REPTILES & AMPHIBIANS

A New Record of the Kallar Torrent Frog, *Micrixalus herrei* Myers 1942, from the Ranni Forest Division, Kerala, India

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The Western Ghats is a global biodiversity hotspot (Myers et al. 2000) with nearly 252 species of amphibians (Dahanukar and Molur 2020), of which nearly 41% are threatened (Luedtke et al. 2023). The endemic monotypic family Micrixalidae (Biju 2001; Biju et al. 2014; Frost 2023), one of nine anuran families in the Western Ghats (Biju et al. 2014; Chandramouli and Dutta 2015; Dinesh et al. 2023), comprises 24 species in the single genus *Micrixalus*. *Micrixalus herrei* was described from Kallar, Trivandrum District, Kerala (Biju et al. 2014), and is known to occur in the states of Kerala and Tamil Nadu with its range reportedly limited to the south of the Shencottah Gap (Table 1; Biju et al. 2014; Amphibia Web 2023). The Shencottah Gap (9 °N), one of the three major discontinuities of the Western Ghats, acts as a biogeographic barrier and plays a substantial role in the region's speciation (Biswas and Karanth 2021); for example, genetic structuring of populations has been observed across the Shencottah Gap for *Indirana* cf. *beddomii* (Nair et al. 2012).

Herein we report the presence of *M. herrei* from the Ranni Forest Division, which is north of the Shencottah Gap (Fig. 1). *Micrixalus herrei* were first observed on 15 June 2023 at Naranamthodu, Kerala, India (9.40616, 76.98539), on 16 June 2023 at Nilackal (9.38436, 77.01598) and Chelikuzhy (9.38888, 76.94833), and a population was recorded from the splash zones of perennial second order streams in semi evergreen forest of the Ranni and Goodrical Ranges in the Ranni Forest Division during fieldwork in March–November 2023. Male frogs (Fig. 2) were collected, euthanized using tricaine methane sulphonate, liver tissues were preserved in 95% ethanol to confirm species identity, and the specimen was fixed in 4% formalin and vouchered in 70% ethanol.

Morphometric measurements followed Biju et al. (2014). Species identification was confirmed by comparing 16S rRNA sequences. Genomic DNA was isolated from the liver tissues using a DNeasy Blood and Tissue Kit (Qiagen). Fragments of mitochondrial 16S rRNA were amplified and sequenced following the procedure described by Gopalan et al. (2014).

Table 1. Known localities for Kallar Torrent Frogs (*Micrixalus herrei*) in Kerala and Tamil Nadu, India. All sites are from Biju et al. (2014) except those from this study, which are marked with asterisks (*).

Glenback Estate, Kiriparai, Kanyakumari District, Tamil Nadu (08.4188, 77.4182; 450 m)
Putherichariurn, Shencottah. Tirunelveli District, Tamil Nadu (08.9833, 77.2944; 140 m)
Kovachal, Shendurney WLS, Kollam District, Kerala (08.8497, 77.1759; 816 m)
Attayar 1, Agasthyamala Hills, Thiruvananthapuram District, Kerala (08.6341, 77.2093; 605 m)
Attayar 2, Agasthyamala Hills, Thiruvananthapuram District, Kerala (08.6545, 77.1905; 680 m)
Chathankod, Kanyakumari District, Tamil Nadu (08.6608, 77.1525; 116 m)
Bonacaud, Thiruvananthapuram District, Kerala (08.6737, 77.1575; 488 m)
Kallar, Ponmudi, Thiruvananthapuram District, Kerala (08.8490, 77.1322; 180 m)
Nilackel, Pathanamthitta District, Kerala (09.38436, 77.01598; 333 m)*
Naranamthodu, Pathanamthita District, Kerala (09.40616, 76.98539; 125 m)*
Chelikuzhy, Pathanamthitta District, Kerala (09.38888, 76.94833; 420 m)*

Reptiles & Amphibians ISSN 2332-4961
https://doi.org/10.17161/randa.v31i1.21567

Sequences were deposited in GenBank under gene accession numbers OR872314 and OR872315.

