Biology Faculty Publications

Biology

5-22-2013

Species boundaries and biogeography of East African torrent frogs of the genus Petropedetes (Amphibia: Anura: Petropeditidae)

Simon P. Loader

F. Sara Ceccarelli

Mark Wilkinson

Michele Menegon

Jean Mariaux

See next page for additional authors

Recommended Citation

Authors

Simon P. Loader, F. Sara Ceccarelli, Mark Wilkinson, Michele Menegon, Jean Mariaux, Rafael O. de Sá, and et al.
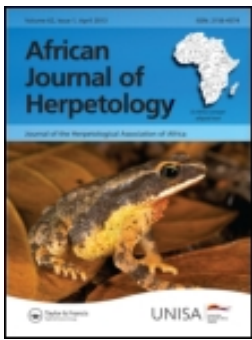
Species boundaries and biogeography of East African torrent frogs of the genus *Petropedetes* (Amphibia: Anura: Petropeditidae)

Simon P. Loader , F. Sara Ceccarelli , Mark Wilkinson , Michele Menegon , Jean Mariaux , Rafael O. de Sá , Kim M. Howell & David J. Gower

Published online: 22 May 2013.

Submit your article to this journal ⎘

Article views: 204

View related articles ⎘

Citing articles: 2 View citing articles ⎘

Species boundaries and biogeography of East African torrent frogs of the genus *Petropedetes* (Amphibia: Anura: Petropeditidae)

Simon P. Loader[1]*, F. Sara Ceccarelli[1],
Mark Wilkinson[2], Michele Menegon[3], Jean Mariaux[4],
Rafael O. de Sá[5], Kim M. Howell[6] & David J. Gower[2]

[1]*Department of Environmental Science (Biogeography), University of Basel, Switzerland;* [2]*Department of Zoology, The Natural History Museum, London, UK;* [3]*Tropical Biodiversity Section, Museo Tridentino di Scienze Naturali, Trento, Italy;* [4]*Department of Invertebrates, Muséum d'Histoire Naturelle, Geneva, Switzerland;* [5]*Department of Biology, University of Richmond, Richmond, VA, USA;* [6]*Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania*

Abstract.—Based on morphology there are currently three described East African species of torrent frogs belonging to the tropical African genus *Petropedetes* (*P. dutoiti*, *P. martiennseni* and *P. yakusini*). Taxonomic uncertainties are evident within this group: a putative new species reported from the Nguru Mountains and the species *P. yakusini* have a fragmented distribution across the Southern Tanzanian highlands. We conducted a molecular systematic study, sampling populations of East African petropedetids occurring in the mountains of Tanzania (*P. martiennseni* and *P. yakusini*), to investigate species boundaries and biogeography. Data provide evidence for the recognition of well-defined genetically distinct populations occurring in each mountain fragment (for *P. yakusini*), and further support for a new undescribed species from the Nguru Mountains. We examine phylogenetic patterns in relation to the biogeographic history of the region.

Key words.—*Arthroleptides*, Eastern Arc Mountains, Mahenge, Nguru, Kanga

Introduction

Torrent frogs of the genus *Petropedetes* Reichenow, 1874 occur in riverine habitats across montane regions of sub-Saharan Africa (Channing & Howell 2006; Barej *et al.* 2010). Of the 12 nominal species, three are found in East Africa and nine in Central and West Africa (Barej *et al.* 2010). Molecular investigations of petropedetids have been sparse thus far, with the main emphasis placed on Central African taxa (e.g. Barej *et al.* 2010) and the generic relationships (Scott 2005). No studies have been made on inter-relationships of East African species and populations. The East African species were formerly placed in the genus *Arthroleptides* Nieden, 1911 "1910" but Scott (2005) considered *Arthroleptides* to be a junior subjective synonym of *Petropedetes* based on a cladistic analysis of molecular and morphological data. In

---

this article we present analyses of mitochondrial DNA sequence data to assess species identity and inter-relationships among East African *Petropedetes sensu* Scott (2005). Furthermore, from a biogeographic perspective, the East African petrope-detids offer an opportunity to examine speciation within the Eastern highlands. Because torrent frog species are strongly associated with riverine and forest habitats, their speciation patterns might provide evidence for (testing) reconstructions of environmental history.

Nieden (1911 "1910") described the first East African torrent frog as *Arthroleptides martiensseni*, based on material presumed to have been collected from Amani in the East Usambara Mountains (Channing *et al.* 2002). Nieden (1911 "1910") noted that his specimens resembled members of the ranid genera *Arthroleptis* and *Petropedetes*, in having the foot morphology and absence of vomerine teeth of the former and the strongly broadened finger and toe tips of the latter. However, Nieden considered the specimens differentiated enough from both of these genera to place them in a separate genus. The second species of East African torrent frog, *A. dutoiti*, distinguished by its reduced webbing, was described by Loveridge (1935) from the volcanic mountain of Elgon, located on the Kenyan and Ugandan border. Since its original description this species has been recorded only once, in 1962, and several recent attempts to locate surviving populations have failed (IUCN 2012). The species is currently considered critically endangered on the IUCN Red List, and might even be extinct (IUCN 2012). Most recently, Channing *et al.* (2002) described a third East African torrent frog, *A. yakusini*, distinguished by its large body size, extensive webbing and reduced supratympanic ridge, from the southern montane region of Tanzania (Uluguru and Udzungwa Mountains), with its type locality in the Udzungwa Mountains National Park. Subsequently the range of this species was extended to the Mahenge Mountains of Tanzania (Loader *et al.* 2004). Menegon *et al.* (2008) reported a new record for the genus from East Africa in the Nguru Mountains of Tanzania but were unable to assign this population to any of the nominal species.

