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A new species of Chameleon (Sauria: Chamaeleonidae: *Kinyongia*) highlights the biological affinities between the Southern Highlands and Eastern Arc Mountains of Tanzania

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Abstract. A new species of chameleon is described from the Livingstone and Udzungwa Mountains of Tanzania. The new species is morphologically most similar to *Kinyongia vanheygeni*. Furthermore, a single, short rostral appendage shows the species similarity to other Eastern Arc endemic *Kinyongia* species (e.g. *K. uthmoelleri*, *K. oxyrhina*, *K. magomberae* and *K. tenuis*). Females of all these species lack any rostral ornamentation and are all very similar morphologically. Males of the new species, on which the morphological diagnosis is based, can be distinguished from other *Kinyongia* by a shorter rostral appendage that bifurcates at the tip. They are easily distinguished from *K. vanheygeni*, otherwise the most similar species, by differences in head scalation and the length and shape of the rostral appendage. The new species is associated with montane rainforest and is known from only four forest fragments of which two are in the Udzungwa and two in the Livingstone Mountains. Phylogenetically, the new species is sister to *K. tenuis* and *K. magomberae,* which together, form a clade that also contains *K. oxyrhina*. The disjunct distribution of the new species, in the Livingstone and Udzungwa mountains, stretches across the 'Makambako Gap' which is a putative biogeographical barrier separating the distinct faunas of the Southern highlands and Eastern Arc Mountains. Evidence from this species however, points to potentially closer biological affinities between the Livingstone and Udzungwa mountains.

Keywords. Southern Highlands, Tanzania, Eastern Afromontane, Biodiversity, Chamaeleonidae, East Africa, new species, reptiles.

INTRODUCTION

Exploration and subsequent research in the past decades have substantially improved our understanding of the biodiversity from the Eastern Afromontane Region (EAR), which is known for its high species richness (Menegon and Davenport, 2008). Collectively the Eastern Arc Mountains (EAM) and Southern Highlands of Tanzania form a system of mountain blocks spanning from southern Kenya, through Tanzania and into Malawi (Lovett and Wasser, 1993). Because of the prevailing climatic influence from the warm Indian Ocean, the EAM receives high orographic rainfall providing a relatively stable climate. This climate stability is thought to have reduced extinction rates for forest endemic taxa (e.g., Tolley et al., 2011; Loader et al., 2014). This, coupled to ele-

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vated speciation rates as a result of specialization due to ecotones between forest and savanna (Caro et al., 2013), has presumably resulted in high diversity and endemism across the EAM (Loader et al., 2015).

Our understanding of more general evolutionary patterns and processes for the EAM, including biogeographic patterns has increased dramatically for some taxonomic groups (e.g., Tolley et al., 2011; Dimitrov et al., 2012; Loader et al., 2014). Despite this, a number of vertebrate species are discovered and described each year (e.g., Rovero et al., 2014), indicating that our knowledge is far from complete in this region. A prime example are chameleons, for which new species are steadily being described (Tolley and Menegon, 2013), or previously named taxa are elevated from synonymy (Tilbury and Emmrich 1996; Menegon et al., 2002; Mariaux and Tilbury, 2006; Tilbury et al., 2006; Mariaux et al., 2008, Tilbury and Tolley, 2009; Menegon et al., 2009; Greenbaum et al., 2012; Branch et al., 2014) and these contributions have subsequently been utilized for revealing broader evolutionary patterns (Tolley et al., 2011; Tolley et al., 2013; Ceccarelli et al., 2014). Essentially, the scientific focus on EAM region has led to a substantial increase in knowledge on the flora and fauna, but the biota of the Southern Highlands, which is separated from the more northern lying EAM by the dry, low-lying Makambako Gap (Lovett and Wasser, 1993), is relatively poorly known. Indeed, the Makambako gap is considered an important turn-over region (e.g., Rovero et al., 2014) and as a result, many biodiversity studies have instead focused on the EAM because of its known biological wealth (e.g. Newmark, 1998; Stanley et al., 1999).

Chameleons in the genus *Kinyongia* (Tilbury et al., 2006) are a prominent group in the EAM because findings have contributed to a broader understanding of species richness, endemism, and biogeography (e.g., Tolley et al., 2011). Twelve of the 16 described species of *Kinyongia* occur on isolated massifs within the EAM, with the remainder found to the northwest in mountainous regions of the Albertine Rift in Democratic Republic of the Congo, Uganda and Rwanda and on isolated volcanoes, such as Kilimanjaro, Meru, Mt. Kenya (Tilbury, 2010; Tolley et al., 2011; Greenbaum et al., 2012). Many species have small distributional ranges, and are usually found on the forested slopes of just one or a few isolated massifs. Their isolated and restricted distributions have provided evidence for a long history of persistence in EAM, and as well as allowed inferences as to the formation and maintenance of refugial areas (Tolley et al., 2011).

