1 **The Amphibian Genomics Consortium: advancing genomic and genetic**

2 **resources for amphibian research and conservation**

3

4 **Authors**

5 Tiffany A. Kosch[1§*], María Torres-Sánchez[2§*], H. Christoph Liedtke[3], Kyle Summers[4],

6 Maximina H. Yun[5,6], Andrew J. Crawford[7,8], Simon T. Maddock[9,10], Md. Sabbir

7 Ahammed[11], Victor L. N. Araújo[7], Lorenzo V. Bertola[12], Gary Bucciarelli[13], Albert

8 Carné[14], Céline M. Carneiro[15], Kin O. Chan[16], Ying Chen[17], Angelica Crottini[18,19,20],

9 Jessica M. da Silva[21,22], Robert D. Denton[23], Carolin Dittrich[24], Gonçalo Espregueira

10 Themudo[25], Katherine A. Farquharson[26,27], Natalie J. Forsdick[28], Edward Gilbert[29,30],

11 Che Jing[31,32], Barbara A. Katzenback[33], Ramachandran Kotharambath[34], Nicholas A.

12 Levis[35], Roberto Márquez[36], Glib Mazepa[37,38], Kevin P. Mulder[39], Hendrik Müller[40],

13 Mary J. O'Connell[41], Pablo Orozco-terWengel[42], Gemma Palomar[43,44], Alice

14 Petzold[45], David W. Pfennig[46], Karin S. Pfennig[46], Michael S. Reichert[47], Jacques

15 Robert[48], Mark D. Scherz[49], Karen Siu-Ting[50,51,52], Anthony A Snead[53], Matthias

16 Stöck[54], Adam M. M. Stuckert[55], Jennifer L. Stynoski[56], Rebecca D. Tarvin[57],

17 Katherina C. Wollenberg Valero[58], and The Amphibian Genomics Consortium (AGC).

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19 §contributed equally to the manuscript

20 *correspondence:

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

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

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**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, UK

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

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

[12]Centre for Tropical Bioinformatics and Molecular Biology, James Cook University, 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

2

50    [16]University of Kansas Biodiversity Institute and Natural History Museum, Lawrence,

51    Kansas 66045, USA

52    [17]Biology Department, Queen's University, Ontario, Canada

53    [18]CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO

54    Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485-661 Vairão,

55    Portugal

56    [19]Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, rua do

57    Campo Alegre s/n, 4169– 007 Porto, Portugal

58    [20]BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus

59    de Vairão, 4485-661 Vairão, Portugal

60    [21]Evolutionary Genomics and Wildlife Management, Foundatonal Biodiversity

61    Science, Kirstenbosch Research Centre, South African National Biodiversity

62    Institute, Newlands 7735, Cape Town, South Africa

63    [22]Centre for Evolutionary Genomics and Wildlife Conservation, Department of

64    Zoology, University of Johannesburg, Auckland Park 2006, Johannesburg, South

65    Africa

66    [23]Department of Biology, Marian University, Indianapolis, IN 46222, USA

67    [24]Rojas Lab, Konrad-Lorenz-Institute of Ethology, Department of Life Science,

68    University of Veterinary Medicine, Vienna, Austria

69    [25]CIIMAR Interdisciplinary Centre of Marine and Environmental Research, University

70    of Porto, Terminal de Cruzeiros do Porto de Leixões, Avenida General Norton de

71    Matos, S/N, Matosinhos, Portugal

72    [26]School of Life and Environmental Sciences, The University of Sydney, Sydney,

73    NSW 2006, Australia

3

74   [27]Australian Research Council Centre of Excellence for Innovations in Peptide and

75   Protein Science, The University of Sydney, Sydney, New South Wales, Australia

76   [28]Manaaki Whenua – Landcare Research, Auckland, New Zealand

77   [29]School of Natural Sciences, The University of Hull, Hull, HU6 7RX, United Kingdom

78   [30]Energy and Environment Institute, The University of Hull, Hull, HU6 7RX, United

79   Kingdom

80   [31] Key Laboratory of Genetic Evolution and Animal Models, and Yunnan Key

81   Laboratory of Biodiversity and Ecological Conservation of Gaoligong Mountain,

82   Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223,

83   China

84   [32] Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences,

85   Yezin, Nay Pyi Taw 05282, Myanmar

86   [33]Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1

87   [34]Herpetology Lab, Dept. of Zoology, Central University of Kerala, Tejaswini Hills,

88   Kasaragod, Kerala, 671320, India

89   [35]Department of Biology, Indiana University, Bloomington, IN 47405, USA

90   [36]Department of Biological Sciences, Virginia Tech. Blacksburg, VA 24060, USA

91   [37]Department of Ecology and Evolution, University of Lausanne, Biophore, 1015,

92   Switzerland

93   [38]Department of Ecology and Genetics, Evolutionary Biology, Norbyvägen 18D,

94   75236 Uppsala, Sweden

95   [39]Wildlife Health Ghent, Faculty of Veterinary Medicine, Ghent University, Merelbeke,

96   Belgium

97   [40]Central Natural Science Collections, Martin Luther University Halle-Wittenberg, D-

98   06108 Halle (Saale), Germany

4

99    [41]School of Life Sciences, Faculty of Medicine and Health Sciences, University of

100   Nottingham, UK

101   [42]School of Biosciences, Cardiff University, Museum Avenue, CF10 3AX Cardiff,

102   United Kingdom

103   [43]Department of Genetics, Physiology, and Microbiology; Faculty of Biological

104   Sciences; Complutense University of Madrid, Madrid, Spain

105   [44]Institute of Environmental Sciences, Faculty of Biology, Jagiellonian University,

106   Kraków, Poland

107   [45]Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht

108   Str.24-25, 14476 Potsdam, Germany

109   [46]Department of Biology, University of North Carolina, Chapel Hill, NC 27599, USA

110   [47]Department of Integrative Biology, Oklahoma State University, Stillwater OK, USA

111   [48]Department of Microbiology and Immunology, University of Rochester Medical

112   Center, Rochester, NY, 14642, USA

113   [49]Natural History Museum of Denmark, University of Copenhagen,

114   Universitetsparken 15, 2100, Copenhagen Ø, Denmark

115   [50]School of Biological Sciences, Queen's University Belfast, Belfast, BT7 1NN,

116   Northern Ireland, United Kingdom

117   [51]Instituto Peruano de Herpetología, Ca. Augusto Salazar Bondy 136, Surco, Lima,

118   Peru

119   [52]Herpetology Lab, The Natural History Museum, London, United Kingdom

120   [53]Department of Biology, New York University, New York, NY, USA

121   [54]Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB),

122   Müggelseedamm 301, D-12587 Berlin, Germany

5

123  [55]Department of Biology and Biochemistry, University of Houston, Houston, Texas,

124  77204, USA

125  [56]Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica

126  [57]Museum of Vertebrate Zoology and Department of Integrative Biology, University of

127  California, Berkeley, CA 94720, USA

128  [58]School of Biology and Environmental Science, University College Dublin, Belfield

129  Campus, Dublin 4, Ireland

130

131  **Abstract**

132  Amphibians represent a diverse group of tetrapods, marked by deep divergence

133  times between their three systematic orders and families. Studying amphibian

134  biology through the genomics lens increases our understanding of the features of

135  this animal class and that of other terrestrial vertebrates. The need for amphibian

136  genomics resources is more urgent than ever due to the increasing threats to this

137  group. Amphibians are one of the most imperiled taxonomic groups, with

138  approximately 41% of species threatened with extinction due to habitat loss,

139  changes in land use patterns, disease, climate change, and their synergistic effects.