We conducted a phylogenetic analysis by comparing sequences obtained in this study with those of 24 *Micrixalus* species retrieved from GenBank (Biju et al. 2014) (Fig. 3) using *Nyctibatrachus* sp. (KJ711394.1) as the outgroup. Sequence alignment was performed using ClustalW, integrated into BIOEDIT (Thompson et al. 1994; Hall 1999). The optimal nucleotide substitution model, determined to be GTR + G + I, was selected through model testing in MEGA ver. 5 (Tamura et al. 2011). The phylogenetic placement of species was ascertained using maximum likelihood

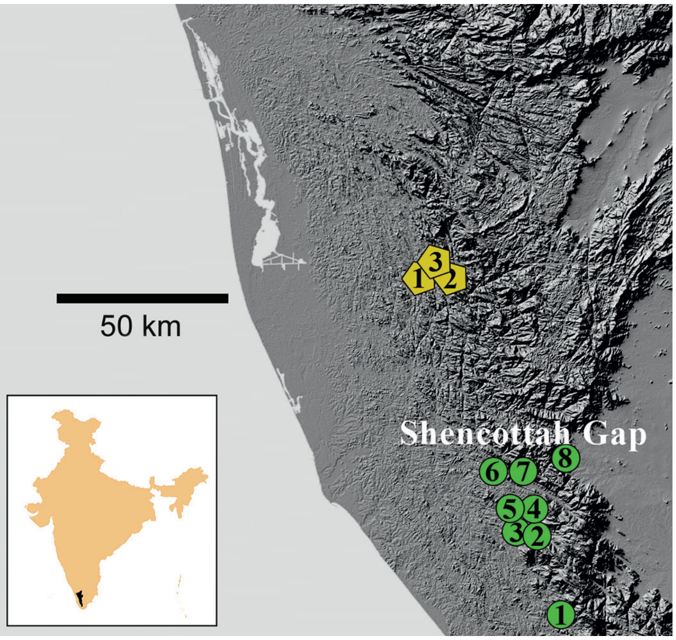

Figure 1. Map showing known localities for the Kallar Torrent Frog (*Micrixalus herrei*) in the southern Western Ghats, Kerala, India. Records previously published by Biju et al. (2014) are marked by green dots (Table 1). The new locality from Ranni Forest Division is indicated by the yellow pentagram. The smaller black area in the inset corresponds to the area shown in the larger map.