New biological surveys in unexplored regions such as southern Tanzania continually reveal the presence of new species, including Matilda's horned viper *Atheris matildae* (Menegon et al., 2011), the two chameleons *Kinyongia vanheygeni* (Necas, 2009) and *K. magomberae* (Menegon et al. 2009), and Africa's only new genus of monkey described in the last 80 years, the kipunji *Rungwecebus kipunji* (Davenport et al., 2006). Unexplored forests to the south of the EAM (e.g. Southern Highlands) are therefore predicted to contain a host of species not yet known to science. In this study, we describe a new chameleon species in the genus *Kinyongia* (Fig. 1) that is found in Afrotemperate forest from both the Livingstone (Southern Highlands) and Udzungwa (EAM) Mountains (Fig. 2). Using both morphological and molecular evidence, we determine the taxonomic placement and evolutionary relationships for this new taxon. Both morphological characters and genetic markers were examined and compared to the other species of *Kinyongia*. Furthermore we examine the biogeographical implications of this new taxon given the phylogenetic hypothesis inferred from the data.

MATERIALS AND METHODS

Material examined

The following specimens (Table 1) were examined from the herpetological collections of the Science Museum of Trento, Trento, Italy (MTSN and MUSE), the Department of Zoology & Wildlife Conservation of the University of Dar es Salaam, Dar es Salaam, Tanzania and the collection of the WCS' Southern Highlands Conservation Project (SHCP), Mbeya, Tanzania: *K. vanheygeni* (MUSE 13523 and MUSE 13524 from Mt. Rungwe), *K. tenuis* (KMH 21325 and KMH 21304 from Nilo Forest Reserve, East Usambara Mts.), *K. oxyrhina* (KMH 28277 from Ukami Forest, Udzungwa Mts.; KMH 28302 from Nyumbanitu Forest, Udzungwa Mts., MTSN 8454 and MTSN 8412 from Nguru South Forest Reserve), *K. tavetana* (MTSN 8658 and MTSN 8661 from Kindoroko Forest Reserve, North Pare Mts.)

Molecular Analysis

To understand the phylogenetic placement the new *Kinyongia* species a phylogenetic analysis was carried out which included 10 individuals from the two mountain ranges, plus multiple representatives from 17 of 19 *Kinyongia* species from published datasets (Menegon et al., 2009; Tolley et al., 2011; Greenbaum et al., 2012). The resulting dataset consisted of 47 individuals, including the outgroup taxa (*Bradypodion pumilum* and *B. melanocephalum*). DNA extraction, PCR amplification, and cycle sequencing of two mitochondrial gene fragments (ND2 and 16S) were carried out following standard procedures using the following primers for ND2: L4437b and H5934 (Macey et al., 1997a, b), and 16S: L2510 and H3080 (Palumbi, 1996). Standard PCR and sequencing were followed for this gene fragment, with PCR annealing temperature at 57°C. All

Fig. 1. *Kinyongia msuyae sp. nov.* from Livingstone Mountains in life. Pictures showing (upper) adult Male, (lower left) close up of male head, (lower right) Adult female.

new sequences were deposited in European Nucleotide Archive (Table 2).

Bayesian inference was used to investigate optimal tree space using MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) for the combined mitochondrial markers (321 characters, partitioned by marker: ND2, 856 bp; 16S, 465 bp), although 18 bases were excluded for 16S due to ambiguous alignment. To investigate which evolutionary model best fit the data, jModeltest was used (Posada, 2008), and the AIC test indicated the same model for all markers was appropriate (GTR + G). Therefore, MrBayes was run specifying six rate categories with uniform priors for the gamma distribution for each of the partitions. To ensure the results were robust for both datasets, the MCMC was run twice in parallel for 10 million and 20 million generations (four chains in each run), with trees sampled every 1000 generations. Burn-in was estimated as 1 million generations (1000 trees), as determined by examination the average standard deviation of split frequencies, the convergence diagnostic (PSRF values ~ 1.0) as well as the log-probabilities and the values of each parameter for stabilization (Ronquist and Huelsenbeck, 2003). In addition, Tracer v1.4.1 (Rambaut and Drummond, 2007) was used to check that the effective sample size (ESS) of all parameters was greater than 200 after burn-in. A 50% majority rule tree was constructed and nodes with ≥ 0.95 posterior probability considered supported.

In addition to the Bayesian analysis, a maximum likelihood (ML) search was run for both datasets using RAxML HPC 7.2.8 (Stamatakis, 2006). The datasets were partitioned as in the Bayesian analysis, with a GTR+I+G model for all markers and rapid bootstrapping halted automatically (Stamatakis et al., 2008) This analysis was run three times to ensure that independent ML searches produced the same topologies. We considered nodes with a bootstrap value of ≥ 70% as supported in this analysis. Both Bayesian and likelihood analyses were run on the CIPRES Science Gateway (Miller et al., 2010; www.phylo.org/sub_sections/portal/). Finally, to provide a rough indica-