140  Amphibian genomics resources have provided a better understanding of ontogenetic

141  diversity, tissue regeneration, diverse life history and reproductive modes, anti-

142  predator strategies, and resilience and adaptive responses. They also serve as

143  critical models for understanding widespread genomic characteristics, including

144  evolutionary genome expansions and contractions given they have the largest range

145  in genome sizes of any animal taxon and multiple mechanisms of genetic sex

146  determination. Despite these features, genome sequencing of amphibians has

147  significantly lagged behind that of other vertebrates, primarily due to the challenges

6

148  of assembling their large, repeat-rich genomes and the relative lack of societal

149  support. The advent of long-read sequencing technologies, along with computational

150  techniques that enhance scaffolding capabilities and streamline computational

151  workload is now enabling the ability to overcome some of these challenges. To

152  promote and accelerate the production and use of amphibian genomics research

153  through international coordination and collaboration, we launched the Amphibian

154  Genomics Consortium (AGC) in early 2023. This burgeoning community already has

155  more than 282 members from 41 countries (6 in Africa, 131 in the Americas, 27 in

156  Asia, 29 in Australasia, and 89 in Europe). The AGC aims to leverage the diverse

157  capabilities of its members to advance genomic resources for amphibians and bridge

158  the implementation gap between biologists, bioinformaticians, and conservation

159  practitioners. Here we evaluate the state of the field of amphibian genomics,

160  highlight previous studies, present challenges to overcome, and outline how the AGC

161  can enable amphibian genomics research to "leap" to the next level.

162  **Keywords**

163  Amphibians, Biodiversity conservation, Comparative genomics, Genomics,

164  Lissamphibia, Metagenomics, Phylogenomics, Population genomics, Taxonomy,

165  Transcriptomics.

166  **State of the field of amphibian genomics**

167  In 2010, the genome of the Western clawed frog (*Xenopus tropicalis*) was

168  sequenced, marking the first genome assembly for Class Amphibia [1]. This species

169  serves as a crucial laboratory model organism for cell biology, molecular genetics,

170  and developmental biology [2]. The first amphibian genome assembly came years

171  after the completion of the first genomes for other vertebrate groups: fishes in 2002

172 (*Fugu rubripes*; [3]), mammals in 2003 (*Homo sapiens*; [4]), birds in 2004 (*Gallus*

173 *gallus*; [5]), and reptiles in 2007 (*Anolis carolinensis*; Anolis Genome Project

174 https://www.broadinstitute.org/anolis/anolis-genome-project). Since then, the

175 generation and annotation of amphibian reference genomes has dramatically lagged

176 behind those of other vertebrates [6], even though amphibians represent nearly 22%

177 of all tetrapods [7]. Nearly 15 years later, amphibians are still the tetrapod class with

178 the lowest number of sequenced genomes (111 genomes of 8648 described

179 amphibian species being the tetrapod class with the second lowest proportion after

180 Reptiles [database records accessed on 1 March 2024], Fig. 1A and Supplementary

181 File 1). This is likely attributable to the size of amphibian genomes, which are

182 generally larger than the genomes of other terrestrial vertebrates (Fig. 1B and Fig.

183 S1; see Supplementary Material for methodological information). Indeed, among all

184 vertebrates, only the genomes of lungfish are larger (up to 130 Gb) than the largest

185 amphibian genomes (up to ~120 Gb in *Necturus lewisi*) [8-10].

186

187 To lower cost and enhance feasibility, early amphibian genome sequencing projects

188 tended to select species with comparatively small genomes (Fig. 1B). This has

189 resulted in the disproportionately fewer sequenced salamander genomes [11]. To

190 date, the largest amphibian genome assemblies belong to three salamander

191 species: *Ambystoma mexicanum* (27.3 Gb assembly; [12]), *Pleurodeles waltl* (20.3

192 Gb; [13]), and *Calotriton arnoldi* (22.8 Gb; [14]). However, these only represent the

193 lower end of the genome size range for this group, with the genomes of *Necturus*

194 salamanders exceeding 100 Gb (Fig. 2) [10].

195

8

196    In addition to their large sizes, amphibian genomes have also been challenging to

197    assemble due to their extensive repeat content (up to 82% [15]). Amphibian

198    transposable elements have expanded and become highly abundant in younger

199    clades, posing challenges for the construction of contiguous genome assemblies

200    [16]. These characteristics of amphibian genomes make sequencing and assembly

201    both costly and technically challenging (e.g., repetitive regions can often lead to

202    fragmented assemblies when using short-read sequencing). However, the advent of

203    new sequencing approaches such as long-read sequencing (e.g., PacBio HiFi and

204    Oxford Nanopore Duplex), Hi-C scaffolding, along with reduced sequencing costs

205    have resolved many of these assembly challenges (e.g., *Nanorana parkeri*; [17]).

206

207    Thus, the number of amphibian genome assemblies has increased rapidly in recent

208    years, reaching 111 currently listed as reference genomes at scaffold level or higher

209    in the National Center for Biotechnology Information (NCBI) genome database (52

210    for Anura, 55 for Urodela, and four for Gymnophiona; NCBI genome database

211    records accessed on 1 March 2024). Despite this rapid increase, the quality of

212    available amphibian genomes varies significantly, only 38 are chromosome-level

213    assemblies, and among these, only 16 are annotated. This indicates that the majority

214    of available assemblies are incomplete or partial. For example, several recently

215    published salamanders genomes of the genus *Desmognathus* have assembly sizes

216    of ~1 Gb while their genome size estimates based on flow cytometry or image

217    densitometry average 14 Gb [18, 19]. Furthermore, the gene content values for

218    many of these incomplete genomes can be as low as 0.7% [15]. Besides the

219    variation in quality, there are substantial taxonomic gaps in genome representation

220    across Amphibia. Notably, 48 of the 77 amphibian families (62%) lack a

9

221    representative genome assembly in the NCBI genome database (Fig. 2B), indicating

222    significant gaps in our understanding (see "The AGC's genome sequencing targets"

223    section and Table 1 for more information about these 48 families).

