Original article

# A new species of chameleon (Sauria: Chamaeleonidae: *Kinyongia*) from the Magombera forest and the Udzungwa Mountains National Park, Tanzania.

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Abstract.—A new species of chameleon Kinyongia magomberae sp. nov. (the Magombera chameleon), is described from Magombera forest and the Udzungwa Mountains National Park, south-central Tanzania. The species status is supported by phylogenetic analyses using mitochondrial and nuclear markers. The new chameleon is similar to *K. tenuis* and the more widespread Eastern Arc endemic *K. oxyrhina* in having a single rostral appendage. However, it can be distinguished from these two species by a shorter rostral appendage, which is different in shape and lacks a movable tip. *K. magomberae* sp. nov. is known from only two forest localities, the lowland and unprotected Magombera forest and the sub-montane Mwanihana forest within the Udzungwa Mountains National Park. Phylogenetically, this taxon is sister to *K. tenuis*, and is part of a clade that also contains *K. oxyrhina*.

Key words.—Chamaeleonidae, Kinyongia, New Species, Phylogeny, Eastern Arc Mountains, mitochondrial and nuclear DNA

The Eastern Arc Mountains of Kenya and Tanzania are within one of the most prominent biodiversitv hotspots on earth (Mittermeier et al. 2004) and remarkably, a growing number of new vertebrates species are discovered there each year (e.g. Blackburn 2009, Doggart et al. 2006; Jones et al. 2005; Menegon et al. 2008; Müller et al. 2005, Rovero et al. 2008). A prime example is the chameleons, for which new species are steadily described, or previously named taxa elevated from synonymy (Tilbury & Emmrich 1996; Menegon et al. 2002; Lutzmann & Necas 2002; Mariaux & Tilbury 2006; Mariaux et al. 2008) and a new East African genus (Kinyongia) has recently been erected (Tilbury et al. 2006). Regardless of this recent upsurge in discovery, each new survey in the Eastern Arc Mountains continues to uncover new species of chameleons (Menegon et al. 2008). Although there are several genera (Chamaeleo, Rieppeleon, Rhampholeon, Trioceros. Kinyongia) of chameleons present within the Eastern Arc (Matthee et al. 2004; Tilbury et al. 2006; Tilbury & Tolley 2009; Spawls et al. 2004), the representatives of the genus Kinyongia are prominent due to their striking morphology and for the high number of species. This group of chameleons underwent significant taxonomical turbulence over the last century (see Mariaux *et al.* 2008), however the addition of molecular systematics to the taxonomy toolbox has recently helped to stabilise the taxonomy, and to resolve the evolutionary relationships of this genus (e.g. Mariaux *et al.* 2008; Tilbury *et al.* 2006).

Ten of the fifteen described species of Kinvongia occur on isolated massifs within the Eastern Arc Mountains, with the remainder found to the northwest in mountainous regions of the Albertine Rift in Burundi, Democratic Republic of the Congo, Uganda and Rwanda (Spawls et al. 2004), and on isolated Kenyan volcanoes. Many species have small distributional ranges, and are usually found on the forested slopes of just one or a few isolated massifs. Given the expansion of the savannah biome and the subsequent contraction of forests over the Pliocene and Pleistocene (Lovett & Wasser, 1993), this biogeographic pattern suggests that each species has undergone a long history of isolation. Such biogeographic patterns are not uncommon to vertebrates in the Eastern Arc (e.g. Blackburn 2009; Bowie et al. 2004; Bowie et al. 2006; Mariaux et al. 2008; Measey et al. 2007), suggesting that many Eastern Arc species have diversified as a consequence of long periods of isolation in forests that were once connected. Within the Eastern Arc, the Udzungwa Mountains are the southernmost massif, lying approximately 300 km northeast of Lake Malawi.

