



On the Phylogeny of the Suffry Red-webbed Treefrog, *Zhangixalus suffry* (Bordoloi, Bortamuli, and Ohler 2007), with Notes on Distribution and Comparisons with the Giant Treefrog, *Zhangixalus smaragdinus* (Blyth 1852), and Other Closely Related Species in Mizoram

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The family Rhacophoridae (Amphibia: Anura) comprises 442 species in 20 genera, 13 of which are represented in India. Ten of these genera occur in Northeast India: *Chirixalus*, *Ghatixalus*, *Kurixalus*, *Nasutixalus*, *Philautus*, *Polypedates*, *Pseudophilautus*, *Raorchestes*, *Zhangixalus*, and *Theloderma*. The genus *Zhangixalus* is represented in India by three species: The Giant Treefrog (*Zhangixalus smaragdinus*),

Patsarlamdam Flying Frog (*Z. burmanus*), and Suffry Red-webbed Treefrog (*Z. suffry*) (Dinesh et al. 2020; Frost 2021). *Zhangixalus suffry* (Fig. 1) was first described by Bordoloi et al. (2007) from an area near the Suffry Tea Estate, Sivsagar, Assam; its known distribution is in Northeast India (Assam, Arunachal Pradesh, Meghalaya, Mizoram, and Nagaland) and Bhutan (Trongsa District) (Bordoloi et al. 2007, 2008;



Fig 1. A Suffry Red-webbed Treefrog (*Zhangixalus suffry*) from Durtlang, Aizawl, Mizoram, India. Photographs by H.T. Lalremsanga.

Lalremsanga 2017; Mukhim et al. 2017; Wangyal et al. 2020; Frost 2021). However, Wildenhues et al. (2010) suggested that “the recently described *R[hacophorus]. suffry* [= *Zhangixalus suffry*] from India ... seems very similar to the red-webbed developmental stage of *R[hacophorus]. maximus* [= *Zhangixalus smaragdinus*] from Vietnam.

We conducted field surveys for *Zhangixalus suffry* throughout Mizoram State using visual and acoustic encounter surveys and collected individuals from 11 localities (Fig. 2). We identified specimens using Bordoloi et al. (2007) and those identities were verified by Annemarie Ohler. Liver tissues were extracted for genetic analysis and specimens were preserved in 10% buffered formalin, transferred to 70% ethanol, and deposited in the Department Museum of Zoology, Mizoram University (MZMU), as MZMU 2027 from Lunglei (22°52'05.56"N, 92°46'28.29"E; 1,180 m asl); MZMU 2047 from Chhingchhip (23°28'45.31"N, 92°51'38.97"E; 1,094 m asl); MZMU 1861 from Chawlhmun Veng, Aizawl (23°44'42.77"N, 92°41'38.14"E; 980 m asl); MZMU 1921 from Durtlang, Aizawl (23°46'27.01"N, 92°43'51.38"E; 1,295 m asl); MZMU 1862 from Khawzawl (23°32'25.71"N, 93°11'07.76"E; 1,105 m asl); MZMU 1653 from MZU Campus, Aizawl (23°44'13.19"N, 92°39'50.49"E; 810

m asl); MZMU 1976 from Hmuifang (23°27'14.48"N, 92°45'13.57"E; 1,464 m asl); MZMU 1866 from Reiek (23°41'30.35"N, 92°36'20.29"E; 1,267 m asl); MZMU 2370a–b from Champhai (23°26'55.64"N, 93°17'53.36"E; 1,324 m asl); MZMU 2399 from Ngentiang; MZMU 2370 from Hlimen (24°14'09.43"N, 92°48'21.72"E; 652 m asl); and MZMU 2370c from Sihphir (23°49'22.82"N, 92°44'09.41"E; 1,242 m asl). We amplified the 16s rRNA gene using primers L02510 (Palumbi 1996) and H03063 (Rassmann 1997) and compared it to 15 sequences from related species obtained from the NCBI database. A Common Asian Toad (*Duttaphrynus melanostictus*) (MW165455) was used as an outgroup. We aligned the sequences using the MUSCLE algorithm in MEGA 7 (Kumar et al. 2016) and constructed a phylogenetic tree by MrBayes (v. 2.7) using the GTR+I+G model (Huelsenbeck and Ronquist 2001) (Fig. 3).

