



<http://dx.doi.org/10.11646/zootaxa.3911.2.6>

<http://zoobank.org/urn:lsid:zoobank.org:pub:2A96080C-856E-417B-AEC2-CA1157222424>

## The Sri Lankan torrent toads (Bufonidae: Adenominae: *Adenomus*): species boundaries assessed using multiple criteria

MADHAVA MEEGASKUMBURA<sup>1,4</sup>, GAYANI SENEVIRATHNE<sup>1</sup>, NAYANA WIJAYATHILAKA<sup>1</sup>,  
BENEETA JAYAWARDENA<sup>1</sup>, CHAMPIKA BANDARA<sup>1</sup>, KELUM MANAMENDRA-ARACHCHI<sup>2</sup>  
& ROHAN PETHIYAGODA<sup>3</sup>

<sup>1</sup>Evolutionary Ecology and Systematics Lab, Faculty of Science and Postgraduate Institute of Science, University of Peradeniya, Sri Lanka

<sup>2</sup>Postgraduate Institute of Archaeology, 407 Bauddhaloka Mawatha, Colombo 7, Sri Lanka

<sup>3</sup>Ichthyology Section, Australian Museum, 6 College Street, Sydney, NSW 2000, Australia

<sup>4</sup>Corresponding author. E-mail: madhava88m@gmail.com

### Abstract

The bufonid genus *Adenomus*, an endemic of the montane and lowland rainforests of central and south-western Sri Lanka, has been considered to comprise of three species, viz. *A. kelaartii*, *A. dasi* and *A. kandianus*, the last of which has been recently highlighted as “the world’s rarest toad”. We conducted a survey across the known range of *Adenomus* and used multiple criteria to delineate species boundaries within the genus. These include: a molecular phylogeny based on a 16S ribosomal RNA gene fragment; an examination of the external morphology of adults and larvae, and the skeletal morphology of adults; a bioacoustic analysis; and ecological niche modelling. We show that *Adenomus* is monophyletic and that it comprises only two species: *A. kelaartii* and *A. kandianus*, with *A. dasi* being a junior synonym of the latter. For the two valid species of *Adenomus*, we provide detailed osteological descriptions; clarify the distribution patterns; and provide genetic data to facilitate their scientific conservation management.

**Key words:** *Adenomus dasi* new synonym, barcoding, bioacoustics, integrative taxonomy, niche modeling, osteology, sucker disc, tadpole

### Introduction

Cope (1861) established the genus *Adenomus* to contain the Sri Lankan toad *A. badioflavus* Cope, 1861, a junior synonym of *Bufo kelaartii* Günther, 1858. The following year, however, Cope (1862) synonymised this genus with *Bufo* Garsault, 1764, in which status it remained until recognized as valid by Manamendra-Arachchi and Pethiyagoda (1998). Frost (2014), however, observed that the validity of *Adenomus* “without discussion of diagnostic features or phylogenetic placement” was “a move that will likely prove controversial”. In addition to describing a further new species, *A. dasi*, from Sri Lanka, Manamendra-Arachchi and Pethiyagoda (1998) included in this genus *Bufo kandianus* Günther, 1872, a species that had not been recorded since its original description. In 2005 *A. kandianus* became one of the 34 amphibians globally declared extinct by the IUCN (Stuart *et al.* 2008), though ‘rediscovered’ shortly thereafter by Wickramasinghe *et al.* (2012) who, despite meagre population data labelled it “the world’s rarest toad”. Alongside the announcement of this rediscovery, Wickramasinghe *et al.* (2012) also speculated that the population they recognized as *A. kandianus* could in fact represent a new species, with the identity of *A. kandianus* remaining in doubt due to lack of genetic data and a lack of clarity as to its precise type locality.

Van Bocxlaer *et al.* (2009) recognized the subfamily Adenominae, which includes the genera *Adenomus*, *Duttaphrynus*, *Ghatophryne*, *Pedostibes* and *Xanthophryne* (Biju *et al.* 2009), the first of which is restricted to Sri Lanka, and the last three of which are endemic to the Western Ghats of India. Based on 2 nuclear and 9 mitochondrial gene fragments, their phylogeny recovered *Adenomus* as the sister group of *Duttaphrynus* + *Xanthophryne*, though Frost (2014) cautioned that the phylogenetic status of *Adenomus* “remains questionable”.

