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Cryptic diversity in pygmy chameleons (Chamaeleonidae: *Rhampholeon*) of the Eastern Arc Mountains of Tanzania, with description of six new species

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Abstract. Previous molecular phylogenetic studies of pygmy chameleons have identified several cases of undescribed cryptic diversity of species, some of which have remained undescribed due to a lack of morphological information. Here, we combine descriptive morphology with principal component analysis, to quantify the overall morphological variation, and phylogenetic analysis to describe six new species of *Rhampholeon* from the Eastern Arc Mountains, including populations found in the Udzungwa, Rubeho, Nguru, Ukaguru, and Nguu Mountains. From our study we detected only limited morphometric variation between species. We distinguish the new species using genetics, combined with assessment of morphological features, and their geographical distribution. We highlight the threats to pygmy chameleons in East Africa from habitat change and exporting live specimens for the wildlife trade. Based on our understanding, we note a few species that we consider at risk of decline – mainly based on their narrow distribution and their apparent popularity in the export market. This study also further underlines the extraordinary biological value of the relatively small forest patches (less than 3000 km^2) of the Eastern Arc, which contain more species of chameleons than any other area in mainland Africa.

Keywords. Afromontane, biodiversity, East Africa, chameleons, new species, reptiles.

INTRODUCTION

Many of the best-known species radiations (e.g., Darwin's finches, Caribbean *Anolis*, African cichlids) are characterised by high species richness with substantial morphological disparity among species. However, species radiations can also be characterised by genetic diversification with little ecological or phenotypic divergence between species. Typically, these species are distributed allopatrically, but in ecologically similar areas (Gittenberger, 1991; Losos and Mahler, 2010). Chameleons in the genus *Rhampholeon* are an example of such a radiation (e.g., Branch et

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al., 2014). They are morphologically conservative, exhibiting limited phenotypic diversity, despite notable genetic divergence that has accumulated over millions of years in isolation (Branch et al., 2014; Hughes et al., 2018). Most *Rhampholeon* species occur in fragmented and ecologically isolated mountains in East Africa, where species have evolved in allopatry (Menegon et al., 2009; Branch et al., 2014; Hughes et al., 2018). The species essentially occur in ecologically similar forests on isolated mountains of the Eastern Arc Mountains, the Albertine Rift, and the sky islands of Mozambique and Malawi. Their phenotypic similarity however has resulted in difficulties diagnosing and identifying species using morphological characters (e.g., Menegon et al., 2002; Fisseha et al., 2013).

One particular radiation demonstrating morphological conservatism is the *Rhampholeon uluguruensis/moyeri* species complex from the Eastern Arc Mountains, which shows large phylogenetic diversity across its distribution (Fisseha et al., 2013). However, external morphological diversity appears to be low. For example, *Rhampholeon uluguruensis* from the Uluguru Mountains (Tilbury and Emmrich, 1996) was the first *Rhampholeon* found in the Eastern Arc Mountains to bear a soft, tuberculated rostral process. This feature is similar to that of *Rhampholeon nchisiensis* (Loveridge, 1953) from the Southern Highlands of Tanzania and Malawi, and of *Rhampholeon boulengeri* (Steindachner, 1911) from the montane forests of the Albertine Rift. Subsequently, the morphological analysis of *Rhampholeon* individuals from the Kihanga and Kitolomero valleys of the Uzungwa Scarp Nature Reserve bore strong resemblance to *R. uluguruensis* but were distinguished by the number of their interorbital scales and the number and arrangement of hemipenial papillae (Menegon et al., 2002). These differences became the basis for describing a new species, *R. moyeri* (Menegon et al., 2002).

Phylogenetic analyses that have incorporated wide geographic coverage across the Eastern Arc Mountains have shown highly divergent lineages within *R. moyeri* with distinct lineages endemic to single mountain blocks (Matthee et al., 2004; Fisseha et al., 2013). Furthermore, *R. uluguruensis,* restricted to the Uluguru Mountains, is deeply divergent from other members of the clade (Fisseha et al., 2013). Moreover, there are two highly divergent clades from the Uzungwa Scarp Nature Reserve populations, both currently assigned to *R. moyeri* suggesting that *R. moyeri* as currently understood is paraphyletic (Fisseha et al., 2013), with specimens in the type series assignable to both these clades. One clade is restricted to the Kitolomero valley and was provisionally designated as *R.* cf. *uluguruensis*, whereas the other clade is from Kihanga (the type locality for *R. moyeri*). In addition, two previously unknown but highly divergent clades were found from the Nguu and the Nguru Mountains, the latter of which is more closely related to *Rhampholeon beraduccii* (Mariaux and Tilbury, 2006) than to the species in the *R. uluguruensis/moyeri* complex. Despite substantial genetic differences shown in these studies, there are few identifiable and diagnosable morphological traits to separate lineages (e.g., Menegon et al., 2002; Mariaux et al., 2006; Fisseha et al., 2013; Branch et al., 2014).

Given the results from phylogenetic studies on *R. uluguruensis/moyeri* complex (e.g., Fisseha et al., 2013), the complex contains a number of unrecognised species. We combine existing data with new data from six candidate species of *Rhampholeon* from the Eastern Arc Mountains in a phylogenetic analysis (including Uzungwa Scarp Nature Reserve in the Udzungwa Mts; Mafwomero Forest Reserve in the Rubeho Mts; Mkingu Nature Reserve in the Nguru Mts; Mount Kanga, in the Nguru landscape; Mamiwa Kisara Forest Reserve in the Ukaguru Mts; and Nguu North and Kilindi Forest Reserves in the Nguu Mts). Using combined data, we provide taxonomic descriptions of six new species and outline, based on their distribution, the conservation status of these new species._

MATERIAL AND METHODS

Fieldwork

Chameleons were collected over several years from seven localities in the Eastern Arc Mountains (Table S1). Where possible, multiple individuals from each population were taken as specimens and fixed in buffered 2–4% formalin, then transferred into 70% ethanol or fixed directly in 80% ethanol for permanent conservation. A small piece of tissue (either liver or muscle) was collected before fixation and stored in 99% ethanol.

Morphometric analyses

Adults of *R. moyeri*, *R. uluguruensis* and each of the new species were measured using digital callipers to the nearest mm: Snout-Vent Length (SVL) – tip of the snout to the anterior edge of the cloaca; Tail Length (TL) – tip of tail to posterior edge of the cloaca; Head Length (HL) - from just behind the tip of the casque to the tip of the snout; Head Width (HW) – maximum width of head; Orbit Diameter (OD) – maximum horizontal width of orbit; Inter-orbital Distance (ID) – minimum width between orbits across crown (Branch et al., 2014); Parietal Crest to Snout (PCS) – distance from the middle of parietal crest to the tip of the snout from a sagittal view. All measurements were taken on the right side of the specimen.

Morphological variation between the species was examined using a multivariate approach in SPSS v.21. Given the small number of individuals per species in the dataset (eight species, total n = 56), the analysis was not partitioned by sex. Using log transformed original variables, a linear regression was run for each morphometric trait using a covariate (SVL) to remove the effect of body size. The resulting residuals were saved and input into a principal component analysis (PCA), to generate linear combinations of variables that explain overall morphological variation. Sampling adequacy for the PCA was assessed using a Kaiser-Mayer-Olkin test, while communalities were assessed to evaluate the contribution of each trait to the analysis (Tabachnick and Fidell, 1996). The varimax rotation of the component matrix was applied to maximize variation across multidimensional space. The first two principal components were extracted, and scores saved for each individual. Only the first PC had an eigenvalue greater than 1 (see Results), so an analysis of variance (ANOVA) was run for only PC1 with species as the fixed factor. Pairwise *ad hoc* Bonferroni tests were run for PC1 to examine pairs of species that differ for this PC.

Phylogenetic analyses

A phylogenetic analysis of the *R. uluguruensis/moyeri* complex was carried out using existing and new sequences from individuals from the type locality for *R. moyeri* (Kihanga) plus six additional localities in Tanzania (Kanga, Kitolomero, Nguru, Nguu, Rubeho, Ukaguru). The analysis included GenBank data from 18 additional described species of the 22 *Rhampholeon*, and all three species from the genus *Rieppeleon* as outgroup taxa (Table S1). DNA was extracted from the new samples using salt extraction (Aljanabi and Martinez, 1997), with PCR amplification, and cycle sequencing of two mitochondrial gene fragments following standard procedures: a 25 μl PCR reaction included 3 μl of 1 mM dNTPs, 3 μl of 25 mM $MgCl_2$, 0.2 μl of 10 pmol forward and reverse primer, 3 μl of Mg^{2+} free buffer solution, 0.1 μl (0.5U) Taq polymerase, and 1-2 μl of 25 ng/μl genomic DNA. Thermal cycling was run with initial denaturation for 4 min at 94 °C followed by: 35 cycles with denaturation for 30 s at 94 °C, annealing for 40 s at 55-57 °C, extension for 40 s at 72 °C, and final extension for 4 min at 72 °C. Primers used for amplification were ND2: L4437b (Macey et al., 1997a) and H5934 (Macey et al., 1997b), and 16S: L2510 and H3080 (Palumbi, 1996). PCR products were run on a 1% agarose gel and visualized under a UV light to verify amplification. Amplicons were sequenced directly using the forward primers at Macrogen (Seoul, Korea). Sequences were edited and aligned using Geneious software v 4.7 (Kearse et al., 2012). All new sequences have been deposited in GenBank (Table S1).

A Bayesian analysis of 1384 characters from the two mitochondrial genes (ND2: 923 bp and 16S: 461 bp) was used to investigate optimal tree space using MrBayes 3.2.2 (Huelsenbeck and Ronquist, 2001). To investigate which evolutionary model best fit the data, jModeltest was used (Posada, 2008). The AIC test specified the most complex model (GTR+I+G) for both markers. Therefore, two unlinked data partitions were created, each specifying six rate categories, including the gamma distribution and invariable sites, with uniform priors for all parameters. For 16S, 42 bases were excluded due to poor alignment. To ensure the results were robust, the MCMC was run twice in parallel for 20 million generations (four chains in each run), with trees sampled every 1000 generations. A 10% burn-in was examined (2 million generations, 2000 trees) in Tracer v1.6 (Rambaut et al., 2014) to check that the effective sample size (ESS) of all parameters met a threshold of 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 using RAxML HPC 7.2.8 (Stamatakis, 2006) on the CIPRES Science Gateway (www.phylo.org/sub_sections/portal/) for the combined dataset. 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.

Pairwise sequence divergence values (net p-distances) were estimated between species for both markers using MEGA v7 (Kumar et al., 2016). In addition, a barcoding approach was used to compare inter- and intra-specific sequence divergences, using SpeciesIdentifier v1.8 (Meier et al., 2006). Pairwise comparisons were generated for all *Rhampholeon* individuals in the phylogeny for the ND2 marker, and a frequency distribution of inter- and intra-specific comparisons was then made.