224

225    Due to the difficulty of assembling genomes, most previous genomic research in

226    amphibians has relied on alternative high-throughput sequencing methodologies,

227    including RNA sequencing (RNA-seq), reduced representation or target-capture

228    approaches, or metagenomic methods (Fig. 3 and Supplementary File 2). For

229    example, RNA sequencing (RNA-Seq) techniques have been used to explore gene

230    expression across more than 300 different amphibian species. Furthermore, a

231    substantial number of *de novo* transcriptomes are available through the NCBI

232    Transcriptome Shotgun Assemblies (TSA) database (79 total: 59 for Anura, 15 for

233    Urodela, and 5 for Gymnophiona). Various reduced-representation (e.g., ddRADseq)

234    and targeted-capture sequencing approaches have also been implemented in recent

235    years to obtain genome-wide sequence information from more than 1,400 amphibian

236    species. All this information—from whole genomes to gene transcript features—has

237    advanced the understanding of amphibian biology and directly contributed to

238    conservation efforts as described below.

239    **Advancing research and conservation through amphibian genomics**

240    Amphibians have many unique characteristics that make them subjects of interest to

241    a wide variety of scientific disciplines, spanning from developmental biology and

242    medical research to ecology and evolution. The rapid development of genomic tools

243    is galvanizing the study of amphibian biology and uncovering important facets of

244    their biology and conservation [20-22]. We highlight some examples here.

245

10

246 <u>Embryogenesis, developmental and regenerative biology</u>

247 Amphibians have played a fundamental role in uncovering development principles

248 [for a detailed review see 23]. Research on anurans has enabled the understanding

249 of critical mechanisms underlying such as the breaking of egg asymmetry [24], axis

250 establishment, and nerve transmission [25]. Notably, the availability of genome

251 assemblies for *Xenopus laevis* and *X. tropicalis* has significantly advanced

252 embryological and developmental biology. This advancement has enabled gene loss-

253 of-function research through the combination of transgenesis with RNA interference,

254 gene editing, and enhanced morpholino design. This has facilitated the in-depth

255 analysis of regulatory and non-coding genomic influences in developmental

256 processes [26, 27]. Consequently, these studies have generated thousands of

257 genomic and transcriptomic resources for these two species [28, 29].

258

259 Yet, there is much more to uncover about amphibian development, especially given

260 the numerous developmental modalities found across amphibian species, which

261 likely demonstrates the highest diversity among vertebrates [30]. This includes direct

262 development (egg to froglet; the first genome of a direct-developing amphibian,

263 *Eleutherodactylus coqui*, was published in 2024 [31]), and phenotypic plasticity [32,

264 33].

265

266 Sexual development and determination are also diverse in amphibians [34],

267 encompassing female and male heterogamety, unique microscopically recognizable

268 sex chromosomes, and species with undifferentiated sex chromosomes. Sex-

269 determining genes across these systems are starting to be explored through high-

270 throughput sequencing [6, 35-38]. For instance, a Y-specific non-coding RNA

11

271    involved in male sex determination in *Bufotes viridis* was identified through the

272    application of multiple omics techniques [38]. Strikingly, some salamanders in the

273    genus *Ambystoma* exist as a single all-female, polyploid lineage that can incorporate

274    new chromosome sets from up to five other sexual species [39]. Transcriptomes

275    from these salamanders have shown that gene expression from their divergent

276    genomes is balanced for some genes but biased for others [40]. Sexual development

277    in amphibians can result in sexually dimorphic features such as nuptial spines, which

278    have been explored using comparative genomics approaches in the frog

279    *Leptobrachium leishanense* [41]. The increasing availability of amphibian genomes

280    will enable a deeper understanding of the molecular mechanisms underlying such

281    ontogenetic diversity.

282

283    Metamorphosis sets many amphibian species apart from amniotes. Transcriptomics

284    has revealed a remarkable turnover in gene expression between larval and adult

285    stages of both frogs [42-45] and salamanders [46, 47]. This represents genomic

286    uncoupling of these life history phases with major macroevolutionary implications

287    [44, 48]. Amphibian omics approaches are rapidly increasing our understanding of

288    the developmental process of metamorphosis, including the role of methylation in

289    gene regulation [49]. Amphibians have also been found to respond to environmental

290    perturbations by altering their behavior or phenotypes in various ways. These include

291    changing developmental rate [32], hybridization with positive fitness effects [50],

292    producing novel trophic morphologies [51], and kin detection to avoid cannibalizing

293    relatives [52-54].

294

12

295 Due to their exceptional tissue repair and regenerative capacities [55, 56],

296 amphibians are leading models for understanding the mechanisms of regeneration.

297 This is particularly true for salamanders, which display the most extensive adult

298 regenerative repertoire among vertebrates, including the ability to regenerate parts of

299 their eyes, brain, heart, jaws, lungs, spinal cord, tail, and entire limbs [56]. Due to

300 new genome assemblies for urodele species, *Ambystoma mexicanum* and the

301 *Pleurodeles waltl*, regeneration can now be studied with transgenesis, advanced

302 imaging, and genome editing. Intensive transcriptomic sequencing for these two

303 salamander species has facilitated gene expression studies, including investigations

304 into regeneration processes and characterization of other genomic features [57].

305 Additionally, a novel mechanism of telomere length maintenance and elongation has

306 recently been described in *P. waltl* [58], potentially linking regenerative capability with

307 longevity. Other amphibian species have also contributed to genomic research on

308 regeneration. For example, databases compiled from gene expression resources of

309 *Notophthalmus viridescens* have enabled comparative studies [59].

310

311 <u>Ecology and evolution</u>

312 Modern amphibians are the sister lineage of all amniotes, making them a valuable

313 resource for studying species relationships and trait evolution. This is exemplified by

314 studies that explore the rapid diversification of frogs [60], the evolution of vision [61],

315 hybridogenesis [62-64], and the evolution of limblessness [65]. Amphibian

316 phylogenomics has addressed many longstanding questions in amphibian evolution

317 [66-69]. Comparative genomic analyses including amphibian groups have also

318 revealed important gaps in our understanding of tetrapod molecular evolution such

319 as chromosomal rearrangements and group-specific gene families that remain

13

320  unclassified to date [65, 70, 71 ]. In this section, we explore how genomics is being

321  applied to understand the diverse ecological and evolutionary features unique to

322  amphibians.

323

324  Like mammals, birds, and reptiles [72-74], some amphibians have evolved the ability

325  to live in high-elevation environments such as the Andes (up to 5400 m) [75, 76] and

326  the Tibetan Plateau (4478 m) [17]. However, unlike other groups, amphibians lack

327  fur, feathers, or scales to protect them from physiological stressors such as UV

328  exposure. This vulnerability makes them an intriguing model for studying the effects

329  of UV radiation, which is relevant not only to humans [17] but also to species

330  impacted by climate change. Amphibians have evolved multiple mechanisms of

331  resisting UV including increasing antioxidant efficiency and gene regulatory changes

332  in defense pathways [17, 77]. There is also evidence from Tibetan anurans that

333  genes that impact other high-elevation traits (e.g., hypoxia resistance, immunity, cold

334  tolerance) have evolved convergently across distantly related families (e.g.,

335  Dicroglossidae, Bufonidae, Megophryidae) [78], and that intraspecific divergence in

336  many of these genes correlates with elevation [79, 80]. Additional genomic

337  signatures of elevation adaptations, including genes regulating resistance to cold,

338  hypoxia, immunity, and reproduction, have been described in ranid species of

339  western North America inhabiting elevations from the sea level to nearly 3000 m [81].