MATERIALS AND METHODS

Taxon sampling

New specimens of East African *Petropedetes sensu* Scott (2005) were obtained by fieldwork in Tanzania between 2000 and 2008 by SPL, MM, RdS, JM and KMH (see Online Supplementary Table 1). Tissue samples (thigh muscle and/or liver) were preserved in 96% ethanol, with voucher specimens fixed in either 10% formalin and stored in 70% ethanol or both fixed and stored in 70% ethanol. We did not sample *P. dutoiti*, which is known only from the type series. Samples were collected from all Eastern Arc Mountain (EAM) localities where *Petropedetes* have previously been reported – East Usambara, Nguru, Uluguru, Udzungwa and Mahenge (Fig. 1 includes type localities). The Magrotto Mountains locality (IUCN 2012) falls biogeographically within (although somewhat isolated from the rest of) the East Usambara Mountains and was not sampled in this study. Attempts were also made to collect *Petropedetes* in places in the EAM where they might potentially occur but have not been reported previously (Malundwe, Rubeho, Nguu, Ukaguru, West Usambara, North and South Pare). For these sites we were not successful in locating any
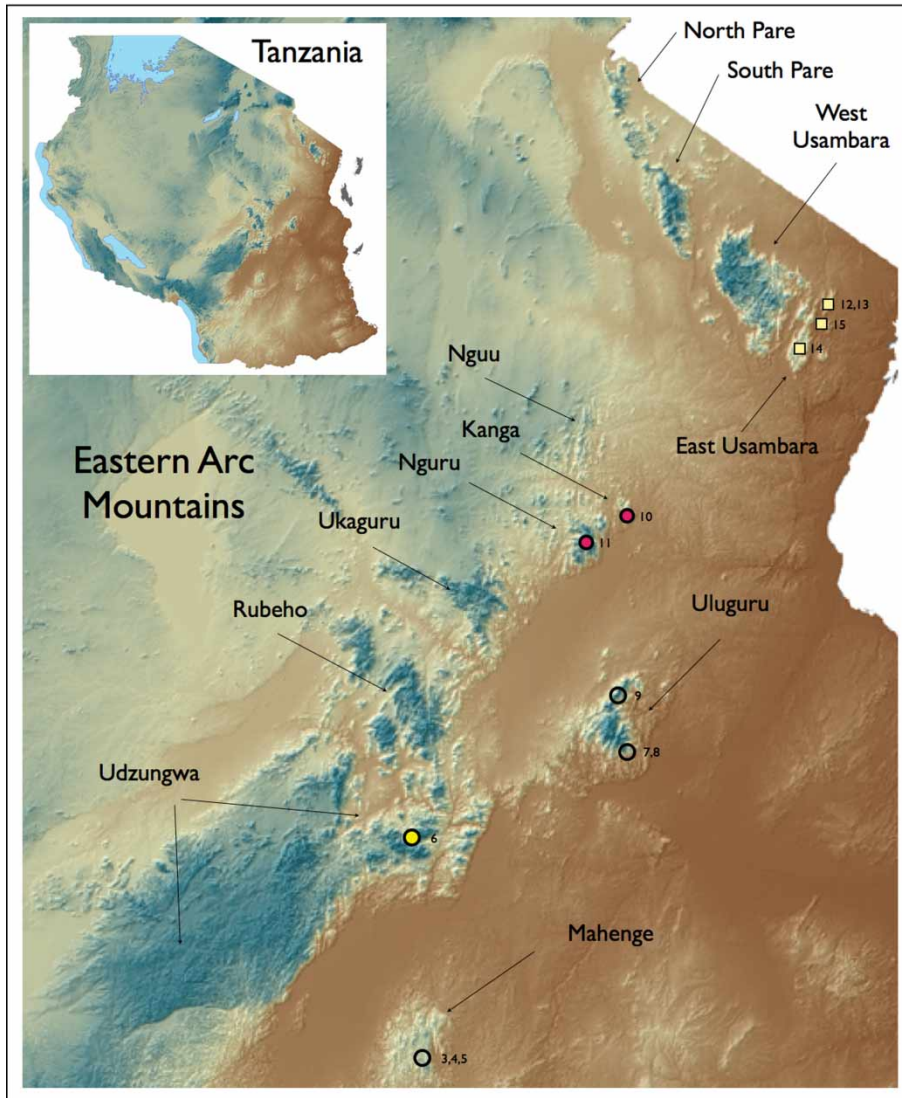
Figure 1. Map of the Eastern Arc Mountains with the distribution of East African torrent frogs. Yellow square = *P. martiensseni* and type locality; open circle = *P. yakusini*; yellow circle = type locality of *P. yakusini*; and red circle = *P.* sp. Nguru. Numbers refer to samples given in Table 1.

additional populations. We know of no specimens from the West Usambara (Loader *et al.* 2011a). *Petropedetes natator* collected from Sierra Leone was used as outgroup.