In 2002, the first specimen - a dead juvenile male - of an apparently undescribed species was collected by F.R. in submontane forest habitat (900 m a.s.l.) in the Mwanihana forest, within the Udzungwa Mountains National Park. Although the specimen was in very poor condition, a preliminary morphological investigation suggested that the specimen belonged to an undescribed species. In 2003, a live male specimen was collected by T.J. in a nearby locality in the same forest (700 m a.s.l.). The third specimen, also a male and designated as the holotype of the new taxon herein described, was observed in 2005 by A.R.M. being predated upon by a twig snake, *Thelotornis mossambicanus*, in Magombera Forest. The snake, disturbed by the presence of the human observer, dropped the already dead chameleon on the forest floor and it was collected the next day by M.M.

All the specimens collected to date, possess a firm, single, laterally compressed, blade-like rostral process on the snout. This is a distinguishing character for a sub-group within Kinyongia that are morphologically similar (K. tenuis, K. oxyrhina). Chameleons of this group are easily recognizable by the following morphological features: small to medium size chameleons, males bear a single laterally compressed blade-like horn, while females are both hornless or with a short horn, tail longer or equal to the body length, absence of dorsal or ventral crest and, in general, a smooth appearance. In this study, we used a combined evidence approach to 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.

### MATERIALS AND METHODS

*Molecular Analysis.*—To determine the taxonomic placement of the new species within the genus *Kinyongia*, a phylogenetic analysis of 31 chameleons from this genus was carried out. The analysis included representatives of almost all described species within the genus, plus two (designated holotype MTSN 8492 and paratype MTSN 8218) specimens of the potentially new species (Table 1). The genus *Kinyongia* has previously been shown to be monophyletic (Tilbury *et al.* 2006), so the outgroup consisted of two individuals from a different genus within the Chamaeleonidae (Table 1: Bradvpodion pumilum and B. melanocephalum). Sequences from 20 of these individuals have been published previously (Table 1). DNA extraction, PCR amplification, and cycle sequencing of two mitochondrial gene fragments were carried out following standard procedures formerly outlined in Tolley et al. (2004) using the following primers for ND2: L4437b (Macey et al. 1997a) and H5934 (Macey et al. 1997b), and 16S: L2510 and H3080 (Palumbi 1996). An 821 bp portion of the nuclear gene RAG1 was amplified and sequenced using primers F118 and R1067 (Matthee et al. 2004). Standard PCR and sequencing were followed for this gene fragment, with PCR annealing temperature at 57° C. All new sequences have been deposited in GenBank (Table 1). Voucher specimens are as listed in Table 1.

A Bayesian analysis of 2 132 characters from the two mitochondrial genes (ND2, 856 bp and 16S, 462 bp) and the one nuclear gene (RAG1, 718 bp) was used to investigate optimal tree space using MrBayes 3.1.0 (Huelsenbeck & Ronquist 2001). Modeltest 3.6 (Posada & Crandall 1998) was initially run to investigate the evolutionary model that best fits the data set. Both the AIC and LRT test specified the most complex model (GTR+I+G) for the combined dataset, so MrBayes was run specifying six rate categories with uniform priors for all parameters. Several data partitions were created, which were unlinked and allowed to run with separate values for the model parameters. A single data partition was created for 16S, although 32 bases (247 - 260 and 274 - 291) were removed due to poor alignment. There were three partitions for ND2: 1st, 2nd, and 3rd codons separately, and three partitions for RAG1: 1st, 2nd, and 3rd codons separately. To confirm that the model used was not over-parameterised, an additional MCMC was run with only 3 partitions (one for each marker). To ensure the results converged on the same topology, the MCMC was run in parallel, twice for each of the two models (for 10 million generations each), with trees sampled every 1 000 generations. The first 1 million generations (1 000 trees) were removed as burn-in, after examination of the average standard deviation of split frequencies (< 0.001), the convergence diagnostic (PSRF values ~ 1.0) as well as the log-probabilities and the values of each parameter for stabilisation (Ronquist & Huelsenbeck 2003). A 50% majority rule tree was constructed and nodes with  $\geq 0.95$  posterior probability considered as supported. A parsimony analysis was also run using the same data set as in the Bayesian analysis. A heuristic search was run with 1 000 random replicates and 100 trees saved each replicate. One thousand bootstrap replicates were run to evaluate confidence in the nodes (100 random addition replicates, saving 100 trees per replicate).