340  While we are beginning to understand the genetic mechanisms of high-elevation

341  adaptation in some Asian and North American frogs, this has yet to be investigated in

342  other high-elevation amphibians, including Andean anurans (e.g., *Telmatobius culeus*

343  [82]) and high-elevation salamanders, such as *Pseudoeurycea gadovii* [83]).

344

14

345    The ability to produce toxins has evolved across all three amphibian orders, where it

346    primarily serves as an anti-predation mechanism. The source of amphibian toxins

347    varies: some species are capable of synthesizing poisonous compounds (e.g.,

348    bufonids, myobatrachids), whereas others sequester toxic substances from their diet

349    (e.g., dendrobatids, mantellids) [84-87] or microbial symbionts (e.g., newts) [88].

350    Since dendrobatid frogs sequester their toxins from prey (e.g., mites and ants), they

351    lack genes encoding these toxins [89, 90]. However, they require genes to facilitate

352    the transport of these toxins to the skin. Recent genomic and proteomic research

353    has identified candidate genes coding for proteins that may serve dual roles in toxin

354    transport and resistance [91-93]. Comparative genomic research has identified

355    specific substitutions that allow toxic amphibian species to effectively mitigate the

356    effects of the sequestered toxins on their own tissues [94-96]. Skin transcriptomes

357    have also proven to be a rich source for data mining and the identification of

358    candidate toxins and antimicrobial peptides in various amphibians, including

359    caecilians [97-101].

360

361    Interactions between toxic amphibians and their predators have resulted in a

362    fascinating variety of co-evolutionary arms races. These include well-characterized

363    systems of toxicity resistance mechanisms in amphibian predators [102-106] and

364    aposematism and mimicry in toxic species [107, 108]. Research on aposematism

365    and mimicry has utilized whole genome, exome capture, and transcriptome

366    sequencing to elucidate the genes underlying the vast diversity of color patterns

367    across populations and species in dendrobatids [109-114]. These approaches have

368    yielded a goldmine of information that can be used to understand the genes, gene

369    networks, and biochemical pathways that underlie variation in coloration in other

15

370 amphibian groups including highly diverged aposematic taxa such as Australian

371 myobatrachid frogs (e.g., *Pseudophryne corroboree)*, Malagasy poison frogs

372 (Mantellidae), caecilians (e.g., *Schistometopum thomense*), and salamanders (e.g.,

373 *Salamandra salamandra*). Indeed, these methods have already enabled the

374 identification of genes and loci involved in coloration in the salamander *S.*

375 *salamandra bernardezi* [115].

376

377 Despite the numerous advances made with amphibian omics in elucidating

378 evolutionary and ecological mechanisms, fully unraveling their genetic basis requires

379 generating a vast number of genomes due to the comparative nature of these fields.

380 Some of the exciting research avenues in amphibians include parental care [116,

381 117], gliding ability [118], lunglessness [119, 120], unusual defense mechanisms,

382 such as the ability of some newts to pierce their ribs through toxin glands in their skin

383 [121, 122], milk production or skin feeding in caecilians [123, 124], and spatial

384 navigation [125].

385

386 Conservation

387 Amphibians are the most endangered class of vertebrates with current estimates

388 suggesting that more than 40% of species are threatened with extinction [126]. The

389 threats amphibians are facing continue to increase [126], creating a clear need to

390 develop innovative and effective methods to conserve them. Paradoxically, current

391 rates of amphibian species description are exponential, and numerous candidate

392 species are being flagged worldwide. This suggests that we are still far from

393 overcoming the amphibian Linnean shortfall, especially in tropical regions [127, 128].

394 Hence, numbers of threatened species are likely underestimated, as undescribed

16

395 species cannot be assessed and are more likely to become extinct [129]. Further, the

396 conservation status of many amphibians remains unknown, especially for tropical

397 species [130] and for a number of soil-dwelling caecilians known only from a few

398 specimens [131]. Generating genomic data is one method to address this challenge,

399 as it can be used to estimate both evolutionary potential and extinction risk [132].

400 Genomes are also vital for understanding species boundaries and the geographic

401 distribution of genetic diversity within species, and for identifying populations under

402 higher risk due to anthropogenic pressures or climate change [20, 21, 133, 134].

403 These features make genomic resources invaluable for developing species

404 conservation action plans [135].

405

406 Amphibian conservation efforts should leverage population genetic theory and the

407 burgeoning field of conservation genomics. This approach enables the quantification

408 of both neutral and adaptive diversity across genomes, thereby facilitating the

409 promotion of adaptive potential or genetic rescue through translocation programs

410 [136-139]. Typically, these studies begin with the genomic characterization of

411 populations across various environmental conditions, assessing population genetic

412 health and disease risk [140, 141]. Omics resources are becoming increasingly

413 important in species detection (e.g., via eDNA [142-144]). They can also support

414 monitoring and surveillance efforts by identifying populations most at risk of declines

415 due to potential genetic threats such as maladaptive alleles, genetic load, inbreeding

416 and outbreeding depression, hybridization, and/or genetic incompatibility [136, 145].

417 Increased monitoring and maintenance of genomic diversity are key targets of many

418 national and international recommendations such as the US Endangered Species

17

419 Act [146], the Kunming-Montreal Global Biodiversity Monitoring Framework [147],

420 and the Amphibian Conservation Action Plan [135].

421

422 A more specific application of amphibian genomics for conservation requires

423 understanding the genetic basis of traits that impact fitness, such as disease

424 resistance or climate change tolerance. This information can be used to promote

425 adaptation using approaches like Targeted Genetic Intervention (TGI), which aims to

426 increase the frequency of adaptive alleles with approaches such as selective

427 breeding, genome editing, or targeted gene flow [148]. Considerable effort has been

428 invested in understanding the genetic basis of resistance to the devastating

429 amphibian disease chytridiomycosis, which has resulted in the identification of

430 multiple candidate genes [149-151] that could be targeted to increase

431 chytridiomycosis resistance with TGI. Additionally, the efficacy of TGI at increasing

432 chytridiomycosis resistance has already been demonstrated in North American

433 mountain yellow-legged frogs (*Rana muscosa* and *R. sierrae*) where translocation of

434 resistant individuals increased recipient population persistence [152]. Despite the

435 obvious appeal of using genetic intervention approaches for conservation, these

436 methods should be evaluated in contained facilities whenever possible and

437 accompanied by long-term monitoring to ensure their efficacy and rule out any

438 unintended impacts [148, 153-155]. Although such conservation interventions require

439 extensive resources, this may be the only effective method for restoring some

440 species to the wild, especially in those threatened by intractable threats such as

441 chytridiomycosis [156].