RESULTS

Morphological characterization of the R. uluguruensis/moyeri *complex*

Species in the *R. uluguruensis/moyeri* complex lack robust diagnostic characters; with the absence of any ossified horn, or crest; tubercles are small and often fragmented; tail is always very short; and the head lacks well defined external features (Fig. 1). The qualitative descriptions of characters given here (e.g., number of tubercles, their position, etc.) often show intraspecific variation on par with interspecific variation. Therefore, these traits are not discussed as diagnostic characters but merely as morphological features characterizing the range of variation across and within these species. There are very few characters that show consistent differences between species (e.g., size and shape of the rostral process, presence/absence of inguinal and axillary pits, angular flexure of the snout or number of hemipenial papillae) and their description could be useful for species identification. In most of the cases there is comprehensive combination of morphological characters that distinguishes one species from the others of the complex.

Adult *Rhampholeon* species range from 35-110 mm in total length. All the species in the complex have short, non-prehensile tails, whereas in other species they can have relatively longer tails. Body colour is usually brown, grey, or green; although colour can lighten and darken, and green individuals can rapidly turn dark brown. *Rhampholeon* seem to have a smaller range of colour variation compared to chameleons in most other genera. There are usually 2 to 3 transverse lines on the flanks that can be dark brown to black, orange, green or blue in colour, larger, darker scattered spots of variable size are often present on the body and limb surface.

Body and limbs. Scales are sub-homogeneous to finely heterogeneous stellate, interlocked, small tubercles. Larger conical tubercles are scattered across body and limbs. Usually, three prominent enlarged cones are spaced along the upper flank, the first (and largest) is situated above the shoulder, the third is located above the sacrum and the second about 2/3 of the way along the flank. Dorsal crest is composed by an undifferentiated row of clustered tubercles in its first third and a clearer crenulation in its central and distal part. Crenu-

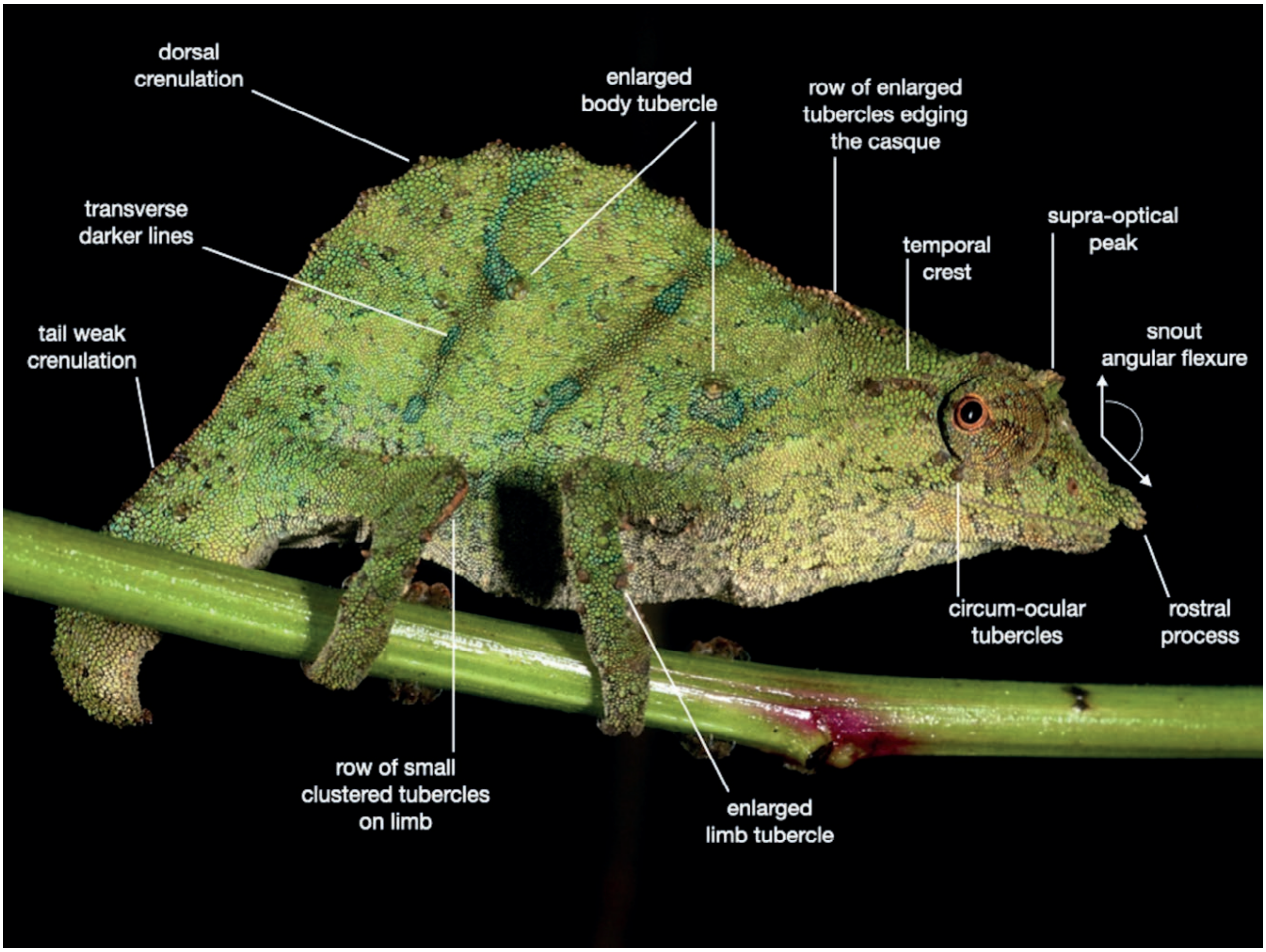

Fig. 1. Main features of external morphology used to describe and differentiate species in the *R. uluguruensis/moyeri* complex.

lation tubercles vary in shape and size from flat polygonal scales to more clearly conical ones. Crenulation may extend onto the tail, although this will be less conspicuous than the dorsal crenulation, or completely absent. There is no gular or ventral crest. There are enlarged spinous tubercles on the distal surface of the forelimbs and a prominent spine is often positioned anteriorly over the mid-radius. A longitudinal ridge of clustered and raised small tubercles, usually paler in colour on the limbs. Claws are always strongly bicuspid. The palms and soles have a smooth 'cobblestoned' appearance, with low palmar accessory spines present, usually close to base of the claws.

Head. Occiput flat. Temporal crest usually strong, formed by a ridge topped with rounded tubercles, lowest anteriorly and ending posteriorly in a large tubercle. Enlarged tubercles are present around the orbit. Parietal crest is usually weakly indicated by a row of round midline granules. Accessory rows of distinct granules may be present and, including the parietal granules form a Y or T shaped arrangement. The superior edge of the casque is edged in a row of enlarged subconical tubercles, which converge at the occiput. Above the eyes, a soft conical cluster of tubercles forms a supra-orbital peak, between the two peaks there is a V shaped row of slightly enlarged tubercles varying in number from 12 to 18. An accessory peaked cluster of tubercles forms distinct protuberances above nasal aperture. In all species in the *R. uluguruensis/moyeri* complex the two canthal ridges merge into the base of a soft, tuberculated rostral process which projects *ca.* 1 to 2 mm over the edge of the snout, in some individuals the rostral process is reduced to a short and pointy projection.

Hemipenes. The everted hemipenes are strongly club-shaped in all species. The bi-lobed distal ends bear two comparable large curved apical horns. Each of the horns possesses up to twelve thorn-like papillae, with the apical three usually arranged in a single row and the basal ones in two rows. The pedicel, truncus and apex cannot be differentiated because calyces and capitate structures are absent. The surface of the hemipenes is relatively smooth.

Morphometric analyses

Morphometric data from 56 individuals were input into the multivariate analyses (Table 1). The log transformed variables were size corrected and these residuals for each character were analysed using a PCA. The Kaiser-Mayer-Olkin test was high (KMO = 0.84), indicating adequate sampling for the PCA. The communality for eye diameter was low, indicating that its contribution to the overall variation in the dataset was negligible, so it was excluded from the analysis (Tabachnick and Fidell, 1996). With the two principal components (PCs) in the analysis, the remaining variables were close to, or higher than 0.70, so they were all retained in the analysis. However, only PC1 had an eigenvalue greater than 1.0 (Eigenvalue = 3.2) with PC2's eigenvalue at 0.65 (Table 2).

Given the small contribution of morphological variation represented by PC2, only PC1 was run for the ANOVA and pairwise comparisons. The ANOVA revealed significant differences between species (F = 6.2, P <0.001), but the pairwise tests indicated that this is due to just two species (*R. nicolai* sp. nov. and *R. sabini* sp. nov.) both having higher values on PC1 than some of the other species (Fig. 2; Table S2), although they are not significantly different from each other. Given that PCS, HL and TL load highly on PC1, this would suggest that these traits are larger for any given body size in *R. nicolai* sp. nov. and *R. sabini* sp. nov. than for other species.

Phylogenetic analyses

Both the Bayesian and maximum likelihood topologies are consistent with other species level phylogenies for this genus (e.g., Branch et al., 2014). In addition, there is a well-supported clade corresponding with the *R. moyeri* complex, which is sister to *R. uluguruensis* (Fig. 3). Within this clade, *R. moyeri* from the type locality (Kihanga) is well-supported and distinct. There are five additional previously unrecognised lineages that represent candidate species from different mountains: Uzungwa Scarp Nature Reserve in the Udzungwa Mts; Mafwomero Forest Reserve in the Rubeho Mts; Mount Kanga, in the Nguru landscape; Mamiwa Kisara Forest Reserve and Mikuvi in the Ukaguru Mts; and Nguu North and Kilindi Forest Reserves in the Nguu Mts. Individuals from Mkingu Nature Reserve in the Nguru Mts are in a separate clade that includes species from central and east Africa (i.e., *R. boulengeri*, *R. hattinghi*, *R. acuminatus*, and *R. beraduccii*; Fig. 3).

The sequence divergence values for all the candidate species in the *R. uluguruensis/moyeri* complex were comparable to that found between other *Rhampholeon* species, ranging from 5.1–14.5% for ND2 and 4.4–12.7% for 16S (Table 3). In addition, the frequency distribution of inter- and intra-specific values for ND2 shows that the candidate species fall within the range of described species (Fig. S1).

Below we describe species which show high molecular sequence divergence and are allopatrically distributed. Despite the morphological conservatism, we also indicate, when possible, the best features that can be used as diagnosable morphological differences.

Table 1. Measurements (mm) of the 52 *Rhampholeon* specimens included for the morphometric analyses. Field ID numbers or voucher accession numbers (MTSN and MUSE = MUSE - Science Museum of Trento, MHNG = Muséum d'Histoire Naturelle, Geneva), sex, SVL (from tip of the snout to the anterior edge of the cloaca), TL (from tip of tail to posterior edge of the cloaca), HL (from just behind the tip of the casque to the tip of the snout), HW (maximum width of head), OD (maximum horizontal width of orbit), ID (minimum width between orbits across crown) and PCS (distance from the middle of parietal crest to the tip of the snout from a sagittal view).