(6°47'N, 80°27'E); WHT 1758, juvenile, 15.8 mm SVL, Seraella (Pottotawela, Knuckles), alt. 450m (7°35'N, 80°45'E); WHT 1759, juvenile, 17.5 mm SVL, Puwakpitiya (Laggala, Knuckles), alt. 450 m (7°34'N, 80°45'E); WHT 1764, ♀ 46.8 mm SVL, Kanneliya (Galle), alt. 150 m (6°15'N, 80°20'E); WHT 1767, ♀ 41.7 mm SVL, Kosmulla (Nelluwa), alt. 320m (6°24'N, 80°23'E); WHT 1768, ♂ 29.3 mm SVL, Mawanana (Nelluwa), alt. 150m (6°22'N, 80°21'E); WHT 1771, ♀ 39.5 mm SVL, Pitadeniya (Watugala), alt. 320m (6°22'N, 80°28'E); WHT 1772, ♀ 28.2 mm SVL, Lanakagama (Dombagoda), alt. 460m (6°21'N, 80°28'E); WHT 1773, ♂ 33.1 mm SVL, Dolahena (Bulutota), alt. 16m (6°26'N, 80°36'E).

## Acknowledgements

We thank James Hanken, SD Biju, Sonali Garg and an anonymous reviewer for suggestions that helped improve this work substantially; Peter K.L. Ng, Kelvin Lim and Tan Heok Hui for facilities at the Raffles Museum of Biodiversity Research, National University of Singapore; Mark Bee (University of Minnesota) for training in Bioacoustics; K.H. Sonali Rangika Premarathne (Postgraduate Institute of Archaeology, University of Kelaniya), for assisting with data compilation; the National Research Council of Sri Lanka (Grant # 11-124) for funding the molecular analysis and providing graduate student support; the Department of Wildlife Conservation and the Forest Department of Sri Lanka for research permits; Nirodha Abayalath, Tharindu Gunatilleke, Waruna Agalawatta and Santhushya Hewapathiranga for fieldwork; Don Church (GWC), James Lewis and Robin Moore for support with Sri Lankan amphibian conservation research. NW thanks the Mohamed bin Zayed Species Conservation Fund (Grant #13056384) for additional research support.

## References

- Altig, R. & McDiarmid, R.W. (1999) Body plan: development and morphology. *In*: McDiarmid, R.W. & Altig, R. (Eds.), *Tadpoles: the biology of anuran larvae*. University of Chicago Press, Chicago, pp. 24–51.
- Biju, S.D., Van Bocxlaer, I., Giri, V.B., Loader, S.P. & Bossuyt, F. (2009) Two new endemic genera and a new species of toad (Anura: Bufonidae) from the Western Ghats of India. *BMC Research Notes*, 2, 241.  
<http://dx.doi.org/10.1186/1756-0500-2-241>
- Bowatte, G. & Meegaskumbura, M. (2011) Morphology and ecology of tadpoles of *Ramanella obscura* (Anura: Microhylidae). *Ceylon Journal of Science (Biological Science)*, 40 (2), 109–120.  
<http://dx.doi.org/10.4038/cjsbs.v40i2.3927>
- Cannatella, D. (1999) Architecture: cranial and axial musculoskeleton. *In*: McDiarmid, R.W. & Altig, R. (Eds.), *Tadpoles: the biology of anuran larvae*. University of Chicago Press, Chicago, pp. 52–91.
- Cope, E.D. (1861) "1860". Descriptions of reptiles from tropical America and Asia. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 12, 368–374.
- Cope, E.D. (1862) Catalogues of the reptiles obtained during the explorations of the Parana, Paraguay, Vermejo and Uruguay Rivers, by Capt. Thos. J. Page, U.S.N.; and of those procured by Lieut. N. Michler, U.S. Top. Eng., Commander of the expedition conducting the survey of the Atrato River. *Proceedings of the Academy of Natural Sciences of Philadelphia*, 14, 346–359.
- De Costa, W.A.J.M. (2008) Climate change in Sri Lanka: myth or reality? Evidence from long-term meteorological data. *Journal of the National Science Foundation of Sri Lanka*, 36, 63–88.
- Duellman, W.E. & Trueb, L. (1986) *Biology of amphibians*. McGraw-Hill, New York, 696 pp.
- Elith, J., Graham, C.H., Anderson, R.P., Dudík, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J., Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J. McC. M., Peterson, A.T., Phillips, S.J., Richardson, K., Scachetti-Pereira, R., Schapire, R.E., Soberón, J., Williams, S., Wisz, M.S. & Zimmermann, N.E. (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography*, 29, 129–151.
- Frost, D.R. (2014) *Amphibian species of the world: an online reference*. Version 6.0 American Museum of Natural History, New York, USA. Electronic database. Available from: <http://research.amnh.org/herpetology/amphibia/index.html> (accessed 10 July 2014).
- Gosner, K.L. (1960) A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 6, 183–190.
- Grillitsch, B., Grillitsch, H., Dubois, A. & Splechtna, H. (1993) The tadpoles of the brown frogs *Rana [graeca] graeca* and *Rana [graeca] italica* (Amphibia, Anura). *Alytes*, 11 (4), 117–139.
- Günther, A.C.L.G. (1858) Neue Batrachier in der Sammlung des britischen Museums. *Archiv für Naturgeschichte*, Berlin, 24,