Our ML phylogenetic reconstruction clearly places *Z. suffry* (MZ702488–MZMU1653; MW658921–MZMU1861; MT808304–MZMU1390) as a sister species to *Z. smaragdinus* (MW658922–MZMU1828) by a well-supported node (bootstrap = 100), and they formed a distinct clade (*Z. smaragdinus* + *Z. suffry*) with respect to congeners. The sequence of the Double-spotted Orange-webbed Treefrog

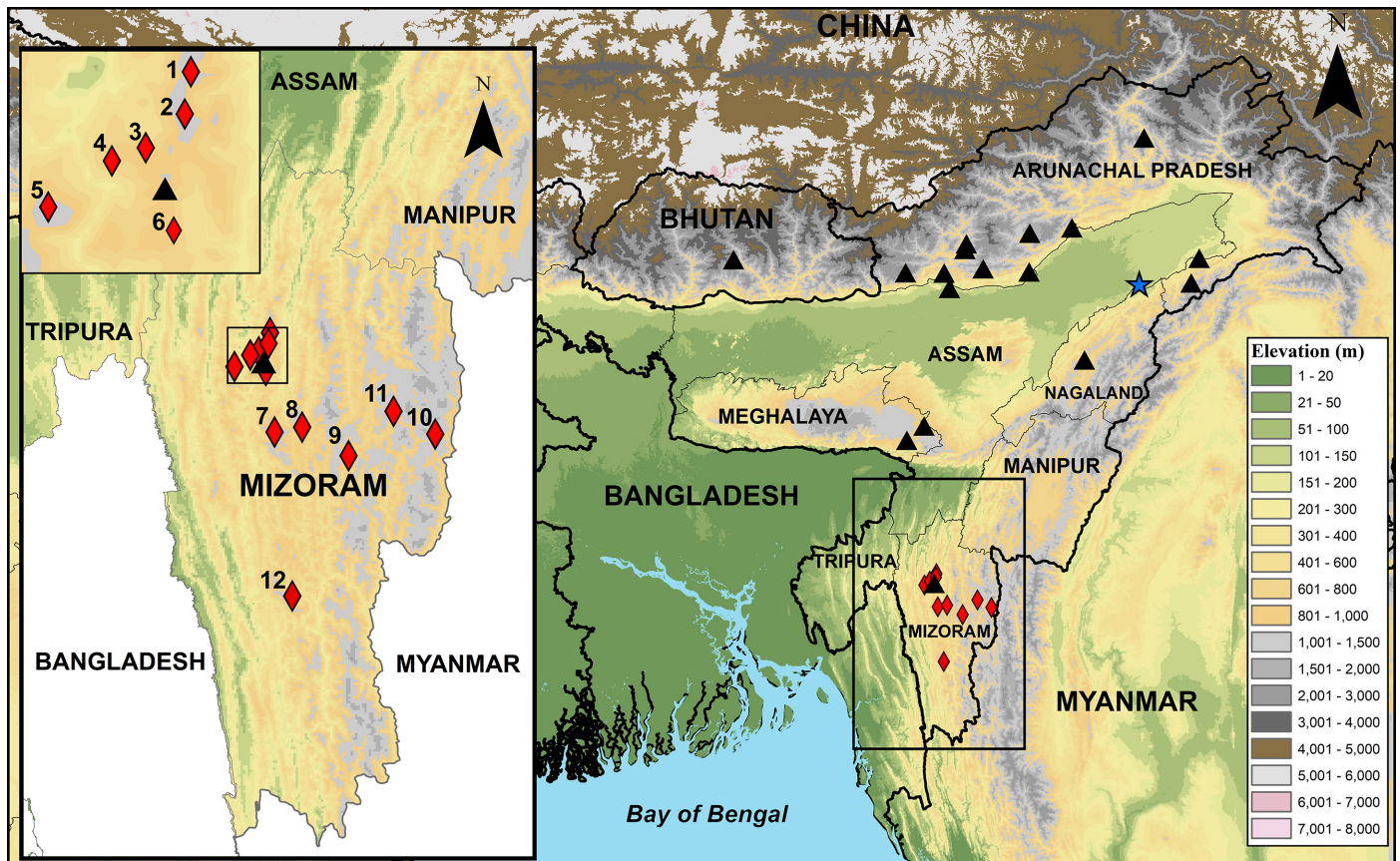


Fig 2. Map of Mizoram, India, showing sampling sites for Suffry Red-webbed Treefrogs (*Zhangixalus suffry*). The type locality is represented by a blue star, previously reported localities are marked in by black triangles, and the 12 new localities by red diamonds (1 = Sihphir; 2 = Durtlang; 3 = Chawlhmun; 4 = MZU; 5 = Reiek; 6 = Hlimen; 7 = Hmuifang; 8 = Chhingchhip; 9 = Ngentiang; 10 = Khawzawl; 11 = Champhai; 12 = Lunglei).