ID number	Species	Sex	SVL	TL	HL	HW	OD	ID	PCS
MUSE 13745	*R. beraducci*	male	35.06	6.63	11.62	6.66	4.10	4.04	4.14
MUSE 13746	*R. beraducci*	male	35.06	6.80	12.12	6.87	3.99	3.98	4.90
MTSN 5379	*R. colemani* sp. nov.	male	37.58	7.04	11.44	7.78	3.93	4.00	5.01
MTSN 5380	*R. colemani* sp. nov.	female	41.50	6.18	12.29	7.07	4.27	4.02	5.38
MTSN 5381	*R. colemani* sp. nov.	female	35.48	6.10	10.51	6.20	4.08	3.80	4.99
MUSE 11033	*R. colemani* sp. nov.	female	29.03	5.44	10.05	5.37	3.53	3.04	4.64
MUSE 11029	*R. colemani* sp. nov.	male	30.13	6.72	9.57	5.96	3.59	2.77	4.31
MUSE 11031	*R. colemani* sp. nov.	male	29.04	4.75	9.01	4.90	3.52	3.06	4.61
MUSE 11032	*R. colemani* sp. nov.	female	29.45	5.35	9.89	5.64	3.62	3.06	4.61
MTSN 8542	*R. waynelotteri* sp. nov.	male	40.03	9.33	13.12	7.49	4.56	3.82	5.98
MTSN 8537	*R. waynelotteri* sp. nov.	male	43.06	9.50	13.70	8.02	4.80	4.12	6.11
MTSN 8540	*R. waynelotteri* sp. nov.	male	43.29	11.74	13.18	7.94	5.00	4.34	6.00
MTSN 8539	*R. waynelotteri* sp. nov.	female	41.80	9.28	14.27	7.78	4.73	4.03	6.23
MTSN 8541	*R. waynelotteri* sp. nov.	female	46.30	8.09	14.96	8.79	5.14	3.89	6.98
MTSN 5235	*R. moyeri*	male	44.89	11.50	13.81	7.57	4.58	4.78	6.13
MTSN 5372	*R. moyeri*	female	52.63	10.62	14.76	8.84	5.57	4.40	7.08
MTSN 5373	*R. moyeri*	female	50.67	11.24	15.21	8.53	4.84	5.45	6.84
MTSN 5374	*R. moyeri*	female	52.36	8.61	15.01	8.36	4.76	4.28	6.99
MTSN 5376	*R. moyeri*	male	37.85	7.57	11.41	6.32	4.12	4.38	5.31
MTSN 5377	*R. moyeri*	male	43.79	9.86	14.00	7.87	4.43	4.55	6.84
MTSN 5378	*R. moyeri*	male	40.09	8.65	13.83	7.87	4.45	4.37	6.57
MTSN 5592	*R. nicolai* sp. nov.	male	45.55	11.01	15.48	9.06	5.33	5.77	6.57
MTSN 5593	*R. nicolai* sp. nov.	female	33.83	6.24	11.46	6.75	3.66	3.77	5.00
MHNG 2624.47	*R. nicolai* sp. nov.	male	36.21	9.44	13.12	7.91	4.66	4.21	5.55
MHNG 2624.56	*R. nicolai* sp. nov.	male	43.10	12.46	14.02	9.70	5.24	4.76	6.44
MHNG 2624.49	*R. nicolai* sp. nov.	male	35.57	8.26	12.49	7.57	4.17	3.74	5.41
MHNG 2624.5	*R. nicolai* sp. nov.	male	34.32	8.49	13.09	8.05	4.51	4.49	5.78
MHNG 2624.86	*R. nicolai* sp. nov.	male	33.75	10.31	12.30	6.97	4.27	3.56	5.87
MHNG 2624.87	*R. nicolai* sp. nov.	male	27.84	9.26	10.46	6.39	3.83	3.47	4.96
MHNG 2624.48	*R. nicolai* sp. nov.	female	36.21	8.98	13.35	7.31	4.13	4.09	5.40
MHNG 2624.57	*R. nicolai* sp. nov.	female	39.09	7.49	14.00	8.21	5.00	4.65	6.19
MUSE 14034	*R. princeeai* sp. nov.	male	37.54	10.14	11.87	7.42	4.21	4.43	5.31
MUSE 14036	*R. princeeai* sp. nov.	male	35.36	10.18	12.05	7.02	4.15	4.84	5.65
MUSE 14033	*R. princeeai* sp. nov.	female	51.79	12.49	16.85	7.23	5.58	5.56	7.08
MUSE 14285 (MTSN 5537)	*R. princeeai* sp. nov.	male	34.04	9.47	11.04	5.71	4.13	4.42	5.41
MUSE 14286 (MTSN 5538)	*R. princeeai* sp. nov.	female	34.96	7.50	11.40	6.64	4.18	4.23	5.44
MTSN 5013	*R. rubeho* sp. nov.	male	41.12	11.12	14.76	8.17	5.00	4.53	6.47
MTSN 5012	*R. rubeho* sp. nov.	female	42.58	8.62	13.86	8.04	4.51	4.72	5.83
MTSN 5014	*R. rubeho* sp. nov.	female	40.23	8.71	13.14	6.95	4.09	4.20	5.68
MTSN 8895	*R. rubeho* sp. nov.	male	48.08	16.68	17.13	10.38	5.72	6.03	7.79
MTSN 8896	*R. rubeho* sp. nov.	male	38.79	11.26	13.49	7.50	4.60	4.46	5.83
MTSN 8899	*R. rubeho* sp. nov.	male	41.07	13.08	13.53	8.03	4.58	4.83	6.12
MTSN 8893	*R. rubeho* sp. nov.	female	42.94	12.29	14.71	8.18	4.67	5.46	6.36
MTSN 8898	*R. rubeho* sp. nov.	female	46.71	10.27	15.26	9.23	4.92	5.81	6.82
MTSN 5092	*R. sabini* sp. nov.	male	37.19	7.05	12.19	7.00	6.03	4.91	5.23
MTSN 5192	*R. sabini* sp. nov.	male	25.41	7.66	11.34	6.91	4.24	3.27	5.54

ID number	Species	Sex	SVL	TL	HL	HW	OD	ID	PCS
MTSN 5193	*R. sabini* sp. nov.	male	36.71	10.26	14.43	8.84	4.85	5.51	5.94
MTSN 5198	*R. sabini* sp. nov.	male	44.60	9.53	14.02	9.04	5.12	5.11	6.44
MTSN 5199	*R. sabini* sp. nov.	male	38.33	7.14	11.56	7.55	4.27	4.31	5.08
MTSN 5195	*R. sabini* sp. nov.	female	38.62	6.46	12.42	7.25	4.65	4.76	6.02
MTSN 5197	*R. sabini* sp. nov.	female	37.99	6.96	12.43	7.58	4.46	4.23	5.42
MTSN 9082	*R. sabini* sp. nov.	female	31.39	5.85	10.41	5.30	3.81	3.34	4.67
MHNG 2817.97	*R. uluguruensis*	male	42.72	10.82	13.17	7.47	4.42	4.14	5.86
MTSN 7764	*R. uluguruensis*	male	46.20	12.10	13.60	7.73	4.95	4.43	6.69
MTSN 7749	*R. uluguruensis*	female	43.10	7.34	12.56	7.91	4.49	4.03	5.71
MTSN 7776	*R. uluguruensis*	female	40.08	7.80	12.49	7.33	4.07	4.22	5.39
MTSN 7755	*R. uluguruensis*	male	45.49	12.28	13.90	7.64	5.06	4.19	6.16
MTSN 7757	*R. uluguruensis*	male	41.86	12.50	13.28	7.81	4.68	4.22	6.59

Table 2. Rotated principal component matrix for PC1 and PC2, with the loadings for each original variable on the principal components for nine species of *Rhampholeon*. Also given is the eigenvalue and % variation for each PC and the significance value from the analysis of variance (ANOVA). Note that the eigenvalue for PC2 is less than 1.

Trait	PC1	PC2
PCS: Parietal crest-snout tip distance	0.90	0.13
HL: Head length	0.73	0.52
TL: Tail length	0.70	0.28
HW: Head width	0.56	0.54
ED: Eye diameter	0.13	0.91
OD: Max horizontal orbit width	0.43	0.62
Eigenvalue	3.2	0.65
% variation	62	13
ANOVA	P < 0.001	NA

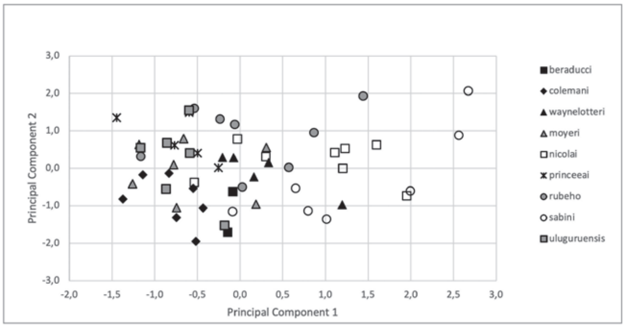

Fig. 2. Scatterplot of the principal component scores on PC1 and PC2 for nine species of *Rhampholeon*.

Species Descriptions

Rhampholeon uluguruensis Tilbury & Emmrich, 1996
Uluguru Pygmy Chameleon
Holotype: ZMB 48421

Synonym

Rhampholeon uluguruensis Tilbury & Emmrich, 1996
Rhampholeon uluguruensis Necas 1999: 284
Rhampholeon (Rhinodigitum) uluguruensis Matthee et al. 2004
Rhampholeon (Rhinodigitum) uluguruensis Tilbury 2010: 199
Rhampholeon (Rhinodigitum) uluguruensis Fisseha et al. 2013
Rhampholeon (Rhinodigitum) uluguruensis Glaw 2015
Rhampholeon (Rhinodigitum) uluguruensis — Spawls et al. 2018: 255

Original diagnosis (verbatim)

A small species with a maximum recorded total length (TL) of 50 mm (largest male 48.5 mm TL, and largest female 50 mm TL; Fig. 4). The tail is short, averaging 24.5% of the TL in males and 20.9% of the TL in females. It has a soft, tuberculated, rostral process and a background scalation of minute sub-homogeneous stellate granules. All specimens have axillary dermal pits, which may be shallow or deep, and in some specimens a mere indication of an inguinal pit is seen. Soles and palms are smooth, claws strongly bicuspid and low accessory palmar/plantar spines are present. Adult males and females have similar SVL. The hemipenis bears two curved apical horns, each with nine papillae comprising a single apical row of three, with six proximal papillae arranged in a double row of three. This combination of characters distinguishes this species from the others of the genus.