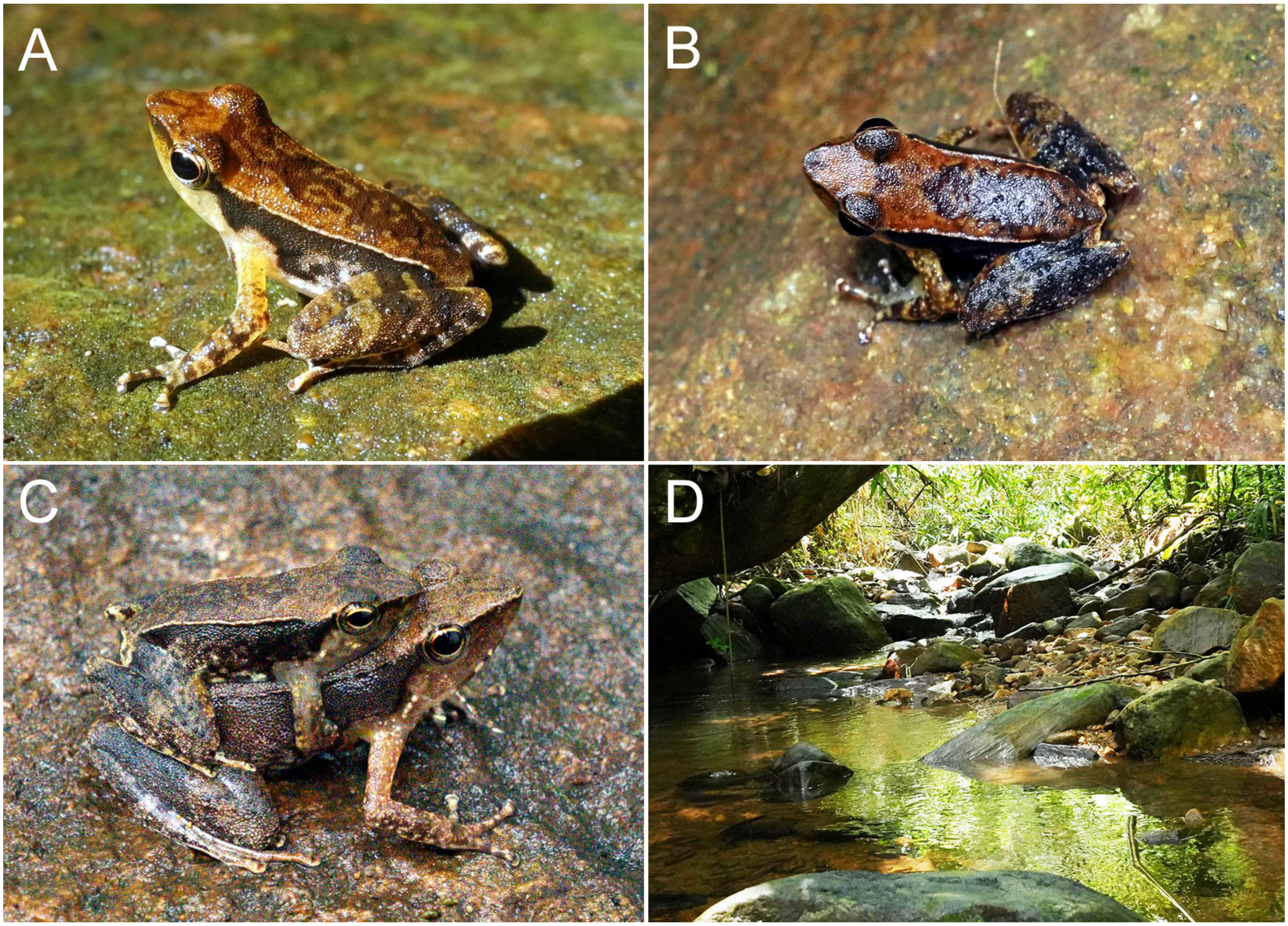

Figure 2. A male Kallar Torrent Frog (*Micrixalus herrei*) (A & B), pair in amplexus (C), and habitat in a second-order stream in Naranamthodu, Kerala, India (D). Photographs by Priya Thomas.

