

THE PYGMY CHAMELEONS OF THE EASTERN ARC RANGE (TANZANIA): EVOLUTIONARY RELATIONSHIPS AND THE DESCRIPTION OF THREE NEW SPECIES OF *RHAMPHOLEON* (SAURIA: CHAMAELEONIDAE)

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The pygmy chameleons of the Eastern Arc Range forests in Tanzania are reviewed on the basis of known and newly collected material. Two new species belonging to *Rhampholeon* (*Rhinodigitum*) and one to *Rh.* (*Rhampholeon*) from the Pare, Nguru and Mahenge Mountains are described. The status and distribution of the other species known in the area are reviewed, and an identification key is provided. The phylogenetic relationships between these taxa are discussed on the basis of small and large mt-rDNA subunits sequences and the relative importance of some morphological characters is evaluated. Hypotheses about the evolution of the group in the area are presented.

Key words: biodiversity hotspot, biogeography, molecular systematics, *Rhampholeon*, *Rieppeleon*

INTRODUCTION

The Eastern Arc Range of Tanzania (EAR) is composed of more than a dozen isolated massifs arrayed in an arc across the north-eastern and central regions of Tanzania (Fig. 1), geologically separated from the Southern Rift Mountains (like the Poroto and Livingstone ranges). The relict montane and sub-montane forests of the EAR (Pócs, 1976) are well known for their extraordinary biodiversity and high level of endemism (Mittermeier *et al.*, 1999; Myers *et al.*, 2000; Newmark, 2002). The East and West Usambara Mountains, which are the closest inland from the coast, are perhaps the best known and most

intensively studied areas of the Eastern Arc. Their herpetological fauna has been explored in detail since the late 1800s by a number of distinguished German and American scientists (e.g. Barbour & Loveridge, 1928; Mertens, 1955; Loveridge, 1957 and references therein). However other massifs of the range, some rather far inland, are comparatively little explored and their fauna is still largely unknown.

Good examples of highly diversified groups of organisms in the area are the pygmy or leaf chameleons of the genera *Rhampholeon* (Günther, 1874) and *Rieppeleon* (Matthee *et al.*, 2004). There are presently 14 species described in these two genera (Tilbury & Mariaux, 2004; Uetz, 2005) and their phylogenetic relationships have been derived from molecular analyses based on one nuclear and two mitochondrial genes by Matthee *et al.* (2004), who also split *Rhampholeon* into three subgenera (*Rhampholeon*, *Rhinodigitum* and *Bicuspis*). *Rieppeleon* is mostly a lowland taxon, while *Rhampholeon* is primarily a montane genus.

Seven species have been reported in the EAR, among which five are endemic (Broadley & Howell, 1991; Tilbury & Emmrich, 1996; Flemming & Bates, 1999; Menegon *et al.*, 2002; Spawls *et al.*, 2002; Matthee *et al.*, 2004; Tilbury & Mariaux, 2004). The external morphological differences between some of these species are subtle and the assessment of characters is often open to subjective interpretation.

Although the genus is well known from the East Usambara forests, specimens of *Rhampholeon* from many sub-montane forests to the west of the range were not known until more recently, when these forests were targeted for biodiversity and other research surveys. Interestingly the EAR endemic species known so far seem to have a restricted distribution, and are found in a few massifs only. Given the high level of morphological similarity and the relative inconspicuousness of these lizards, we thought it possible that undescribed taxa might be found in the few remaining forests of the



FIG.1. Geographical position of the Eastern Arc Range with massif names and sampling localities (map modified from the Bugwood Network, 2002).

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more remote EAR massifs, and that the diversity of the groups could in fact be more important than recorded until now. An accurate systematic coverage of the group would also allow a better understanding of the evolutionary relationships of its members, as well as their precise geographical distribution.

During the course of several expeditions between 1999 and 2002 we collected numerous reptiles and amphibians in the EAR, and studied their evolutionary relationships as well as their parasitofauna. In the collection were 65 pygmy chameleons belonging to nine species, only six of which have been previously described. A few other specimens collected earlier by other workers in the Usambaras and Pares, tissue samples provided by M. Menegon (Trento, Italy) and comparative material from outside the EAR are also included in this study.

In this contribution we describe three new species, discuss the taxonomic status of several taxa and, more generally, discuss the distribution of the pygmy chameleons in the EAR, as well as the evolutionary relationships of these taxa as derived from mitochondrial DNA sequences.

MATERIALS AND METHODS

SPECIMENS AND LOCALITIES

Collection data for all unpublished material are given in Appendix 1 (specimens) and Appendix 2 (localities). All specimens mentioned in the text are adults unless specified otherwise. Abbreviations: FR: Forest Reserve; *Rh.*: *Rhampholeon*; *Ri.*: *Rieppeleon*.

List of acronyms used for Collections – BMNH: British Museum, Natural History, London; KMH: Collection Kim Howell, Dar es Salaam; MHNG: Muséum d'Histoire Naturelle de Genève, Geneva; MNHN: Muséum National d'Histoire Naturelle, Paris; MTSN: Museo Tridentino di Scienze Naturali, Trento; NMB: National Museum, Bloemfontein; NMZB: National Museum of Zimbabwe, Bulawayo; PEM: Port Elizabeth Museum; UDSM: University of Dar es Salaam.

METHODS

Pygmy chameleons were caught at night by hand during torchlight searches. Most specimens were dissected in the field for parasitological investigations within 24 hours of capture. During this process all abdominal soft organs, including gonads, were removed and examined for parasites (except for *Rh. viridis* specimens with field tags other than TZ). A small piece of tissue (usually liver) of each animal was removed and conserved in 80% ethanol for further molecular studies. The specimens were then labelled and fixed in 2-4% buffered formaldehyde for one week to one month, then transferred to 70% ethanol for long-term conservation. They are kept at the MHNG, UDSM, NMZB and PEM. All measurements for morphological studies were made on alcohol-preserved material.

For molecular analyses, DNA extractions were made out of liver samples with the DNeasy Tissue kit®

(Qiagen) according to the manufacturer's instructions. DNA fragments were amplified in a Techgene thermocycler (Techne). A 0.56 kbp fragment of the 16S rDNA was amplified using the universal primers L2510 and H3059 designed by Palumbi *et al.* (1991). PCR conditions were as follows: 94° for 3 min, then (93°, 45 seconds; 55°, 45 seconds; 72°, 1 min) × 37 cycles, and final extension 72° for 5 min.

For 12S, we used the primers 12S1 (CTAGGATTAGATACCCTACTATGC) and 12S2 (GATGAGGGTGACGGCGGTGTG) that are modified versions of the universal primers designed by Kocher *et al.* (1989). PCR conditions were as for 16S except that annealing temperature was 60°. PCR products were checked on a 1% agarose gel, then purified with the QUIAquick® purification kit (Qiagen) and resuspended in a final volume of 30 µl.

Cycle sequencing reactions on both strands were performed using the BigDye® cycle sequencing kit (Applied Biosystems), and sequences were obtained with an ABI 377 automated sequencer. All sequences are deposited with EMBL under accession numbers AJ609595, AJ609597 to AJ609600, and AM55644 to AM55698.

Sequences were treated and aligned with Sequencher™ v. 4.1.2 (Gene Codes Corp.), and minor corrections were done by hand. The final matrix was analysed with PAUP* v4.0b10 (Swofford, 2002), including tests for base composition heterogeneity and for checking the compatibility of partitions using the PHT test (Farris *et al.*, 1994). Evolutionary relationships were inferred under the parsimony criterion. Heuristic parsimony analyses (100 repeats, random addition order) were performed on the whole matrix, with the following settings: uninformative characters excluded, characters unordered and unweighted, gaps treated as missing (or fifth base), multistate treated as uncertainty. Nodal support was estimated with 1000 bootstrap pseudoreplicates (each with five repeats). Comparative maximum likelihood (ML) heuristic analyses (with 50 repeats) were performed on the same matrix. The best-fit model was determined by Modeltest v. 3.6, using the Likelihood Ratio Test (LRT) (Posada & Crandall, 1998). ML bootstrapping was limited to 100 pseudoreplicates (each with three repeats).

RESULTS

DIVERSITY AND DISTRIBUTION OF PYGMY CHAMELEONS IN THE EAR

Up until recently, only seven species (*Rh. boulengeri*, *Ri. brachyurus*, *Ri. brevicaudatus*, *Ri. kerstenii*, *Rh. moyeri*, *Rh. temporalis* and *Rh. uluguruensis*) were reported from the EAR (Broadley & Howell, 1991; Spawls *et al.*, 2002; Uetz, 2005). To this list we must add *Rh. spinosus*, which was transferred to *Rhampholeon* by Tilbury & Mariaux (2004). These taxa were reported from six mountain chains only (East and West Usambara, Nguru, Uluguru, Udzungwa and Ukaguru) until Loader *et al.* (2004b) reported *Ri.*

TABLE 1. Known distribution of pygmy chameleons in the EAR, by main massif (+ indicates presence). a, associated with non-montane elevations around the bases of these mountain ranges; b, specimens found by M. Menegon (Italy), see Menegon *et al.* (2003); c, *boulengeri*-like specimens from the Nguru appear to belong to the *uluguruensis* complex (see text); d, described as *Rh. moyeri*.

| | <i>Ri.</i> <i>brachyurus</i> | <i>Ri.</i> <i>brevicaudatus</i> | <i>Ri.</i> <i>kerstenii</i> | <i>Rh.</i> <i>spinus</i> | <i>Rh.</i> <i>temporalis</i> | <i>Rh.</i> <i>uluguruensis</i> complex | <i>Rh.</i> <i>acuminatus</i> | <i>Rh.</i> <i>viridis</i> | <i>Rh.</i> <i>beraduccii</i> |
|------------|---------------------------------|------------------------------------|--------------------------------|-----------------------------|---------------------------------|--|---------------------------------|------------------------------|---------------------------------|
| N Pare | | | + a | | | | | | + |
| S Pare | | | + a | | | | | | + |
| W Usambara | | | + a | + | | | | | + |
| E Usambara | | + | + a | + | + | | | | |
| Nguu | + a | | + a | | | + b | | | |
| Nguru | + a | | + a | | | + c | + | | |
| Ukaguru | + a | | | | | + | | | |
| Rubeho | | | | | | + d | | | |
| Uluguru | | + | | | | + | | | |
| Udzungwa | | + | | | | + d | | | |
| Mahenge | | + | | | | | | | + |

brevicaudatus and another undetermined species from the isolated Mahenge mountains. This situation is summarized in Table 1. In the course of the present work we found almost all these taxa again, and also noted new localities and range extension for several species. Although recently discovered in north-western Tanzania, we have discounted the presence of *Rh. boulengeri* in the EAR, its occurrence being based on misidentified specimens. We also report three completely new taxa, bringing the total number of described pygmy chameleons in the EAR to 10; however, the exact status of these species is not always certain, as discussed below. A key allowing the identification of all EAR pygmy chameleons is provided in Appendix 3. One other species (*Rhampholeon nchisiensis*) occurs in Tanzania, inhabiting the forests of the Poroto and Livingstone mountains and the Tukuyu volcanic complex. Since these mountain ranges are not included in the EAR, further discussion of this species is not provided.