18

**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

19

467    are currently developments underway, https://www.cbd.int/dsi-gr) but must also be

468    considered going forward [see for example 163]. Moreover, voucher specimens and

469    duplicate tissue samples should be deposited in local museums or preferred partners

470    of the local communities [164, 165].

471

472    Amphibian fieldwork often involves overcoming numerous hurdles such as

473    navigating socio-political conflicts and accessing remote field sites. These

474    challenges may be exacerbated in developing economies or for researchers with

475    limited access to high volume funding streams. In addition, inadequate infrastructure

476    for accessing field sites, and the need to time of fieldwork to coincide with the often

477    highly seasonal and cryptic activities of amphibians [166] can further complicate the

478    task. Attention should also be directed towards overcoming inequities that may pose

479    additional obstacles to fieldwork for underrepresented groups [161].

480

481    Once amphibians are collected, selecting the tissue sample to obtain sufficient high-

482    molecular-weight DNA (HMW, reaching 100 Kb or ultra HMW, reaching 1 Mb) can be

483    challenging due to the small body sizes of most amphibians (e.g., < 30 g). For

484    generating reference genomes, blood is one of the most recommended sample

485    types for amphibian genomics [167]. However, the blood volume of most amphibians

486    is too small for non-lethal collection [168]. This presents a significant challenge

487    because obtaining the required quantity of HMW DNA often necessitates lethal

488    sampling, which may not always be legally permitted or ethically advisable, especially

489    for threatened species or those in captive collections [169]. Non-lethal sampling

490    approaches, such as buccal swabs or toe or tail clips, are increasingly viable for

491    various genomic applications, including low-coverage whole genome sequencing or

20

492    targeted sequencing approaches [170, 171]. Another alternative is to use tadpoles

493    instead of adults as was done to generate the genome of *Taudactylus pleione* [172].

494

495    Most tissue sampling protocols for reference genomes or transcriptome sequencing

496    recommend harvesting samples from fresh tissue, followed immediately by flash

497    freezing in liquid nitrogen (LN2) and storing at -80°C until extraction

498    (https://www.vertebrategenomelab.org/resources/guidelines). Maintaining ultracold

499    storage and the cold chain during transport from remote collection sites can be

500    challenging. After being adequately charged with LN2, dry vapor shippers can keep

501    samples ultra-cold for a week or more in the field. While LN2 is the gold standard,

502    other preservatives like 95% EtOH, 20-25% DMSO in EDTA may adequately

503    preserve DNA for long-read sequencing, although efficacy may vary depending on

504    the tissue type [167, 173]. Other options include lysis buffers or commercial products

505    such as Zymo DNA/RNA shield (Zymo Research, USA). However, it remains

506    essential to test the impact of these preservatives on sequencing outcomes

507    beforehand, specifically for the given taxon and tissue type, since preservation

508    methods may inhibit downstream approaches, such as Hi-C library construction [167,

509    174].

510

511    While these challenges apply to any researcher or organization, they become

512    particularly challenging when the sequencing work occurs outside the species'

513    country of origin. This difficulty arises not only because genetic material is prone to

514    degradation but also because of regulations on moving biological samples across

515    political borders [175, 176]. The global genomics community should strive to ensure

516    that sequencing projects occur within the country of origin of the samples and

21

517 discourage 'parachute' or 'helicopter science' [177, 178]. Oxford Nanopore

518 Technology (ONT) may be promising solution, providing comparatively affordable

519 access to equipment and reagents for ultra-long read sequencing that can even be

520 done directly in the field [179]. However, working with non-model organisms requires

521 prior optimization, and the startup costs for this infrastructure remain prohibitive for

522 many scientists from low-income countries. Moving forward, the goal should be to

523 apply these technologies in collaboration with local researchers. For example,

524 programs like the In Situ Laboratories Initiative (https://insitulabs.org/hubs/) aim to

525 provide affordable access to high-tech laboratories in remote biodiverse areas).

526 Such collaboration should proceed from finding shared interests, developing ideas,

527 realizing the shared benefits from research outputs, and focusing on capacity-

528 building efforts [180].

529

530 Working with museum specimens [the burgeoning field of "museomics"; 181] is

531 another promising avenue of research allowing to access to past amphibian

532 biodiversity. However, there are several additional challenges associated with DNA

533 degradation, preservation methods, and contamination that need to be overcome

534 [182-184]. This is particularly relevant for wet-preserved amphibian specimens, as

535 retrieving DNA can be challenging due to the often unknown fixation and

536 preservation methods that can alter nucleotide integrity. Methodological advances in

537 laboratory protocols [e.g., 185, 186, 187] and the development of sequencing

538 strategies, such as 'Barcode Fishing' [188, 189], have made significant progress in

539 addressing these challenges, including the ability to sequence extinct species [190,

540 191]. In the current era, even limited sequences from taxonomic type specimens are

541 of unparalleled importance, especially for species identification using genetic data,

22

542 by those applying methods like eDNA and metagenomics [192]. Museomics has also

543 revolutionized amphibian taxonomy by integrating DNA from name-bearing types,

544 overcoming impediments like uncertainty in taxonomic names, species complexes,

545 and cryptic species [188, 189, 193, 194].

546

547 As mentioned earlier, the large genome sizes of many amphibians affect

548 sequencing, computing, and storage costs, as well as computational requirements

549 for data analysis [6, 195]. However, the greater challenges lie in the methodological

550 and theoretical limitations of assembling large genomes. Additionally, access to

551 cluster computing for genome assembly remains limited for many scientists,

552 particularly those from low-income countries. Genome size in amphibians is

553 correlated with increased intron lengths and repeat content [196], posing challenges

554 for assembly, especially when using with short-read sequencing technologies or

555 during contig joining processes. Regions misassembled due to low complexity have

556 previously resulted in a significant loss of sequence information (e.g., by as much as

557 16%) through the collapsing of repetitive sequences [197]. Repetitive regions can

558 also result in the formation of problematic chimeras during assembly, where two

559 distant contigs are erroneously joined due to a shared repeat sequence [198]. These

560 problems are exacerbated when repeats are longer than sequencing reads. Further

561 challenges are posed by polyploidy [199], which has evolved repeatedly in

562 amphibians [200, 201]. This can make developing haplotype-specific assemblies

563 challenging and may require dramatically increased sequencing and computational

564 efforts [199, 202]. Thus, the development of long-read and 3C technology (i.e., Hi-C

565 scaffolding) is especially important for assembling amphibian genomes [198, 203].

566 Recent technological advances, including long-read techniques such as PacBio HiFi,

23

567 ONT, as well as scaffolding methods such as Hi-C and optical mapping, now make it

568 feasible and affordable to generate reference genomes for most frogs, caecilians,

569 and increasingly for salamanders [203].