#### RESULTS

Molecular Analysis.-The Bayesian analysis showed a number of clades supported by  $\geq$ 95% posterior probability (pp) that correspond with previously described species (Fig. 1). Specimens from the Udzungwa Mountains and Magombera formed a well-supported clade (100 pp), verifying the specific status of this taxon. Although sequence divergence values should not be used as a means to define species (Ferguson 2002), values between this taxon and its sister species (K. tenuis) were comparatively high (2.9% 16S, 8.8% ND2, <1% RAG). These values are similar to that found between other species in this genus (Tilbury *et al.* 2004) as well as among other chameleons (Tolley et al. 2004, 2006). The parsimony analysis (tree not shown) produced two equally parsimonious trees (1883 steps, CI 0.58, RI 0.76) that differed only in terminal branch swapping. The parsimony trees had the same overall topology and supported nodes (>90% bootstrap) as the Bayesian analysis, with a few exceptions where

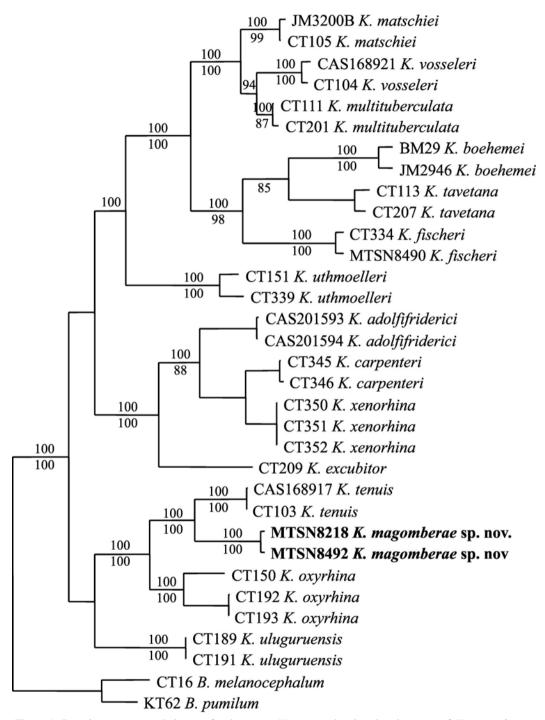


Figure 1. Bayesian consensus phylogram for the genus *Kinyongia*, showing the placement of *K. magomberae*. Posterior probabilites for each node are given above the branches, while parsimony bootstrap values are given below.

the Bayesian analysis performed slightly better. Regardless, the placement of the new taxon was fully supported in both analyses.

*Material examined.*—The following specimens were examined from the herpetological collections of Museo Tridentino di Scienze Naturali, Trento, Italy and the Department of Zoology and Marine Biology of the University of Dar es Salaam, Dar es Salaam, Tanzania: K. tenuis (KMH 21325 and KMH 21304 from Nilo Forest Reserve, East Usambara Mts.). K. oxvrhina (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.).

### TAXONOMY

*Kinyongia magomberae* **sp. nov.** (Fig. 2)

*Holotype.*—An adult male in the Museo Tridentino di Scienze Naturali, Trento, MTSN 8492 collected in April 2005 in Magombera Forest by Michele Menegon.

*Type locality.*—Magombera Forest, 270 m above sea level, Kilombero District, Morogoro Region, South Eastern Tanzania (4°66' S, 38°25' E).

*Paratypes.*—MTSN 8218, adult male, collected in 2003 by Trevor Jones in Mizimu, Mwanihana Forest, Udzungwa Mountains National Park.

*Referred material.*—MTSN 5897 juvenile, found dead (in poor condition) by Francesco Rovero, July 2002 above Mizimu, at about 900 m asl in Mwanihana Forest, Udzungwa Mountains National Park.