RIEPPELEON BRACHYURUS (GÜNTHER, 1893)

Although known from a diverse geographical range within Tanzania, none of the reported localities could be considered to be montane. It has been collected from the Miombo woodlands at the base of several massifs of the EAR including the Nguru, Ukaguru and near the Nguu.

RIEPPELEON BREVICAUDATUS (MATSCHIE 1892)

This species is the most widely encountered in the EAR mountains. It is known from many lowland forests extending from south-eastern Kenya into Tanzania, penetrating into sub-montane forest in the East Usambara, Uluguru, Nguru and Udzungwa up to 1300 m (Spawls *et al.*, 2002). Loader *et al.* (2004b) recently reported its presence in the Mahenge and found it in all the above-mentioned massifs up to a maximal altitude of about 1200 m in the Uluguru, which is compatible with the recent observations of Emmett (2004). Although *Ri. brevicaudatus* is common in the East Usambara we were unable to confirm its presence in the

West Usambara, and, until further collecting proves otherwise we regard *Ri. brevicaudatus* as being absent from this massif.

RIEPPELEON KERSTENII (PETERS, 1868)

Although not a montane species, this pygmy chameleon is widely distributed in north-eastern Tanzania and may be found in acacia scrub and grasslands on the lower slopes of several of the EAR massifs including Uluguru, Nguru, Nguu, Ukaguru, Usambaras and Pares ranges. It is likely that its range extends into the foothills of the Rubeho as well.

RHAMPHOLEON (RHINODIGITUM) BOULENGERI (STEINDACHNER, 1911)

This species is currently considered to be widely distributed in eastern central Africa, including Burundi, Rwanda, Uganda, the eastern Democratic Republic of Congo and in remnant forest patches in western Kenya up to 2000 m. At least one report from Tanzania (Kange Estate, Nguru mountains) is known (Witte, 1965), and we have recently become aware of a new Tanzanian record from Minziro Forest in Bukoba (J. Beraducci, *in litt.*, 2005). The homogeneity of this widely distributed species has not to date been tested and it is considered possible that *boulengeri* could represent a species complex. A population of pygmy chameleons from the upper reaches of the forests on the Nguru mountains bears a striking morphological resemblance to *boulengeri* and has an almost identical hemipenal structure (Tilbury, unpublished observations). A further population of pygmy chameleons from the Nguu mountains is likewise very similar to *boulengeri* (Menegon *et al.*, 2003).

We collected *boulengeri*-like specimens from the Nguru and included them in our molecular analysis together with specimens from the core populations of the species (DRC, Rwanda) in order to check their conspecificity. The results of the mtDNA analysis clearly show that the Nguru specimens, as well as those from the Nguu, are unequivocally members of the

uluguruensis complex (see below). We therefore consider that *Rh. boulengeri* is absent from the EAR.

RHAMPHOLEON (RHINODIGITUM) MOYERI (MENEGON ET AL., 2002)

This recently described species (Menegon *et al.*, 2002) is currently only known from two localities in the Udzungwa but DNA analyses suggest that the population present in the Rubeho mountains is also of this form (Matthee *et al.*, 2004). It may be relevant to note that the only morphological characters allowing the differentiation between *Rh. moyeri* and *Rh. uluguruensis* are found at the level of the hemipenis (10-12 vs. 9 papillae) and the interorbitals (15-19 vs. 11-13 tubercles) (Menegon *et al.*, 2002), and should be considered with caution given the variation known to exist at least for the second criterion. We included two specimens from *Rh. moyeri* provided by M. Menegon in our mtDNA analysis and obtained equivocal results, as the two samples did not form a clade in our tree (Fig. 12). The specimen from Kihanga is shown to be related to *uluguruensis*, but the specimen from Kitolomero is basal to the *uluguruensis* complex, thus, in theory, possibly justifying its specific status. In any case the differentiation of this species with other members of the *uluguruensis* complex remains at best difficult (see also discussion).

RHAMPHOLEON (RHAMPHOLEON) SPINOSUS (MATSCHIE, 1892)

This species was recently transferred to *Rhampholeon* (from *Bradypodion*) by Tilbury & Mariaux (2004). It is an endemic of the West and East Usambara, and although somewhat more common in the former mountains, it is rare and vulnerable to environmental changes in both places. We found it between about 1000 and 1500 m. Its position in our tree, as well as its morphological characteristics, makes it a member of the *Rh.* (*Rhampholeon*) subgenus (Matthee *et al.*, 2004, appendix C).

RHAMPHOLEON (RHAMPHOLEON) TEMPORALIS (MATSCHIE, 1892)

A poorly known endemic of the East Usambara (and a few neighbouring relict forests) found at up to 1400 m in the East Usambara (Emmett, 2004). Although this species is surrounded by habitats rich in other species of pygmy chameleon, a recent comparative DNA study of the pygmy chameleons (Matthee *et al.*, 2004) showed that its closest relative was the West African species *Rhampholeon spectrum*. Our augmented database shows however that both *Rh. spinosus* and *Rh. viridis* n. sp. (described below) are closer relatives of *Rh. temporalis*, thus demonstrating the radiation of the *Rh.* (*Rhampholeon*) lineage in the easternmost extremity of the EAR.

The morphological homogeneity of this lineage is reinforced by the hemipenis anatomy of its members. The hemipenes of *Rh. temporalis* and *Rh. spectrum* are fig-

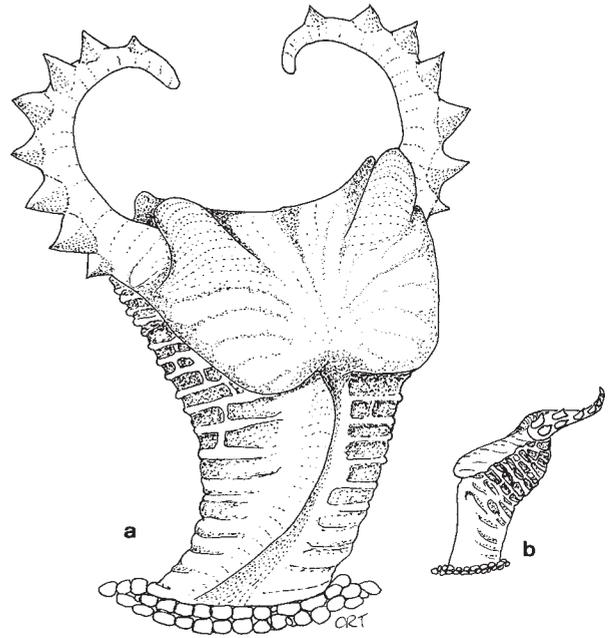


FIG. 2. *Rhampholeon temporalis* (BM 1988.641). Left hemipenis: a, sulcal view; b, lateral view.

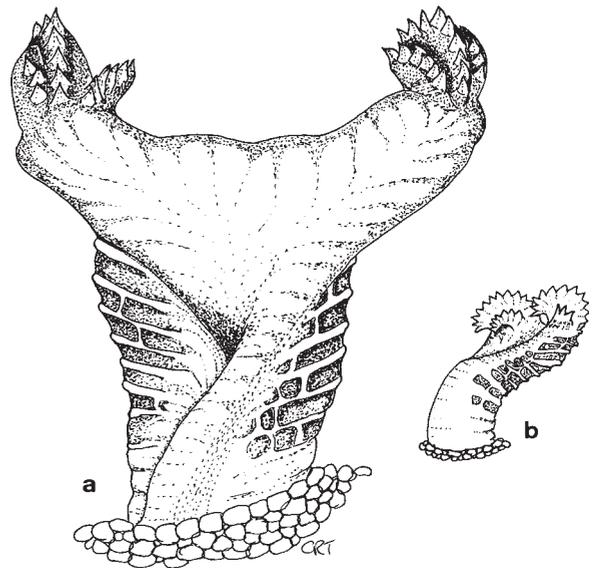


FIG. 3. *Rhampholeon spectrum* (PEM R 15701). Right hemipenis: a, sulcal view; b, lateral view.

ured here for comparative purposes to emphasize the unique morphology within the subgenus *Rhampholeon* (*Rhampholeon*) (Figs 2-3). The hemipenes of the subgenus would appear to combine features commonly associated with the typical chameleons, viz: elongate and calyculate truncus with a capitate apex, and the paired apical structures more typical of *Rhampholeon*. *Rh. spectrum* has a rather more complex hemipenal apex indicative of its divergent history. In this species a dual arrangement of short blunt apical horns each flanked laterally by a denticulate crest and medially by a smaller blunt horn (Fig. 3) is much more evocative of the apical rotulae seen in typical chameleons.



FIG. 4. *Rhampholeon (Rhinodigitum) beraduccii* n. sp. Male; Sali, Mahenge mountains.