Phylogenetics

An alignment of concatenated partial 12S, 16S and cytochrome b (*cytb*) sequences was assembled based on newly generated data (see Online Supplementary Table 2).

The methods for extraction, amplification and sequencing are as reported by Loader et al. (2004, 2011b). Details of voucher specimens and GenBank accession numbers are given in Online Supplementary Table 1. Sequences were aligned using MUSCLE (Edgar 2004) with default settings in the bioinformatics tool suite Geneious Pro 5.5.4. Alignment ambiguities were identified and removed by eye. For the protein coding partition (*cytb*) TranslatorX (Abascal *et al.* 2010) was used to translate nucleotides to amino acid sequences and determine codon position. For each gene partition the best-fit models of nucleotide substitution were identified using the Akaike information criterion (AIC; Akaike 1973) as implemented in jModeltest version 0.1.1 (Posada 2008). Best-fit models were estimated for each gene, and for each *cytb* codon position, and for combined datasets (see Online Supplementary Table 2). Six datasets were examined in detail (see Online Supplementary Table 3).

The datasets were analysed using maximum likelihood (ML) and Bayesian inference (BI) using the Bioportal at the University of Oslo (http://www.bioportal. uio.no). ML analyses were conducted with RAxML version 7.0.4 (Stamatakis 2006) using the rapid hill climbing algorithm (Stamatakis *et al.* 2007). BI was carried out with MrBayes version 3.2.1 (Ronquist *et al.* 2012) running four simultaneous Markov chains for 10 million generations, sampling every 1 000 generations, and discarding the first one million generations as burn-in to prevent sampling before reaching stationarity by using Tracer v1.5 (Rambaut & Drummond 2007). Two independent BI runs were performed to identify convergence. Bayes Factors were calculated using Tracer v1.5 (Rambaut & Drummond 2007). For both ML and BI analyses, model parameters were independently optimised for each partition ('un-link' option in effect). Support for internal branches was evaluated by non-parametric bootstrapping (Felsenstein 1985) with 1 000 replicates performed with RAxML (ML), and by posterior probabilities (BI).

RESULTS AND DISCUSSION

All PCR amplifications from DNA templates yielded products of the expected size and were subsequently analysed in Sequencher 3.1.1 (Gene Codes Inc., Ann Arbor, MI, USA) or CodonCode Aligner (CodonCode Corporation, Dedham, MA, USA). Transitions vs transversions were plotted for each gene partition and none showed saturation (not shown). The phylogenetic results show highly congruent patterns of relationships among all the datasets (support values given in Online Supplementary Table 5) and the various methods of analysis, with little ambiguity in the relative positions of all taxa. A summary tree is shown in Fig. 2 from partition dataset 4, the most appropriate partition scheme for the analysis as judged using Bayes Factors (see Online Supplementary Tables 1–2). Pairwise differences are given in Online Supplementary Table 4.

Taxonomy and species boundaries

Our molecular data provide good support for the distinctiveness of two (*P. martiensseni* and *P. yakusini*) of the three nominal species of East African *Petropedetes* that we were able to sample. The substantial differences (> 20%, see
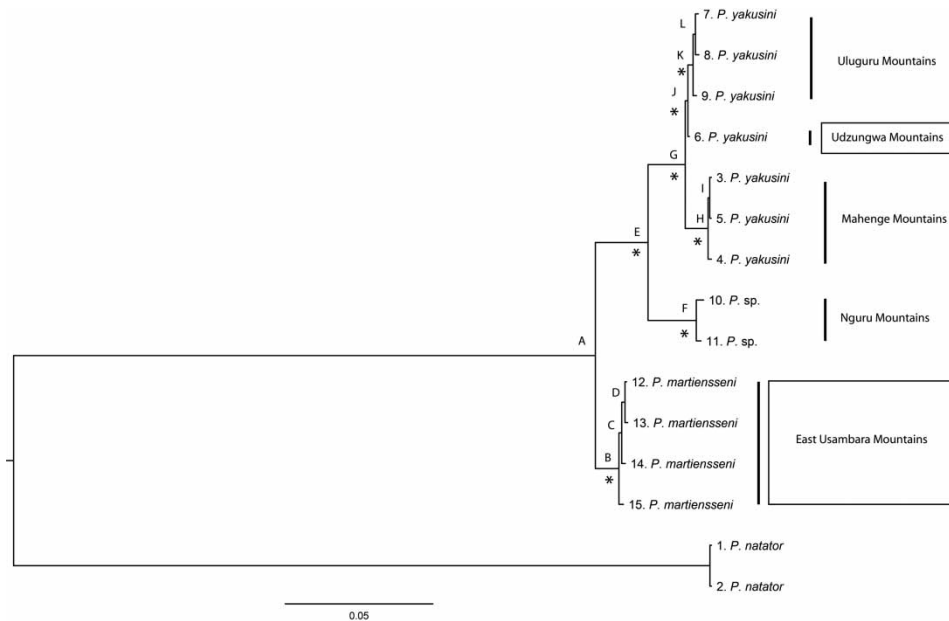
Figure 2. Maximum likelihood tree from partitioned dataset 4. Online Supplementary Tables 2–4 provide details on datasets analysed. Quantitative support values for nodes A–L are given in Online Supplementary Table 4. *Asterisks on branches indicate support values > 95 (BPP) and > 80 (ML Bootstrap). Boxes indicate topotypic material for the two nominal species.