Amendments to the original diagnosis

Rhampholeon uluguruensis differs from other *Rhampholeon* for the ND2 gene by having the amino acid pheny-

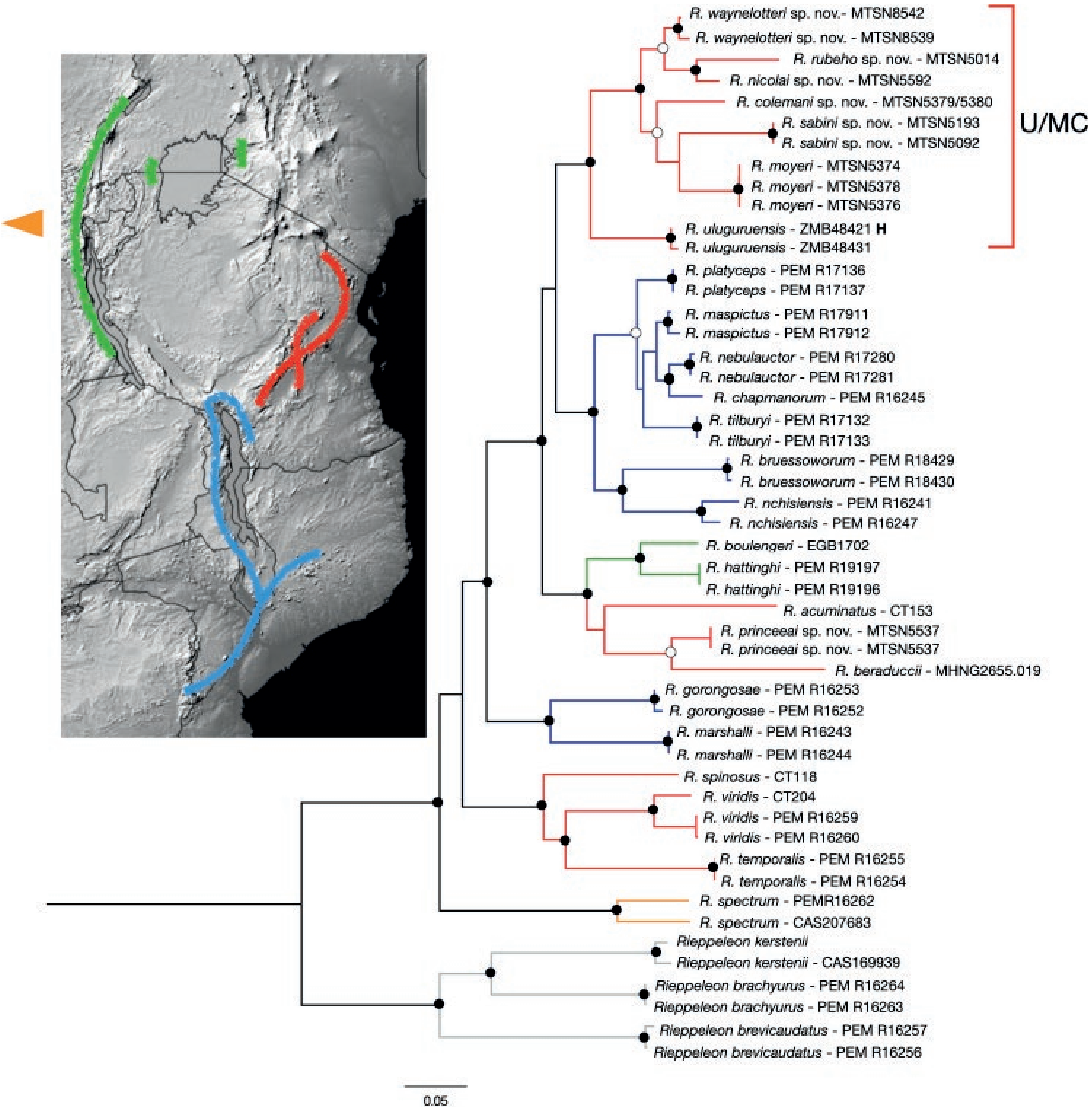

Fig. 3. Maximum likelihood topology for the *Rhampholeon uluguruensis/moyeri* complex (indicated by U/MC), and other members of the genus. Nodes supported by both maximum likelihood (≥70% bootstrap) and Bayesian (≥0.95 posterior probability) analyses are denoted with black circles, those supported by either maximum likelihood or Bayesian alone are denoted with white circles. Branches on the tree are colour coded to match the general distribution regions shown on the map (green: Albertine Rift; blue: The Southern Rift; red: The Eastern Arc Mountains). Orange arrow indicates that *R. spectrum* occurs outside the area represented in the map.

lalanine at codon position 466–468. For the 16S gene, it differs by having a G at position 896.

DISTRIBUTION AND REMARKS

Restricted to the Uluguru Mountains, including Uluguru Nature Reserve, and Mkungwe Forest Reserve.

Rhampholeon moyeri Menegon, Salvidio & Tilbury 2002
Moyer's Pygmy Chameleon
Holotype: MTSN 006TA

SYNONYMS

Rhampholeon moyeri Menegon, Salvidio & Tilbury 2002

Table 3. Uncorrected net p-distances for a) ND2 and b) 16S between species of *Rhampholeon* included in the phylogenetic analysis. Values within a species are indicated on the diagonal. Within-species values that were not estimated (only one sequence available for that taxon) are denoted by 'na'.

a)	*Rhampholeon*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	*moyeri*	0.000																							
2	*princeeai*	0.145	na																						
3	*colemani*	0.111	0.118	na																					
4	*sabini*	0.121	0.000	0.093	na																				
5	*rubeho*	0.065	0.152	0.081	0.119	na																			
6	*waynelotteri*	0.069	0.129	0.079	0.107	0.056	0.013																		
7	*nicolai*	0.085	0.147	0.086	0.116	0.058	0.051	na																	
8	*boulengeri*	0.194	0.230	0.185	0.200	0.171	0.163	0.189	na																
9	*bruessoworum*	0.184	0.228	0.178	0.173	0.172	0.168	0.183	0.158	0.006															
10	*chapmanorum*	0.186	0.216	0.172	0.191	0.161	0.156	0.176	0.166	0.137	na														
11	*gorongosae*	0.218	0.217	0.195	0.214	0.197	0.186	0.200	0.167	0.196	0.191	0.008													
12	*hattinghi*	0.189	0.225	0.175	0.193	0.168	0.158	0.174	0.095	0.171	0.181	0.176	0.000												
13	*marshalli*	0.227	0.259	0.218	0.229	0.215	0.191	0.210	0.177	0.197	0.194	0.140	0.196	0.001											
14	*maspictus*	0.162	0.187	0.150	0.157	0.139	0.130	0.150	0.150	0.107	0.051	0.181	0.162	0.162	0.023										
15	*nchisiensis*	0.129	0.178	0.132	0.130	0.128	0.119	0.124	0.123	0.110	0.120	0.168	0.141	0.159	0.092	0.046									
16	*nebulauctor*	0.174	0.200	0.160	0.171	0.138	0.136	0.151	0.155	0.113	0.052	0.192	0.147	0.176	0.042	0.106	0.006								
17	*platyceps*	0.187	0.177	0.162	0.159	0.158	0.148	0.164	0.149	0.120	0.091	0.167	0.164	0.181	0.057	0.128	0.069	0.001							
18	*spectrum*	0.167	0.183	0.151	0.163	0.155	0.141	0.152	0.140	0.158	0.179	0.140	0.140	0.165	0.154	0.158	0.160	0.169	0.105						
19	*spinosus*	0.239	0.270	0.223	0.222	0.221	0.211	0.208	0.215	0.212	0.219	0.189	0.212	0.209	0.195	0.198	0.207	0.216	0.159	na					
20	*temporalis*	0.226	0.220	0.228	0.220	0.231	0.217	0.237	0.208	0.205	0.219	0.204	0.222	0.210	0.201	0.197	0.216	0.222	0.176	0.186	0.001				
21	*tilburyi*	0.180	0.186	0.170	0.183	0.160	0.143	0.159	0.149	0.146	0.101	0.192	0.169	0.195	0.063	0.129	0.065	0.100	0.166	0.215	0.220	0.000			
22	*uluguruensis*	0.135	0.166	0.115	0.120	0.124	0.123	0.142	0.173	0.162	0.171	0.179	0.157	0.193	0.146	0.140	0.161	0.142	0.166	0.192	0.206	0.163	0.007		
23	*viridis* (North)	0.204	0.235	0.204	0.198	0.195	0.188	0.203	0.196	0.183	0.218	0.187	0.205	0.204	0.194	0.190	0.205	0.202	0.140	0.166	0.161	0.216	0.206	na	
24	*viridis* (South)	0.209	0.239	0.188	0.209	0.195	0.171	0.191	0.191	0.201	0.231	0.181	0.196	0.205	0.192	0.207	0.207	0.216	0.163	0.165	0.175	0.224	0.202	0.070	0.002

b)	*Rhampholeon*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	*moyeri*	0.000																							
2	*princeeai*	0.084	0.000																						
3	*colemani*	0.048	0.087	na																					
4	*sabini*	0.044	0.078	0.068	na																				
5	*rubeho*	0.119	0.104	0.109	0.127	na																			
6	*acuminatus*	0.102	0.071	0.100	0.094	0.134	na																		
7	*beraduccii*	0.111	0.046	0.101	0.110	0.130	0.085	na																	
8	*boulengeri*	0.075	0.067	0.080	0.076	0.121	0.073	0.080	na																
9	*bruessoworum*	0.081	0.080	0.086	0.083	0.125	0.089	0.111	0.083	0.000															
10	*chapmanorum*	0.053	0.067	0.060	0.068	0.106	0.085	0.094	0.075	0.065	na														

b)	*Rhampholeon*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
11	*gorongosae*	0.092	0.087	0.097	0.101	0.107	0.098	0.106	0.106	0.091	0.075	0.002													
12	*hattinghi*	0.066	0.061	0.070	0.071	0.107	0.066	0.078	0.017	0.078	0.065	0.095	0.000												
13	*marshalli*	0.089	0.096	0.089	0.100	0.101	0.112	0.115	0.108	0.091	0.075	0.067	0.092	na											
14	*maspictus*	0.058	0.066	0.061	0.074	0.108	0.084	0.091	0.076	0.068	0.008	0.074	0.066	0.076	0.000										
15	*nchisiensis*	0.072	0.067	0.067	0.086	0.103	0.074	0.095	0.080	0.051	0.038	0.082	0.070	0.073	0.041	0.024									
16	*nebulauctor*	0.052	0.073	0.063	0.069	0.114	0.075	0.104	0.080	0.064	0.006	0.078	0.070	0.081	0.008	0.046	0.000								
17	*platyceps*	0.053	0.058	0.051	0.068	0.099	0.080	0.084	0.065	0.060	0.010	0.072	0.056	0.067	0.008	0.034	0.011	0.000							
18	*spectrum*	0.078	0.085	0.087	0.086	0.088	0.110	0.100	0.092	0.082	0.065	0.074	0.083	0.060	0.063	0.064	0.065	0.058	0.048						
19	*spinosus*	0.095	0.100	0.098	0.101	0.132	0.112	0.129	0.112	0.094	0.080	0.083	0.110	0.095	0.080	0.084	0.082	0.073	0.088	na					
20	*temporalis*	0.094	0.089	0.101	0.115	0.133	0.090	0.111	0.099	0.092	0.079	0.080	0.097	0.101	0.078	0.076	0.081	0.070	0.091	0.070	na				
21	*tilburyi*	0.060	0.060	0.058	0.068	0.106	0.085	0.087	0.070	0.068	0.017	0.075	0.059	0.079	0.013	0.043	0.017	0.012	0.067	0.080	0.075	0.000			
22	*uluguruensis*	0.060	0.058	0.055	0.071	0.101	0.078	0.089	0.065	0.055	0.043	0.075	0.061	0.072	0.042	0.041	0.041	0.034	0.063	0.085	0.077	0.043	0.002		
23	*viridis* (North)	0.110	0.112	0.115	0.115	0.120	0.130	0.141	0.117	0.101	0.092	0.102	0.111	0.102	0.088	0.095	0.093	0.085	0.085	0.088	0.080	0.090	0.100	na	
24	*viridis* (South)	0.101	0.108	0.104	0.115	0.113	0.126	0.132	0.106	0.093	0.082	0.094	0.107	0.094	0.080	0.083	0.087	0.075	0.078	0.083	0.070	0.087	0.091	0.024	0.002