- Günther, A.C.L.G. (1872) Descriptions of some Ceylonese reptiles and batrachians. *Annals and Magazine of Natural History*, Series 4, 9, 85–88.
- Haas, A. & Das, I. (2008) Larval identities of *Ansonia hanitschi* Inger, 1960 (Amphibia: Bufonidae) and *Polypedates colletti* (Boulenger, 1890) (Amphibia: Rhacophoridae) from East Malaysia (Borneo). *Salamandra*, 44, 2 85–100.
- Haas, W., Lehr, E. & Kohler, G. (1997) The tadpole of *Bufo kelaartii* Günther, 1859, from Sri Lanka. *Lyriocephalus*, 3, 2–6.
- Hernandez, P.A., Graham, C.H., Master, L.L. & Albert, D.L. (2006) The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, 29 (5), 773–775.  
doi: 10.1111/j.0906-7590.2006.04700.x.
- Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, 25, 1965–1978.  
<http://dx.doi.org/10.1002/joc.1276>
- Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17, 754–755.  
<http://dx.doi.org/10.1093/bioinformatics/17.8.754>
- Huelsenbeck, J.P., Ronquist, F., Neilsen, R. & Bollback, J.P. (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, 294, 2310–2314.  
<http://dx.doi.org/10.1126/science.1065889>
- Lobo, J.M., Jimenez-Valverde, A. & Real, R. (2008) AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography*, 17, 145–151.  
<http://dx.doi.org/10.1111/j.1466-8238.2007.00358.x>
- Manamendra-Arachchi, K. & Pethiyagoda, R. (1998) A synopsis of the Sri Lankan Bufonidae (Amphibia: Anura) with description of new species. *Journal of South Asian Natural History*, 3, 213–248.
- Manamendra-Arachchi, K. & Pethiyagoda, R. (2006) *Sri Lankawe ubhayajevee* [*The amphibian fauna of Sri Lanka*]. WHT Publications, Colombo, 440 pp., 88 pls. [in Sinhala]
- Orozco-Terwengel, P., Andreone, F., Louis, E. & Vences, M. (2013) Mitochondrial introgressive hybridization following a demographic expansion in the tomato frogs of Madagascar, genus *Dyscophus*. *Molecular Ecology*, 22 (24), 6074–6090.  
<http://dx.doi.org/10.1111/mec.12558>
- Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C. & Mable, B.K. (Eds.), *Molecular systematics*. Sinauer Associates, Sunderland, pp. 205–248.
- Phillips, S.J., Dudík, M. & Schapire, R.E. (2004) A maximum entropy approach to species distribution modeling. In: *Proceedings of the Twenty-First International Conference on Machine Learning*, pp. 655–662.  
<http://dx.doi.org/10.1145/1015330.1015412>
- Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, 190, 231–259.  
<http://dx.doi.org/10.1016/j.ecolmodel.2005.03.026>
- Rambaut, A. & Drummond, A.J. (2007) Tracer, version 1.6. Available from: <http://beast.bio.ed.ac.uk/Tracer> (accessed 9 December 2014)
- Stuart, S., Hoffmann, M., Chanson, J., Cox, N., Berridge, R., Ramani, P. & Young, B. (2008) *Threatened amphibians of the world*. Lynx Edicions, Barcelona, 758 pp.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731–2739.  
<http://dx.doi.org/10.1093/molbev/msr121>
- Van Bocxlaer, I., Biju, S.D., Loader, S.P. & Bossuyt, F. (2009) Toad radiation reveals into-India dispersal as a source of endemism in the Western Ghats-Sri Lanka biodiversity hotspot. *BMC Evolutionary Biology*, 9, 131  
<http://dx.doi.org/10.1186/1471-2148-9-131>
- Taylor, W.R. & Van Dyke, G.C. (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. *Cybium* 9, 107–119.
- Vences, M., Thomas, M., Van der Meijden, A., Chiari, Y. & Vieites, D.R. (2005) Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology*, 2, 5.  
<http://dx.doi.org/10.1186/1742-9994-2-5>
- Wickramasinghe, L.J.M., Vidanapathirana, D.R. & Wickramasinghe, N. (2012) Back from the dead: the world's rarest toad *Adenomus kandianus* rediscovered in Sri Lanka. *Zootaxa*, 3347, 63–68.
- Zweifel, R.G. (1968) Effects of temperature, body size, and hybridization on mating calls of toads, *Bufo a. americanus* and *Bufo woodhousei fowleri*. *Copeia*, 1968, 269–285.  
<http://dx.doi.org/10.2307/1441753>