Online Supplementary Table 4) between the sampled East and West African *Petropedetes* are greater than between many pairs of congeneric frog species (e.g. Vences *et al*. 2005) and indicate that Scott's (2005) synonymy of *Arthroleptides* with *Petropedetes* might have been premature. Further work is in progress to address the issue (M.F. Barej, pers. comm, 2012) beyond the speculation made here.

There is very little variation among specimens of *P. martiensseni* from across its sampled range in the East Usambara Mountains (Amani Nature Reserve (NR) in the South to Nilo NR in the north). In contrast, among mountains there is clear geographical structure within populations referred to *P. yakusini*, with genetically distinct populations in each of the Uluguru, Udzungwa and Mahenge Mountains. However, the difference between Uluguru and Udzungwa populations is small (0.5%) despite the sampled localities being approximately 200 km apart (Fig. 1). The name *P. yakusini* would appear to be appropriate for both the Uluguru and Udzungwa populations based on the morphological (Channing *et al*. 2002) and molecular (outlined here) similarities of these populations. Herein, we apply the name *P. yakusini* to populations in both mountains.

Data provided in this study suggest that the Mahenge population of *Petropedetes yakusini* is perhaps a distinct lineage but with relatively low divergence (1.3% different from the Udzungwa population). Further morphological studies are necessary to identify diagnostic characters for the taxonomic recognition of this population at the species level. Other reptile and amphibian species are known to be endemic to the Mahenge mountain block (Menegon *et al*. 2011).

The recently discovered *Petropedetes* population from the Nguru Mountains is more closely related to *P. yakusini* than to *P. martiensseni* but is substantially different genetically (pairwise distance > 6%) from the former. We noted phenotypic differences between Nguru specimens and congeners, particularly in colour and body shape (see Online Supplementary Fig. 1 and Supplementary Table 6 for specimens examined). Compared to the more robust forms of *P. yakusini* (see Online Supplementary Fig. 1A, B) and *P. martiensseni*, the Nguru specimens are more gracile (see Online Supplementary Fig. 1C, D). The absence of a thickened supratympanic ridge and greater snout–vent length (of 35 mm) indicate that the population cannot be ascribed to the other East African species, *P. dutoiti* (Channing & Howell 2006). Given this large genetic distance and morphological differences, the Nguru population is likely to be a distinct new species. The Nguru Mountains are increasingly becoming a hotspot of amphibian diversity in East Africa, with numerous single-site endemic species known previously (Menegon et al. 2008) and additions recently described (e.g. Poynton *et al.* 2008; Loader et al. 2010).

Biogeography

The rifting of East Africa, initiated approximately 30 mya, is hypothesised to be causally associated with the start of the separation of the forests of East and Central/West Africa (Kingdon 1989; Lovett 1993; Maley 1996). It has been speculated that rifting of East Africa is key to understanding the distinctive faunas and floras of these regions of Africa. Based on our preliminary genetic findings, the separation of West and East African *Petropedetes* is substantial (24%, see Online Supplementary Table 4) and perhaps fits with the early origin of the separation of the forests of East and Central/West Africa. It is notable that sister lineages of montane West-East African amphibians show large genetic differences across this range (e.g. Loader *et al.* 2007; this study). Further sampling of *Petropedetes*, additional data (notably nuclear gene sequences) and molecular dating analyses are required to clarify patterns within this genus and how these compare with spatiotemporal patterns of divergence for other taxa.

The biogeographic history of the EAM has been speculated to have occurred over a long time period and to be complex, potentially involving multiple abiotic events (Lovett 1993). However, the main process that is thought to have promoted the diversification of organisms in this region is the gradual isolation of each Eastern Arc Mountain fragment, correlated with the orogenic activity here (Lovett 1993). More recent contacts and separations between forests across mountains in the EAM may have been caused by climatic changes in the region. The strong geographical structure of genetic variation among *Petropedetes* populations presented here indicates likely long-term isolation of some lineages in particular regions. The northern species *P. martiensseni* is clearly distinct genetically and, if pairwise differences (minimally 7.5%) are roughly proportional to time, likely to have undergone a substantial period of isolation. Similarly, the Nguru population appears to have been isolated for a prolonged period. Phylogenetic splits between northern and southern Eastern Arc samples have been noted previously for some other moist forest occurring organisms (e.g. Möller & Cronk 1997; Roy 1997; Lindqvist & Albert 2001; Gravlund 2002; Bowie *et al.* 2004; Blackburn & Measey 2009; Lawson 2010).