*Diagnosis.*—A small chameleon, lacking any distinctive colours or pattern, with a tail longer

than the snout/vent length. It has a very short, scaly, bone -based rostral appendage, laterally compressed, and immoveable. The appendage is plated with subequal rounded tubercules. In the lateral view it appears sub-triangular in shape, with crenulated edges and it originates above the nostrils at the conjunction of canthi rostrales. In the males examined it extends not more than 2 mm beyond the anterior margin of the rostral scale. To date no females have been collected. The Magombera chameleon can easily be distinguished from most other known Kinyongia by the combination of a long tail and the single flattened rostral appendage. However, K. magomberae does resemble K. oxyrhina Klaver & Bohme 1988 and especially K. tenuis (Matschie, 1892) in size, body and head shape and by possession of a single, bonebased rostral appendage in males. It differs from K. oxyrhina and K. tenuis in the shape of the rostral appendage being very short, subtriangular and formed by subequal rounded tubercules in K. magomberae, but elogated, projecting forward and formed by heterogeneous scales both in K. tenuis and K. oxyrhina.

Description of the holotype.--Adult male. Casque elongated, not raised, covered by flattened polygonal scales, giving it a smooth appearance. Parietal crest formed by seven tuberculated scales, very faint temporal and orbital crests present. Nostril laterally directed, positioned halfway between tip of snout and the anterior rim of the eye, and separated from upper labials by two rows of granular scales. Canthi rostrales converge above and before the nostrils in forming a single, short, sub-triangular rostral appendage, protruding beyond the rostral scale by 2 mm. The rostral process is completely ossified and covered by sub-equal, convex scales, the superior edge is crenulated. Upper labials 14, lower labials 13 on each side. The gular region is lined with 18 shallow grooves. There is no gular crest. No signs of dorsal, or ventral crests. Scales on body flat and homogeneous. Scales on limbs subequal, rounded, and flattened. Tail longer than the snout/vent length, laterally compressed, and covered by quadrangular scales. The holotype specimen has a hole about 6 mm wide on the right flank, probably due to insects activity while lying on the forest floor. Hemipenes: uneverted.

*Colour in preservative.*—The overall colour is whitish-grey with few, scattered black speck-ling.

*Paratype variation.*—The paratype, a male, shows no relevant morphological variation compared to the holotype. Variation in scutella-

tion and body proportions for the type series are shown in Table 1.

*Colour in life.*—There are very few available photos or observations of this species in life. *K. magomberae* appears to be an overall pale chameleon, often homogeneously greyish or brown. A live specimen found hiding on the forest floor was white with black transverse bands. The best photo available (Fig. 2) shows an individual (not collected) with an overall grey colouration. The tip of the snout, rostral appendage and limbs are green, the top of the casque and eyes red-brown. There are scattered blue spots, especially on the tail, shoulders,

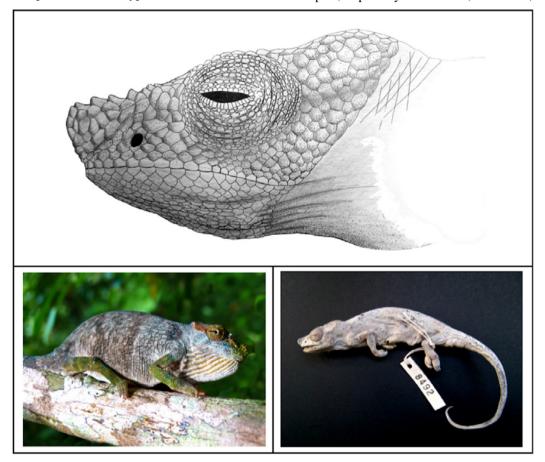


Figure 2. Top: Head scalation of the holotype MTSN 8492, Bottom right: photo of the holotype Bottom left: photo of a living specimen of *Kinyongia magomberae* showing colouration.