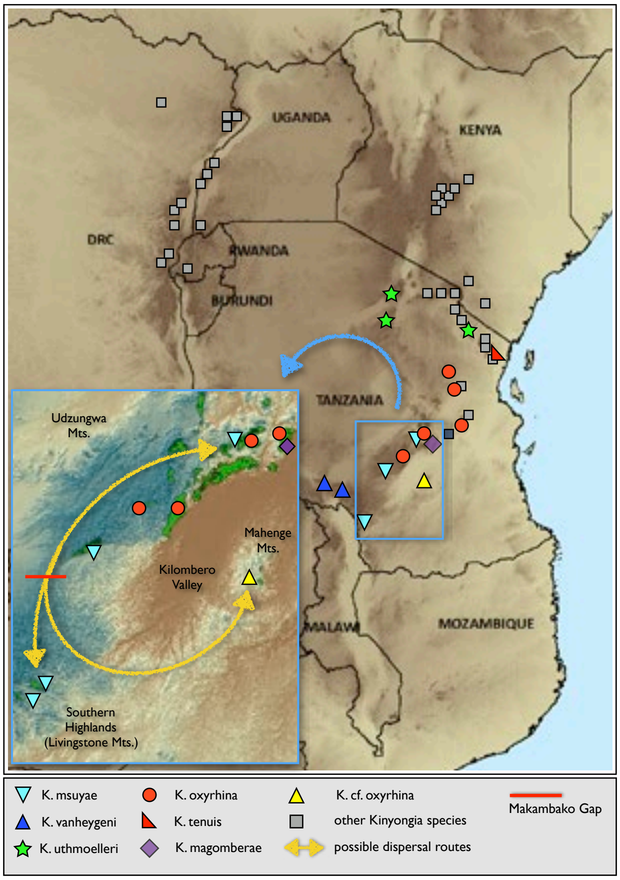

Fig. 2. Distribution of *Kinyongia* species in the Eastern Afromontane Region. Inset map shows enlargement of Udzungwa and Southern Highlands region of Tanzania with possible dispersal routes of montane associated species.

tion of the degree of divergence between species, uncorrected p-distances were estimated in MEGA 5.05 for 16S which had the most complete taxon sampling (Tamura et al., 2011).

RESULTS

Molecular Analysis

The likelihood and Bayesian searches produced the same topology and supported nodes (Fig. 3). The phylogenetic analysis showed that the *Kinyongia* sampled from the Livingstone and the Udzungwa mountains form a well-supported clade. The uncorrected net sequence divergence (p-distance) between this clade and other closely related species of *Kinyongia* range from 1.4-3.7% (Table 3), which is similar to within species values of other chameleons (e.g. Menegon et al., 2009; Tilbury and Tolley; 2009, Greenbaum et al., 2012, Tolley et al., 2012; Branch et al., 2014). Although sequence divergence between chameleons from Livingstone and Udzungwa (< 1.3% for the 16S marker) are close to the lowest values found between some other *Kinyongia* (e.g. *K. tenuis* and *K. magomberae*; Table 3), additional samples from Udzungwa would be needed to obtain a more accurate estimate of sequence divergence between the mountain ranges. Given the genetic distinctiveness, we take the opportunity to describe the individuals from Livingstone and Udzungwa Mountains as a new species.

Taxonomy

Kinyongia msuyae sp. nov (Fig. 1; 3)

Holotype: Adult male in the Science Museum of Trento, MTSN 9374 collected in Mdandu Forest Reserve, Livingstone Mountains in January 2011 by Michele Menegon, Tim Davenport, Simon Loader, Sandra Dürrenberger, Sandra Rudolf and Sophy Machaga

Type locality: Mdandu Forest Reserve, Livingstone Mountains 1900 m above sea level, Mbeya Region, South Eastern Tanzania (-9.769549621; 34.78832024)

Table 1. Biometrics of the holotype and paratypes. Continuous measurements given in mm.

	Total Length	SVL	TL	Head length	Head Width	Casque Lenght	Casque Eye	Snouth Lenght	Eye Diameter	Eye-eye Gap	Upper Labials	Rostral process
MTSN 9374	150.74	69.56	81.18	23.54	10.12	16.68	10.78	7.02	6.61	5.38	20	4.62
MTSN 9375	142.3	65.02	77.28	23.18	10.42	15.11	10.29	8.14	6.69	5.39	20	4.4
MTSN 9898	103.77	43.84	59.93	17.55	18.21	10.58	7.98	6.39	4.88	4.52	18	3.13
MTSN 9373	129.44	57.46	71.98	19.64	9.29	13	8.04	6.58	5.38	5.37	14	no
MTSN 9377	103.5	52.69	50.81	15.75	7.77	10.11	6.12	5.99	4.53	4.41	14	no
MTSN 7497	124.46	61.99	62.47	21.08	11.13	13.39	8.23	7.07	7.75	5.93	13	no
MTSN 9378	97.36	40.2	57.16	13.31	6.59	7.79	6.21	4.37	4.61	3.6	14	no
MUSE 13521	141. 89	68.35	73.54	22.57	11.03	14.99	10.03	7.56	7.06	5.41	19	3.63
MUSE 13522	145.53	64.25	81.28	22.37	10.31	15.66	10.36	7.14	6.39	5.01	17	4.87