Such consistent results suggest a substantial and prolonged barrier to dispersal between these regions, and support the notion that isolation of these forest regions might have promoted biotic diversification here.

Phylogeographic patterns of *P. yakusini* suggest relatively recent gene flow of forest stream adapted amphibian species across currently low-lying savanna habitats (e.g. Uluguru and Udzungwa). Pleistocene fluctuations might account for connecting habitats in recent times (Hamilton 1976). Interestingly, some specimens of *P. yakusini* have been recorded from low elevations (in miombo woodland below the Kihansi gorge ca. 400–500 m a.s.l. in the Udzungwa Mountains – K.M. Howell, pers. comm.), which suggests the species may not be so strongly dependent upon montane forest habitats (IUCN 2012). The work outlined here further underlines the progress being made over recent years in revealing the biogeographic patterns of amphibians, and organisms more widely, in this fascinating region of Africa.

REFERENCES

ABASCAL, F., R. ZARDOYA & M.J. TELFORD. 2010. TranslatorX: multiple alignment of nucleotide sequences guided by amino acid translations. Nuc. Acids Res. 38: W7–W13.

AKAIKE, H. 1973. Information theory and an extension of the maximum likelihood principle. Pp. 267–281. In B.N. PETROV & F. CSAKI (Eds.) Second International Symposium on Information Theory. Akademiai Kiado, Budapest.

BAREJ, M.F., M.-O. RÖDEL, L.N. GONWOUO, O.S.G. PAUWELS, W. BÖHME & A. SCHMITZ. 2010. Review of the genus *Petropedetes* Reichenow, 1874 in Central Africa with the description of three new species (Amphibia: Anura: Petropedetidae). Zootaxa. 2340: 1–49.

BLACKBURN, D.C. & G.J. MEASEY. 2009. Dispersal to or from an African biodiversity hotspot? Mol. Ecol. 18: 1904–1915.

BOWIE, R.C.K., J. FJELDSÅ, S.J. HACKETT & T.M. CROWE. 2004. Molecular evolution in space and through time: mtDNA phylogeography of the Olive Sunbird (*Nectarinia olivacea/obscura*) throughout continental Africa. Mol. Phyl. Evol. 33: 56–76.

CHANNING, A. & K.M. HOWELL. 2006. Amphibians of East Africa. Cornell University Press, Ithaca, NY.

CHANNING, A., D.C. MOYER & K.M. HOWELL. 2002. Description of a new torrent frog in the genus *Arthroleptides* from Tanzania (Ranidae). Alytes 20: 13–27.

EDGAR, R.C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinformatics 5: 113.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): 783–791.

GRAVLUND, P. 2002. Molecular phylogeny of Tornier's cat snake (*Crotaphopeltis tornieri*), endemic to East African mountain forests: biogeography, vicariance events and problematic species boundaries. J. Zool. Syst. Evol. Res. 40(1): 46–56.

HAMILTON, A.C. 1976. The significance of patterns of distribution shown by forest plants and animals in tropical Africa for the reconstruction of Upper Pleistocene palaeoenvironments: a review. Palaeoecol. A. 9: 63–97.

IUCN, CONSERVATION INTERNATIONAL & NATURESERVE (2012). Global Amphibian Assessment. http://www.iucnredlist.org/amphibians (accessed 1 September 2012).

KINGDON, J. 1989. Island Africa. Princeton University Press, Princeton, NJ.

LAWSON, L.P. 2010. The discordance of diversification: evolution in the tropical-montane frogs of the Eastern Arc Mountains of Tanzania. Mol. Ecol. 19: 4046–4060.

LINDQVIST, C. & V.A. ALBERT. 2001. A high elevation ancestry for the Usambara Mountains and lowland populations of African violets (Saintpaulia, Gesneriaceae). Syst. and Geogr. Plants 71: 37–44.

LOADER, S.P., D.J. GOWER, B.T. CLARKE, K.M. HOWELL, N. DOGGART, M.-O. RÖDEL, R.O. DE SÁ, B.L. COHEN & M. WILKINSON. 2004. Phylogenetic relationships of African microhylid frogs inferred from DNA sequences of mitochondrial 12S and 16S rRNA genes. Org. Divers. Evol. 4: 227–235.

LOADER, S.P., H. MÜLLER, D.J. GOWER & M. MENEGON. 2010. Two new species of *Callulina* (Amphibia: Anura: Brevicipitidae) from the Nguru Mountains, Tanzania. Zootaxa. 2694: 26–42.

LOADER, S.P., D. PISANI, J.A. COTTON, D.J. GOWER, J.J. DAY & M. WILKINSON. 2007. Relative timescales reveal multiple origins of parallel disjunct distributions of African caecilian amphibians. Biol. Letters 3: 505–508.

LOADER, S.P., J.C. POYNTON, L. LAWSON, D. BLACKBURN & M. MENEGON. 2011a. Amphibian diversity in the north-western elements of the Eastern Arc Mountains, Tanzania, with the description of new *Arthroleptis* (Anura: Arthroleptidae) species. Fieldiana Life and Earth Sciences 4: 90–102.