RHAMPHOLEON (RHINODIGITUM) ULUGURUENSIS
TILBURY & EMMRICH 1995

Tilbury and Emmrich (1996) described this species from the Uluguru. We have since recorded this taxon from other neighbouring massifs with slight morphological variations, and our sampling from the Uluguru (in the isolated Mkungwe massif), Rubeho, Nguru and Ukaguru mountains, as well as additional samples from very similar specimens from the Nguu (provided by M. Menegon) allowed for a test of the validity and limits of this taxon (see below). With the latter specimens, all EAR massifs are now known to harbour a pygmy chameleon fauna.

RHAMPHOLEON (RHINODIGITUM) BERADUCCII SP. NOV.
(FIGS 4-5)

Holotype. MHNG 2655.019 (field tag TZ 343), female. Tanzania, Morogoro region, Mahenge Mountains, Sali FR [8°57'57.4" S, 36°41'17.9" E], about 1000 m, 9 October 2001. Collected by J. Mariaux & S. Loader.

Paratypes. Two males, MHNG 2655.020–021 (TZ 344, TZ 345) same locality and date.

Etymology. The new species is named in honour of Joe Beraducci, Arusha, Tanzania, as an appreciation for his generous assistance and help provided to us and to numerous other scientists working in the EAR.

Diagnosis. Chamaeleonidae, *Rhampholeon (Rhinodigitum)*. With the characters of the subgenus. A tiny brown chameleon with snout–vent length (SVL) 20.5–28 mm, maximum total length (TL) 36 mm, and a very short tail, 19–22% of TL. The smallest known *Rhampholeon*. 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 sub-homogeneous granules, but conspicuous shoulder spine present. Deep axillary and inguinal pits present. Claws bicuspid with small accessory spines.

Description of the holotype. Head (Fig. 4): Casque discrete, flat, with smooth edges. Weak temporal crest, first a horizontal line then forming an upward angle, without marked ornamentation except for three larger



FIG. 5. *Rhampholeon (Rhinodigitum) beraduccii* n. sp. (paratype, MHNG 2655.021). Head detail. Adult male; Sali, Mahenge mountains.

tubercles just behind the eyes. No parietal crest. Supra-orbital ridge well marked, peaking anteriorly in flattened, short and thick horn-like clusters of tubercles. Two larger tubercles on inferior orbital rim. Supra-orbital peaks connected by an interorbital ridge composed of 14 small granular tubercles marking a prominent frontal line. Rostral ridge well marked, forming a small bump over the nostrils and joining anteriorly in a 1.5 mm long triangular, pointed rostral appendage, about 12 small granules long and seven wide at its base. Nares opening posteriorly. No gular or mental appendages.

Body: TL 36 mm, (SVL 28 mm, tail length 8 mm. Tail 22% of TL. Dorsal crest weakly undulating – almost smooth, without clusters of spines, smooth on the lumbar area, and again weakly undulated on the tail. Deep axillary and inguinal pits present.

Flank scalation homogeneous, composed of small stellate granules with occasional slightly larger ones. One conspicuous enlarged dark tubercle over shoulder and another one on upper mid-flank. Scalation somewhat more irregular on the limbs. One or two larger tubercles on forearms. Claws strongly bicuspid with small accessory plantar spines.

Variation in paratypes. Males, TL 28–29, and tail 19–20% of TL. Very similar to holotype. Interorbital ridge up to 16 granules. Lateral crest more developed. Up to four tubercles behind and below the eye. Rostral appendage with slightly curved lower border. Tubercles on limbs a little more developed although still discrete. The hemipenal morphology is unknown at present.

Colour in life (Fig. 5). Generally yellowish to pale brown with various darker spots, especially on the back. May present two thin blackish diagonal lines on the flanks (antero-dorsal to postero-ventral).

Differential diagnosis. *Rh. beraduccii* can be differentiated from other members of the genus by its smaller size, small optical peaks and the shape of its rostral appendage. Furthermore, members of the similar *uluguruensis* group, including *Rh. moyeri*, do not show inguinal pits, which are clearly marked in *Rh. beraduccii*.

Distribution and ecology. *Rh. beraduccii* is to date only known from the vicinity of Sali in the Mahenge



FIG. 6. *Rhampholeon (Rhinodigitum) acuminatus* n. sp. Adult male; Nguru South FR, Nguru mountains.

mountains, an isolated massif separated from the Udzungwa range by the Kilombero valley. All animals were found alone, on low shrubs or herbs, within a few centimetres of the ground, in open land, in the immediate vicinity of the village.

Remarks. No faunistic surveys of the Mahenge were available until 2004 when Loader *et al.* (2004b) reported the presence of *Ri. brevicaudatus* as well as of *Rh. cf. moyeri* around Sali FR. Although morphologically close to *moyeri*, specimens from the latter group are clearly distinct from the other members of the *uluguruensis* complex. Meanwhile our mtDNA analysis confirmed that the Mahenge specimens were unequivocally distinct from *moyeri* or *uluguruensis*. The very small size of our specimens might also indicate that only juveniles or immature animals were collected. Although this cannot be completely excluded, we note that our specimens were collected in several distinct locations around Sali and, although more specimens were spotted, no significantly larger individuals were seen. Even if *Rh. beraduccii* is remarkably small, other tiny chameleons are known; *Ri. brachyurus*, for example, does not reach 6 cm, and *Brookesia minima* is of a size similar to the new species. Thus, on the basis of both our morphological and molecular evidence, and despite a very limited sampling, we propose to designate this material as a new species.



FIG. 7. *Rhampholeon (Rhampholeon) viridis* n. sp. Adult male; Chome FR, South Pare mountains.

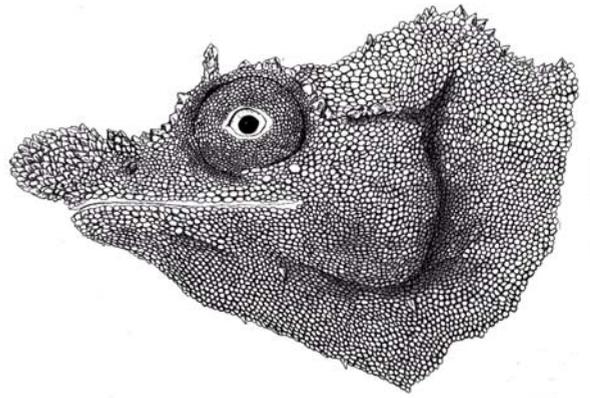


FIG. 8. *Rhampholeon (Rhinodigitum) acuminatus* n. sp. (Holotype, MHNG 2645.001). Head detail.

RHAMPHOLEON (RHINODIGITUM) ACUMINATUS SP. NOV.
(Figs 6, 8, 9)

Holotype. MHNG 2645.001 (field tag TZ 414), male. Tanzania, Morogoro region, Nguru mountains, Nguru South Catchment FR, Komkore Forest above Ubili village [6°2'29" S; 37°30'40.5" E], 1500–1600 m, 21 October 2000. Collected by J. Mariaux & S. Loader.

Paratypes. Three males, MHNG 2645.002–004 (TZ 412, 413, 417), two females MHNG 2645.005–006 (TZ 415, 416), and one male PEM-R 16271. All same locality and date.

Other material. Three specimens collected by David Moyer (Iringa, Tanzania), 25–26 August 1997, 6 km SW of Ubili, 1500 m. These specimens were the first ever recorded for the species but can no longer be localized and are presumed to be lost.

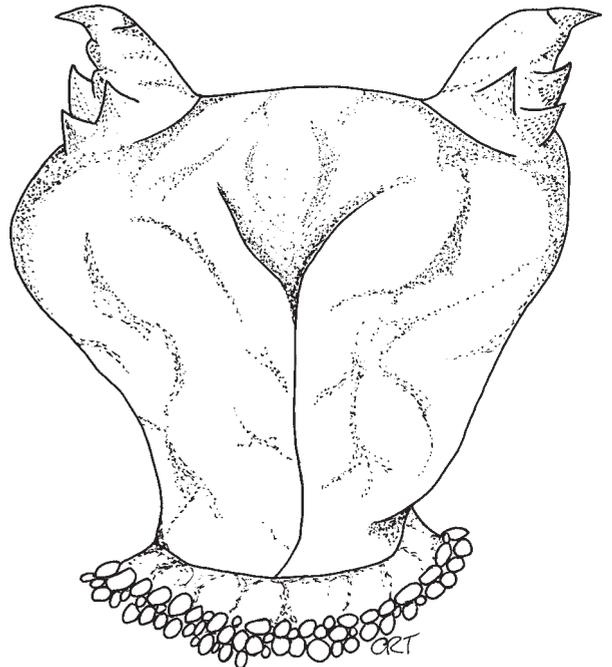


FIG. 9. *Rhampholeon (Rhinodigitum) acuminatus* n. sp. (PEM-R 16271). Hemipenis: sulcal view.

Etymology. From Latin *acuminare* (to sharpen), in reference to the numerous sharp spines found on the head and body.

Diagnosis. 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. 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.

Description of the holotype. Head (Fig. 8): Elongated with a particularly prominent pyramidal casque formed by upward extensions of the posterior orbital/lateral crests. The parietal region of the head is slightly concave. Sharply acuminate vertical spines are distributed along the lateral edges of the casque (three on each side) and one at the peak. Weak postero-orbital transversal crest. No parietal crest. Supra-orbital ridge strong, marked by large rounded tubercles in its posterior half, peaking anteriorly in prominent, thin, horn-like tufts of tubercles 4–5 rows of tubercles high, just posterior to a markedly enlarged tubercle. Supra-orbital peaks connected by a row of 12 flattened tubercular plates. Orbits almost touching each other anteriorly, only separated by 1–2 granules. Temporal crest prominent, composed of a fin-like triangular ridge formed of 6–8 tubercles in a horizontal line behind the postorbital rim, bending upwards posteriorly. 2–3 enlarged tubercles below the eyeballs. Pre-orbital ridge well marked peaking above the nostril in a short conical cluster, 2–3 tubercles high. Rostral appendage oval (12 granules along its maximal length \times 10 granules at its maximum height, 4×3.5 mm), with its longer axis horizontal, 6–7 tubercles wide at its base, becoming 2–3 tubercles thick anteriorly. Nares opening posteriorly. No submental appendage. No gular crest but a few randomly distributed spinous tubercles along the mandible and the upper throat.