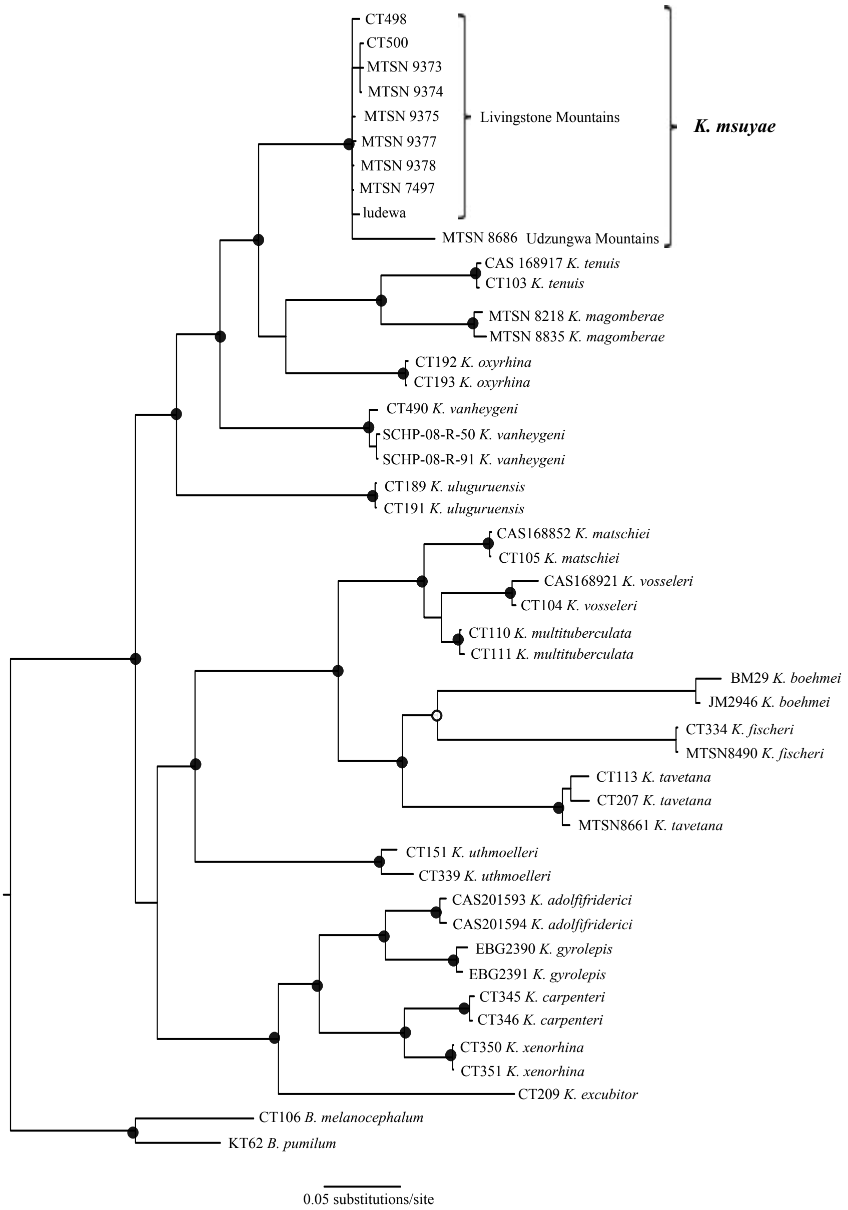

Fig. 3. The best scoring maximum likelihood tree for *Kinyongia*, with nodes supported by maximum likelihood (bootstrap > 70%) and Bayesian (posterior probabilities > 0.95) analyses indicated by black circles. Grey circle indicate support with Bayesian posterior probabilities only.

Paratype: MTSN 9375, adult male, MTSN 9373, MTSN 9377, adult females; MTSN 9378, juvenile, same data as holotype. MTSN 7497 collected in Sakara Nyumo Forest Reserve (Livingstone Mountains) in January 2011 by Michele Menegon, Tim Davenport, Simon Loader and Sophy Machaga; MTSN 8686 (adult male) collected in Kigogo Forest Reserve, Udzungwa Mountains in February 2006 by Michele Menegon;

Referred material: MUSE 13521 (Field number CAM 1013) collected in Kigogo Forest Reserve by Charles A. Msuya. MUSE 13522 (Field number KMH 28302), adult male, collected in Nyumbanitu Forest Reserve by Louis Hansen.

Diagnosis: A small, elongated chameleon, lacking distinctive colours or pattern, with a tail longer than the snout-vent length. It has a short, bone-based rostral appendage formed by a converging, scaly elongation of the *canthi rostrales*, the areas bound by the two *canthi* is concave and covered in flattened scales. The tips of these elongations are free and they appear like a double-tipped

Table 2. Museum, GenBank and European Nucleotide Archive accession numbers (16S, ND2) for *Kinyongia* used in this study (CAS = California Academy of Sciences; MSTN = Science Museum of Trento (formerly Museo Tridentino di Scienze Naturali); PEM = Port Elizabeth Museum (Bayworld). N/A: sequences not available.