LOADER, S.P., M. WILKINSON, J. COTTON, G.J. MEASEY, M. MENEGON, K.M. HOWELL, H. MÜLLER & D.J. GOWER. 2011b. Molecular phylogenetics of *Boulengerula* (Amphibia: Gymnophiona: Caeciliidae) and implications for taxonomy, biogeography and conservation. Herpetol. J. 21: 5–16.

LOVERIDGE, A. 1935. Scientific results of an expedition to rain forest regions in eastern Africa. Bulletin Mus. Comp. Zool., Harv. 79: 3–19.

LOVETT, J.C. 1993. Climatic history and forest distribution in eastern Africa. Pp. 23–29. In L.C. LOVETT & S.K. WASSER (Eds.) Biogeography and Ecology of the Rain Forests of Eastern Africa. Cambridge University Press, Cambridge.

MALEY, J. 1996. The African rain forest – main characteristics of changes in vegetation and climate from the Upper Cretaceous to the Quaternary. P. Roy. Soc. Edinb. B. 104: 31–73.

MENEGON, M., C. BRACEBRIDGE, N. OWEN & S.P. LOADER. 2011. Amphibians and reptiles of Mahenge Mountains, with comments on biogeography, diversity, and conservation. Fieldiana Life and Earth Sciences 4: 103–111.

MENEGON, M., N.H. DOGGART & N. OWEN. 2008. The Nguru mountains of Tanzania, an outstanding hotspot of herpetofaunal diversity. Acta Herpetol. Firenze 3: 107–127.

MÖLLER, M. & Q.C.B. CRONK. 1997. Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. Am. J. Bot. 84(7): 956–965.

NIEDEN, F. 1911 "1910". Verziechnis der bei Amani in Deutschostafrika vorkommenden Reptilien und Amphibien. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin 1910: 441–452.

POSADA, D. 2008. jModeltest: phylogenetic model averaging. Mol. Biol. Evol. 25: 1253–1256.

POYNTON, J.C., M. MENEGON & S.P. LOADER. 2008. A new giant species of *Arthroleptis* (Amphibia: Anura) from the forests of the Nguru Mountains, Tanzania. Afr. J Herpetol. 57(2): 63–74.

RAMBAUT, A. & A.J. DRUMMOND. 2007. Tracer v1.4. http://beast.bio.ed.ac.uk/Tracer (accessed 1 December 2009).

RONQUIST, F., M. TESLENKO, P. VAN DER MARK, D.L. AYRES, A. DARLING, S. HÖHNA, B. LARGET, L. LIU, M.A. SUCHARD & J.P. HUELSENBECK. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61(3): 539–542.

Roy, M.S. 1997. Recent diversification in African greenbuls (Pycnonotidae: *Andropadus*) supports a montane speciation model. Proc. Roy. Soc. London B 264: 1337–1344.

Scott, E. 2005. A phylogeny of ranid frogs (Anura: Ranoidea: Ranidae), based on a simultaneous analysis of morphological and molecular data. Cladistics. 21: 507–574.

Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22(21): 2688–2690.

Stamatakis, A., F. Blagojevic, D. Nikolopoulos & C. Antonopoulos. 2007. Exploring new search algorithms and hardware for phylogenetics: RAxML meets the IBM cell. J. VLSI Signal Process. 48: 271–286.

Vences, M., M. Thomas, A. van der Meijden, Y. Chiari & D.R. Vieites. 2005. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Frontiers in Zoology* 2: 5.

Species boundaries and biogeography of East African torrent frogs of the genus *Petropedetes* (Amphibia: Anura: Petropeditidae)

SIMON P. LOADER[1]*, F. SARA CECCARELLI[1], MARK WILKINSON[2], MICHELE MENEGON[3], JEAN MARIAUX[4], RAFAEL O. DE SÁ[5], KIM M. HOWELL[6]& DAVID J. GOWER[2]

[1]*Department of Environmental Science (Biogeography), University of Basel, Switzerland;* [2]*Department of Zoology, The Natural History Museum, London, UK;* [3]*Tropical Biodiversity Section, MuseoTridentino di ScienzeNaturali, Trento, Italy;* [4]*Department of Invertebrates, Muséumd'HistoireNaturelle, Geneva, Switzerland;*[5]*Department of Biology, University of Richmond, Richmond, VA, USA;* [6]*Department of Zoology and Marine Biology, University of Dar es Salaam, Dar es Salaam, Tanzania*

ONLINE SUPPLEMENTARY MATERIAL

Supplementary Table 1.Details of East African Petropedetes and West African Petropedetesoutgroup samples used in analyses plus GenBank accession codes. Collection abbreviations: BMNH (The Natural History Museum, London, UK);MTSN (MuseoTridentino di ScienzeNaturali, Trento Italy);MW (Field Series destined for accessioning in The Natural History Museum in brackets); and RDS (Rafael de Sá Field Series). Vouchers were identified through comparisons with published descriptions and type material. FR = Forest Reserve;NR = Nature Reserve. Type localities (or likely within 20 km) for

nominate species indicated by asterisks *. Locality Tanzania unless otherwise stated (see Figure 1 for map overview).