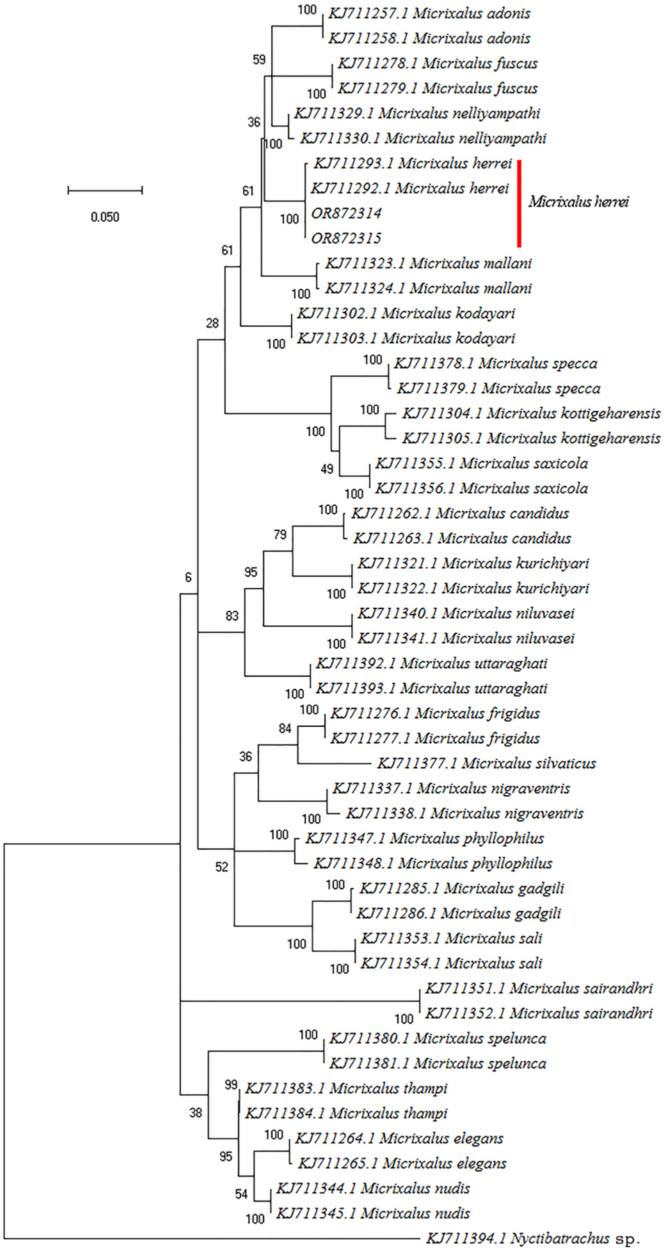

Figure 3. Phylogenetic tree (ML) showing relationships of the Kallar Torrent Frog (*Micrixalus herrei*) with sister species and *Nyctibatrachus* sp. as the outgroup; the tree is constructed based on 16S rRNA sequences, and the phylogenetic positions of species were determined using the maximum likelihood method as implemented in MEGA ver. 5 (Tamura et al. 2011).

Table 2. Morphometric measurements (in mm) of Kallar Torrent Frogs (*Micrixalus herrei*) in this study. Abbreviations: SVL = snout-vent length, HW = head width, HL = head length, TYD = diameter of the tympanum, SL = snout length, IUE = inter upper eyelid width, UEW = maximum upper eyelid width, MN = distance from rear of mandible to nostril, EN = distance from eye to nostril, NS = distance from nostril to snout, EL = eye length, FAL = forearm length, HAL = hand length, TL = thigh length, SHL = shank length, FOL = foot length.

	SVL	HW	HL	TYD	SL	IUE	UEW	MN	EN	NS	EL	FAL	HAL	TL	SHL	FOL
BCMZPT1	18.13	6.24	6.24	1.14	2.26	2.29	1.39	4.48	2.12	0.68	2.53	3.74	4.92	8.70	8.70	7.85
BCMZPT2	20.81	6.46	6.48	1.11	2.77	2.11	1.31	5.47	1.81	0.74	2.58	3.34	5.10	9.21	9.21	9.12
BCMZPT3	17.98	5.98	5.98	0.94	1.81	2.09	1.22	5.38	1.52	0.67	1.88	3.42	4.52	9.43	9.43	7.72
BCMZPT4	18.36	6.11	6.11	1.13	2.24	1.46	1.46	4.85	1.69	0.73	2.31	3.42	4.91	9.09	9.09	8.51
BCMZPT5	18.62	6.17	6.17	0.98	2.4	1.36	1.05	4.16	2.13	0.70	2.94	2.45	5.00	8.80	8.80	8.62
Mean	18.78	6.19	6.20	1.06	2.30	1.86	1.29	4.87	1.85	0.70	2.45	3.27	4.89	9.05	9.05	8.36
SD	1.04	0.18	0.19	0.09	0.35	0.42	0.16	0.56	0.27	0.03	0.39	0.49	0.22	0.30	0.30	0.58

implemented in MEGA ver. 5. Branch support was assessed with 1,000 bootstrap replicates (Felsenstein 1985). Pairwise genetic distances between species were computed using the Kimura 2-parameter method in MEGA ver. 5.