Species	Locality	ID	Specimen	16S	ND2
B. melanocephalum	KwaZulu-Natal, South Africa	CT016	N/A	AY289813	HF570475
B. pumilum	Western Cape, South Africa	KT62	N/A	AY756639	AY756689
K. adlofifriderici	Bwindi N.P., Uganda	CAS201593	CAS201593	DQ923820	EF014304
K. adlofifriderici	Bwindi N.P., Uganda	CAS201594	CAS201594	GQ221944	GQ221965
K. boehmei	Taita Hills, Kenya	BM29	N/A	GQ221942	GQ221963
K. boehmei	Taita Hills, Kenya	JM2946	N/A	GQ221948	GQ221969
K. carpenteri	Rwenzori Mtns, Uganda	CT345	PEM R16572	DQ923821	EF014305
K. carpenteri	Rwenzori Mtns, Uganda	CT346	PEM R16573	DQ923822	EF014306
K. excubitor	Mount Kenya, Kenya	CT209	PEM R16571	DQ923823	EF014307
K. fischeri	Nguru Mountains, Tanzania	CT334	PEM R16566	DQ923829	EF014313
K. fischeri	Nguru Mountains, Tanzania	MTSN 8490	MTSN 8490	GQ221951	GQ221971
K. gyrolepis	Lendu Plateau, DRC	UTEP 20341	UTEP20341	JN602059	JN602049
K. gyrolepis	Lendu Plateau, DRC	UTEP 20342	UTEP 20342	JN602055	JN602050
K. magomberae	Udzungwa Mountains, Tanzania	MTSN 8218	MTSN 8218	GQ221950	GQ221970
K. magomberae	Magombera Forest, Tanzania	MTSN 8492	MTSN 8492	GQ221952	GQ221972
K. matschiei	East Usambara Mtns, Tanzania	CAS 168852	CAS 168852	FR716605	FR716641
K. matschiei	East Usambara Mtns, Tanzania	CT105	N/A	GQ221946	GQ221967
K. multituberculata	West Usambara Mtns, Tanzania	CT110	PEM R5735	DQ923824	EF014308
K. multituberculata	West Usambara Mtns, Tanzania	CT111	N/A	GQ221947	GQ221968
K. msuyae	Livingstone Mountains, Tanzania	CT498	N/A	LN997632	
K. msuyae	Livingstone Mountains, Tanzania	CT500	N/A	LN997633	LN997642
K. msuyae	Livingstone Mountains, Tanzania	Ludewa	N/A	LN997638	
K. msuyae	Livingstone Mountains, Tanzania	MTSN7497	MTSN7497	LN997637	LN997643
K. msuyae	Udzungwa Mountains, Tanzania	MTSN8686	MTSN8686	LN997639	
K. msuyae	Livingstone Mountains, Tanzania	MTSN9373	MTSN9373	LN997634	LN997644
K. msuyae	Livingstone Mountains, Tanzania	MTSN9374	MTSN9374	LN997635	LN997645
K. msuyae	Livingstone Mountains, Tanzania	MTSN9375	MTSN9375	LN997636	LN997646
K. msuyae	Livingstone Mountains, Tanzania	MTSN9377	MTSN9377		LN997647
K. msuyae	Livingstone Mountains, Tanzania	MTSN9378	MTSN9378		LN997648
K. oxyrhina	Uluguru Mountains, Tanzania	CT192	PEM R16569	DQ923831	EF014315
K. oxyrhina	Uluguru Mountains, Tanzania	CT193	PEM R16552	DQ923832	EF014316
K. tavetana	Mount Kilimanjaro, Tanzania	CT113	PEM R5736	DQ991233	FJ717801
K. tavetana	Mount Meru, Tanzania	CT207	PEM R16563	DQ923833	EF014317
K. tavetana	North Pare Mountains, Tanzania	MTSN 8661	MTSN 8661	FR716615	FR716649
K. tenuis	East Usambara Mtns, Tanzania	CAS 168917	CAS 168917	DQ923834	EF014318
K. tenuis	East Usambara Mtns, Tanzania	CT103	PEM R5731	DQ923835	EF014319
K. uluguruensis	Uluguru Mountains, Tanzania	CT189	PEM R16565	DQ923825	EF014309
K. uluguruensis	Uluguru Mountains, Tanzania	CT191	PEM R16557	DQ923826	EF014310
K. uthmoelleri	South Pare Mtns, Tanzania	CT151	PEM R16585	DQ923836	EF014320
K. uthmoelleri	Mount Hanang, Tanzania	CT339	N/A	DQ923837	EF014321
K. vanheygeni	Poroto Mountains, Tanzania	CT490		LN997631	LN997649
K. vanheygeni	Poroto Mountains, Tanzania	SCHP-08-R-50		LN997640	LN997650
K. vanheygeni	Poroto Mountains, Tanzania	SCHP-08-R-91		LN997641	LN997651
K. vosseleri	East Usambara Mtns, Tanzania	CAS 168921	CAS 168921	GQ221943	GQ221964
K. vosseleri	East Usambara Mtns, Tanzania	CT104	N/A	GQ221945	GQ221966
K. xenorhina	Rwenzori Mtns, Uganda	CT350	PEM R16570	DQ923838	EF014322
K. xenorhina	Rwenzori Mtns, Uganda	CT351	PEM R15568	DQ923839	EF014323

Table 3. Sequence divergence (p-distances) for *Kinyongia* within species (on diagonal) and between selected species for 16S (lower matrix). Populations of K. *msuyae* from Livingstone and Udzungwa are given separately. N/A = not available.