Rhampholeon (Rhinodigitum) moyeri Matthee et al. 2004
Rhampholeon (Rhinodigitum) moyeri Tilbury 2010: 181
Rhampholeon (Rhinodigitum) uluguruensis Fisseha et al. 2013
Rhampholeon (Rhinodigitum) moyeri Glaw 2015
Rhampholeon (Rhinodigitum) moyeri Spawls et al. 2018: 252

Original diagnosis (verbatim)

A small chameleon with a maximum total length of 64 mm (maximum SVL = 51 mm) of which the tail comprises an average of 23% in males and 17% in females (Fig. 5). Adult males and females have similar SVL. A soft-tuberculated rostral dermal process and soft supra-optic peaks are present. Between 14 and 19 scales span the interorbital region between the bases of the peaks, with between 20 and 27 scales from peak to peak. The casque is flat posteriorly with a short indistinct parietal crest. The temporal crest is distinct. Deep axillary pits are present but there are no inguinal pits. Background body scalation is of fine sub-homogeneous stellate granules with scattered enlarged rounded tubercles. Claws are strongly bicuspid with low accessory palmar and plantar spines and smooth "cobblestoned" palms and soles. Hemipenis has two large incurving apical horns with prominent thorn-like papillae arranged in two proximal rows of four (occasionally five) papillae, followed by a single terminal row of four (occasionally five) papillae. The above characters distinguish *R. moyeri* from all the other known species of *Rhampholeon*.

Amendments to the original diagnosis

In our specimens, inguinal pits are sometimes faintly indicated, and the maximum number of hemipenial papillae is more than five but never exceeding ten. It also differs from other *Rhampholeon* for the ND2 gene by having the amino acid alanine at position 365–367, glutamine at 454–456, valine at position 475–477, valine at 478–480 and alanine at 619–621.

Distribution and remarks

Udzungwa Mountains. In the Uzungwa Scarp Nature Reserve, the species might occur sympatrically with *R. colemani* with some degree of elevational segregation between the taxa. Further research is required to properly assess the actual distribution of the two species within the Reserve. The individuals from the Kitolomero valley, smaller in size with males having up to 12 hemipenial papillae and originally included in the *R. moyeri* type series, belong to a distinct taxon (see *R. colemani* sp. nov. description).

Fig. 4. *Rhampholeon uluguruensis* from type locality, in life.

Rhampholeon beraduccii Mariaux & Tilbury 2006
Beraducci's Pygmy Chameleon
Holotype: MHNG 2655.019

SYNONYMS

Rhampholeon (Rhinodigitum) beraduccii Mariaux & Tilbury 2006
Rhampholeon (Rhinodigitum) beraduccii Tilbury 2010: 167
Rhampholeon (Rhinodigitum) beraduccii Glaw 2015
Rhampholeon (Rhinodigitum) beraduccii Spawls et al. 2018: 250

ORIGINAL DIAGNOSIS (VERBATIM)

Chamaeleonidae, *Rhampholeon* (*Rhinodigitum*). With the characters of the subgenus. A tiny brown chameleon with snout–vent length (SVL) 20.5–28 mm, maximum total

Fig. 5. *Rhampholeon moyeri* in life.

length (TL) 36 mm, and a very short tail, 19–22% of TL (Fig. 6). The smallest known *Rhampholeon* and the smallest chameleon from continental Africa. Head with a well-developed nasal process and short supra-optical peaks. Head flat with very slightly marked crests, temporal crest very weak. Dorsal keel weakly undulated. Body with subhomogeneous granules, but conspicuous shoulder spine present. Deep axillary and inguinal pits present. Claws bicuspid with small accessory spines.

Amendments to the original diagnosis

Rhampholeon beraduccii has not been sequenced for the ND2 gene. For the 16S gene, it differs by having a G at positions 965 and 1008 and from all other species except *R. acuminatus* at position 1269 by having an A. It differs at position 1271 by having a G.

Distribution

Restricted to Sali Forest Reserve, in the Mahenge Mountains.

Fig. 6. *Rhampholeon beraduccii* from type locality, in life.

Fig. 7. *Rhampholeon acuminatus* from type locality, in life.

Rhampholeon acuminatus Mariaux & Tilbury 2006
Nguru Spiny Pygmy Chameleon
Holotype: MHNG 2645.001

Synonyms
Rhampholeon (Rhinodigitum) acuminatus Mariaux & Tilbury 2006
Rhampholeon (Rhinodigitum) acuminatus Tilbury 2010: 161
Rhampholeon (Rhinodigitum) acuminatus Glaw 2015
Rhampholeon (Rhinodigitum) acuminatus Spawls et al. 2018: 249

Original diagnosis (verbatim)
Chamaeleonidae, *Rhampholeon* (*Rhinodigitum*). With the characters of the subgenus. A small chameleon with SVL 47–57 mm (maximum TL 82 mm) and a tail 25–30% of TL (Fig. 7). Adults are unmistakable due to their large discoid and vertically flattened rostral process (up to 5 × 3 mm) projecting forward off the rostrum (Figs 6-7), spinous supra-orbital and other cranial projections, prominent casque, exaggerated dorsal crest and numerous spines on the body, limbs and tail. No axillary or inguinal pits. Claws bicuspid. Parietal peritoneum unpigmented.

Amendments to the original diagnosis
Rhampholeon acuminatus has not been sequenced for the ND2 gene. For the 16S gene, it differs by having an A at position 917 and from all other species except *R. beraduccii* at position 1269 by having an A.

Distribution
Restricted to Mkingu Nature Reserve in the Nguru mountains.

***Rhampholeon colemani* sp. nov.**
Uzungwa Scarp's Pygmy Chameleon
Holotype: Adult male in the MUSE, the Science Museum of Trento, MTSN 5379 (formerly MTSN 001TA) collected in the Uzungwa Scarp Nature Reserve on the 8 January 1999 by Michele Menegon and Sebastiano Salvidio and formerly included in the paratype series of *Rhampholeon moyeri* (Fig. 8 and Table S3).

Type locality
Kitolomero valley, a locality in the Uzungwa Scarp Nature Reserve at about 1200 m a.s.l. (-8.3975; 35.9786) Kilombero District, Morogoro Region of Tanzania.

Paratypes
Two adult females – MTSN 5380, MTSN 5381, collected in the Uzungwa Scarp Forest Reserve on 8 January 1999 by Michele Menegon and Sebastiano Salvidio were formerly included in the paratype series of *Rhampholeon moyeri*. Two additional females, MUSE 11032 and MUSE 11033, and two adult males, MUSE 11029 and MUSE 11031, collected at the same locality on 17 November 2013 by Michele Menegon, Elena Tonelli and Anna Sustersic.

Diagnosis
A very small chameleon (slightly larger in size than *R. beraduccii* which is the smallest chameleon in continental Africa), with a maximum total length of 44 mm (maximum SVL = 35.5 mm) of which the tail comprises an average of 19% in females and 24% in males. Adult females and males have similar SVL. Overall morphology similar to other species in the complex, differing by its smaller size (only *R. beraduccii*, with a max recorded size of 28 mm SVL and 36 mm total length, is smaller), a pronounced angular flexure of the snout (59° in *R. colemani*, 44° in *R.*

Fig. 8. *Rhampholeon colemani* from type locality, in life.

moyeri, the other species all <41°), by the ratio between tail length and orbital diameter, by having hemipenis with two long apical horns, curving inwardly toward the sulcal surface and each horn bearing up to 12 prominent thornlike papillae arranged in two proximal rows of 3 to 4, with an additional spine at base of the horn and with a row of 4 distal papillae. It also differs from other *Rhampholeon* for the ND2 gene by having the amino acid isoleucine at position 463–465, valine at position 577–579 and threonine at position 583–585 (Table S3), and by a known distribution restricted to a single locality in the sub-montane rainforest of the Uzungwa Scarp Nature Reserve.

Description of Holotype

Snout-vent 37.58 mm, tail 8.8 mm. Body habitus leaf like - typical of all other *Rhampholeon* (*Rhinodigitum*) species.

Head: occiput flat, parietal crest indistinct. The lateral crests are studded with several prominent tubercles. The two supra-orbital ridges are connected to each other by a series of 18 inter-orbital tubercles arranged in a shallow V across the top of the head with the last tubercle being the most prominent. A tuft of tubercles forms a small peak on the supra-orbital rim. The two peaks are connected between the orbits by an interorbital row of 19 scales counted from peak to peak. The canthal ridges are formed by a row of enlarged, relatively smooth tubercles which merge anteriorly on the tip of the snout and extend into a small soft tuberculated rostral process extending 1.2 mm off the tip of the snout. A distinct temporal crest arises from the mid post-orbital rim and consists of 5 tubercles on the right and 6 tubercles on the left, of which the most posterior is the largest. The nares open infero-posteriorly. There are no gular appendages or crests.

Body: the dorsal crest is crenulated over the anterior two-thirds of the dorsal keel, fading to smooth over the sacrum. The crenulations are formed by a cluster of mucronate tubercles sited above each dorsal spinous process. The tail is very weakly crenulate, more so over the distal third. A single deep pit is present in each axilla, but there is no trace of a pit in the inguinal region. The soles of the feet present a relatively smooth, non-spinous cobblestoned appearance. Low palmar spines are present at the bases of the digits. The claws are strongly bicuspid.

Scalation: body covered with a subhomogeneous background of small stellate edged tubercles. A more or less regular scattering of larger (2–4 times larger in diameter) subconical/rounded tubercles occurs on the sides of the body. A particularly large tubercle is sited just above the shoulder. The forearms have a scattering of enlarged spinous tubercles, two prominent spines along the radius. The belly is smooth with small homogeneous scales. The scales on the circular eyeball are relatively heterogeneous.

Hemipenis: stout and truncated. No calyces or capitate structures are present and sulcal lips smooth. Two long apical horns are present, curving inwardly toward the sulcal surface. Each horn is adorned with 10 to 12 prominent thornlike papillae arranged in two proximal rows of 3 to 4, with an additional spine at base of the horn and with a row of 4 distal papillae.

Distribution

The species is only known from the type locality, at 1200 m a.s.l., in the Uzungwa Scarp Nature Reserve, where it might occur sympatrically with *R. moyeri* with possible elevational segregation. Further research is needed to properly assess the distribution of the two species.

Etymology

Rhampholeon colemani is named in honour of Carter Coleman, who for more than 25 years has raised funds and campaigned for the conservation of Tanzania's forests. In 1991 he revived the Tanzania Forest Conservation Group based in Tanzania, and went on to establish the African Rainforest Conservancy in the USA and the African Rainforest Trust in the United Kingdom, organisations that remain dedicated to conserving Tanzania's high biodiversity forests

***Rhampholeon sabini* sp. nov.**

Nguu North Pygmy Chameleon

Holotype

Adult male in the MUSE, the Science Museum of Trento, MTSN 5092 collected in the Nguu North Forest Reserve at 1200 m a.s.l. on 22 March 2002 by Michele Menegon (Fig. 9 and Table S2).