Morphological measurements of adult males aligned with measurements in Biju et al. (2014) (Table 2) The sequences obtained from the current study clustered with sequences of voucher specimens of *M. herrei* with high boot strap values (Table 3). An integrated approach using both morphological data and DNA barcoding sequences (Abraham et al. 2015) confirmed the presence of *M. herrei* in the Ranni Forest Division. This new record, which extends the range of the species more than 133 km north of the Shencottah Gap, calls for an examination of the genetic structuring of *M. herrei* populations on either side of the gap. All three sites mentioned in the study are en route to the forest shrine of Sabarimala, which experiences heavy pilgrimage in certain seasons, and hence is subjected to disturbance on a large scale from pilgrimage activities. Biotic interference is reflected in the degradation of the forest and the opening of the canopy (Kattany and Jose

2013). Consequently, studying the distribution, microhabitats, and population structure of this ancient endemic lineage of frogs is of great importance.

Table 3. Pairwise genetic distances (%) between *Micrixalus herrei* and related species calculated using the Kimura 2-parameter method in MEGA ver. 5 (Tamura et al. 2011).

KJ711293.1_*Micrixalus_herrei*		
KJ711292.1_*Micrixalus_herrei*	0.0019	
OR872314	0.0019	0.0000
OR872315	0.0019	0.0000
KJ711257.1_*Micrixalus_adonis*	0.0482	0.0461
KJ711258.1_*Micrixalus_adonis*	0.0482	0.0461
KJ711262.1_*Micrixalus_candidus*	0.0912	0.0891
KJ711263.1_*Micrixalus_candidus*	0.0937	0.0915
KJ711264.1_*Micrixalus_elegans*	0.0854	0.0833
KJ711265.1_*Micrixalus_elegans*	0.0857	0.0835
KJ711276.1_*Micrixalus_frigidus*	0.1010	0.0988
KJ711277.1_*Micrixalus_frigidus*	0.1010	0.0988
KJ711278.1_*Micrixalus_fuscus*	0.0612	0.0591
KJ711279.1_*Micrixalus_fuscus*	0.0612	0.0591
KJ711285.1_*Micrixalus_gadgili*	0.1023	0.1001
KJ711286.1_*Micrixalus_gadgili*	0.1022	0.1000
KJ711302.1_*Micrixalus_kodayari*	0.0611	0.0590
KJ711303.1_*Micrixalus_kodayari*	0.0611	0.0590
KJ711304.1_*Micrixalus_kottigeharensis*	0.0823	0.0802
KJ711305.1_*Micrixalus_kottigeharensis*	0.0868	0.0846
KJ711321.1_*Micrixalus_kurichiyari*	0.1002	0.0980
KJ711322.1_*Micrixalus_kurichiyari*	0.1002	0.0980
KJ711323.1_*Micrixalus_mallani*	0.0500	0.0480
KJ711324.1_*Micrixalus_mallani*	0.0500	0.0480
KJ711329.1_*Micrixalus_nelliyampathi*	0.0338	0.0318
KJ711330.1_*Micrixalus_nelliyampathi*	0.0379	0.0358
KJ711337.1_*Micrixalus_nigraventris*	0.0988	0.0966
KJ711338.1_*Micrixalus_nigraventris*	0.1011	0.0989
KJ711340.1_*Micrixalus_niluvasei*	0.1034	0.1012
KJ711341.1_*Micrixalus_niluvasei*	0.1034	0.1012
KJ711344.1_*Micrixalus_nudis*	0.0786	0.0765
KJ711345.1_*Micrixalus_nudis*	0.0788	0.0766
KJ711347.1_*Micrixalus_phyllophilus*	0.0870	0.0849
KJ711348.1_*Micrixalus_phyllophilus*	0.0869	0.0848
KJ711351.1_*Micrixalus_sairandhri*	0.1114	0.1091
KJ711352.1_*Micrixalus_sairandhri*	0.1114	0.1091
KJ711353.1_*Micrixalus_sali*	0.1005	0.0983
KJ711354.1_*Micrixalus_sali*	0.1005	0.0983
KJ711355.1_*Micrixalus_saxicola*	0.0847	0.0826
KJ711356.1_*Micrixalus_saxicola*	0.0847	0.0826
KJ711377.1_*Micrixalus_silvaticus*	0.1028	0.1006
KJ711378.1_*Micrixalus_specca*	0.0892	0.0870
KJ711379.1_*Micrixalus_specca*	0.0914	0.0893
KJ711380.1_*Micrixalus_spelunca*	0.0920	0.0942
KJ711381.1_*Micrixalus_spelunca*	0.0920	0.0942
KJ711383.1_*Micrixalus_thampi*	0.0720	0.0698
KJ711384.1_*Micrixalus_thampi*	0.0720	0.0698
KJ711392.1_*Micrixalus_uttaraghati*	0.0761	0.0739
KJ711393.1_*Micrixalus_uttaraghati*	0.0761	0.0739
KJ711394.1_*Nyctibatrachus*_sp.	0.2033	0.2035