Body: TL 82 mm, SVL 57 mm, tail length 25 mm. Tail 30% of TL. Dorsal crest preceded anteriorly by three paired simple tubercles commencing at the nape, followed by nine prominent pyramidal clusters of tubercles each positioned over a vertebral body, the most prominent in the middle of the back, separated from each other by 4–6 granules, becoming smooth on the lumbar area, then followed by 14 smaller clusters distributed along the length of the tail. Largest cluster formed of about 10 tubercles. No axillary or inguinal pits. Flank scalation homogeneous, composed of small interlocking stellate granules with about 10–12 enlarged spiny tubercles, half a dozen of them forming an indistinct row on the upper flank. Tail with a row of prominent isolated spinous tubercles along the inferolateral side of the tail on each side. One inconspicuous spine above the shoulder. Forearms with 3–4, and fore-

legs with 1–2, large isolated spinous tubercles. Upper arms and legs with 1–2 spines. Claws bicuspid. Palms and soles smooth but 1–2 small accessory plantar spines present at the base of each claw. The holotype hemipenis is not everted.

Variation in paratypes. Males: TL 63–71 mm, and tail 25–30% of TL. Casque sometimes with only two lateral spines on each edge, the most basal one more prominent than in holotype. Some specimens have a noticeable interorbital ridge of up to 8–12 tubercles across the ridge to the bases of the supra-orbital horns. Parietal crest always absent but some very small irregular lines may be present on top of head. Temporal crests may be reduced to only 3–4 tubercles on a straight line, the most posterior one being the most prominent. Up to six large spines on forearms and forelegs, up to three on upper arms, and four on upper legs. Up to about 20 small body spines per flank.

Females. TL 67–69 mm, and tail 25–28% of TL. Variation as for males.

Hemipenis. PEM-R 16271 (Fig. 9). Short, bag-like. No truncal calyces. Apex adorned with two short outwardly curved horns. Each horn has a cluster of three prominent thorn-like papillae at the base and 1–2 other papillae along the outer curvature of the horn.

Eggs. Both female specimens with four eggs (up to 11×6 mm).

Colour in life (Fig. 6). Background colour varies from rather bright shades of green, especially on head, to light brown. Generally paler on lower parts with feet pale yellow and belly almost white. Blue patches may be present on casque and shoulders, and occasionally yellow to orange spots at the level of eyes. Very small regular dark spots sometimes present on the body, seen especially in chameleons with a greenish background. Two prominent wide dark antero-dorsal to postero-ventral parallel lines are almost always visible.

Differential diagnosis. Among the pygmy chameleons, only *Rh. spinosus* presents a similar rostral process. However, *Rh. spinosus* has a more rounded rostral process, numerous spiny tubercles on the gular region, a slender overall appearance, and a significantly longer tail (up to more than 40% of TL); furthermore it is not sympatric with *Rh. acuminatus*. Although several other species, like *Rh. uluguruensis* and related taxa, also have rather conspicuous naso-rostral processes, these are more cylindrical and much smaller. Furthermore these species do not show the characteristic body spines seen in *Rh. acuminatus*, thus making confusion unlikely.

Distribution and ecology. So far *Rh. acuminatus* is known from a single population in an Afro-montane rainforest between 1500 and 1600 m above the village of Ubili in the Nguru mountains. The species seems to be locally abundant. Six specimens have been collected for the present description; another six have been transferred to a reptile park in Arusha to attempt captive breeding. Most animals have been found between 50 cm and 2 m high on large ferns and shrubs, although several

have been spotted up to an estimated 3-4 m high. This spatial distribution is rather unusual for pygmy chameleons, which generally stay closer to the ground. Interestingly it is comparable in its arboreal inclination to the morphologically similar *Rh. spinosus* from the Usambara mountains. The living specimens laid 2-4 eggs, hatching in January (J. Beraducci, Arusha, *in litt.*).

RHAMPHOLEON (RHAMPHOLEON) VIRIDIS SP. NOV. (FIGS 7, 10, 11)

Holotype. NMZB 16905 (field tag CT 119), male; allotype NMZB 16906 (CT 120), female. Tanga region, South Pare mountains, from a patch of forest next to the Hingili stream, just north of the Shengena Mountain FR [4°14' 50" S, 37°59'28" E], 1450 m, 4 July 2001. Collected by Colin and Douglas Tilbury.

Paratypes. One male UDSM 1641, and one female UDSM 1642, same data as holotype.

Other material. One male BMNH 1982.1426 (KMH 1514), West Usambara, Mazumbai FR, 02 June 1980, collected by Kim Howell; one female NMZB 16700 (KMH 19586), South Pare, Chome FR, 1800 m, and one male NMZB 14059 (KMH 7935), North Pare, Ngofi Peak, Minja FR, 31 July 1993, collected by N. Cordeiro; two males NMB 7913 & 7914, South Pare, forest above Kisiwani, 18 April 1996, collected by Alexander Flemming; four males MHNG 2617.090, 093 and 2619.031-2 (TZ 139, 140, 142, 147) and two females MHNG 2617.091-092 (TZ 145-146). South Pare, Chome FR, 1840-2070 m, 29-30 September 2000; four males MHNG 2624.059, 2624.074, 2624.076-07 (TZ 495, 510, 512-3) and one female MHNG 2624.075 (TZ 511) North Pare, Kindoroko FR, 1600-1700 m, 10 May 2002.

Rh. temporalis examined for comparative purposes (all from East Usambara): NMZB 14820, female, and NMZB 14821 (KMH 12178), male, Bamba FR; NMZB 16362 (KMH 17875), female, Kwamkoro/Kwamsambia FR; NMZB 14068 (KMH 11224), juvenile male, and NMZB 14069, male, Magrotto Hill, Muheza; KMH 21313, male; BMNH 1935.4.1.35, male, and BMNH 1974.526, juvenile female, Amani; BMNH 1988.641-643 male, Monga estate; MHNG 2617.034, female, Lutindi Peak.

Note. The 16S rRNA sequences (AY524868-9) of the specimens listed as *Rh. sp. nova* by Matthee *et al.* (2004) are 99-100% identical to our sequences of *Rh. viridis*. Therefore their material from the South Pare can safely be identified as *Rh. viridis*.

Etymology. The specific name derives from Latin *viridis* (green) and refers to the rich green colour of the males.

Diagnosis (Fig. 7). *Chamaeleonidae*, *Rhampholeon* (*Rhampholeon*). With the characters of the subgenus. A small chameleon (maximum TL 89 mm) with a tail 34-46% of TL in males and 33-34% in females. Low casque. Small rostral process represented by a bulge barely projecting over the front of the snout, barely visible in males, somewhat larger in females. Temporal

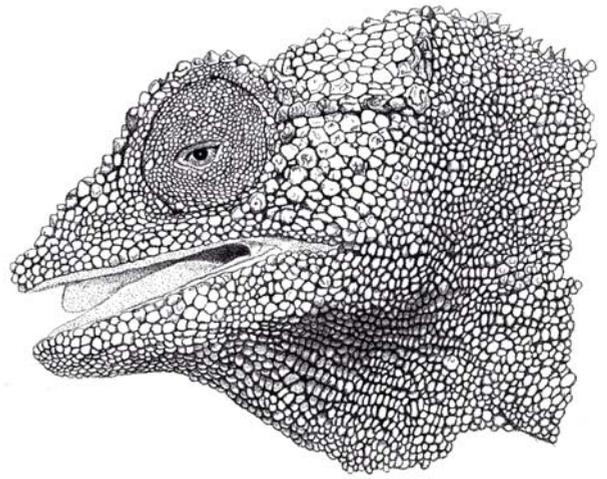


FIG. 10. *Rhampholeon (Rhampholeon) viridis* n. sp. (MHNG 2624.059). Head detail.

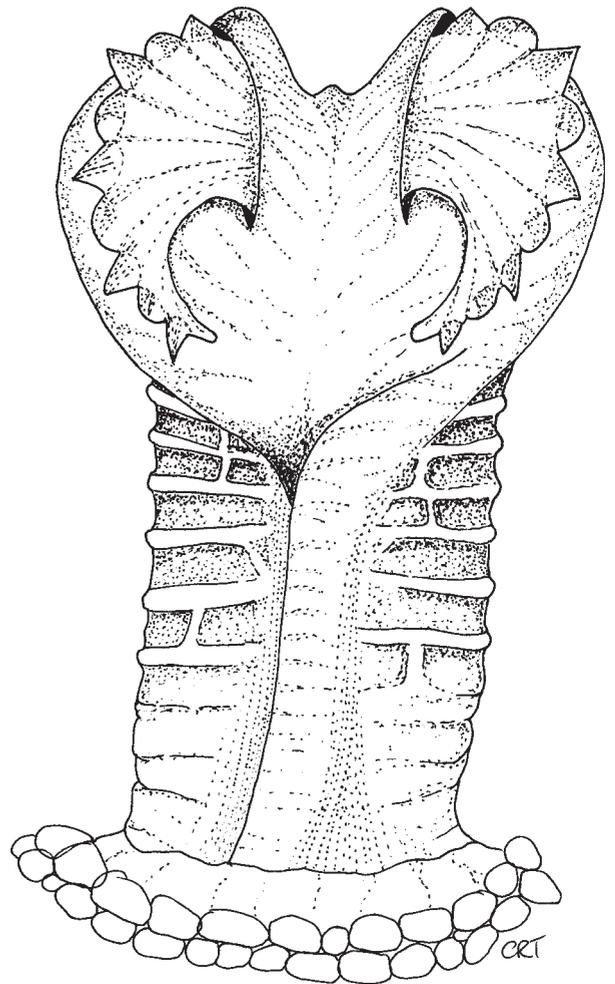


FIG. 11. *Rhampholeon (Rhampholeon) viridis* n. sp. (MHNG 2624.059). Hemipenis, sulcal view.

crest is distinct. Dorsal keel variable in outline from almost smooth to strongly crenulated. Hemipenis with prominent calyces on the truncus and broad paired apical horns arising from mucosal folds bearing up to nine papillae typically alternating rounded and sharp papillae on the outer edge of the horn. Axillary pits and inguinal pits present, the latter less distinct. Claws simple. There may be one or two slightly enlarged accessory plantar tubercles present at the base of the claws. Soles of feet smooth/cobblestoned as opposed to spinous. The male hemipenis is distinct from other species of *Rhampholeon*. The specimens from North Pare bear typical reddish patches.