Type locality

Nguu North Forest Reserve, 1200 m a.s.l. (-5.4803; 37.4753), Handeni District, Tanga Region, Tanzania.

Paratype

Four adult males – MTSN 5192, MTSN 5193, MTSN 5198, MTSN 5199, two adult females MTSN 5195, MTSN 5197, collected in Nguu North Forest Reserve, Nguu Mountains between 15–22 March 2002 by Michele Menegon and one adult female MTSN 9082, collected in Kilindi Forest Reserve on the 7 February 2008 by Michele Menegon.

Diagnosis

A small chameleon with a maximum total length of 54.13 mm (maximum SVL = 44.17 mm) of which the tail comprises an average of 18% in females and 26% in males. Adult females and males have similar SVL. Overall morphology is similar to other species in the complex, but in this species, the relative size of the head and tail is significantly larger than in other *Rhampholoen* species analysed, see Fig. 2 and related text. It also differs by a marked mitochondrial sequence divergence, more specifically for the ND2 gene by having the amino acid valine at position 460–462, leucine at position 487–489, proline at position 583–585, threonine at position 619–621, methionine at position 643–645, threonine at position 664–666 and threonine at position 667–669 (Table S3) and by its current distribution restricted to the sub-montane rainforest of Nguu and Kilindi forest reserves. Note that without locality or genetic information, preserved individuals of the

Fig. 9. *Rhampholeon sabini* from type locality, in life.

new species cannot be univocally distinguished on the ground of their morphology.

Description of Holotype

Snout-vent 37.19 mm, tail 7.05 mm. Body habitus leaf like - typical of all other *Rhampholeon* (*Rhinodigitum*) species.

Head: occiput flat, parietal crest formed by 5 enlarged tubercles. The lateral crests are studded with several prominent tubercles. No inter-orbital tubercles arranged in a shallow V across the top of the head. A tuft of tubercles forms a small peak on the supra-orbital rim. The two peaks are connected between the orbits by an interorbital row of 23 scales counted from peak to peak. The canthal ridges are formed by a row of enlarged, relatively smooth tubercles which merge anteriorly on the tip of the snout and extend into a small soft tuberculated rostral process extending 1.2 mm off the tip of the snout. An indistinct temporal crest arises from the mid post-orbital rim and consists of some tubercles slightly larger than the surrounding ones of which the most posterior is the largest. The nares open infero-posteriorly. There are no gular appendages or crests.

Body: the dorsal crest is crenulated over the anterior two-thirds of the dorsal keel, fading to smooth over the sacrum. The crenulations are formed by a cluster of mucronate tubercles sited above each dorsal spinous process. The tail shows no crenulation.

A single deep pit is present in each axilla, but there is no trace of a pit in the inguinal region. The soles of the feet present a relatively smooth, non-spinous cobblestoned appearance. Low palmar spines are present at the bases of the digits. The claws are strongly bicuspid.

Scalation: body covered with a subhomogeneous background of small stellate edged tubercles. A more-or-less regular scattering of larger (2–4 times larger in diameter) subconical/rounded tubercles occurs on the sides of the body. A particularly large tubercle is sited just above the shoulder. The forearms have a scattering of enlarged spinous tubercles, two prominent spines along the radius. The belly is smooth with small homogeneous scales. The scales on the circular eyeball are relatively heterogeneous.

Hemipenis: stout and truncated. No calyces or capitate structures are present and sulcal lips smooth. Two long apical horns are present, curving inwardly toward the sulcal surface. Each horn is adorned with 7 to 8 prominent thornlike papillae arranged in two proximal rows of two, with an additional spine at base of the horn and with a row of three distal papillae.

Distribution

The species is known to occur in the Nguu North and Kilindi Forest Reserves only, Handeni district, Tanga Region, Tanzania.

Etymology

The species name is a patronym for Andy Sabin who provides financial support to many organizations and is actively engaged with community and environmental programs around the world. As an extension of his lifelong fascination with reptiles and amphibians and dedication to environmental education.

Rhampholeon rubeho **sp. nov**

Rubeho's Pigmy Chameleon

Holotype

Adult male in the MUSE, the Science Museum of Trento, MTSN 5013 collected in the Mafwomero Forest Reserve on 22 March 2002 by Michele Menegon, Andrew Perkin and Lucinda Lawson (Fig. 10 and Table S2).

Type locality

Mafwomero Forest Reserve in the Rubeho Mountains, at 1970 m a.s.l. (-6.8981; 36.5653), Mpwapwa District, Dodoma Region of Tanzania.

Paratypes

Two adult females MTSN 5012, MTSN 5014, collected in the Mafwomero Forest Reserve on 8 January 1999 by Michele Menegon, Andrew Perkin and Lucinda Lawson.

Additional material

Three adult males – MTSN 8895, MTSN 8896, MTSN 8899 and two adult females – MTSN 8893, MTSN 8898 collected in Ilole Forest Reserve by Michele Menegon on 21 September 2006.

Diagnosis

A small chameleon with a maximum total length of 63 mm (maximum SVL = 46.8 mm) of which the tail comprises an average of 24% in females and 30% in males. Adult females and males have similar SVL. Overall morphology is similar to the other species in the complex, but it differs by a marked mitochondrial sequence divergence from other *Rhampholeon* more specifically for the ND2 gene by having the amino acid valine at position 421–423 and leucine at 469–471. For the 16S gene, it differs by having a T at position 1080, T at position 1123 and T at position 1125 (Table S3) and by its' restricted distribution in forest fragments of the Rubeho Mts. Without locality or genetic information, preserved individuals of the new species cannot be univocally distinguished on the basis of their morphology.

Description of Holotype

Snout-vent 41.12 mm, tail 11.12 mm. Body habitus leaf like - typical of all other *Rhampholeon* (*Rhinodigitum*) species.

Head: occiput flat, parietal crest formed by 5 enlarged tubercles. The lateral crests are studded with several prominent tubercles. The two supra-orbital ridges are connected to each other by a series of 18 inter-orbital tubercles arranged in a shallow V across the top of the head with the last tubercle being the most prominent. A tuft of tubercles forms a small peak on the supra-orbital rim. The two peaks are connected between the orbits by an interorbital row of 19 scales counted from peak to peak. Posteriorly to the interorbital row there's an additional, less distinct, one. The canthal ridges are formed

Fig. 10. *Rhampholeon rubeho* from type locality, in life.

by a row of enlarged, relatively smooth tubercles which merge anteriorly on the tip of the snout and extend into a small soft tuberculated rostral process extending 1 mm off the tip of the snout. A distinct temporal crest arises from the mid post-orbital rim and consists of 7 tubercles on both sides, of which the most posterior is the largest. The nares open infero-posteriorly. There are no gular appendages or crests.

Body: the dorsal crest is crenulated over the anterior two-thirds of the dorsal keel, fading to smooth over the sacrum. The crenulations are formed by a cluster of mucronate tubercles sited above each dorsal spinous process. The tail shows no crenulation. A single deep pit is present in each axilla, but there is no trace of a pit in the inguinal region. The soles of the feet present a relatively smooth, non-spinous cobblestoned appearance. Low palmar spines are present at the bases of the digits. The claws are strongly bicuspid.

Scalation: body covered with a subhomogeneous background of small stellate edged tubercles. A more-or-less regular scattering of larger (2–4 times larger in diameter) subconical/rounded tubercles occurs on the sides of the body. A particularly large tubercle is sited just above the shoulder. The forearms have a scattering of enlarged spinous tubercles, two prominent spines along the radius. The belly is smooth with small homogeneous scales. The scales on the circular eyeball are relatively heterogeneous.

Hemipenis: stout and truncated. No calyces or capitate structures are present and sulcal lips smooth. Two long apical horns are present, curving inwardly toward the sulcal surface. Each horn is adorned with 8 to 9 prominent thornlike papillae arranged in 3 basal ones and a row of 5 to 6 distal papillae.

Distribution

This species is known from Mafwomero Forest Reserve. However, there is an isolated population of *Rhampholeon* in the Ilole Forest Reserve, on the southern end of the Rubeho massif, about 50 km south from Mafwomero Forest Reserve. Individuals from the Ilole population were not included in the phylogenetic analysis, so we tentatively assign this population to *R. rubeho*. Inclusion in a phylogenetic analysis is needed to assess its taxonomic position.

Etymology

The species is named after the mountain block (Rubeho) where the type series was collected and where it is considered to be restricted. The specific epithet is considered to be a noun in apposition.

***Rhampholeon nicolai* sp. nov**

Nicola's Pigmy Chameleon

Holotype

Adult male in the MUSE, the Science Museum of Trento, MTSN 5592 collected in the Mamiwa Kisara North Forest Reserve on the 25th of January 2004 by Michele Menegon (Fig. 11 and Table S2).

Type locality

Mamiwa Kisara North Forest Reserve in the Ukaguru Mountains, at 1970 m a.s.l. (-6.3716; 36.9248), Gairo District, Morogoro Region of Tanzania.

Paratypes

One adult female – MTSN 5593 from same locality as the holotype. Four adult males – MHNG 2624.47, MHNG 2624.56, MHNG 2624.49, MHNG 2624.50 and two adult females – MHNG 2624.48, MHNG 2624.57 collected by Jean Mariaux in Ikwamba Forest Reserve (-6.3453; 36.9770) on 4–5th May 2002.

Diagnosis

A small chameleon with a maximum total length of 60.1 mm (maximum SVL = 47.44 mm) of which the tail comprises an average of 22% in females and 29% in males. Adult females and males have similar SVL. Overall morphology is similar to other species in the complex, but in this species, the relative size of the head and tail is significantly larger than in the other *Rhampholeon* species analysed, see Fig. 2 and related text. It also differs from other *Rhampholeon* for the ND2 gene by having the methionine codon ATC at position 445–447, and the amino acid threonine at position 454–456 (Table S3) and by its restricted distribution to the forest fragments in the Ukaguru Mts. Without locality or genetic information, preserved individuals of the new species cannot be unequivocally distinguished on the ground of their morphology alone.

Description of Holotype

Snout-vent 45.55 mm, tail 11.01 mm. Body habitus leaf like - typical of all other *Rhampholeon* (*Rhinodigitum*) species.

Head: occiput flat, parietal crest indicated by a few low tubercles. The lateral crests are studded with several prominent tubercles. No inter-orbital tubercles arranged in a shallow V across the top of the head. A tuft of flat roundish tubercles forms a small peak on the supraorbital rim, and additional cluster of enlarged tubercles for a second less raised peak on the orbital rim, posterior to the main one. The two main peaks are connected between the orbits by an interorbital row of 18 scales counted from peak to peak. The canthal ridges are formed by a row of enlarged, relatively smooth tubercles which merge anteriorly on the tip of the snout and extend into a small soft tuberculated rostral process extending 2 mm off the tip of the snout. A distinct Y shaped temporal crest arises from the mid post-orbital rim and consists of 12 tubercles on the right and 13 tubercles on the left, of which the most posterior is the largest. The nares open infero-posteriorly. There are no gular appendages or crests.