570

571 Gene and repeat annotation resources in amphibians are also under-developed.

572 Although this may not pose a significant issue for well-conserved genes, where

573 researchers can retrieve annotation information from orthologous regions using

574 existing databases like UniProt [204], it often results in missed or poorly annotated

575 genes, particularly for highly polymorphic genes or genes lacking representation in

576 model taxa. Hence, the ability to study features such as gene evolution [67], repeats

577 [15, 196], immune genes [205], and genetic sex determination [206] is still limited

578 and requires caution to prevent overinterpretation.

579

580 Although key tools for understanding functional genomics exist, tools and protocols

581 for gene editing, transgenesis, *in vitro* fertilization, are rare or non-existent for most

582 amphibians [148, 207], with the exception of some model species (e.g., *Xenopus*

583 *laevis*, *X. tropicalis*, and *Ambystoma mexicanum*) [56, 208-211]. Immortal cell lines

584 have been successfully generated for some amphibians [212] and protocols have

585 been established to facilitate the initiation of spontaneously arising cell lines for a

586 subset of anurans [213]. However, establishing cell cultures for most species

587 requires extensive problem-solving and expertise [212].

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

589 The AGC was launched in March 2023 to address the aforementioned knowledge

590 gaps through technological advances and international cooperation. The mission of

591 the AGC is to increase collaboration and communication among amphibian

24

592    researchers internationally and across scientific disciplines, to increase amphibian

593    genomics resources, and to apply genomic data and functional resources to help

594    bridge the implementation gaps between genome biologists, other scientists and

595    conservation practitioners. The leadership structure of the AGC consists of a director,

596    two co-directors, and a 10-member board. The board was carefully chosen to ensure

597    gender equality, diversity of scientific disciplines, career stages, and representation

598    from various geographic regions.

599

600    Currently the AGC organizes monthly virtual meetings and a monthly virtual seminar

601    series. They maintain a website (https://mvs.unimelb.edu.au/amphibian-genomics-

602    consortium), facilitate active discussion groups, and have hosted social events at

603    several scientific conferences. Additionally, the AGC is preparing to host a full-day

604    symposium titled "Beyond the reference: genomics for amphibian research and

605    conservation" at the World Congress of Herpetology in August 2024 in Kuching,

606    Malaysia.

607

608    The first actions of the AGC include raising funds for genome sequencing,

609    developing technical resources and best practices guidelines, improving amphibian

610    genome annotation, supporting travel for students and early career researchers, and

611    conducting virtual and in-person computational workshops. The AGC plans to secure

612    funding to sequence high-priority amphibian species (see The AGC's genome

613    sequencing targets section and Table 1). Additionally, the AGC aims to facilitate

614    amphibian sample collection for broader taxonomic consortia. The AGC is already

615    affiliated with the Earth BioGenome Project (EBP) and AmphibiaWeb

616  (https://amphibiaweb.org), reinforcing its commitment to advancing amphibian

617  genomics and conservation efforts.

618

619  AGC membership

620  At the time of the submission of this work, the AGC had 282 members from 41

621  countries (Fig. 4). Although the membership is geographically diverse, there remains

622  some disparity across regions. Efforts will be intensified to attract members from

623  underrepresented countries, particularly regions known for high amphibian diversity

624  and/or endemism such as Central and South America, and Southeast Asia. We

625  promote equity between members by providing additional support and opportunities

626  to those from developing countries and underrepresented groups. This includes

627  eliminating membership fees, scheduling online meetings at alternating times to

628  accommodate global time zones, facilitating discussion groups on the cloud-based

629  collaboration platform Discord, and translating AGC correspondence into multiple

630  languages. Furthermore, we are also committed to fostering knowledge and skills

631  transfer to all emerging scientists worldwide, and we actively encourage early career

632  researchers to join the initiative and participate in governance.

633

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

635  The AGC leadership designed a 23-question survey to investigate consortium

636  members' experiences in amphibian genomics (questions can be found in

637  Supplementary Table S1). The survey was distributed using the Qualtrics XM

638  platform and remained active from the 4th of March to the 27th of December 2023.

639  We collected responses from a total of 133 AGC members from 32 countries with

640  different expertise in sequencing approaches and bioinformatics techniques, who

26

641 primarily work on the ecology and evolution of anurans. Overall, respondents

642 emphasized the urgency of filling knowledge gaps in amphibian genomics due to the

643 current conservation crisis, pinpointing the necessity to expand the number of high-

644 quality chromosome-level amphibian genomes. Additionally, there was strong

645 agreement among survey respondents that the generation of new genomics

646 resources needs to be coupled with the improvement and accessibility of annotation

647 processes. A better development of sharing computational expertise among

648 members and resources internationally was also underscored. More than half of the

649 survey participants said they use sequencing technologies for their studies (70 of the

650 133). About half of the respondents said their main work activities were "genomics

651 lab work" or "computational analyses" (48% and 57%, respectively).

652

653 To evaluate consortium members' experience in amphibian genomics, we applied a

654 principal components analysis to the quantitative responses. Bioinformatic

655 competencies and perceived challenges of the AGC respondents were grouped in

656 two dimensions, respectively (Fig. 5A and Fig. S2; see Supplementary Material for

657 methodological information). To explain the variation of these two new variables, we

658 used the scientific expertise of AGC members, the funding success, and two

659 variables related to the country of main affiliation of the respondent: the number of

660 amphibian species and gross domestic expenditure on R&D (GERD) per capita, as

661 explanatory variables. Amphibian genomics expertise and identified challenges

662 varied substantially among respondents. The number of amphibian species and

663 GERD per capita of the respondent's main affiliation country did not capture this

664 variation (Fig. 5B and Fig. S3; see Supplementary Material for methodological

665 information). Instead, genomics funding success and years of scientific expertise

27

666   were, as expected, positively correlated and both variables and were associated with

667   a reduction in the perceived challenges associated with amphibian genomics.

668

669   <u>The AGC's genome sequencing targets</u>

670   Following the efforts of genomics consortia for other tetrapod groups [e.g., 214], and

671   previous research efforts [21], we identified amphibian families without any

672   representative genomes and selected one representative species per family for our

673   sequencing priority list (Fig. 2B and Table 1). We propose 48 candidate species

674   based on their IUCN Red List category, ecological and evolutionary distinctiveness,

675   and the availability of other genomics records, especially transcriptomics. This list

676   includes 38 anurans, four urodeles, and six caecilians. We aim to build upon the

677   efforts of existing genomics consortia such as the Vertebrate Genomes Project

678   (VGP), hence, we included in our sequencing target list species with draft genomes

679   in the GenomeArk (https://www.genomeark.org/).