No.	Specimens	Species	Locality	Genbank Accession: 12S, 16S, and *cytb*
1.	(MW 6025)	*Petropedetesnatator*	Sierra Leone	JX546938, JX546953, JX546968,
2.	(MW 6045)	*Petropedetesnatator*	Sierra Leone	JX546939, JX546954, JX546969
3.	BM2005.1382 (MW 1844)	*Petropedetesyakusini*	Mahenge, Sali FR	JX546946, JX546963, JX546972
4.	BM2005.1383 (MW 1852)	*Petropedetesyakusini*	Mahenge, Sali FR	JX546944, JX546966, JX546974
5.	BM2005.1384 (MW 1854)	*Petropedetesyakusini*	Mahenge, Sali FR	JX546952, JX546967, JX546973
6.	BM2005.567 (KMH 22148)	*Petropedetesyakusini*	Udzungwa, West Kilombero Scarp FR *	JX546941, JX546965, JX546980
7.	BM2005.013 (KMH 21533)	*Petropedetesyakusini*	Uluguru, Kasanga FR	JX546942, JX546964, JX546982
8.	BM2005.013	*Petropedetesyakusini*	Uluguru, Kasanga FR	JX546945, JX546961,

				JX546981
9.	RdS 5862	*Petropedetesyakusini*	Uluguru, Uluguru North FR	JX546940, JX546962, JX546971
10.	MTSN 8359	*Petropedetes*sp.	Nguru, Kanga FR	JX546948, JX546960, JX546976
11.	MTSN 8255	*Petropedetes*sp.	Nguru, Nguru South FR	JX546947, JX546959, JX546975
12.	BM2002.576 (KMH 21215)	*Petropedetesmartiensseni*	East Usambara, Nilo FR	JX546951, JX546956, JX546977
13.	BM2002.575 (KMH 21188)	*Petropedetesmartiensseni*	East Usambara, Nilo FR	JX546943, JX546958, JX546978
14.	RDS 5946	*Petropedetesmartiensseni*	East Usambara, Amani NR *	JX546949, JX546957, JX546979
15.	BM2000.826 (KMH 22294)	*Petropedetesmartiensseni*	East Usambara, Kwamgumi FR	JX546950, JX546955, JX546970,

Supplementary Table 2.Partitions and their model selection.Parsimony-uninformative (PU), parsimony-informative (PI), total number of characters (Total), Base frequencies (Base), Number of substitution types (Nst), Rate Matrix (Rmat), Shape of the gamma distribution (Shape), and Proportion of invariable sites (Pinv).

Partitions	Total	PU	PI	Model Selection (AIC in jModeltest)
Combined	1809	1298	511	TIM2+G (base=(0.2892 0.2462 0.1613 0.3032) nst=6 rmat=(5.0455 10.6677 5.0455 1.0000 34.8192 1.0000) rates=gamma shape=0.3190 ncat=4 pinvar=0)
12S	428	304	124	TIM2+G (base=(0.3051 0.2592 0.1795 0.2562) nst=6 rmat=(3.2407 2.5053 3.2407 1.0000 15.2509 1.0000) rates=gamma shape=0.3960 ncat=4 pinvar=0)
16S	559	432	127	TIM2+G (base=(0.3019 0.2350 0.1926 0.2705) nst=6 rmat=(8.8899 30.2307 8.8899 1.0000 95.3580 1.0000) rates=gamma shape=0.2270 ncat=4 pinvar=0)
cytb (all)	822	562	260	TIM2+G (base=(0.2727 0.2476 0.1298 0.3500) nst=6 rmat=(4.7789 12.8423 4.7789 1.0000 32.1764 1.0000) rates=gamma shape=0.3430 ncat=4 pinvar=0)

1st Pos. (*cytb*)	274	227	47	K80+G (base=equal nst=2 tratio=8.4358 rates=gamma shape=0.0670 ncat=4 pinvar=0)
2nd Pos. (*cytb*)	274	251	23	TPM2uf (base=(0.2227 0.2564 0.1412 0.3797) nst=6 rmat=(6.5008 10.4552 6.5008 1.0000 10.4552 1.0000) rates=equal pinvar=0)
3rd Pos. (*cytb*)	274	84	190	TIM2+I (base=(0.3109 0.2610 0.0318 0.3963) nst=6 rmat=(0.2144 8.9459 0.2144 1.0000 3.5566 1.0000) rates=equal pinvar=0.0680)
1st and 2nd Pos. (*cytb*)	548	478	70	TPM2uf+G (base=(0.2522 0.2432 0.1824 0.3223) nst=6 rmat=(6.4175 20.1514 6.4175 1.0000 20.1514 1.0000) rates=gamma shape=0.1940 ncat=4 pinvar=0)

Supplementary Table 3.Datasets with partitions used, ML and BI best tree results.