Table 1. Sample numbers, collecting localities, voucher accession numbers (PE Tridentino di Scienze Naturali, and GenBank accession numbers (ND2, 16S, RAC tion not available. * indicates new data. Ug - Uganda, Ky - Kenya, Tz - Tanzania	cting localities, v nd GenBank acce w data. Ug - Uga	Table 1. Sample numbers, collecting localities, voucher accession numbers (PEM=Port Elizabeth Museum, CAS = California Academy of Sciences, MTSN = Museo Tridentino di Scienze Naturali, and GenBank accession numbers (ND2, 16S, RAG1) for chameleons used in this study. (T) = Topotype, NA = data, specimen, or information not available. * indicates new data. Ug - Uganda, Ky - Kenya, Tz - Tanzania.	n, CAS = Califo t this study. (T) =	mia Academy o Topotype, NA =	f Sciences, MT = data, specime1	SN = Museo 1, or informa-
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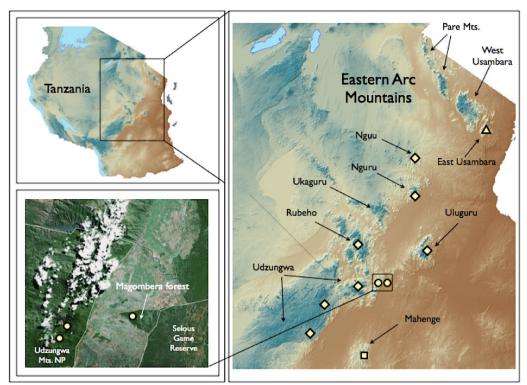


Figure 3. Maps showing the Tanzanian Eastern Arc Mountains with collection localities of the three currently known species of single-blade horned *Kinyongia* species (circle - *K. magomberae*, diamond - *K. oxyrhina*, triangle - *K. tenuis*, square *Kinyongia* sp.). The location of the collecting sites of *K. magomberae* within the Udzungwa Mountains National Park and in Magombera forest are also shown (bottom left).

limbs and head. The gular grooves are orange in colour.

*Etymology.*—The Magombera chameleon *Kinyongia magomberae* is named after the type locality, Magombera forest, a currently unprotected lowland forest of great biological importance.

Habitat and distribution.—Kinyongia magomberae has a limited range, occurring in only two forests in the Udzungwa Mountains range: Magombera Forest, in the Kilombero valley, and at low and medium altitude in the Mwanihana forest, within the Udzungwa Mountains National Park (UMNP) as shown in Fig. 3. Magombera is an unprotected forest fragment of 11.89 km<sup>2</sup>, approximately 6 km

east of UMNP. Its vegetation is a unique mix of lowland forest, coastal forest, and woodland species including dominant canopy trees Lettowianthus stellatus, Isoberlinia scheffleri, Erythrophleum suaveolens and Xylopia parviflora, and areas of swamp forest dominated by Syzygium sp. Mwanihana is a large forest block (177 km<sup>2</sup>) inside UMNP, and is the eastern forested escarpment with continuous vegetation cover from 300 m to over 2 000 m a.s.l. The sites where K. magomberae has been found are within the sub-montane vegetation zone, consisting of a mosaic of semi-evergreen and evergreen forest species, dominated by Parinari excelsa (details of vegetation in Lovett et al. 1988).

K. magomberae is the third species of single

blade-horned chameleon known from Tanzania. The sister species of K. magomberae is K. tenuis (Matschie, 1892) which is known to occur in the Usambara Mountains, Magrotto Hill of Tanzania, and the Shimba Hills in southeastern Kenya. K. oxyrhina (Klaver & Böhme, 1988) was previously described from the Uluguru and Udzungwa Mountains only. However, recent herpetological surveys in the Eastern Arc Mountains have resulted in several additional distribution records of chameleons assignable to of K. oxyrhina based on morphology. The species appears to be quite widespread in the central and southern Eastern Arc Mountains, ranging from Nguu Mountains in the north to Lulanda forest on the Udzungwa Mountains in the south (Menegon & Salvidio 2004; Menegon et al. 2008; UDSM collection, this paper). A molecular-based taxonomic assessment of the several isolated population of chameleons assigned to the species K. oxyrhina would be of great importance in order to understand their evolutionary relationship and detect the presence of cryptic taxa. A fourth *Kinyongia* species, still undescribed, is known from a single female collected on Mahenge Mountains.