680   **Conclusion and outlook**

681   Amphibians are declining faster than they can be discovered [215]. Our hope is that

682   the recent advancements in technology (e.g., long-read sequencing, computational

683   tools) and integration of the research community to from the Amphibian Genomics

684   Consortium (AGC) will ignite research to revolutionize amphibian conservation and

685   our understanding of their fascinating biology, ecology and evolution. By supporting

686   amphibian genomics research and uniting amphibian researchers worldwide, the

687   AGC aims to propel amphibian genomics research into the future.

688

689   Moving forward, the AGC is committed to supporting amphibian sequencing

690   initiatives worldwide, with a particular emphasis on families lacking representation

28

691  and species from biodiverse countries (Table 1). Local sequencing initiatives will be

692  given priority whenever feasible to promote the development of *in situ* research

693  efforts and facilities. Additionally, we aim to provide funding and training opportunities

694  to facilitate collaboration among underrepresented groups, molecular and organismal

695  biologists, bioinformaticians, and conservation practitioners. We aspire to stimulate

696  public and scientific interest in amphibian research and enhance conservation efforts

697  for this intriguing and highly endangered group of vertebrates.

698

699  **List of Abbreviations**

700  ABS: access and benefit-sharing

701  AGC: Amphibian Genomics Consortium

702  CBD: Convention on Biological Diversity

703  DSI: digital sequence information

704  EBP: Earth BioGenome Project

705  GERD: gross domestic expenditure on research and development

706  GoaT: Genomes on a Tree

707  HMW: High molecular weight DNA

708  IPLC: Indigenous peoples and local communities

709  IUCN: International Union for Conservation of Nature

710  ONT: Oxford Nanopore Technology

711  VGP: Vertebrate Genomes Project

712  **Declarations**

713  Ethics approval and consent to participate

714  Not applicable.

29

715

## Consent for publication

717 Not applicable.

718

## Availability of data and materials

720 Not applicable.

721

## Competing interests

723 The authors declare no competing interests.

724

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731

## Authors' contributions

733 T.A.K. and M.T.-S. drafted the manuscript. T.A.K., M.T.-S., H.C.L., K.S., M.H.Y.,

734 S.T.M., A.J.C. contributed text to the first draft, M.T.-S. and T.A.K. analyzed the data

735 and created the figures, members of the Amphibian Genomics Consortium (AGC)

736 reviewed later drafts. All authors reviewed the manuscript.

737

30

31

756 **Table 1.** Amphibian Genomics Consortium (AGC) sequencing priority list. Table of

757 amphibian families without any sequenced genomes. For each family, AGC

758 proposed a candidate species based on its IUCN Red List category (LC: Least

759 Concern, NT: Near Threatened, VU: Vulnerable, EN: Endangered, CR: Critically

760 Endangered, and NA: Not evaluated), ecological and evolutionary distinctiveness,

761 and availability of other genomic records. This table shows the amphibian order to

762 which each family belongs and its number of genera (#G) and described extant

763 species (#S) as well as distribution region. *Species with available draft genome

764 assemblies in the GenomeArk (https://www.genomeark.org/).

765

| 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 |

32

| 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 |

33

| 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 | |

766
767
768

34

769 **Figures**

770
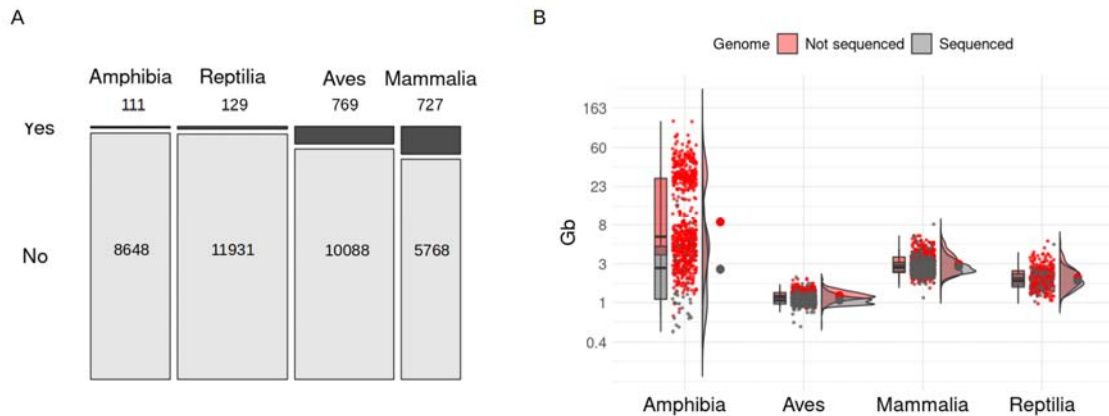


771 **Figure 1**. **Estimated genome size across tetrapod classes in relation to**

772 **sequenced genomes.** (A) Mosaic plot representing the percentage of species with

773 sequenced genomes as a proportion of the number of described species for each

774 tetrapod class (Yes: % species with sequenced genome; No: % species without

775 sequenced genome). (B) Combined box and density plot with raw data as points

776 comparing genome size of species with sequenced genome (gray; genome sizes

777 from NCBI genome assemblies) versus a subset of species without a sequenced

778 genome (red; genome sizes from the Animal Genome Size Database) for each

779 tetrapod class. The y-axis is log-transformed to facilitate visualization. Information