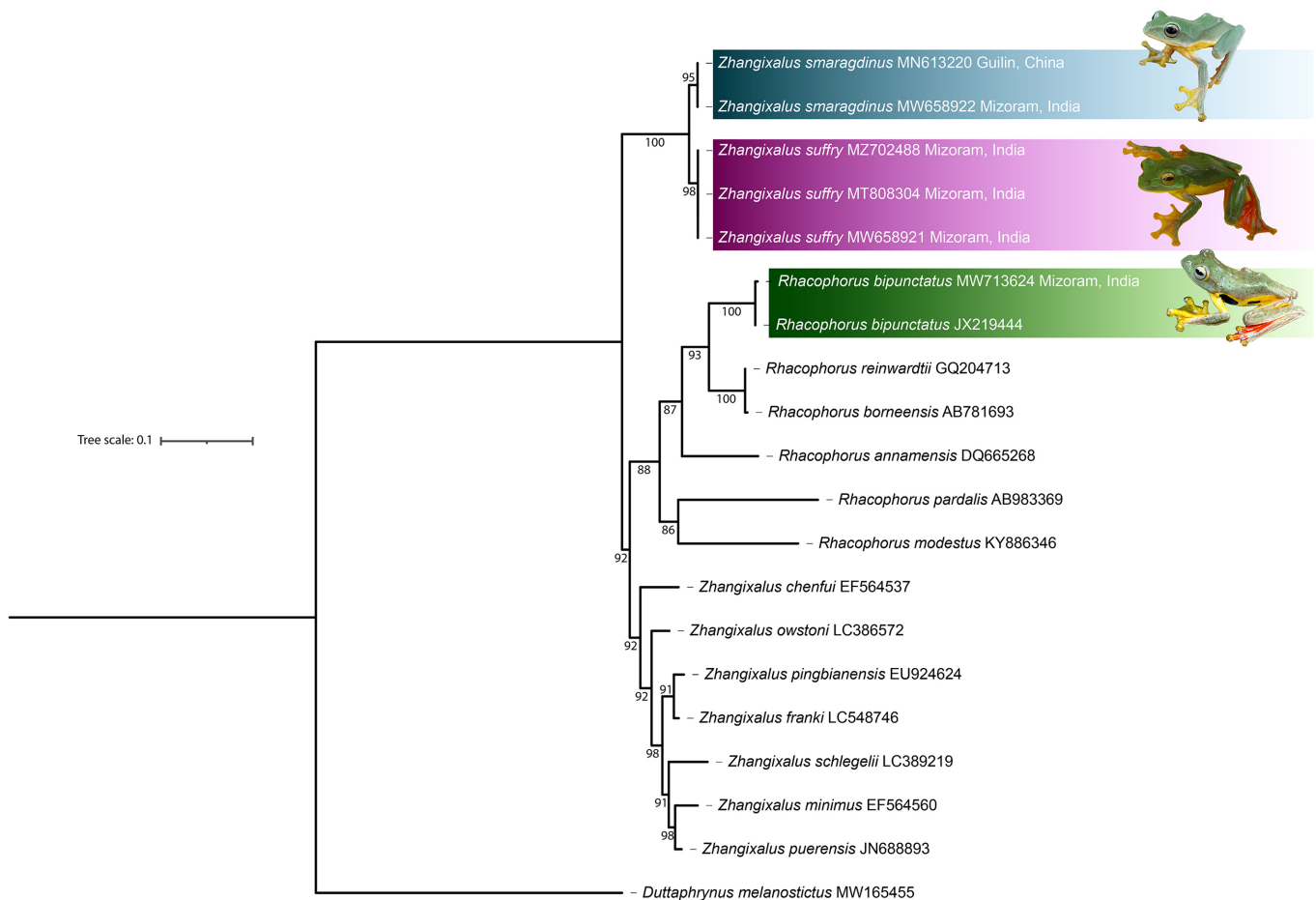


Fig. 3. Maximum-likelihood phylogram (16s rRNA) of the Suffry Red-webbed Treefrog (*Zhangixalus suffry*) and related species. Numbers at nodes represent bootstrap support. The Common Asian Toad (*Duttaphrynus melanostictus*) (MW165455) was used as the outgroup.

(*Rhacophorus bipunctatus*) also grouped with a conspecific (bootstrap = 100) from Chin State, Myanmar (JX219444), and the species is sister to the group consisting of Reinwardt's Treefrog (*Rhacophorus reinwardtii*) and the Borneo Treefrog (*Rhacophorus borneensis*) (bootstrap = 93). Average interspecific K2p genetic distance was 10% (Table 1). The lowest was between *R. borneensis* and *R. reinwardtii* (0.2%) and the highest between *Z. smaragdinus* and *Rhacophorus pardalis* (17%). Low divergence was seen between *Z. suffry* and *Z. smaragdinus* (1.6%) and high divergence between *Z. suffry* and *R. bipunctatus* (12.7%). Also, minimal within-species divergence (0.0%) was evident in the *R. bipunctatus* (MW713624–MZMU1814) sequences from this study (MW713624) and that from Myanmar (JX219444).

Suffry Red-webbed Treefrogs (SVL = 35.58–65.33 mm) from Mizoram were much smaller than the sympatric congener, *Z. smaragdinus* (SVL = 47.88–76.90 mm). Females of *Z. suffry* were usually smaller than males (SVL = 35.58–49.00 mm; SVL = 41.00–65.33 mm), whereas females of *Z. smaragdinus* were usually larger than males (SVL = 66.48–76.90 mm; SVL = 47.88–70.14 mm). The ratios of tibia length/snout vent length (TL/SVL) and inner metatarsal