Body: the dorsal crest is crenulated over the anterior two-thirds of the dorsal keel, fading to smooth over the sacrum. The crenulations are formed by a cluster of mucronate tubercles sited above each dorsal spinous process. The tail is very weakly crenulate, more so over the

Fig. 11. *Rhampholeon nicolai* from type locality.

distal third. A single deep pit is present in each axilla, but there is no trace of a pit in the inguinal region. The soles of the feet present a relatively smooth, non-spinous cobblestoned appearance. Low palmar spines are present at the bases of the digits. The claws are strongly bicuspid.

Scalation: body covered with a subhomogeneous background of small stellate edged tubercles. A more or less regular scattering of larger (2–4 times larger in diameter) subconical/rounded tubercles occurs on the sides of the body. A particularly large tubercle is sited just above the shoulder. The forearms have a scattering of enlarged spinous tubercles, two prominent spines along the radius. The belly is smooth with small homogeneous scales. The scales on the circular eyeball are relatively heterogeneous.

Hemipenis: stout and truncated. No calyces or capitate structures are present and sulcal lips smooth. Two long apical horns are present, curving inwardly toward the sulcal surface. Each horn is adorned with 7 to 8 prominent thornlike papillae arranged in 3 basal ones and a row of 4 to 5 distal papillae.

Distribution

Rhampholeon nicolai occurs in the Mamiwa Kisara North and Mamiwa Kisara South forest reserves, which are contiguous in the Ukaguru mountains. In addition, specimens were recorded from Ikwamba Forest Reserve, adjacent to Mamiwa-Kisara North Forest Reserve. *Rhampholeon* individuals from Mikuvi forest have been recorded, and we tentatively assign this population to *R. nicolai*. However, these specimens will need to be properly evaluated to verify the taxonomic status of this population.

Etymology

This species is named after Nicola Colangelo, an entrepreneur and industrialist who worked in Tanzania for most of his life. He supported conservation initiatives and organisations, and before many others argued for the sustainable utilisation of natural resources. He was passionate about conservation for its intrinsic importance but also for a sustainable development of economies, such as low impact tourism activities.

Rhampholeon waynelotteri **sp. nov**

Wayne's Pygmy Chameleon

Holotype

Adult male in the MUSE, the Science Museum of Trento, MTSN 8542, collected in the Kanga Forest Reserve on the 22 March 2004 by Michele Menegon (Fig. 12 and Table S2).

Type locality

Kanga Forest Reserve in the Nguru Mountains landscape, at 1280 m a.s.l., (-5.9168; 37.7056), Mvomero District, Morogoro Region of Tanzania.

Paratypes

Four adult males – MTSN 8537, MTSN 8538, MTSN 8540, MTSN 8543 and two adult females – MTSN 8539, MTSN 8541 with the same locality as holotype.

Diagnosis

A small chameleon with a maximum total length of 55 mm (maximum SVL = 46.2 mm) of which the tail comprises an average of 21% in females and 26% in males. Adult females and males have similar SVL. Overall morphology is similar to other species in the complex. It differs from other *Rhampholeon* for the ND2 gene by having the amino acid histidine at position 436–438, threonine at position 445–447 and leucine at position 478–489 (Table S3) and by the restricted distribution in the forest of Mt. Kanga and Mkingu Nature reserve. In Mkingu, it is sympatric with *R. acuminatus* from which it can be easily distinguished by the lack of a large, discoid and vertically flattened rostral process and supra-orbital and cranial spinous projections, and with *R. princeeai* sp. nov. from which it is distinguished by the lack of a triangular, platform like rostral process. Aside from the two sympatric species, without locality or genetic information, preserved individuals of the new species cannot be univocally distinguished on the ground of their morphology

Description of Holotype

Snout-vent 40.03 mm, tail 9.33 mm. Body habitus leaf like - typical of all other *Rhampholeon* (*Rhinodigitum*) species.

Head: occiput flat, parietal crest indicated by a few low tubercles. No inter-orbital tubercles arranged in a shallow V across the top of the head. A tuft of tubercles forms a small peak on the supra-orbital rim. The two peaks are connected between the orbits by an interorbital row of 18 scales counted from peak to peak. The canthal ridges are formed by a row of enlarged, relatively smooth tubercles which merge anteriorly on the tip of the snout and extend into a small soft tuberculated rostral process extending 1 mm off the tip of the snout. A distinct temporal crest arises from the mid post-orbital rim and consists of 5 tubercles on the right and 4 tubercles on the left, of which the most posterior is the largest. The nares open infero-posteriorly. There are no gular appendages or crests.

Body: the dorsal crest is crenulated over the anterior two-thirds of the dorsal keel, fading to smooth over the sacrum. The crenulations are formed by a cluster of mucronate tubercles sited above each dorsal spinous process. The tail is very weakly crenulate, more so over the distal third. A single deep pit is present in each axilla, but there is no trace of a pit in the inguinal region. The soles of the feet present a relatively smooth, non-spinous cobblestoned appearance. Low palmar spines are present at the bases of the digits. The claws are strongly bicuspid.

Scalation: body covered with a subhomogeneous background of small stellate edged tubercles. A more-or-less regular scattering of larger (2-4 times larger in diameter) subconical/rounded tubercles occurs on the sides of the body. A particularly large tubercle is sited just above the shoulder. The forearms have a scattering of enlarged spinous tubercles, two prominent spines along the radius. The belly is smooth with small homogeneous scales. The

Fig. 12. *Rhampholeon waynelotteri* from type locality (top) and from Mkingu Nature Reserve (bottom left and right), in life.

scales on the circular eyeball are relatively heterogeneous. Hemipenis: not fully everted.

Distribution

Known only from Kanga and Nguru mountains. The population from Nguru, which has not been included in the phylogenetic analysis, is separated from the one in Kanga by a lowland corridor of about 8 km wide. On the basis of their geographic proximity and morphological similarities, we tentatively assign the Nguru population to this species. Further phylogenetic analysis is needed to confirm its taxonomic placement.

Etymology

This species is named after and dedicated to Wayne Lotter, in recognition to his ground-breaking work in developing a holistic and strategic intelligence-based approach to anti-poaching. He helped successfully reverse the high rates of elephant poaching in Tanzania, during 2010's. Unfortunately, he died on 16 August 2017 bravely fighting for the cause he was most passionate about. The specific epithet is patronym in the genitive masculine singular.

Rhampholeon princeeai **sp. nov**

Princeeai's Pygmy Chameleon

Holotype

Adult male in the MUSE, the Science Museum of Trento, MUSE 14034 (field tag MW 06838) collected in the Nguru South Forest Reserve, now part of Mkingu Nature Reserve, on 13 January 2008 by David Gower, Simon Loader, Hendrik Mueller and Mark Wilkinson (Fig. 13 and Table S2).

Type locality

Mkingu Nature Reserve in the Nguru Mountains, at 1870 m a.s.l. (-6.0655; 37.4907), Mvomero District, Morogoro Region of Tanzania.

Paratypes

One adult male – MUSE 14036 (field tag MW 06914), one adult female, MUSE 14033 (field tag MW 06837) and one juvenile – MUSE 14035 (field tag MW0 06839) same locality as the holotype. One adult male – MTSN 5537 and one adult female – MTSN 5538 (field tags respectively KMH 35703 and KMH 35704) collected by members of Frontier Tanzania on 7 September 2002.

Diagnosis

A small chameleon with a maximum total length of 46 mm (maximum SVL = 35.5 mm) of which the tail comprises an average of 28% in females and 29% in males. Adult females and males have similar SVL. Despite an overall morphology similar to other species in the complex, *R. princeeai* can be distinguished from all the other *Rhampholeon* by the shape and structure of the rostral process, which is expanded at the base, forming a scaly triangular, platform formed by 9 transverse and 11 longitudinal scales and by having a shallow pit in the inguinal region. It also differentiated by a marked mitochondrial sequence divergence, more specifically for the 16S gene by having a T at position 891, C at 1021, G at 1075, C at 1080, A at 1125, C at 1126 and T at 1135 (Table S3), and its' known distribution restricted to the high elevation areas of the Mkingu Nature Reserve.

Description of Holotype

Snout-vent 37.54 mm, tail 10.14 mm. Body habitus leaf like - typical of all other *Rhampholeon* (*Rhinodigitum*) species.

Head: occiput slightly elevated, a Y shaped parietal crest indicated by 3 short series of elevated, polygonal tubercles. The lateral crests are studded with several prominent tubercles and form a tuft of tubercles over the occiput. A tuft of conical, pointed tubercles forms a small peak on the supra-orbital rim. The two peaks are connected between the orbits by an interorbital row of 22 scales counted from peak to peak. The canthal ridges are formed by a row of enlarged, conical tubercles which merge anteriorly on the tip of the snout and extend into a triangular, tuberculated rostral process extending 1.3 mm off the tip of the snout with the base almost as wide as the length. A distinct temporal crest arises from the mid post-orbital rim and consists of 6 tubercles of which the most posterior is the largest. The nares open infero-posteriorly. There are no gular appendages or crests.

Body: the dorsal crest is crenulated over the anterior two-thirds of the dorsal keel, fading to smooth over the sacrum. The crenulations are formed by a cluster of mucronate tubercles sited above each dorsal spinous process. The tail shows no crenulation. A single deep pit is present in each axilla, a shallower pit is present in the inguinal region. The soles of the feet present a relatively smooth, non-spinous cobblestoned appearance. Low palmar spines are present at the bases of the digits. The claws are strongly bicuspid.

Scalation: body covered with a subhomogeneous background of small stellate edged tubercles. A more or less regular scattering of larger (2–4 times larger in diameter) subconical/rounded tubercles occurs on the sides of the body. A particularly large tubercle is sited just above the shoulder. The forearms have a scattering of enlarged spinous tubercles, two prominent spines along the radius. The belly is smooth with small homogeneous scales. The scales on the circular eyeball are relatively heterogeneous.

Hemipenis: not everted.

Distribution

Rhampholeon princeeai is only known from the type locality, presumably restricted to the montane forest above 1800 m a.s.l. Within the nature reserve, *R. princeeai* is syntopic with *R. acuminatus* and occurs sympatrically with the Nguru population of *R. waynelotteri*.

Etymology

The species is named after Richard Williams, better known by his stage name Prince Ea, an American activist and inspirational spoken word artist, poet, rapper and filmmaker. From 2014 he has shifted his focus from music to creating motivational and inspirational spoken word films and content covering a wide range

Fig. 13. *Rhampholeon princeeai* from Mkingu Nature Reserve, in life.

of topics such as deforestation and the reckless destruction of our environment for which we are all responsible. The specific epithet is considered to be a noun in apposition.