## **DISCUSSION**

The new taxon, Kinyongia magomberae, is a well-supported lineage with sequence divergence values comparable to that found between most other Kinyongia species, thus supporting the species designation. Kinvongia magomberae is the sister taxon of K. tenuis, which is found within a restricted range across north-eastern Tanzania and south-eastern Kenva (East Usambara Mountains, Magrotto Hill and Shimba Hills). These two closely related species are both associated with submontane forest of the Eastern Arc Mountains, although they are separated by a gap of about 380 km of arid savannah. It appears that the history of the blade-horned chameleons may be the result of the isolation of populations on forested mountain massifs. Presumably, a common ancestor to these species was more widespread throughout Tanzania and southern Kenya, but climateinduced changes in forest cover have led to fragmentation of the forest habitat. Similar biogeographic patterns in relation to expansion and contraction of forest have been suggested as an explanation for other taxa with similar distributions e.g. birds and frogs (e.g.

**MTSN 8492 MTSN 8218** Character Snout-Vent Length (SVL): tip of the snout to the anterior edge of the cloaca 72.0 71.0 Tail Length (Tail): tip of tail to posterior edge of the cloaca 73.5 72.0 Total Length (TL): combined SVL and Tail Length 145.5 143.0 Head length (HL): from tip of the casque to the tip of the snout 20.5 19.7 Head width (HW): maximum width of head 7.8 8.0 (usually in the middle of the casque; Raw 1976) Casque Length (CL): minimum diagonal distance from angle of the jaw 12.7 12.3 (mouth closed) to highest point of casque Casque-Eye (CE): minimum diagonal distance from orbit to posterior 9.0 8.0 the tip of the casque 7.0 6.8 Snout Length (SL): from tip of snout to anterior margin of orbit Eye Diameter (Eye): maximum horizontal width of orbit. 6.0 5.8 Eye-Eye gap (EE): minimum width between orbits across crown. 3.6 3.2 Upper labials (UL): from rostral to (but not including) the enlarged 14 13 sub-ocular tubercle.

Table 2. Biometrics of the holotype and paratype. Continuous measurements given in mm.

Blackburn 2009; Bowie *et al.* 2004; Bowie *et al.* 2006; Measey *et al.* 2007).

Conservation.-Whilst the new species does not face any tangible threat within the Udzungwa Mountains National Park, the situation is much more worrying for Magombera Forest. This is among the last few remnants of a once continuous tropical lowland forest, long recognised for its high biodiversity value (Rodgers et al. 1979; Struhsaker & Leland 1980; Vollesen 1980). In addition to the occurrence of the regional endemic chameleon described here, Magombera has several notable features of conservation importance (Marshall 2008). Primarily, an astounding 41.2 % of large trees are either IUCN Red-Listed or restricted to East Africa. Line transect surveys have also recorded the highest encounter rate of the IUCN Endangered Udzungwa red colobus (Procolobus gordonorum) anywhere (Marshall 2008). The forest is also an important dry season refuge for elephants from the adjacent Selous Game Reserve, and is home to the Kilombero valley endemic frog Hyperolius reesi.

Despite the outstanding importance of this forest fragment, Magombera is still unprotected due to its controversial management history (Marshall 2008). Early ecological surveys in the 1970s led management authorities to agree that the area would be annexed into the Selous Game Reserve. However after degazettement of its Forest Reserve status in 1981 it was never formally annexed. The vulnerable status of Magombera Forest was re-emphasised during a 2004 WWF/CEPF workshop in Morogoro, Tanzania, discussing priorities for the Udzungwa Mountains and surrounding area. This followed threats of deforestation and subsequent development of housing for squatters from adjacent agricultural land. Considerable concern was raised by the local and international conservation community and in 2007 effort to annex Magombera forest into the

Selous Game Reserve were resumed.

Meanwhile, threats to Magombera Forest continue (Marshall 2008). Some deforestation during construction of the TAZARA railroad has reduced canopy cover and the vegetation structure is now typical of high under-storey disturbance (A.R.M. pers. obs.). One of the primary current threats is pole-cutting by locals, and this practice is known to negatively affect forest composition and even lead to local extinctions of some species (e.g. Burgess et al. 2000, Obiri et al. 2002). Given projected increases in pole-cutting, understorey trees could disappear by as soon as 2011 (Marshall 2008). Other threats include annual bushfires that prevent regeneration, but encourage growth of constricting climbers, firewood collection, timber felling and hunting. Although Kinyongia magomberae is not directly threatened by these activities, disruption to the forest matrix will likely have negative consequences on population density and resilience of this newly discovered chameleon.

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