tubercle length/snout-vent length (IMT/SVL) in *Z. smaragdinus* and *Z. suffry* were 0.49–0.53 versus 0.46–0.51 and 0.04–0.06 versus 0.03–0.05, respectively; the ratio of head length/snout-vent length (HL/SVL) in *Z. suffry* (0.34–0.40 mm) overlapped with that of *Z. smaragdinus* (0.31–0.43 mm); the ratio of femur length/snout-vent length (FL/SVL) of *Z. suffry* was generally less than that of *Z. smaragdinus* (0.44–0.52 vs. 0.49–0.53); the ratio of head length/head width (HL/HW) of *Z. suffry* (0.88–1.05) was less than that of *Z. smaragdinus* (0.99–1.20); and the ratio of head width/snout-vent length (HW/SVL) was consistently greater in *Z. suffry* (0.37–0.39) than in *Z. smaragdinus* (0.31–0.36). Apart from these dissimilarities, *Z. smaragdinus* is bluish dorsally and brown laterally with a clearly defined border, the venter is brown, the thighs dark brown, and webbing between fingers and toes is uniformly grayish. Size of these species, a relatively broader head, complete webbing between fingers, and color pattern differences readily distinguish the two species. Paraphyly among *Zhangixalus* species in the study's dataset reinforces the idea that the monophyletic clade consisting of *Z. suffry* + *Z. smaragdinus* is an independently evolving clade comprising the deeply divergent lineage of *Z. suffry*. Recognition of