Description of the holotype. Head (Fig. 10): Casque flat, not elevated above the nape. No parietal crest present. The supra-orbital crest is composed of sub-conical to conical tubercles. The supra-orbital ridge is relatively smooth with no supra-optic peak. The orbital ridges are connected across the top of the head by a row of 14 tubercles. The canthal ridge is sharply delineated, terminating anteriorly at the base of a rostral bulge. This bulge, which is covered with sub-conical tubercles, projects forward barely clearing the tip of the snout. The nares open infero-posteriorly from within a low nasal bulge. The gular region is smooth and unadorned with spines or tufts of scales. A prominent temporal crest is present formed by a row of seven conical tubercles, the most posterior of which is by far the largest. The temporal crest continues upwards as a well-marked posterior temporal or squamosal crest of enlarged conical to subconical tubercles to the apex of the casque. The skin of the eyeball is clad with small relatively homogeneous rounded tubercles.

Body: TL 68.5 mm (SVL 44.5 mm + tail 24 mm), the tail comprising 35% of the TL. The dorsal keel is only weakly crenulated. A low cluster of slightly enlarged tubercles is present over each vertebral spinous process commencing from above the shoulder area, fading over the sacrum and re-appearing along the tail. The cluster of tubercles may be centred on either a single low cone or a pair of smaller cones. The flanks are clad in tightly packed sub-homogeneous granules with scattered enlarged conical tubercles. The granules are largely rounded but there are scattered clusters of stellate granules. There is no enlarged tubercle above the shoulder. A vague row of four slightly enlarged tubercles is present along the infero-lateral aspect of the proximal half of the tail. The claws of the feet are simple with no evidence of cusp formation. The tubercles on the soles of the feet are rounded to give the appearance of a cobblestoned surface. There are no prominent accessory plantar spines present at the bases of the toes, rather low plantar tubercles. Deep wide-mouthed dermal pits/invaginations are present in both axillae and the inguinal regions. The hemipenes are not fully everted.

Variation in paratypes and other material. Head: Narrow occipital concave surface may be present. Temporal crest typically with 4-5 large conical postocular tubercles, then typically three more on an upward line,

with the lowest one being the most prominent. Vestigial parietal crest, sometimes formed by three ridges. One isolated tubercle above jaw articulation. Interorbital ridge a shallow V, formed of 8-14 granules. A small but distinct rounded rostral appendage in females (1×1 mm), less marked or absent in males.

Body: TL 63-89 mm, SVL 38-47 mm, tail 25-32 mm, tail 34-46% of TL for males. TL 65-72 mm, SVL 43-48 mm, tail 22-24 mm, tail 33-34% of TL for females. Thus males slightly larger, but females with a shorter tail. Dorsal keel weakly to strongly crenulated in males (9-12 clusters) may be almost smooth in females. Axillary pits present, but only less conspicuous inguinal depressions. Flanks of one specimen with a few enlarged pyramidal clusters of tubercles on each side. Claws simple. Two gravid female with four eggs each ($10-11 \times 4.5-6$ mm).

Hemipenis. (Fig. 11) (MHNG 2624.059). Hemipenial truncus with prominent calyces on the asulcal (posterior) aspect becoming smooth in the para-sulcal zone. The sulcal lips are smooth. Apex capitate. A pair of apical horns arise from between prominent mucosal folds sited towards the asulcal side of the apex and which curve inwardly over the apical plateau. The outer margins of the horns are adorned with a series of alternating thorn like and button like papillae – nine on one horn and six on the other.

Colour in life (Fig. 7). When first seen in undisturbed conditions the males of this species have a background colour of emerald green. Two thin dark stripes are angled postero-inferiorly over the flanks from the dorsal keel. North Pare specimens harbour several characteristic reddish/rusty coloured patches on the head, belly, tail and around the main lateral cones, and occasionally one thin transversal reddish stripe from above the shoulder to inguinal region, or some whitish areas on shoulder and occiput.

Differential diagnosis. The simple claws of this species immediately place this form within the group of pygmy chameleons that only includes *Rh. spinosus* and *Rh. temporalis*. The former species differs from *Rh. viridis* by the prominent ovoid rostro-nasal projection found in both sexes. Apart from the striking hemipenial differences between males of *viridis* and *temporalis* (breadth of the apical horns and shape of the papillae on the horns, see Figs 2 and 11), they appear very similar in external morphology. Differences between the two are subtle but may be seen in the more pronounced dorsal crest and the conspicuous temporal crest of *viridis*. Perhaps the best distinguishing feature between them is that the accessory plantar spines in *temporalis* are usually well developed and prominent but are inconspicuous to rudimentary in *viridis*.

Distribution and ecology. This species inhabits the undergrowth and lower story vegetation of the submontane evergreen forests of the South and North Pare mountains. Its occurrence in the West Usambara is based on a single specimen in the British Museum collected in 1980 but its presence in these mountains has

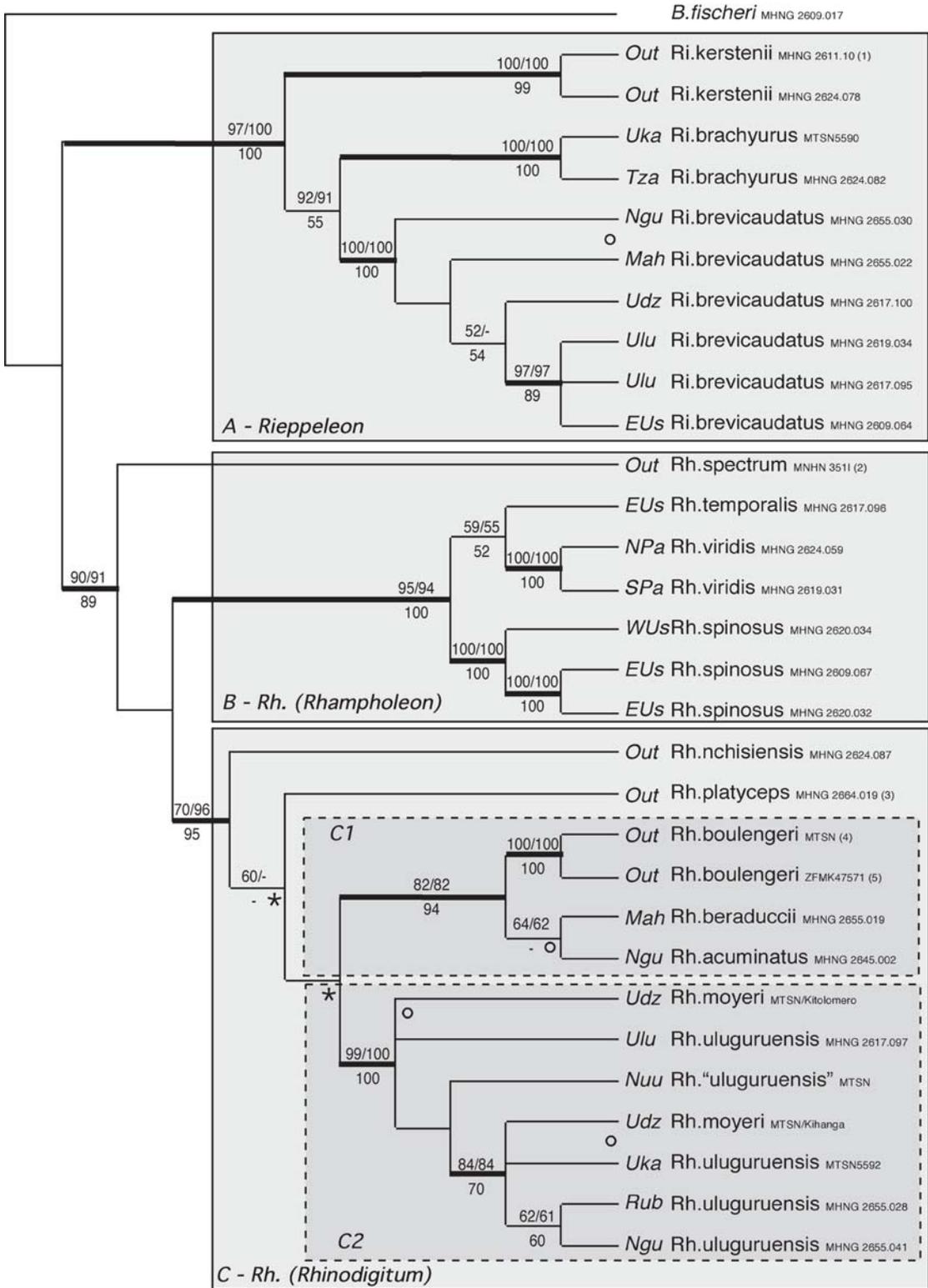


FIG. 12. Molecular tree. Parsimony analysis, gaps treated as missing. Strict consensus of four shortest trees. Numbers above branches are bootstrap values over 50% for heuristic parsimony searches, 1000 repeats (with gaps treated as missing or fifth base) and below branches bootstrap of ML searches (100 repeats). Branches leading to strongly supported nodes by all methods (over 70% bootstrap) are in bold. * Indicates different results found in the analysis where gaps were treated as fifth base (branches collapsed), and ° indicates such variations in the ML best tree (branches collapsed; or sister group relationship between *Ri. brevicaudatus* MHNG 255.022 and 030, between *Rh. moyeri*, Kihanga and *uluguruensis* MTSN5592; or basal position of *Rh. moyeri*, Kitolomero in the clade). Massif of origin of specimens is abbreviated as follows: NPa: North Pare; Spa: South Pare; WUs: West Usambara; EUs: East Usambara; Nuu: Nguu; Ngu: Nguru; Ulu: Uluguru; Uka: Ukaguru; Rub: Rubeho; Udz: Udzungwa; Mah: Mahenge, Tza: unknown origin in Tanzania; Out indicates an origin from outside the EAR. (1) Captivity, (2) Cameroon, (3) Mozambique, (4) Rwanda, (5) Democratic Republic of Congo.

not since been reconfirmed. The holotype was collected at an altitude of 1450 m, but specimens have been found up to 2070 m in the South Pare and 1700 m in the North Pare. These forests are typical examples of the Afro-montane forests that are dominated by emergent trees such as *Albizia gummifera*, *Macaranga kilimandscharica*, *Xymalos monospora*, *Ocotea usambarensis*, *Podocarpus latifolius* and *Chrysophyllum gorongosanum*.