	1	2	3	4	5
1 *K. msuyae* (Livingstone)	0.0006				
2 *K. msuyae* (Udzungwa)	0.0129	N/A			
3 *K. tenuis*	0.0262	0.0292	0.0000		
4 *K. oxyrhina*	0.0208	0.0235	0.0246	0.0000	
5 *K. magomberae*	0.0269	0.0370	0.0137	0.0186	0.0000

short horn protruding over the snout by 3 to 5 mm. The appendage is plated with subequal rounded tubercules. Laterally, the appendage continues from the supra-orbital crest, formed by low peaked tubercles, becoming more serrated over the anterior rim, from where it continues forward as a scaly rostral short horn. In the males examined it extends between 3 and 4 mm beyond the anterior margin of the rostral scale. Females lack any rostral appendage and have a lower casque.

K. msuyae does resemble *K. vanheygeni* Necas, 2009 and, to a lesser extent, *K. uthmoelleri* (Müller, 1938) in size, general body and head shape and by possession of a single, bone-based rostral appendage in males. It differs from *K. vanheygeni* in the length of the rostral appendage being longer, formed by more than ten scales and pointing straight forward (less than ten scales and slightly pointing upward in *K. vanheygeni*), from *K. uthmoelleri* by having a horn-like longer rostral appendage (canthal scales in *K. uthmoelleri* males meet to form a 'rostral wall', or protruding in form of a very short rostral projection).

Kinyongia msuyae can easily be distinguished from the other known *Kinyongia* species by the combination of the following characters: (1) presence of rostral process in males formed by the partial fusion of the *canthi rostrales* and protruding forward over the snout by 3 to 5 mm. (2) tail longer than SVL in both sexes, and (3) gular, ventral and dorsal crest absent.

Description of the holotype

Adult male. Total length 162.4 mm, SVL 73.7, Tail length 88.7. Casque elongated, posteriorly raised, covered by flattened polygonal scales, giving it a smooth appearance. Parietal crest formed by a series of low peaked tuberculated scales, temporal and orbital crests present. No occipital lobes. Nostril posteriorly directed, positioned halfway between tip of snout and the anterior rim of the eye, and separated from upper labials by two to three rows of flattened scales. Canthi rostrales converge above and before the nostrils in forming a single, short, rostral appendage with two tips, giving the appearance of two very short horns protruding beyond the rostral scale by 4.5 mm. The rostral process is completely ossified and covered by sub-equal, convex scales, the superior edge is serrated. Upper labials 16, lower labials 15 on each side. The sides of gular region is lined with 6 shallow grooves on each side. There is no dorsal or gular crest, while the central part of the gular region has no groove. No signs of dorsal, or ventral crests. Scales on body flat and homogeneous, arranged is small clusters, those on the upper part of the dorsum are more quadrangular and arranged in vertical rows. Scales on limbs sub-equal, rounded, and flattened. Tail longer than the snout/vent length, laterally compressed, and covered by quadrangular scales arranged in vertical rows. Hemipenes: unenverted.

Colour in preservative: The overall colour is whitish-grey with few paler areas

Paratype variation: Paratypes show no relevant morphological variation compared to the holotype. Variation in scutellation and body proportions for the type series and referred material are shown in Table 2.

Colour in life: *K. msuyae* is an overall brown to green chameleon, sometimes with broad pale transversal bands and scattered blue spots formed by single scales or clusters of several scales. Females have often a larger round spot of contrasting colour on the flanks (Fig. 1). The tip of the snout, rostral appendage and limbs and top of the casque are often brownish to grey.

Distribution: Refer to Figure 2.

Etymology: The species is named after and dedicated to Charles A. Msuya, a pioneer of Tanzanian herpetology, who collected the first known specimen attributable to this species and has spent most of his life studying Tanzanian wildlife.

DISCUSSION

The phylogenetic analyses, sequence divergence estimates, and morphological assessment suggest that there is a previously unknown but distinctive species of *Kinyongia* from the Livingstone and Udzungwa mountains, which we describe as *Kinyongia msuyae*. This species is sister to chameleons found in the Eastern Arc Mountains (i.e. *K. tenuis* from Usambara Mountains, *K. magomberae* from Udzungwa Mountains, and *K. oxyrhina* from Uluguru Mountains). There was no clear morphological differentiation between the population on the Udzungwa and that from Livingstone Mountains, despite these two mountain ranges being separated by the Makambako Gap (ca. 150 km apart). Sequence divergence between chameleons from these localities less than what is normally found between species. Population level differences may exist, but additional sampling would be required to confirm that hypothesis.

The close relationship among populations has some important biogeographic implications. The Southern Highlands have long been regarded as isolated and not part of the Eastern Arc Mountains, with the Makambako gap considered inhospitable, preventing dispersal. However, recent molecular data has started to alter this view. Phylogenetic analyses for the shrew, *Myosorex*, suggest that the Makambako Gap is of little consequence in the historical biogeography of the genus (Stanley and Esseltyn, 2010). Similarly, there is little morphological variation among populations of the murid rodent *Hylomyscus arcimontensis* on either side of the Makambako Gap (Carleton and Stanley, 2005), and the newly discovered kipunji monkey (*Rungwecebus kipunji*) has populations on both sides of the gap (Jones et al., 2005; Davenport et al., 2006). There are also some commonalities in the avifauna among populations on the Nyika Plateau, Mount Rungwe, and the southern Udzungwas, with no evidence of the Makambako Gap having a biogeographic influence (Stuart et al., 1993). Furthermore the Southern Highlands might have served as a dispersal route for amphibians, connecting the Udzungwa and the Mahenge Mts, the two southernmost mountain blocks of the Eastern Arc (Menegon et al., 2011; Loader et al., 2014).