Table 1. Uncorrected K2p distances of the Suffy Red-webbed Treefrog (*Zhangixalus suffryi*) and related species based on partial 16S rRNA gene sequences. Sequences from frogs in this study are indicated by bold type.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>Zhangixalus suffryi</i> MW658921		0.000	0.000	0.006	0.006	0.013	0.015	0.016	0.014	0.013	0.015	0.015	0.018	0.018	0.018	0.018	0.020	0.024	0.020	0.029
<i>Zhangixalus suffryi</i> MT808304	0.000		0.000	0.006	0.006	0.013	0.015	0.016	0.014	0.013	0.015	0.015	0.018	0.018	0.018	0.018	0.020	0.024	0.020	0.029
<i>Zhangixalus suffryi</i> MZ702488	0.000	0.000	0.000	0.006	0.006	0.013	0.015	0.016	0.014	0.013	0.015	0.015	0.018	0.018	0.018	0.018	0.020	0.024	0.020	0.029
<i>Zhangixalus smaragdinus</i> MW658922	0.016	0.016	0.016		0.000	0.014	0.015	0.017	0.014	0.013	0.014	0.015	0.018	0.018	0.018	0.018	0.020	0.023	0.021	0.029
<i>Zhangixalus smaragdinus</i> MIN613220	0.016	0.016	0.016	0.000		0.014	0.015	0.017	0.014	0.013	0.014	0.015	0.018	0.018	0.018	0.018	0.020	0.023	0.021	0.029
<i>Zhangixalus franki</i> LC548746	0.085	0.085	0.085	0.088	0.088		0.008	0.010	0.006	0.010	0.008	0.010	0.016	0.016	0.015	0.015	0.016	0.017	0.016	0.028
<i>Zhangixalus oustoni</i> LC386572	0.090	0.090	0.090	0.087	0.087	0.035		0.013	0.010	0.012	0.008	0.010	0.018	0.018	0.016	0.016	0.016	0.018	0.017	0.030
<i>Zhangixalus chenfu</i> EF564537	0.090	0.090	0.090	0.090	0.090	0.052	0.054		0.012	0.013	0.011	0.012	0.017	0.017	0.018	0.018	0.016	0.016	0.018	0.030
<i>Zhangixalus pingbianensis</i> EU924624	0.090	0.090	0.090	0.088	0.088	0.016	0.045	0.065		0.010	0.009	0.010	0.016	0.016	0.015	0.015	0.015	0.018	0.015	0.028
<i>Zhangixalus schlegelii</i> LC389219	0.093	0.093	0.093	0.090	0.090	0.050	0.057	0.072	0.052		0.010	0.011	0.018	0.018	0.017	0.017	0.016	0.018	0.019	0.027
<i>Zhangixalus puerensis</i> JN688893	0.096	0.096	0.096	0.088	0.088	0.033	0.035	0.057	0.035	0.042		0.007	0.016	0.016	0.015	0.015	0.016	0.017	0.017	0.028
<i>Zhangixalus minimus</i> EF564560	0.104	0.104	0.104	0.099	0.099	0.040	0.047	0.075	0.042	0.054	0.025		0.018	0.018	0.016	0.016	0.016	0.019	0.019	0.027
<i>Rhacophorus borneensis</i> AB781693	0.121	0.121	0.121	0.118	0.118	0.095	0.092	0.104	0.102	0.108	0.105	0.116		0.002	0.015	0.015	0.016	0.020	0.019	0.030
<i>Rhacophorus reinwardtii</i> GQ204713	0.121	0.121	0.121	0.118	0.118	0.095	0.092	0.104	0.102	0.108	0.105	0.116	0.002		0.015	0.015	0.016	0.019	0.019	0.030
<i>Rhacophorus bipunctatus</i> JX219444	0.127	0.127	0.127	0.124	0.124	0.090	0.087	0.107	0.100	0.113	0.105	0.111	0.070	0.067		0.002	0.016	0.019	0.019	0.029
<i>Rhacophorus bipunctatus</i> MW713624	0.127	0.127	0.127	0.124	0.124	0.090	0.087	0.107	0.100	0.113	0.105	0.111	0.072	0.070	0.002		0.017	0.019	0.019	0.029
<i>Rhacophorus annamensis</i> DQ665268	0.135	0.135	0.135	0.138	0.138	0.111	0.105	0.107	0.108	0.105	0.119	0.119	0.102	0.102	0.108	0.111		0.018	0.018	0.030
<i>Rhacophorus modestus</i> KY886346	0.151	0.151	0.151	0.157	0.157	0.126	0.122	0.122	0.126	0.131	0.123	0.137	0.127	0.124	0.125	0.125	0.127		0.019	0.029
<i>Rhacophorus pardalis</i> AB983369	0.167	0.167	0.167	0.170	0.170	0.136	0.126	0.135	0.138	0.158	0.144	0.147	0.132	0.132	0.149	0.152	0.127	0.145		0.029
<i>Duttapbrynus melanostictus</i> MW165455	0.250	0.250	0.250	0.247	0.247	0.237	0.233	0.262	0.233	0.234	0.234	0.224	0.259	0.256	0.240	0.240	0.265	0.247	0.277	

