

Bonn zoological Bulletin	Volume 57	Issue 1	pp. 19–29	Bonn, April 2010
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## A new chameleon of the *Trioceros bitaeniatus* complex from Mt. Hanang, Tanzania, East Africa (Squamata: Chamaeleonidae)

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**Abstract.** A new species of *Trioceros* from the montane forests of Mount Hanang in Northwest Tanzania is described. The only known population of this species is found on Mt. Hanang in Tanzania around 2800 m a.s.l. This chameleon bears close morphological resemblance to *Trioceros sternfeldi* (Rand, 1963) from northern Tanzania. In order to infer phylogenetic relationships within the group, morphological and genetic data (mitochondrial 16S and 12S rRNA genes) were analysed. The new species could be compared with specimens of different populations of *Trioceros sternfeldi* (Mts. Meru, Kilimanjaro and Ngorongoro) and several other representatives of the family (*T. hoehnelii*, *T. ellioti*, *T. rudis rudis*, *T. bitaeniatus*). Results of maximum parsimony, maximum likelihood and Bayesian analyses consistently placed *Trioceros* n. sp. from Mt. Hanang as a single, independent terminal clade with high genetic divergences towards all other included members of the group.

**Key words.** Squamata, Chamaeleonidae, *Trioceros*, Africa, molecular phylogeny, biogeography

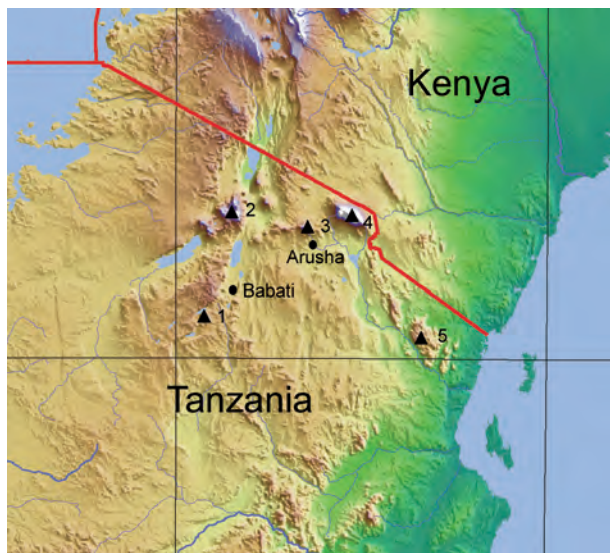
### INTRODUCTION

Mt. Hanang represents one of the numerous ancient and isolated volcanic mountains which characterize the landscape of eastern Africa. In northern Tanzania Mts. Kilimanjaro, Meru, and the Ngorongoro Crater are three of the better known extinct volcanoes which are frequently visited by tourists and lie within some of Tanzania's most famous national parks. Several biologists identified these forested East African mountains as centers of endemism for chameleons of the “*rudis* group”, a subgroup of the *Trioceros bitaeniatus* species complex (Rand 1963; Pilley 2000; Nečas et al. 2003). The *Trioceros bitaeniatus* complex comprises various taxa of small, hornless, ovoviviparous East African chameleons from the highland regions of eastern Africa, (Uganda, Kenya, Tanzania, southern Ethiopia and Sudan). All members of the group were placed into the separate subgenus *Trioceros* by Klaver & Böhme (1986) which was recently elevated to full generic rank by Tilbury & Tolley (2009). In contrast to other species of the *bitaeniatus* group, chameleons of the *rudis* subgroup are rather stocky animals with short, broad heads and heterogeneous scalation. They belong to a series isolated populations restricted to mountain forests ranging from the Ruwenzori in the north-west, south to the mountains east of Lake Malawi with outliers on Mt. Kilimanjaro, Mt. Meru and Ngorongoro in northern Tanzania. The Tanzanian forms of this subgroup were initial-

ly assigned to a *rudis* subspecies (*Chamaeleo rudis sternfeldi*) by Rand (1963), but this was long believed to be of rather specific than subspecific rank by several authors (e.g. Nečas et al. 2003). The independent taxonomic status of this form was subsequently demonstrated on the ground of morphological (Klaver & Böhme 1997; Krause 2006) and genetic data (Krause 2006).