When handled these chameleons produced an easily felt “buzzing” vibration, particularly if touched lightly on the back. At the time of collection in early July, an adult male and female chameleon were found sleeping within a few centimetres of each other, indicating pairing off and thus possible recent past or potentially future mating activity.

Mating. A single pair was observed mating in Kindoroko FR (North Pare) on 10 May 2002 at 7 pm. Exact duration of the copulation was not recorded but was longer than three hours. The pair was on a narrow branch about one metre high; the male was on the back, slightly to the right and parallel to the female.

Parasitology. All specimens from North Pare were parasitized both by intestinal nematodes and acanthocephalans. The acanthocephalans have been found to represent a new species of *Acanthocephalus* (*Pseudacanthocephalus*) recently described by Smales (2005). In the South Pare all specimens but one harboured *Cylindrotaenia* sp. (Nematotaeniidae) tapeworms.

ADDITIONAL SPECIES

Rhampholeon (*Rhinodigitum*) *nchisiensis* (Loveridge, 1953), which is present in Tanzania but not in the EAR, *Rhampholeon* (*Rhampholeon*) *spectrum* (Buchholz, 1874), and *Rhampholeon* (*Rhinodigitum*) *platyceps* (Günther, 1892), which are not found in Tanzania, have opportunistically been included in our molecular analysis because of their close geographical distribution and possible relatedness to our species of interest.

MOLECULAR SYSTEMATICS

Results. Sequences for 12S (414 bp) and 16S (523 bp) were obtained for 45 *Rhampholeon/Rieppeleon* and one outgroup (*Bradypodion fischeri*). A PHT found both partitions to be compatible, and those sequences were thus concatenated in a single matrix 938 characters long, from which 44 positions were removed for analyses due to uncertain alignment. Distances between in- and outgroups averaged 13.7% (11.9–15.5%). Interspecific distances within the ingroup averaged 11.0% (7.3–15.4%), and intraspecific distances varied between 0 and 3.5%, and up to 5.9% within the *uluguruensis* complex (which was treated as a single species in this case). Distances between members of *Rieppeleon* and *Rhampholeon* (14.1% on average) were as high as between any pygmy chameleon and the outgroup. Among sequences from conspecific speci-

mens distances of 0 to less than 0.35% (0–3 changes) were found between 6 out of 10 *Ri. brevicaudatus*, 4 out of 8 *Rh. uluguruensis*, 3 out of 4 *Rh. viridis* and all *Rh. beraduccii* (2), *Rh. acuminatus* (2) and *Rh. “uluguruensis”* from Nguu (4). Removing these identical, or nearly identical, sequences from the analyses reduced the final matrix to 31 taxa and 894 positions.

A heuristic parsimony search of this dataset returned nearly identical results whether gaps were treated as missing (four shortest trees, L 906, CI 0.433, RI 0.704) of “fifth base” (six shortest trees, L 1001, CI 0.447, RI 0.721) (Fig. 12): a first well-supported basal clade comprises three species, a basal *Ri. kerstenii* sister taxon of *Ri. brachyurus* and *Ri. brevicaudatus* (clade A, corresponding to the genus *Rieppeleon*). The second clade is rooted, although very weakly, by *Rh. spectrum*, which is the sister taxon of a large clade comprising *Rh. temporalis*, *Rh. viridis* and *Rh. spinosus* on one side (group B, together with *Rh. spectrum*) and the rest of the ingroup on the other (clade C), corresponding respectively to Matthee *et al.*'s (2004) subgenera *Rhampholeon* and *Rhinodigitum*. Technically, the subgenus *Rh. (Rhampholeon)* is thus paraphyletic. Within the latter group relationships are less clearly resolved, although two subgroups, one with *Rh. boulengeri*, *Rh. acuminatus* and *Rh. beraduccii* (C1) and the other with *Rh. uluguruensis*, including *Rh. moyeri* and *Nguu* specimens (C2) are supported. The positions of *Rh. platyceps* and *Rh. nchisiensis* are uncertain within *Rh. (Rhinodigitum)*, although both the parsimony analysis with gaps treated as missing and the ML analysis place them basal.

The maximum likelihood best-fit model was SYM+I+G with the following parameters: Base=e qual, Nst=6, Rmat=(0.6538 5.2133 0.5304 0.0728 3.7009), Rates=gamma, Shape=0.7680, Pinvar=0.4361. The analysis gave similar results as parsimony except for the clade C1, in which *Rh. acuminatus* is basal, and a few minor details within *Ri. brevicaudatus* and the *Rh. uluguruensis* complex (see caption to Fig. 12).

DISCUSSION

Field studies of the pygmy chameleons are few and our understanding of the group is weak. A single recent paper addressed the overall systematics and evolution of the group (Matthee *et al.*, 2004), and, to our knowledge, no paper dealing specifically with the taxa from the EAR has been published apart from occasional species descriptions and a recent comparative study on the ecology of *Rh. temporalis* and *Ri. brevicaudatus* in the East Usambara (Emmett, 2004).

At the morphological level, the descriptions presented herein clearly demonstrate the frailty of external morphology for differentiating species, and the important role that hemipenial analysis can play in discriminating between most species in this group. For example whilst the hemipenial differences are striking, the external morphological differences between *Rh. viridis* sp. n. and *Rh. temporalis* are subtle and to a cer-

tain extent subjective. The two species are sister taxa and *Rh. temporalis* differs only by showing more derived character states in the degree of development of the rostral process and plantar spines.

However, the problem of external similarity is not always simply solved just by examining the soft tissues. The phenotype and hemipenes of a population of Nguru pygmy chameleons (shown here to belong to the *uluguruensis* complex) cannot reliably differentiate this population from typical *Rh. boulengeri* from Rwanda. Notwithstanding the observation that the nearest recognized population of *boulengeri* is over 600 km away, the question must be asked as to why the Nguru population should not be assigned to *boulengeri* (see also Menegon *et al.*, 2003). Tolley *et al.* (2004) demonstrated that phenotype in chameleons (the Southern African *Bradypodion*) was a relatively plastic expression sensitive to environmental selection pressures. We consider it likely that this observation in *Bradypodion* is mirrored in the pygmy chameleons and particularly in the EAR species. For example, on comparing *Rh. boulengeri* and *Rh. uluguruensis* within their stable forest habitats, it would appear that their external phenotype has been under hardly any environmental pressure to evolve. Similarly, and despite their importance, the relatively simple hemipenis structures observed in these species are not sufficient to characterize them. Their evolutionary differentiation has been rather at the genetic level as indicated by a sequence divergence of about 9%, a level that is clearly within the interspecific range in our data set. Although morphology easily indicates a placement within the subgenus *Rhinodigitum*, it does not allow for specific differentiation.

At the molecular level, Matthee *et al.* (2004) published a complete analysis of the pygmy chameleons and demonstrated their basic organization in two main clades that they proposed to consider as distinct genera, *Rieppeleon* and *Rhampholeon*, in accordance with the earlier observations of Rieppel (1987) and Tilbury (1992). They also distinguished three distinct and well supported lineages (*Rhinodigitum*, *Bicuspis* and *Rhampholeon*) within *Rhampholeon*. Our sampling is different from the one of Matthee *et al.* (2004), in two main ways: first, we do not include members of their “*Bicuspis*” lineage whose members are not found in the EAR (and in Tanzania), and second, a few new species described herein are added to the dataset. Nevertheless our results (Fig. 12) confirm most of Matthee *et al.*'s (2004) observations. We find strong support for the *Rhampholeon* and *Rieppeleon* lineages. In the latter one, though, our data (as well as unpublished preliminary cytochrome b sequences) support a (*Ri. brachyurus* – *Ri. brevicaudatus*) sister-group relationship instead of (*Ri. kerstenii* – *Ri. brachyurus*) as in Matthee *et al.* (2004). This node, however, was relatively weakly supported by their 16S data, and their RAG1 data suggested the same clustering as found here.

Within *Rhampholeon sensu stricto* a possible important difference lies with the position of the West African

species *Rh. spectrum*, which is a sister group of the *temporalis/viridis* (Pare Mountain) clade in Matthee *et al.* (2004), and is basal to the whole *Rhampholeon* genus in our work, even if bootstrap support for this position is weak. Matthee *et al.* (2004) explained that the close relationship between *Rh. spectrum* and *Rh. temporalis* was the result of historic climatic changes that resulted in the desiccation of the pan-African forests about 25 million years ago. The position of *Rh. spectrum* in our analysis is speculative given the weak support of this node, which might be due to saturation in this case. We must also note that our sequence is 3–4% different from the Equatorial Guinea sequences from Matthee *et al.* (2004), which may explain the slightly different position of this taxon on our tree. In any case, assuming our placement of *Rh. spectrum* is correct, this would suggest that this species might be the most ancient sister group to all other *Rhampholeon*, an interesting hypothesis given the wide distribution of this taxon. This would also imply that *Rh. (Rhampholeon)* is paraphyletic and might therefore have further taxonomical consequences. Globally, the diversification of the genus in the easternmost extremity of the EAR (i.e. at least with *Rh. temporalis*, *Rh. spinosus* and *Rh. viridis*) is more extensive than previously thought. In Tanzania, *Rh. (Rhampholeon)* is restricted to the eastern/northeastern EAR.