the proposed lineage is important because species groups with considerable genetic diversity may reflect multiple evolutionary lineages with low interspecific genetic diversity (Neves et al. 2020). Moreover, phylogenetic studies in other vertebrate taxa also emphasized the existence of independently evolving cryptic lineages within some paraphyletic taxonomically recognized species (see Durand et al. 2012a, 2012b; Durand and Borsa 2015; Xia et al. 2016). However, further assessment based on more ecological data, reproductive behavior, morphology, as well as multiple mitochondrial and nuclear loci will be crucial for delineating the precise taxonomic status of *Z. suffry*. Despite questions raised by Wildenhues et al. (2010), the data presented herein clearly indicate that *Z. suffry* and *Z. smaragdinus* are distinct species.

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Literature Cited

- Bordoloi, S., T. Bortamuli, and A. Ohler. 2007. Systematics of the genus *Rhacophorus* (Amphibia, Anura): Identity of red-webbed forms and description of a new species from Assam. *Zootaxa* 1653: 1–20. <http://dx.doi.org/10.5281/zenodo.179813>.
- Bordoloi, S., S. Sengupta, A. Ohler, and I. Agarwal. 2008. *Rhacophorus suffry*. *The IUCN Red List of Threatened Species 2008*: e.T136092A4231921. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T136092A4231921.en>.
- Dinesh, K.P., C. Radhakrishnan, B.H. Channakeshavamurthy, P. Deepak, and N.U. Kulkarni. 2020. *A Checklist of Amphibians of India with IUCN Conservation Status*. Version 3.0. https://www.amphibians.org/wp-content/uploads/2020/05/2020_Indian_Amphibian_checklist.pdf.
- Durand, J.D. and P. Borsa. 2015. Mitochondrial phylogeny of grey mullets (Acanthopterygii: Mugilidae) suggests high proportion of cryptic species. *Comptes Rendus Biologies* 338: 266–277. <http://dx.doi.org/10.1016/j.crvi.2015.01.007>.
- Durand, J.D., W.J. Chen, K.N. Shen, C. Fu, and P. Borsa. 2012a. Genus-level taxonomic changes implied by the mitochondrial phylogeny of grey mullets (Teleostei: Mugilidae). *Comptes Rendus Biologies* 335: 687–697. <http://dx.doi.org/10.1016/j.crvi.2012.09.005>.
- Durand, J.D., K.N. Shen, W.J. Chen, B.W. Jamandre, H. Blel, K. Diop, and P. Borsa. 2012b. Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): Molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. *Molecular Phylogenetics and Evolution* 64: 73–92. <http://dx.doi.org/10.1016/j.ympev.2012.03.006>.
- Frost, D.R. 2021. *Amphibian Species of the World: An Online Reference*. Version 6.1. American Museum of Natural History, New York, New York, USA. <<https://amphibiansoftheworld.amnh.org/index.php>>. <https://doi.org/10.5531/db.vz.0001>.