780 about sequenced genomes and genome sizes was obtained from the NCBI Genome

781 Browser, the Animal Genome Size database, and amphibian records from [11, 19].
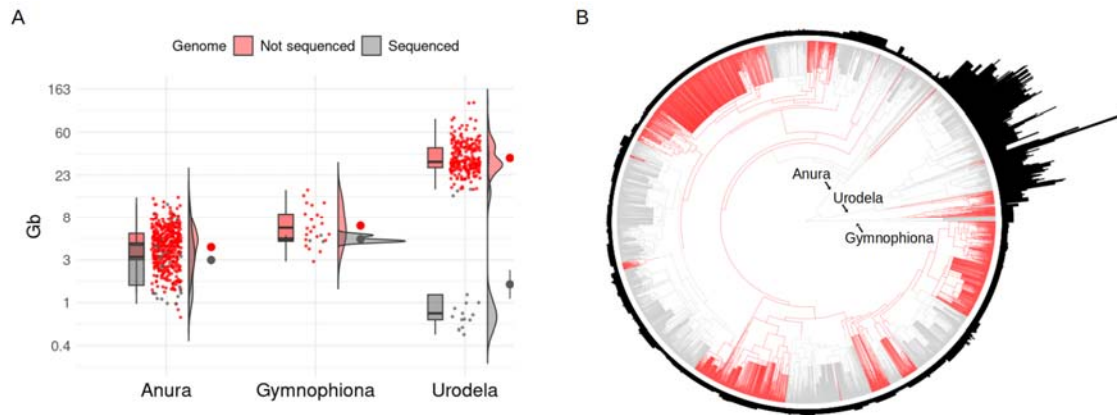
**Figure 2. Estimated genome size across amphibian orders in relation to sequenced genomes**. (A) Combined box and density plot with raw data as points showing genome size of species with sequenced genome (gray color; genome sizes from NCBI genome assemblies) versus a subset of species without available 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.
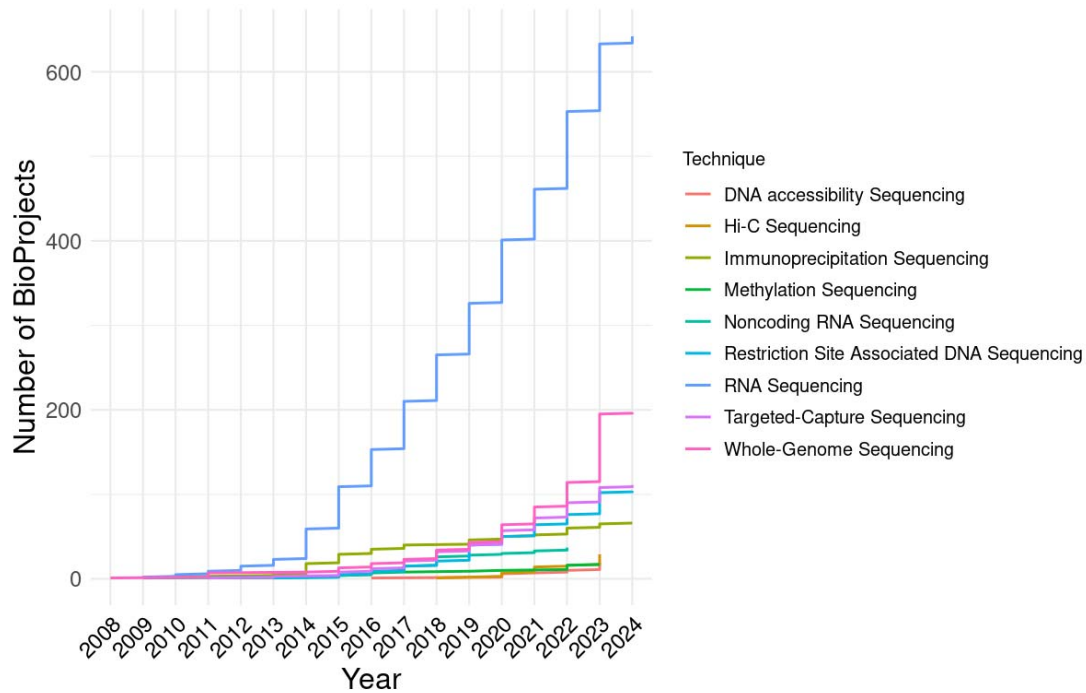
**Figure 3. Main sequencing techniques applied to amphibian genomics studies**.

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;

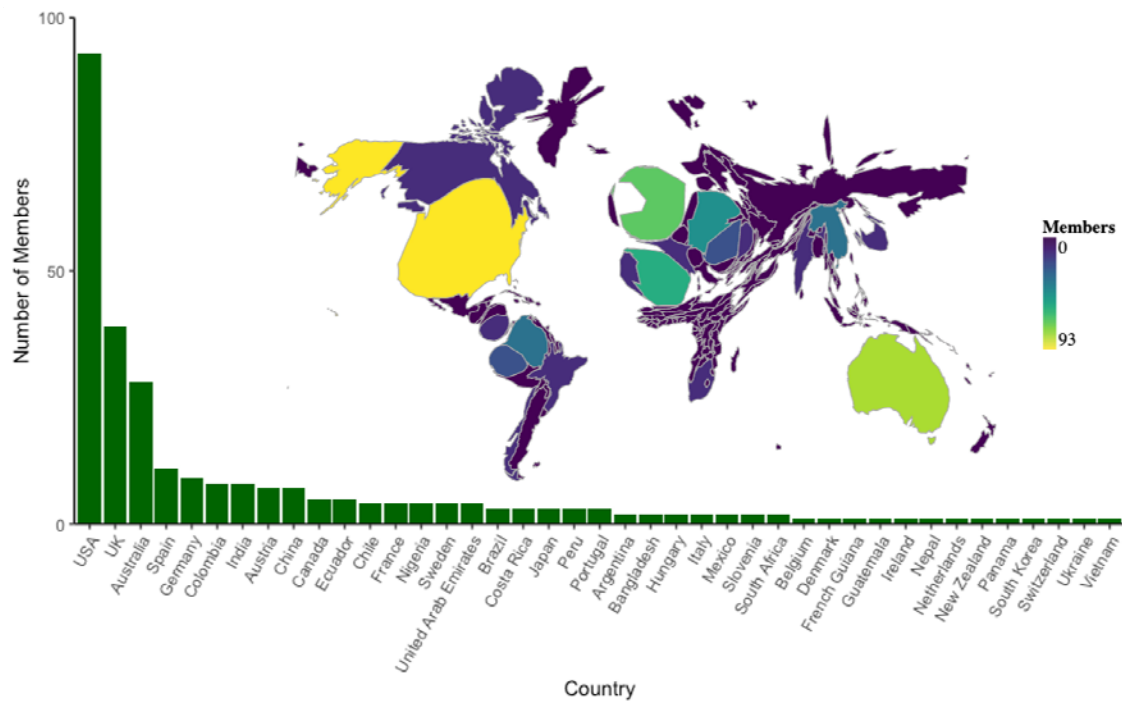Amplicon sequencing was included with Targeted-Capture Sequencing; Noncoding

RNA Sequencing includes: miRNA-Seq and ncRNA-Seq). BioProject information

was obtained from the NCBI Sequence Read Archive (SRA, accessed 1 March

2024).
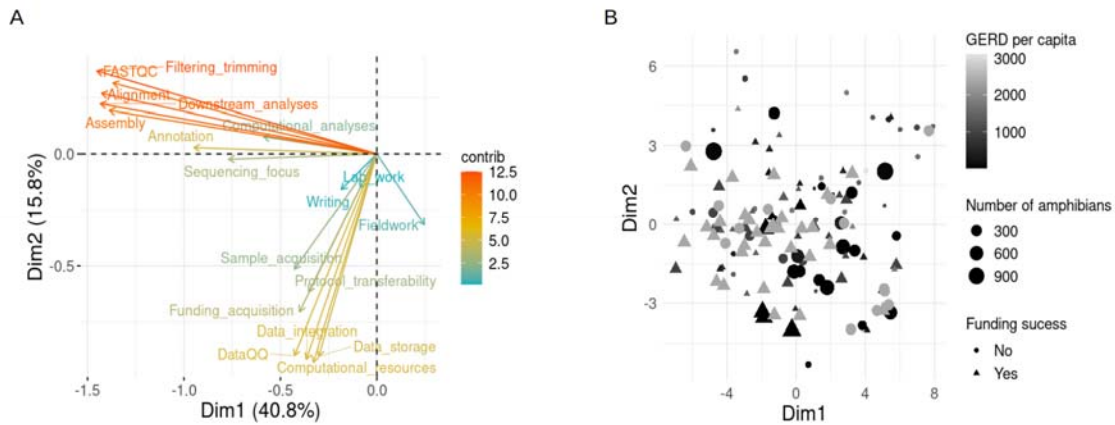
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**Figure 4. Amphibian Genomics Consortium (AGC) membership by country**.

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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