Interestingly, from a biogeographic perspective, the most suitable dispersal route for forest endemics from Udzungwa and Mahenge mountains – both part of the Eastern Arc Mountains - does not appear to be the shortest straight line distance, which would require crossing the Kilombero Valley (an ancient, deep, wide valley). Instead, the continuous ridge of highlands connecting the southern Udzungwa, through the Southern Highlands/Livingstone Mountains via the Makambako Gap and then northeast to the Mahenge Mountains may have remained more suitable over historical times, potentially with forested areas (see Fig. 2).

The description of *Kinyongia msuyae* provides a tantalizing piece of evidence suggesting strong biogeographical affinities between the Southern Highlands (i.e. Livingstone Mountains) and the Eastern Arc Mountains (i.e. Udzungwa Mountains). The Makambako Gap may not be a turn-over region of high significance between the EAM and the Southern Highlands, rejecting previous claims of its biogeographical importance as a barrier. Instead, it is likely that some taxa can or have crossed this barrier, or that the gap was formerly less dry, forming a corridor between Udzungwa and the Southern Highlands. Our increased understanding of the Southern Highlands is revealing that the region is more species rich than had been supposed, possibly similar in scale to some of the Eastern Arc Mountain forests.

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REFERENCES

Branch, W.R., Bayliss, J., Tolley, K.A. (2014): Pygmy chameleons of the *Rhampholeon platyceps* complex (Squamata: Chamaeleonidae): Description of four new species from isolated 'sky islands' of northern Mozambique. Zootaxa **3817**: 1-36.

Carleton M.D., Stanley W.T. (2012): Species limits within the *Praomys delectorum* group (Rodentia: Muridae: Murinae) of East Africa: a morphometric reassessment and biogeographical implications. Zool. J. Linn. Soc. **165**: 420-469.

Caro L.M., Caycedo-Rosales P.C., Bowie R.C.K., Slabbekoorn H., Cadena C.D. (2013): Ecological speciation along an elevational gradient in a tropical passerine bird? J. Evol. Biol. **26**: 357-374.

Davenport T., Stanley W.T., Sargis E.J., De Luca D.W., Mpunga N.E., Machaga S.J., Olson L.E. (2006): A new genus of African monkey, *Rungwecebus*: Morphology, ecology, and molecular phylogenetics. Science **312**: 1378-1381.

Dimitrov D., Nogues-Bravo D., & Scharff N. (2012) Why Do Tropical Mountains Support Exceptionally High Biodiversity? The Eastern Arc Mountains and the Drivers of *Saintpaulia* Diversity. PLoS ONE **7**: e48908.

Greenbaum, E., Tolley, K.A., Joma, A., Kusamba, C. (2012): A new species of chameleon (Sauria: Chamaeleonidae: *Kinyongia*) from the northern Albertine Rift, Central Africa. Herpetologica **68**: 60-75.

Huelsenbeck, J.P.,Ronquist, F. (2001): MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics **17:** 754-755.

Jones T., Ehardt C.L., Butynski T.M., Davenport T.R.B., Mpunga N.E., Machaga S.J., & De Luca D.W. (2005) The Highland Mangabey *Lophocebus kipunji*: A New Species of African Monkey. Science **308**: 1161-1164.

Loader, S.P., Ceccarelli, S., Menegon, M., Howell, K.M., Kassahun, R., Mengistu, A.A., Saber, S.A., Gebresenbet, F., S_a, R., Davenport, T.R.B., Larson, J.G., Mueller, H., Wilkinson, M., Gower, D.J. (2014): Persistence and stability of Eastern Afromontane forests: evidence from brevicipitid frogs. J. Biog. **41**: 1781-1792.

Loader, S.P., Lawson L., Portik D. M., Menegon M. (2015): Three new species of spiny throated reed frogs (Anura: Hyperoliidae) from evergreen forests of Tanzania. BMC Res. Notes **8**: 167.

Lovett, J.C., Wasser S.K. (1993): Biogeography and ecology of the rain forest of Eastern Africa. Cambridge University Press.

Macey, J.R., Larson, A., Ananjeva, N.B, Papenfuss, T.J. (1997a): Evolutionary shifts in three major structural features of the mitochondrial genome among iguanian lizards. J. Mol. Evol. **44**: 660-674.

Macey, J.R., Larson, A., Ananjeva, N.B., Papenfuss, T.J. (1997b): Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. Mol. Biol. Evol. **14**: 91-104.

Mariaux J., Lutzmann N., Stipala J. (2008): The two horned chameleons of East Africa. Zool. J. Linn. Soc. **152**: 367-391.

Mariaux J.,Tilbury C.R. (2006): The pygmy chameleons of the Eastern Arc Range (Tanzania): Evolutionary relationships and the description of three new species of *Rhampholeon* (Sauria : Chamaeleonidae). Herpetol. J. **16**: 315-331.