DISCUSSION

Morphological and phylogenetic diversity

The genus *Rhampholeon* is a morphologically conservative group, and species delimitations have relied heavily on molecular phylogenetic evidence (e.g., Fisseha et al., 2013). Our new phylogeny suggests that there are

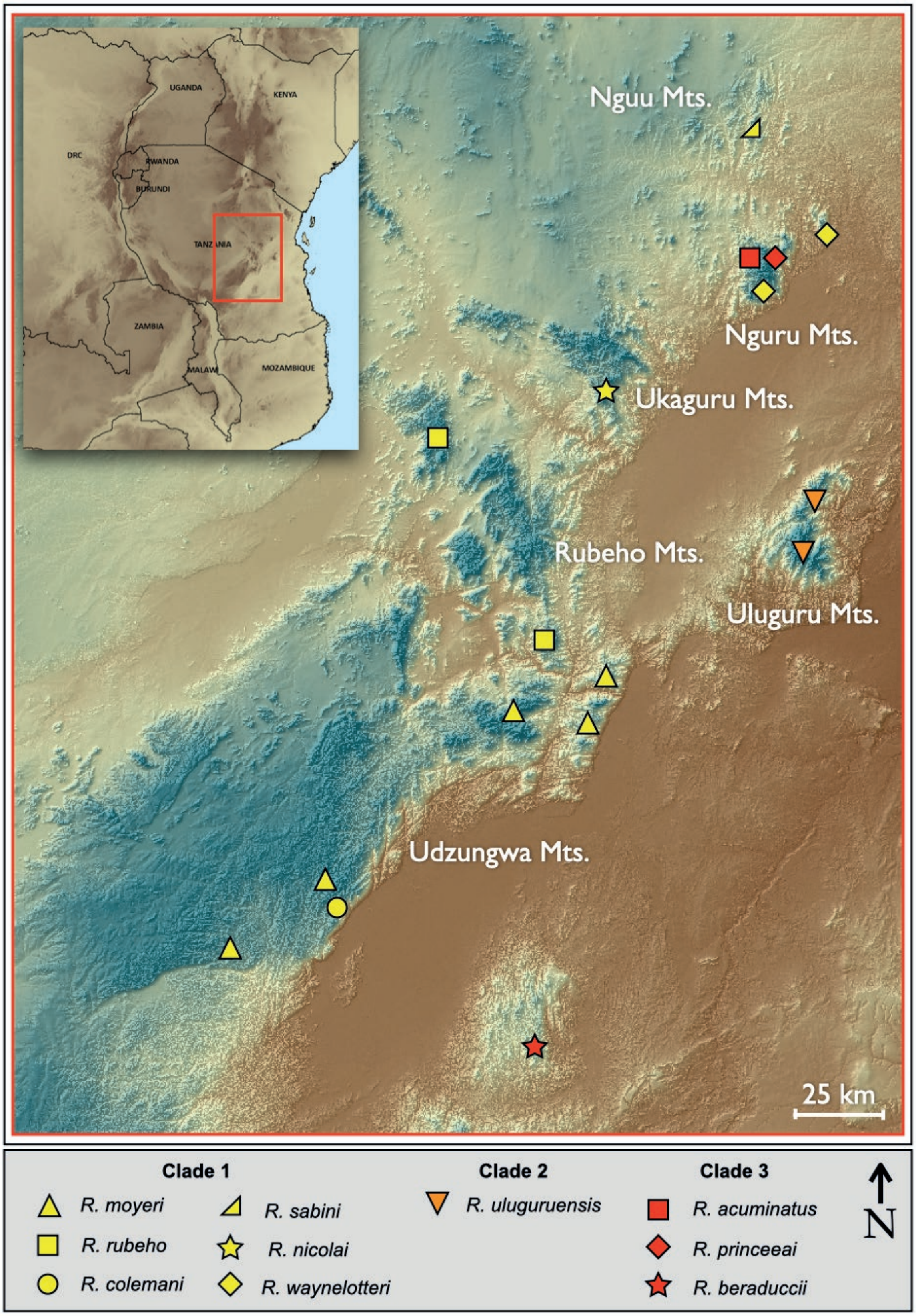

Fig. 14. Distribution of *Rhampholeon* species across the Eastern Arc Mountains of Tanzania. Inset map shows the study area.

at least seven species within the *Rhampholeon uluguruensis/moyeri* complex, of which six are undescribed. This conclusion is supported by the comparative barcoding approaches, where species divergences among these new species, ranging from 5 to 16%, show a difference comparable to other described *Rhampholeon* species (see Fig. S1). In *R. colemani* and *R. princeeai,* the clear genetic differences are supported by discrete morphological differences. The large phylogenetic differences are in contrast with the limited morphometric differences exhibited between most species. When morphological differences are lacking for species recognition, genetic and distributional information can be key characteristics for assisting in diagnosing species.

Our integrative taxonomic approach is broadly aligned with the General Lineage Concept (GLC) whereby species are considered as independently evolving metapopulation lineages (de Queiroz, 1998; 2007). Under the GLC, species distinctions are based on multiple criteria, such as pre- and post-zygotic isolation, monophyly, ecology and morphology. However, the GLC does not require all criteria to be fully met but does entail evaluating multiple lines of evidence to determine the distinction of a species. As outlined, we evaluated genetic and morphological data, providing a test of two lines of evidence. All the species have a similar ecological niche, inhabiting a leaf litter microhabitat in Afrotemperate forest. Despite this, they are geographically isolated by barriers of unsuitable habitat (savanna) which species are unable to inhabit or cross. Given the isolation of forests has been in place for a substantial amount of time (e.g., Tolley et al., 2013; Loader et al., 2014), we speculate that these species have likely been on separate evolutionary trajectories but largely nonchanging ecologically and phenotypically. In addition to morphology and species ecology, this is further supported by divergence estimates, which indicates that *R. uluguruensis* and the *R. moyeri* complex diverged more than 20 million years ago (Tolley et al., 2013).

The description of these six new species of pygmy chameleons increases the number of *Rhampholeon* to twenty-six. Although this is a substantial increase in species number, there is no doubt that additional *Rhampholeon* species remain to be described given that much of central and eastern Africa is poorly surveyed (Tolley et al., 2016). The number of described *Rhampholeon* has doubled in just the last 15 years, most of which are endemic to specific mountain chains in the Eastern Arc of Tanzania. At present, nearly every Tanzanian mountain chain (excluding Malundwe) has an endemic *Rhampholeon* species.

Conservation status

Chameleons have a high proportion of threatened species according to the IUCN Red List (see also Tolley et al., 2016). This high proportion of threatened species is linked to their habitats being increasingly impacted by human activities (e.g., Hall et al., 2009; Tolley et al., 2016; see Global Forest Watch). Although habitat loss, including fire, is the primary trigger for the elevated extinction risk in this group (Jenkins et al., 2013; Tolley et al., 2016), many species are also threatened by illegal or unsustainable harvesting for the pet trade (Jenkins et al., 2013). However, our understanding of the factors contributing to declines of individual species is poor. Our limited knowledge of species ecology and the absence of any information on population demographic trends prevents assessment of population declines or trends.

As is the case with many reptiles, the evaluation of chameleon species for their IUCN Red List status is based primarily on the extent of habitat loss within their inferred distributions. In order to infer these distributions, the spatial distribution of recorded localities is used, and for poorly sampled species, the estimation of distribution is difficult and could result in under-estimates of range size. The degree to which the inferred ranges overlap with protected areas as well as land cover change (e.g., habitat loss) allows for an assessment of extinction risk. However, the impact of habitat loss on chameleon populations (e.g., demographic decline trends) has not been assessed for any species. Clearly, a priority would be to monitor the population demographics of threatened species to better understand chameleon demographic trends given their susceptibility to environmental change and, at the same time, investigate the real supply of chameleons on the international market. For example, at reptile trade fairs in Europe, it is not uncommon to find various species of pygmy chameleons of the genus *Rhampholeon* for sale. However, given that *Rhampholeon* was only recently included on the Convention on International Trade in Endangered Species (CITES) list in 2017, some of these individuals could have entered the pet trade prior to their listing and be of captive bred stock. Thus, it is possible that wild caught and illegally exported individuals could be laundered as captive bred from animals that were in trade prior to the CITES listing.

The genus *Rhampholeon* includes a number of species with small, and fragmented ranges, with more than 30% of species considered threatened with extinction (https://www.iucnredlist.org/). Based on our field experience in East Africa, *Rhampholeon* species do not occur outside intact forest areas, and are restricted to the remaining, small, closed canopy forest fragments. The loss of forest is the primary factor for their high threat status. For the new species described here, we anticipate that most are at risk of extinction given that they have small distributions that have been impacted by habitat loss. Among them, *R. sabini, R. colemani* and *R. waynelotteri* mainly occur in submontane forests, where deforestation is particularly acute, unlike the higher elevations, e.g., >1500m (Hamunyela et al., 2020). In particular, *R. colemani* has a very small range (about 36 km^2) in a single forest patch. Although the entire distribution is within the Uzungwa Scarp Nature Reserve, an area that is officially protected, the forest habitat has declined in quality due to anthropogenic impacts that are continuing at present. It is unlikely that this species can be considered secure in that protected area. More broadly, other *Rhampholeon* that occur in Tanzania are also at risk of extinction, particularly *R. spinosus* (Endangered) and *R. acuminatus* (Critically Endangered) due to their highly fragmented and small distributions, that are impacted by habitat loss and have limited protection (in the Usambara and Nguru Mountains, respectively). While the high elevation montane forest region of the Eastern Arc has undergone less habitat loss than lower elevation forests, increasing deforestation levels are evident more recently (Hamunyela et al., 2020). For example, from 2001 to 2020, Mkingu Nature Reserve and Kanga Forest Reserve combined lost 1180 ha of humid primary forest and the total area of humid primary forest in decreased by 5.2% in this time period (data from Global Forest Watch, 2021). These increased impacts are likely to intensify the threats to high elevation *Rhampholeon* populations and species.

Without effective forest conservation measures, market-driven agricultural development is likely to trigger an expansion of cropland at the expense of forests to meet the increased demand for the agricultural products promoted, with negative impact on biodiversity, carbon sequestration and water services (Hamunyela et al., 2020). Based on recent estimations there are approximately 296,000 ha of closed canopy natural forest left in the Eastern Arc Mountains, that is more highly fragmented than previously estimated (Koskikala et al., 2020). In general, forest fragmentation causes multiple impacts for ecological resilience of tropical forests through direct habitat loss, reduction of fragment size, increase in edge effects, and spatial isolation, while fragmented forests are also prone to further deforestation (Koskikala et al., 2020). From a conservation perspective, small natural forest fragments are considered less important compared to large and intact natural forest patches (Pimm et al., 2013). However, small fragments are likely to harbor irreplaceable unique or rare biodiversity values compared to an area of the same size in intact landscape (Tulloch et al., 2016). In Tanzania, commercial and subsistence agriculture is the main driver of deforestation, and aside from protected areas, there is no clear policy limiting the conversion of forests to agricultural land, which would require a greater inter-sectoral coordination between the agriculture, livestock, land, energy and forest sectors (Doggart et al., 2020).

Our knowledge of the species diversity of *Rhampholeon* has improved greatly, particularly for Tanzania, but we still know very little about their basic ecology and life history. Furthermore, their geographic distributions are poorly known as most species are known from just a few records from even fewer localities. It is unclear if *Rhampholeon* species from Tanzania are significantly buffered from extinction risk, despite many occurring in protected areas. For example, *R. acuminatus* from the officially protected Nguru Mountains is considered Critically Endangered suggesting that formal protection of the habitat has not safeguarded this species from extinction. An improved quantitative assessment of demographic trends for these threatened species is needed to understand whether protected areas are functioning as adequate buffers for these species. Overall, there is concern regarding the long-term survival of East African pygmy chameleons but limited data on their biology, distribution and population prevents empirically based solutions to mitigate declines and their future survival.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 12978

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