We confirm the existence of two main EAR lineages within *Rh. (Rhinodigitum)*, one comprising *Rh. boulengeri* and other species, and the other with *Rh. uluguruensis*, and find the subgenus to be rooted with the non-EAR species *Rh. nchisiensis* and *Rh. platyceps* (although again with weak support). All species included in this subgenus are found in the western/southwestern part of the EAR (and beyond). Interestingly, we show that both newly described *Rh. beraduccii* and *Rh. acuminatus* are more closely related to *Rh. boulengeri* than to the *uluguruensis* complex. Given the overall similarity of *Rh. beraduccii* with the members of the *uluguruensis* complex at the level of the head and appendages, this is again an indication that these morphological characters can be deceptive, and that very similar morphologies may have evolved independently. Convergences can also occur for characters looking very original, like the discoid rostral appendages of *Rh. spinosus* and *Rh. acuminatus*.

We have not found any decisive argument to resolve the status of the taxa included in the *uluguruensis* complex. Relatively high genetic distances between its components obviously plead for a heterogeneous assemblage encompassing more than a single species; however, no satisfactory nomenclatural system can be derived yet. The status of both *Rh. moyeri* and of the new Nguru specimens remains equivocal within this group.

Faunistically, it appears that the genus is more species rich in the EAR than previously expected, and that a combination of ancient colonization and recent, or ongoing, speciation processes can explain this situation. In

the eastern EAR (East and West Usambaras and South and North Pares), at least six species of pygmy chameleon are described. These species belong to both pygmy chameleon genera and are representative of the most ancient lineages of these lizards in our sampling, thus suggesting that the colonization of the EAR started in this geographical area. In parallel with these ancient events it seems that a further diversification of *Rh. (Rhampholeon)* is still ongoing and is facilitated by the complete separation of the East and West Usambara as well as the North and South Pare by lowland valleys. In the former mountain we observe a rather high DNA distance (3.5%) between *Rh. spinosus* specimens sampled on each side of the Lwengera valley (as compared to 0.7% between two specimens from the East Usambara). In the Pare, *Rh. viridis* from the southern massif are clearly distinguishable at the mtDNA level (as well as, to a certain extent, morphologically) from conspecific specimens from the northern massif. In one of the few comparable studies in the area, Johanson & Willassen (1997), working on the Helicopsychidae (caddis flies), also reached the conclusion that the East and West Usambara formed distinct endemic areas. On the other hand, Gravlund (2002), who studied the snake *Crotaphopeltis tornieri* (Colubridae), found no evidence that populations on both sides of the Lwengera valley were genetically distinct. He nevertheless concluded that they were most probably isolated.

A similar scenario might explain the situation in the western EAR with a fauna originating from the south or west of the area and a recent differentiation within the Uluguru–Nguru–Rubeho–Udzungwa massifs. The best example of such a scenario would be the “*uluguruensis*” complex where morphology is minimally useful for the identification of lineages but for which mtDNA shows that some populations (i.e. *Rh. moyeri* from Kitolomero or the Nguu specimens) are relatively well characterized. As many isolated forest reserves in this area have not yet been fully explored, especially in the Rubeho/Ukaguru massifs, we should expect the discovery of more populations in this group.

These hypotheses of relatively recent diversification in the mountains are corroborated by similar observations made for various groups of animals such as birds (Roy, 1997), insects (Johanson & Willassen, 1997) or amphibians (Loader *et al.*, 2004a) and support an important role of the “mountain speciation model” (Fjeldså & Lovett, 1997). In any case, the fact that no montane species of pygmy chameleons is shared between the eastern and western parts of the EAR tends to confirm the status of distinct “Evolutionary Significant Units” for the distinct mountains blocks of the range, as suggested by Gravlund (2002). This, added to the fact that the distribution area of most taxa discussed herein is, most probably, very reduced, should imply the strongest possible protection for the remaining forests in the EAR.

ACKNOWLEDGEMENTS

Kim Howell, Gamba Nkwengulila, Joe Beraducci, David Moyer, Kathryn Doody, Nike Doggart and all the “Frontier project” people provided precious advice and assistance in Tanzania. Claude Vaucher, Simon Loader, Douglas Tilbury and Eric Ayo – the only human able to spot a *Rhampholeon* from a moving car – provided invaluable help in the field. Alexander Flemming allowed access to specimens in the Bloemfontein National Museum; Wolfgang Böhme (Bonn), Klaas-Douwe Dijkstra (Leiden), Ivan Ineich (Paris) and Michele Menegon (Trento) supplied complementary samples for molecular analyses. Corinne Charvet made the line drawings of the heads. Janik Pralong and José Fahrni provided excellent technical assistance in the laboratory. Last but not least, Donald and Sheila Broadley of the National Museum of Zimbabwe wholeheartedly supported and encouraged our pursuit of the pygmy chameleons. The Swiss Foundation for Scientific Research (grant 3100-55710.98) and the Museum of Natural History, Geneva provided financial support, and the Tanzanian Commission for Science and Technology (Costech) issued the research clearances (99-280-NA-97-128). All are warmly thanked for their support.

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APPENDIX 1 : NEWLY COLLECTED SPECIMENS

| Location | Species | Field no. | No. | Date | Locality | Sex |
|----------|------------------------------|-----------|----------|----------|-------------|-----|
| MHNG | <i>Rh. acuminatus</i> n. sp. | TZ412 | 2645.002 | 21.10.01 | Komkore | M |
| | <i>Rh. acuminatus</i> n. sp. | TZ413 | 2645.003 | 21.10.01 | Komkore | M |
| | <i>Rh. acuminatus</i> n. sp. | TZ414 | 2645.001 | 21.10.01 | Komkore | F |
| | <i>Rh. acuminatus</i> n. sp. | TZ415 | 2645.005 | 21.10.01 | Komkore | F |
| | <i>Rh. acuminatus</i> n. sp. | TZ416 | 2645.006 | 21.10.01 | Komkore | |
| | <i>Rh. acuminatus</i> n. sp. | TZ417 | 2645.004 | 21.10.01 | Komkore | M |
| | <i>Rh. beraduccii</i> n. sp. | TZ343 | 2655.019 | 09.10.01 | Sali | F |
| | <i>Rh. beraduccii</i> n. sp. | TZ344 | 2655.020 | 09.10.01 | Sali | juv |
| | <i>Rh. beraduccii</i> n. sp. | TZ345 | 2655.021 | 09.10.01 | Sali | M |
| | <i>Ri. brachyurus</i> | TZ331 | 2655.018 | 2001 | Ngurus | |
| | <i>Ri. brachyurus</i> | TZ525 | 2624.082 | 14.05.02 | Tanzania | |
| | <i>Ri. brachyurus</i> | TZ526 | 2624.083 | 14.05.02 | Tanzania | |
| | <i>Ri. brachyurus</i> | TZ527 | 2624.084 | 14.05.02 | Tanzania | |
| | <i>Ri. brevicaudatus</i> | TZ053 | 2609.062 | 28.11.99 | Amani | juv |
| | <i>Ri. brevicaudatus</i> | TZ054 | 2609.063 | 28.11.99 | Amani | F |
| | <i>Ri. brevicaudatus</i> | TZ060 | 2609.064 | 28.11.99 | Amani | M |
| | <i>Ri. brevicaudatus</i> | TZ061 | 2609.065 | 28.11.99 | Amani | F |
| | <i>Ri. brevicaudatus</i> | TZ173 | 2617.087 | 02.10.00 | Nilo | |
| | <i>Ri. brevicaudatus</i> | TZ174 | 2617.088 | 02.10.00 | Nilo | |
| | <i>Ri. brevicaudatus</i> | TZ175 | 2617.089 | 02.10.00 | Nilo | M |
| | <i>Ri. brevicaudatus</i> | TZ176 | 2619.033 | 02.10.00 | Nilo | M |
| | <i>Ri. brevicaudatus</i> | TZ185 | 2617.094 | 03.10.00 | Nilo | |
| | <i>Ri. brevicaudatus</i> | TZ220 | 2619.034 | 09.10.00 | Tegetero | M |
| | <i>Ri. brevicaudatus</i> | TZ221 | 2617.095 | 09.10.00 | Tegetero | F |
| | <i>Ri. brevicaudatus</i> | TZ284 | 2619.035 | 20.10.00 | Kihansi | F |
| | <i>Ri. brevicaudatus</i> | TZ291 | 2617.100 | 21.10.00 | Kihansi | F |
| | <i>Ri. brevicaudatus</i> | TZ292 | 2618.001 | 21.10.00 | Kihansi | F? |
| | <i>Ri. brevicaudatus</i> | TZ293 | 2618.002 | 21.10.00 | Kihansi | F |
| | <i>Ri. brevicaudatus</i> | TZ346 | 2655.022 | 09.10.01 | Sali | F |
| | <i>Ri. brevicaudatus</i> | TZ347 | 2655.023 | 09.10.01 | Sali | M |
| | <i>Ri. brevicaudatus</i> | TZ348 | 2655.024 | 09.10.01 | Sali | F |
| | <i>Ri. brevicaudatus</i> | TZ349 | 2655.025 | 09.10.01 | Sali | F |
| | <i>Ri. brevicaudatus</i> | TZ350 | 2655.026 | 09.10.01 | Sali | F |
| | <i>Ri. brevicaudatus</i> | TZ374 | 2655.027 | 09.10.01 | Sali | M |
| | <i>Ri. brevicaudatus</i> | TZ405 | 2655.030 | 20.10.01 | Komkore | F |
| | <i>Ri. brevicaudatus</i> | TZ406 | 2655.031 | 20.10.01 | Komkore | M |
| | <i>Ri. brevicaudatus</i> | TZ422 | 2655.038 | 21.10.01 | Komkore | F |
| | <i>Rh. brevicaudatus</i> | TZ432 | 2655.044 | 22.10.01 | Komkore | M |
| | <i>Ri. kerstenii</i> | TZ517 | 2624.078 | 13.05.02 | Masai plain | M |
| | <i>Ri. kerstenii</i> | TZ518 | 2624.079 | 13.05.02 | Masai plain | M |
| | <i>Ri. kerstenii</i> | TZ530 | 2624.085 | 14.05.02 | Tanzania | |
| | <i>Rh. nchisiensis</i> | TZ531 | 2624.086 | 14.05.02 | Poroto Mtns | M |
| | <i>Rh. nchisiensis</i> | TZ532 | 2624.087 | 14.05.02 | Poroto Mtns | |
| | <i>Rh. spinosus</i> | TZ24 | 2609.067 | 27.11.99 | Amani | |
| | <i>Rh. spinosus</i> | TZ329 | 2620.032 | 06.10.01 | E. Usambara | |
| | <i>Rh. spinosus</i> | TZ438 | 2620.034 | 26.10.01 | Mazumbai | |
| | <i>Rh. spinosus</i> | TZ440 | 2620.036 | 26.10.01 | Mazumbai | |
| | <i>Rh. temporalis</i> | TZ197 | 2617.096 | 01.10.00 | Lutindi Pk | F |
| | <i>Rh. temporalis</i> | TZ198 | not kept | 01.10.00 | Lutindi Pk | juv |
| | <i>Rh. uluguruensis</i> | TZ267 | 2617.097 | 12.10.00 | Mkungwe | M |
| | <i>Rh. uluguruensis</i> | TZ268 | 2617.098 | 12.10.00 | Mkungwe | M |
| | <i>Rh. uluguruensis</i> | TZ269 | 2619.036 | 12.10.00 | Mkungwe | M |
| | <i>Rh. uluguruensis</i> | TZ270 | 2617.099 | 12.10.00 | Mkungwe | M |
| | <i>Rh. uluguruensis</i> | TZ394 | 2655.028 | 17.10.01 | Mafwomero | M |
| | <i>Rh. uluguruensis</i> | TZ395 | 2655.029 | 17.10.01 | Mafwomero | M |