Menegon, M., Tolley, K.A., Jones, T., Rovero, F., Marshall, A.R., Tilbury, C.R. (2009): A new species of chameleon (Sauria: Chamaeleonidae: Kinyongia) from the Magombera Forest and the Udzungwa Mountains National Park, Tanzania. Afr. J. Herpetol. **58**: 59-70.

Menegon, M., Salvidio, S., Tilbury, C. (2002): A new dwarf forest chameleon from the Udzungwa Mountains of Tanzania, East Africa, (Squamata*: Rhampholeon,* Günther 1874). J. Herpetol. **36**: 51-57.

Menegon, M., Davenport, T.R.B. (2008): The amphibian fauna of the Eastern Arc Mountains of Kenya and Tanzania. In: Threatened Amphibians of the World. Stuart, S. Hoffmann, M., Chanson, J., Cox, N., Berridge, R., Ramani P., Young, B. Eds, Lynx Editions, Barcelona.

Miller, M.A., Pfeiffer, W., Schwartz, T. (2010): Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, pp 1-8. IEEE Xplore Digital Library, New Orleans, LA.

Necas P. (2009): Ein neues Chamäleon der Gattung *Kinyongia* Tilbury, Tolley & Branch 2006 aus den Poroto-Bergen, Süd-Tansania (Reptilia: Sauria: Chamaeleonidae). Sauria **31**: 41-48.

Newmark W.D. (1998): Forest Area, Fragmentation, and Loss in the Eastern Arc Mountains: Implications For the Conservation of Biological Diversity. J. East Afr. Nat. Hist. **87**: 29-36.

Palumbi, S.R. (1996): Nucleic Acids II: The polymerase chain reaction. In: Molecular systematics, pp. 205-247. Hillis, D.M., Moritz, C. Mable, B.K., Sinauer Associates, Sunderland, MA.

Posada, D. (2008): Jmodeltest: Phylogenetic model averaging. Mol. Biol. Evol. **25**: 1253-1256.

Rambaut, A., Drummond, A.J. (2007): Tracer. Version 1.4. http://tree.bio.ed.ac.uk/software/tracer/

Ronquist, F., Huelsenbeck, J.P. (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics **19**: 1572-1574.

Rovero F., Menegon M., Fjeldså J., Collett L., Doggart N., Leonard C., Norton G., Owen N., Perkin A., Spitale D., Ahrends A., Burgess N.D. (2014): Targeted vertebrate surveys enhance the faunal importance and improve explanatory models within the Eastern Arc Mountains of Kenya and Tanzania. Diver. Distrib. **20**: 1438-1449.

Stamatakis, A. (2006): RaxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics **22**: 2688-2690.

Stamatakis, A., Hoover, P., Rougemont, J. (2008): A rapid bootstrap algorithm for the RaxML web servers. Syst. Biol. **57**: 758-771.

Stanley W.T., Esselstyn J.A. (2010): Biogeography and diversity among montane populations of mouse shrew (Soricidae: Myosorex) in Tanzania. Biol. J. Linn. Soc. **100**: 669-680.

Stanley W.T., Rogers M.A., Hutterer R. (1999): A new species of *Congosorex* from the Eastern Arc Mountains, Tanzania, with significant biogeographical implications. J. Zool. **265**: 269-280.

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. Mol. Biol. Evol. **28**: 2731-2739.

Tilbury, C.R. (2010): Chameleons of Africa - An Atlas. Including the Chameleons of Europe, the Middle East and Asia. Edition Chimaira, Frankfurt am Main.

Tilbury, C.R., Tolley, K.A. (2009): A re-appraisal of the systematics of the African genus *Chamaeleo* (Reptilia: Chamaeleonidae). Zootaxa **2079**: 57-68.

Tilbury, C.R., Tolley, K.A. (2009): A new species of dwarf chameleon (Sauria; Chamaeleonidae) from KwaZulu-Natal, South Africa with notes on recent climatic shifts and their influence on speciation in the genus. Zootaxa **2226**: 43-57.

Tilbury C.R., Tolley K.A., Branch W.R. (2006): A review of the systematics of the genus Bradypodion (Sauria: Chamaeleonidae), with the description of two new genera. Zootaxa, **1363**: 23-38.

Tilbury C.R., Emmrich D. (1996): A new dwarf forest chameleon (Squamata: *Rhampholeon* Günther 1874) from Tanzania, East Africa with notes on its infrageneric and Zoogeographic relationships. Trop. Zool. **9**: 61-71.

Tolley K., Menegon M. (2013): Evolution and biogeography of Chameleons. In: The Biology of Chameleons, pp. 131-150. Tolley, K, Herrell, A. Eds, University of California Press, Berkeley.

Tolley, K.A., Tilbury, C.R., Measey, G.J., Menegon, M., Branch, W.R., Matthee, C.A. (2011): Ancient forest fragmentation or recent radiation? Testing the refugial speciation model with East Africa's most diverse clade of endemic chameleons, *Kinyongia*. J. Biogeogr. **38**: 1748-1760.

Tolley, K.A., Townsend, T.M., Vences, M. (2013): Large-scale phylogeny of chameleons suggests African origins and Eocene diversification. Pr. R. Soc. London, B. **280**: 20130184.