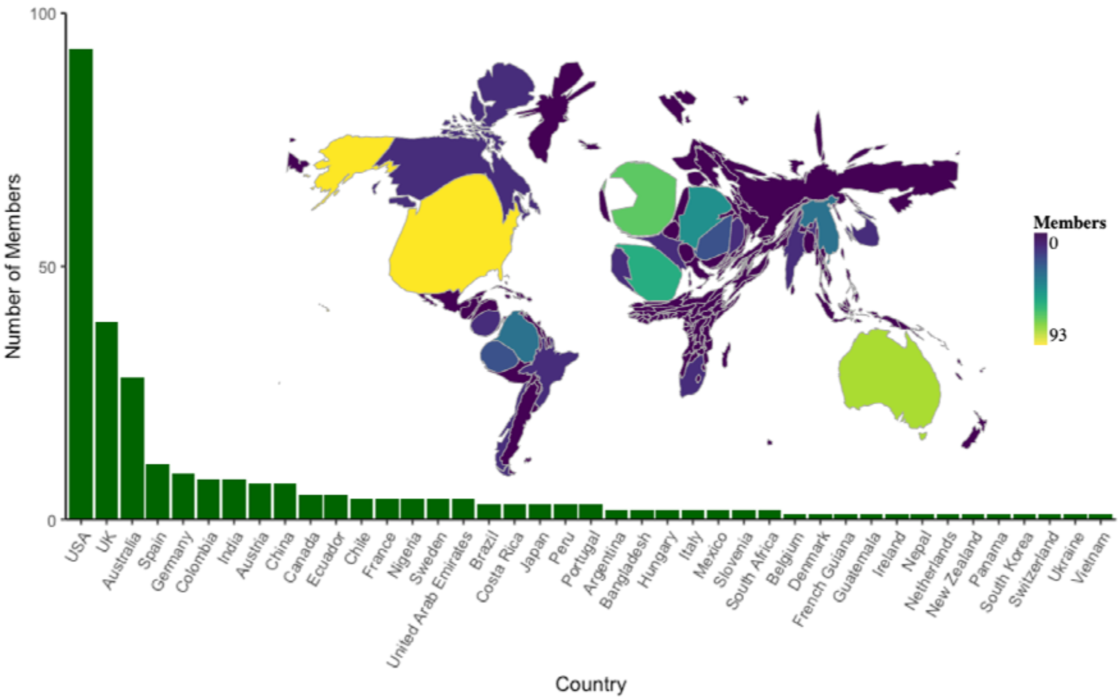

Figure 4. Amphibian Genomics Consortium (AGC) membership by country. Inset map showing the size of each country scaled by number of members in the AGC.

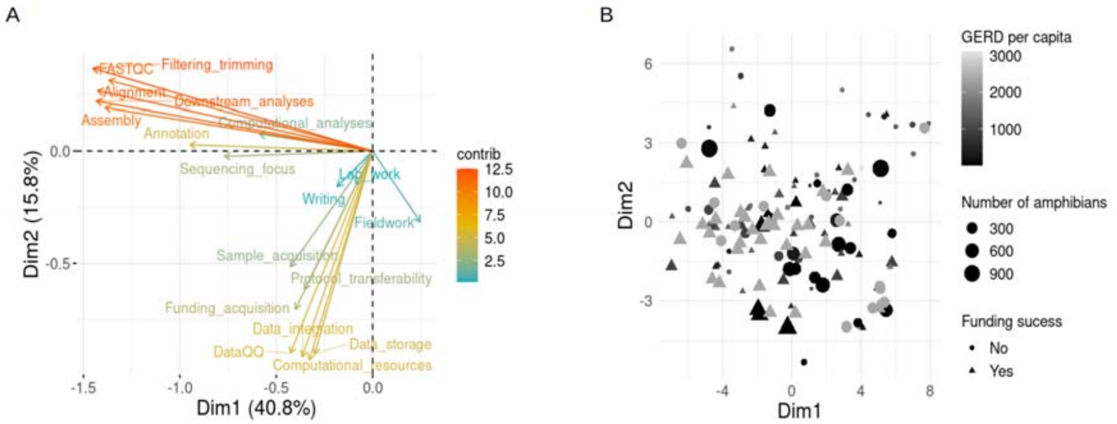

Figure 5. Sequencing competencies and identified challenges of the members of the Amphibian Genomics Consortium (AGC). (A) Representation of the contribution of the AGC survey quantitative questions to the first dimensions after computing a principal component analysis (PCA). Bioinformatic competencies 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.

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