| Location | Species | Field no. | No. | Date | Locality | Sex |
|---------------------------|------------------------------|-----------|----------|-----------|-----------|-----|
| MHNG (cont...) | <i>Rh. uluguruensis</i> | TZ427 | 2655.039 | 21.10.01 | Komkore | F |
| | <i>Rh. uluguruensis</i> | TZ428 | 2655.040 | 21.10.01 | Komkore | M |
| | <i>Rh. uluguruensis</i> | TZ429 | 2655.041 | 21.10.01 | Komkore | M |
| | <i>Rh. uluguruensis</i> | TZ430 | 2655.042 | 21.10.01 | Komkore | juv |
| | <i>Rh. uluguruensis</i> | TZ431 | 2655.043 | 21.10.01 | Komkore | M |
| | <i>Rh. uluguruensis</i> | TZ481 | 2624.047 | 04.05.02 | Ikwamba | M |
| | <i>Rh. uluguruensis</i> | TZ482 | 2624.048 | 04.05.02 | Ikwamba | F |
| | <i>Rh. uluguruensis</i> | TZ483 | 2624.049 | 04.05.02 | Ikwamba | M |
| | <i>Rh. uluguruensis</i> | TZ484 | 2624.050 | 04.05.02 | Ikwamba | M |
| | <i>Rh. uluguruensis</i> | TZ492 | 2624.056 | 05.05.02 | Mandenge | M |
| | <i>Rh. uluguruensis</i> | TZ493 | 2624.057 | 05.05.02 | Mandenge | F |
| | <i>Rh. viridis</i> n. sp. | TZ139 | 2617.090 | 29.09.00 | Chome | M |
| | <i>Rh. viridis</i> n. sp. | TZ140 | 2619.032 | 29.09.00 | Chome | M |
| | <i>Rh. viridis</i> n. sp. | TZ142 | 2619.031 | 30.09.00 | Chome | M |
| | <i>Rh. viridis</i> n. sp. | TZ145 | 2617.091 | 30.09.00 | Chome | F |
| | <i>Rh. viridis</i> n. sp. | TZ146 | 2617.092 | 30.09.00 | Chome | F |
| | <i>Rh. viridis</i> n. sp. | TZ147 | 2617.093 | 30.09.00 | Chome | M |
| | <i>Rh. viridis</i> n. sp. | TZ495 | 2624.059 | 10.05.02 | Kindoroko | M |
| | <i>Rh. viridis</i> n. sp. | TZ510 | 2624.074 | 10.05.02 | Kindoroko | |
| | <i>Rh. viridis</i> n. sp. | TZ511 | 2624.075 | 10.05.02 | Kindoroko | M |
| <i>Rh. viridis</i> n. sp. | TZ512 | 2624.076 | 10.05.02 | Kindoroko | F | |
| <i>Rh. viridis</i> n. sp. | TZ513 | 2624.077 | 10.05.02 | Kindoroko | M | |
| NMZB | <i>Rh. viridis</i> n. sp. | CT119 | 16905 | 04.07.01 | Shengena | M |
| | <i>Rh. viridis</i> n. sp. | CT120 | 16906 | 04.07.01 | Shengena | F |
| | <i>Rh. viridis</i> n. sp. | KMH19586 | 19586 | - | Mazumbai | F |
| | <i>Rh. viridis</i> n. sp. | KMH7935 | 14059 | 31.07.93 | Ngofi Pk | M |
| USDM | <i>Rh. viridis</i> n. sp. | - | 1641 | 04.07.01 | Shengena | M |
| | <i>Rh. viridis</i> n. sp. | - | 1642 | 04.07.01 | Shengena | F |
| NMB | <i>Rh. viridis</i> n. sp. | - | 7913 | 18.04.96 | Kisiwani | M |
| | <i>Rh. viridis</i> n. sp. | - | 7914 | 18.04.96 | Kisiwani | M |
| PEM-R | <i>Rh. acuminatus</i> n. sp. | - | 16271 | 21.10.01 | Komkore | M |

APPENDIX 2 : LOCALITIES

The specimens included in this study come from the following localities [locality, mountain (region), coordinates, altitude]:

Kindoroko FR, North Pare (Kilimanjaro), 3°43'44" S, 37°39'16" E, 1600 m; Ngofi Pk, Minja FR, North Pare (Kilimanjaro), 3°36' S, 37°43' E; Chome FR, South Pare (Kilimanjaro), 4°17'29" S, 37°55'16" E, 1850 m; Shengena FR (Hingili Stream), South Pare (Kilimanjaro), 4°14'50" S, 37°59'28" E; above Kisiwani, South Pare (Kilimanjaro), 4°7' S, 38°5' E; Mazumbai FR, West Usambara (Tanga), 4°48'45" S, 38°30'13" E, 1500 m; Amani, East Usambara (Tanga), 5°5'58" S, 38°37'55" E, 1000 m; Nilo FR, East Usambara (Tanga), 4°54'38" S, 38°39'49" E, 750 m; Lutindi Pk, East Usambara (Tanga), 4°53' S, 38°38' E, 1300 m; Komkore (above Ubili), Nguru (Morogoro), 6°2'51" S, 37°31'43" E, 1000 m; Mafwomero FR (above Mbuga), Rubeho (Dodoma), 6°56'27" S, 36°35'14" E, 1900 m; Tegetero, Uluguru (Morogoro), 6°56'30" S, 37°43'11" E, 1000–1200 m; Mkungwe, Uluguru (Morogoro), 6°52'41" S, 37°55'15" E, 1000 m; Mandenge, Ukaguru (Dodoma), 6°21'14" S, 36°57'54" E, 1600 m; Ikwamba FR, Ukaguru (Dodoma), 6°20'31" S, 36°58'58" E, 1500 m; Kihansi gorges, Udzungwa (Morogoro), 8°35'10" S, 35°51'2" E, 800 m; Sali, Mahenge (Morogoro), 8°57'57" S, 36°41'18" E, 900–1000 m. Additional comparative material comes from: Bamba FR, Magrotto Hill, Kwamkoro/Kwamsambia and Monga Estate, all East Usambara; Kitolomero, Udzungwa; Kihanga, Udzungwa; Mamiwa Kisara FR, Ukaguru; Nguu FR, Nguu; Ukalini Forest, Namuli, Mozambique; Cyangugu/Cyamudongo Forest, Rwanda; Cameroon; and Irangi, Kivu, Democratic Republic of Congo.

APPENDIX 3: KEY TO THE PYGMY CHAMELEONS OF THE EASTERN ARC RANGE

- 1a. Soles of feet covered with sharply pointed/ spinous (acuminate) tubercles..... 2
- 1b. Soles of feet covered with sub-conical to rounded tubercles..... 4
- 2a. A single small beardlike tuft of tubercles present under chin/mentum..... *Rieppeleon brevicaudatus*
- 2b. Gular region either smooth or with conical tubercles scattered or in divergent rows.....3
- 3a. Tail very short, averaging less than 20% of the total length of the chameleon..... *Rieppeleon brachyurus*
- 3b. Tail averaging 25–30% of the total length of the chameleon, supracilliary process in adult males*Rieppeleon kerstenii*
- 4a. Claws of feet are simple non-bicuspid..... 5
- 4b. Claws of feet are strongly bicuspid..... 7
- 5a. Rostral process prominent cushion-like (East and West Usambara Mountains)..... *Rhampholeon (Rh) spinosus*
- 5b. Rostral process short stubby or indistinct..... 6
- 6a. Accessory plantar spines well developed and prominent (Eastern Usambara)..... *Rhampholeon (Rh) temporalis*
- 6b. Accessory plantar spines weak or indistinct, (Pare Mtns and West Usambara)..... *Rhampholeon (Rh) viridis*
- 7a. Deep dermal pits in the groin/inguinal region.....*Rhampholeon (Rhin) beraduccii*
- 7b. Groin/inguinal dermal pits absent or indistinct..... 8
- 8a. Axillary dermal pits prominent..... 9
- 8b. Axillary dermal pits absent.....*Rhampholeon (Rhin) acuminatus*
- 9a. 11–13 tubercles between bases of supra-optic peaks (Uluguru, Nguru, Ukaguru, Nguu Mountains)*Rhampholeon (Rhin) uluguruensis*
- 9b. 15–19 tubercles between bases of supra-optic peaks (Udzungwa, Rubeho Mtns) *Rhampholeon (Rhin) moyeri*