Acknowledgements

We thank the Kerala Forest and Wildlife Department for granting permission to conduct this field study (KFDHQ-1089/2020-CWW/WD10). The first author acknowledges the Rajiv Gandhi Centre for Biotechnology Trivandrum, for providing facilities for DNA barcoding and Nirmala College, Muvattupuzha, for laboratory facilities.

Literature Cited

Abraham, R.K., J.K. Mathew, V.P. Cyriac, A. Zachariah, D.V. Raju, and A. Zachariah. 2015. A novel third species of the Western Ghats endemic genus *Ghatixalus* (Anura: Rhacophoridae), with description of its tadpole. *Zootaxa* 4048: 101–113. https://doi.org/10.11646/zootaxa.4048.1.6.

Amphibia Web. 2023. *Micrixalus herrei* Myers, 1942. University of California, Berkeley, California, USA. <https://amphibiaweb.org/species/8171>.

Biju, S.D. 2001. A synopsis to the frog fauna of the Western Ghats, India. *Indian Society for Conservation Biology-Occasional Publication* 1: 1–24.

Biju, S.D., S. Garg, K.V. Gururaja, Y.S. Shouche, and S.A. Walujkar. 2014. DNA barcoding reveals unprecedented diversity in dancing frogs of India (Micrixalidae, *Micrixalus*): a taxonomic revision with description of 14 new species. *Ceylon Journal of Science (Biological Sci*ences) 43: 1–87. https://dx.doi.org/10.4038/cjsbs.v43i1.6850.

Biswas, A. and K.P. Karanth. 2021. Role of geographical gaps in the Western Ghats in shaping intra- and interspecific genetic diversity. *Journal of Indian Institute of Science* 101: 151–164. https://doi.org/10.1007/s41745-021-00241-5.

Chandramouli, S.R. and S.K. Dutta. 2015. Comparative osteology of anuran genera in the Western Ghats, Peninsular India. *Alytes* 32: 67–81.

Dahanukar, N. and S. Molur. 2020. *JoTT Checklist of Amphibians of the Western Ghats* (v1.0). <https://threatenedtaxa.org/index.php/JoTT/checklists/amphibians/westernghats>.

Dinesh, K.P., C. Radhakrishnan, P. Deepak, and N.U. Kulkarni. 2023. *A Checklist of Indian Amphibians with Common Names for the Country and their IUCN Conservation Status*. Version 5.0. <http://zsi.gov.in>.

Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 39: 783–791. https://doi.org/10.2307/2408678.

Frost, D.R. 2023. *Amphibian Species of the World: An Online Reference*. Version 6.2. American Museum of Natural History, New York, New York, USA. https://doi.org/10.5531/db.vz.0001. <http://research.amnh.org/herpetology/amphibia/index.html>.

Gopalan, S.V., S. George, and D. Evans. 2020. A new record of the Myristica Swamp Treefrog, *Mercurana myristicapalustris* (Anura: Rhacophoridae), from the Vazhachal Reserve Forest, Kerala, India. *Reptiles & Amphibians* 27: 446–449. https://doi.org/10.17161/randa.v27i3.14874.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Kattany, V. and B. Jose. 2013. Ecological impact assessment of Sabarimala Forest in the Western Ghat Kerala with special reference to pilgrimage activities. *Journal of Ecotoxicology & Environmental Monitoring* 23: 259–268.