- Huelsenbeck, J.P. and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755. <http://dx.doi.org/10.1093/bioinformatics/17.8.754>.
- Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870–1874. <https://doi.org/10.1093/molbev/msw054>.
- Lalremangsa, H.T. 2017. Geographic distribution. *Rhacophorus suffry* (Suffry Red-webbed Tree Frog). *Herpetological Review* 48: 120–121.
- Mukhim, D.K.B., B. Saikia, I. Imam, I.J. Kharkongor, D. Sarma, and T. Arbenz. 2017. *Rhacophorus suffry* Bordoloi, Bortamuli and Ohler, 2007. A New Record from Meghalaya, North-East India. *Journal of the Bombay Natural History Society* 114: 2017. <http://dx.doi.org/10.17087/jbnhs/2017/v114/112222>.
- Neves, J.M., J.P. Almeida, M.J. Sturaro, N.N. FabrÉ, R.J. Pereira, and T. Mott. 2020. Deep genetic divergence and paraphyly in cryptic species of *Mugil* fishes (Actinopterygii: Mugilidae). *Systematics and Biodiversity* 18: 116–128. <https://doi.org/10.1080/14772000.2020.1729892>.
- Palumbi, S.R. 1996. Nucleic acids II: The polymerase chain reaction, pp. 205–247. In: D.M. Hillis, C. Moritz, and B.K. Mable (eds.), *Molecular Systematics*. 2nd edition. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.
- Rassmann, K. 1997. Evolutionary age of the Galápagos iguanas predates the age of the present Galápagos Islands. *Molecular Phylogenetics and Evolution* 7: 158–172. <https://doi.org/10.1006/MPEV.1996.0386>.
- Wangyal, J.T., D.S. Bower, S. Sherub, S. Tshewang, D. Wangdi, K. Rinchen, S. Puntsho, C. Tashi, B.K. Koirala, G.S. Bhandari, Y. Phuntsho, T.P. Koirala, B.B. Ghallay, L. Chaida, J. Tenzin, R.B. Powrel, R. Tshewang, O.N. Raika, S. Jamtsho, Kinley, G. Gyeltshen, S. Tashi, D. Nidup, N. Wangdi, Phuentsho, L. Norbu, K. Wangdi, T. Wangchuk, P. Tobgay, T. Dorji, and I. Das. 2020. New herpetofaunal records from the Kingdom of Bhutan obtained through citizen science. *Herpetological Review* 51: 790–798.
- Wildenhues, M.J., A. Gawor, T.Q. Nguyen, T.T. Nguyen, A. Schmitz, and T. Ziegler. 2010. First description of larval and juvenile stages of *Rhacophorus maximus* Günther, 1859 “1858” (Anura: Rhacophoridae) from Vietnam. *Revue Suisse de Zoologie* 117: 679–696. <http://dx.doi.org/10.5962/bhl.part.118618>.
- Xia, R., J.D. Durand, and C. Fu. 2016. Multilocus resolution of Mugilidae phylogeny (Teleostei: Mugiliformes): Implications for the family’s taxonomy. *Molecular Phylogenetics and Evolution* 96: 161–177. <http://dx.doi.org/10.1016/j.ympev.2015.12.010>.