Datasets	Number of partitions, and name	Harmonic Mean (BI), Log Likelihood (ML)
1. 12S + 16S + $cytb$	1, combined	-4985.36
		-4948.88
2. 12S + 16S + $cytb$	2, 12S+16s, $cytb$	-4966.41
		-4915.42
3. 12S + 16S + $cytb$	3, all separate	-5018.42
		-4897.92
4. 12S + 16S + $cytb$ 1st Pos. + $cytb$ 2nd Pos. + $cytb$ 3rd Pos.	5, all separate with codon positions ($cytb$)	-4748.25
		-4692.22
5. 12S + 16S + $cytb$ 1st /2nd Pos. + $cytb$ 3rd Pos.	4, all separate but 1st /2nd position as one partition	-4758.12
		-4709.09
6. 12S + 16S + $cytb$ 1st Pos. + $cytb$ 2nd Pos.	4, all separate with codon positions ($cytb$) with 3rd position removed	-3650.84
		-3588.10

Supplementary Table 4.Uncorrected pairwise distances (p-distance) for 12S, 16S and *cytb* sequence data, MA = Mahenge, UDZ = Udzungwa, ULU = Uluguru, KA = Kanga, NGU = Nguru, EUS = East Usamabra.

Locality			MA			UDZ	ULU			KA	NGU	EUS		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *P. natator*														
2. *P. natator*	0													
3. *P.* cf.*yakusini*	0.246	0.246												
4. *P.* cf.*yakusini*	0.247	0.247	0.001											
5. *P.* cf.*yakusini*	0.246	0.246	0	0.001										
6. *P. yakusini*	0.249	0.249	0.013	0.013	0.012									
7. *P. yakusini*	0.25	0.249	0.015	0.015	0.015	0.005								
8. *P. yakusini*	0.251	0.251	0.015	0.015	0.015	0.005	0.001							
9. *P. yakusini*	0.25	0.25	0.015	0.015	0.014	0.005	0.002	0.001						
10. *P.* cf.*yakusini*	0.244	0.244	0.064	0.065	0.064	0.06	0.06	0.06	0.059					
11. *P.* cf.*yakusini*	0.244	0.244	0.065	0.063	0.063	0.059	0.059	0.058	0.058	0.002				
12. *P. martiensseni*	0.246	0.245	0.081	0.082	0.081	0.078	0.081	0.081	0.081	0.082	0.081			
13. *P. martiensseni*	0.242	0.241	0.081	0.081	0.081	0.078	0.081	0.08	0.08	0.082	0.081	0		
14. *P. martiensseni*	0.242	0.242	0.081	0.081	0.081	0.079	0.082	0.08	0.081	0.081	0.08	0.001	0.001	
15. *P. martiensseni*	0.243	0.242	0.08	0.079	0.079	0.076	0.079	0.078	0.078	0.079	0.078	0.001	0.002	0.001

Supplementary Table 5.Bayesian posterior probability, and maximum likelihoodbootstrap results for nodes A–Q labelled in Figure 2 for each partitioned dataset (1–6).

	Dataset 1	Dataset 2	Dataset 3	Dataset 4	Dataset 5	Dataset 6
A	1/100	1/100	1/100	1/100	1/100	1/100
B	1/87	0.97/-	1/-	0.98/-	0.92/-	0.84/-
C	0.4/70	0.48/-	0.58/-	0.45/-	0.42/-	0.42/-
D	0.55/91	0.65/87	0.85/85	0.51/89	0.51/88	0.5/90
E	0.98/89	0.99/93	1/94	1/95	1/96	0.98/97
F	1/100	1/100	1/99	1/100	1/100	1/99
G	1/98	1/97	1/98	1/96	1/97	1/99
H	1/100	1/100	1/100	1/100	1/99	1/100
I	0.35/84	0.34/87	0.34/92	0.35/90	0.35/90	0.36/88
J	1/99	0.99/98	1/98	0.94/96	0.99/97	0.47/71
K	1/100	1/100	1/100	1/99	1/100	0.99/94
L	0.43/66	0.44/62	0.43/69	0.43/64	0.34/68	0.49/77

Supplementary Table 6.Specimens examinedin the study. Collection abbreviations: BMNH (The Natural History Museum, London, UK);MTSN (MuseoTridentino di ScienzeNaturali, Trento Italy).

Species	Specimens
Petropedetesmartiensseni	BMNH 2000.416-.418, 2000.412, 2000.406, 2000.455-.456, 2000.513-.514, 2000.738, 2002.320-.322, 2000.826, 2002.804, 2002.906, 2002.466-.467, 2002.574-.578, 2005.433-.436, 2005.1237, 2005.1505
Petropedetesyakusini(Uluguru)	BMNH2005.012-.014
Petropedetesyakusini(Mahenge)	BMNH2005.1382-.1384
Petropedetesyakusini(Udzungwa)	MTSN 5448-5466, 5469, 5677, 5610-5613, 5669-5676, 8476, 8479, BMNH2005.567
Petropetes sp. Nguru(Nguru)	MTSN 8255, 8276
Petropetes sp. Nguru(Kanga)	MTSN 8359, 8364, 8378, 8382, 8384, 8518

Supplementary Figure 1.*Petropedetes*spp in life at various localities.(A) *Petropedetes yakusini*Mahenge, (B) *Petropedetes yakusini*Udzungwa, (C) *Petropedetes sp*Kanga and (D) *Petropedetes sp*Nguru South.