Luedtke, J.A., J. Chanson, K. Neam, L. Hobin, A.O. Maciel, A. Catenazzi, A. Borzée, A. Hamidy, A. Aowphol, A. Jean, Á. Sosa-Bartuano, A. Fong G., A. de Silva, A. Fouquet, A. Angulo, A.A. Kidov, A. Muñoz Saravia, A.C. Diesmos, A. Tominaga, B. Shrestha, B. Gratwicke, B. Tjaturadi, C.C. Martínez Rivera, C.R. Vásquez Almazán, C. Señaris, S.R. Chandramouli, C. Strüssmann, C.F. Cortez Fernández, C. Azat, C.J. Hoskin, C. Hilton-Taylor, D.L. Whyte, D.J. Gower, D.H. Olson, D.F. Cisneros-Heredia, D.J. Santana, E. Nagombi, E. Najafi-Majd, E.S.H. Quah, F. Bolaños, F. Xie, F. Brusquetti, F.S. Álvarez, F. Andreone, F. Glaw, F.E. Castañeda, F. Kraus, G. Parra-Olea, G. Chaves, G.F. Medina-Rangel, G. González-Durán, H.M. Ortega-Andrade, I.F. Machado, I. Das, I. Ribeiro Dias, J.N. Urbina-Cardona, J. Crnobrnja-Isailović J.-H. Yang, J. Jianping, J. Tshelthrim Wangyal, J.J.L. Rowley, J. Measey, K. Vasudevan, K.O. Chan, K.V. Gururaja, K. Ovaska, L.C. Warr, L. Canseco-

Márquez, L. Felipe Toledo, L.M. Díaz, M.M.H. Khan, M. Meegaskumbura, M.E. Acevedo, M. Felgueiras Napoli, M.A. Ponce, M.Vaira, M. Lampo, M.H. Yánez-Muñoz, M.D. Scherz, M.-O. Rödel, M. Matsui, M. Fildor, M.D. Kusrini, M.F. Ahmed, M. Rais, N'G.G. Kouamé, N. García, N.L. Gonwouo, P.A. Burrowes, P.Y. Imbun, P. Wagner, P.J.R. Kok, R.L. Joglar, R.J. Auguste, R. Albuquerque Brandão, R. Ibáñez, R. von May, S.B. Hedges, S.D. Biju, S.R. Ganesh, S. Wren, S. Das, S.V. Flechas, S.L. Ashpole, S.J. Robleto-Hernández, S.P. Loader, S.J. Incháustegui, S. Garg, S. Phimmachak, S.J. Richards, T. Slimani, T. Osborne-Naikatini, T.P.F. Abreu-Jardim, T.H. Condez, T.R. De Carvalho, T.P. Cutajar, T.W. Pierson, T.Q. Nguyen, U. Kaya, Z. Yuan, B. Long, P. Langhammer, and S.N. Stuart. 2023. Ongoing declines for the world's amphibians in the face of emerging threats. *Nature* 622: 308–314. https://doi.org/10.1038/s41586-023-06578-4.

Myers, G.S. 1942. A new frog of the genus *Micrixalus* from Travancore. *Proceedings of the Biological Society of Washington* 55: 71–74.

Myers, N., R.A. Mittermeier, C.G. Mittermeier, G.A. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. *Nature* 403: 853–858. https://doi.org/ 10.1038/35002501.

Nair, A., S.V. Gopalan, S. George, K.S. Kumar, T. Shikano, and J. Merilä. 2012. Genetic variation and differentiation in *Indirana beddomii* frogs endemic to the Western Ghats biodiversity hotspot. *Conservation Genetics* 13: 1459–1467. https://doi.org/10.1007/s10592-012-0389-z.

Re:wild, Synchronicity Earth, IUCN SSC Amphibian Specialist Group. 2023. *State of the World's Amphibians: The Second Global Amphibian Assessment*. Re:wild, Austin, Texas, USA.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731–2739. https://doi.org/10.1093/molbev/msr121.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680. https://doi.org/10.1093/2Fnar/2F22.22.4673.