The speciation of the *bitaeniatus* group as a whole seems closely related to the formation of the East African rift valleys when volcanism resulted in a separation of the ancestral populations. This can therefore be regarded as a major factor that gave rise to present day species (Pilley 2000; Wasser & Lovett 1993).

Three specimens of a curious chameleon from the *Trioceros bitaeniatus*- species complex were collected on Mt. Hanang at 2800 m altitude in March 2002, morphologically similar to *T. sternfeldi* (Rand, 1963) but with a very distinct colouration. This form is known only from the Mt. Hanang Catchment Forest Reserve (4° 25' to 4° 35' S and 35° 20' to 35° 25' E), situated about 4 km from the district capital Katesh and 70 km from the town Babati in the administrative region of Arusha in northern Tanzania (Figure 1).



**Fig. 1.** Map of Tanzania with distribution of the discussed species. 1=Mt. Hanang: *T. hanangensis* n. sp.; 2=Ngorongoro: *T. sternfeldi*; 3=Mt. Meru: *T. sternfeldi*; 4= Mt. Kilimanjaro: *T. sternfeldi*; 5=Usambara Mts.: *T. sternfeldi*.

## MATERIALS AND METHODS

All specimens were photographed *in situ* to record their natural coloration and habitus. Eversion of hemipenes in freshly dead specimens was done in the by injecting 70 % Alcohol into the organs (Glaw et al. 1999). The collected voucher specimens were deposited in the Zoologisches Forschungsmuseum Alexander Koenig in Bonn (ZFMK). Additional material was examined from the Zoologische Staatssammlung München (ZSM).

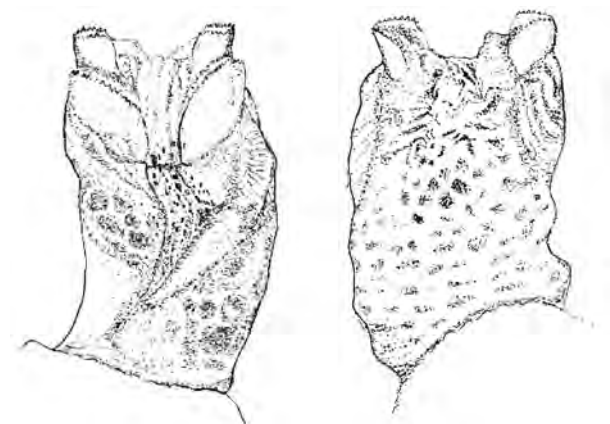
In order to test for morphological variation of the paratypes, chameleon body measurements were obtained with a digital caliper and evaluated with morphometric analyses methods (Krause 2006). Statistical analysis was performed using the GLM Multivariate procedure as implemented in SPSS 12 which provides analysis of variance for multiple dependent variables by one or more factor variables or covariates. In the present study the morphometric measurements (Krause 2006) excluding body length were chosen as dependent variables and the morphometric variable “snout-vent-length” was included as covariate to exclude the effect of body size on the other measured parameters. After an overall f-test has shown significance, post hoc tests (Scheffé) were applied to evaluate differences among the specific means. Probability values less than 0,05 were assumed to infer biological significance.

Molecular data could be obtained from tissue samples (muscle or liver, fresh or preserved in 98% ethanol) of the following taxa: *Trioceros* n. sp., (Mt. Hanang, Tanzania), *Trioceros rudis* (Uganda), *Trioceros sternfeldi* (Mt. Kilimanjaro, Mt. Meru, Tanzania). In order to analyse the phylogenetic relationships within the *bitaeniatus* group the following taxa were also included in the analysis: *T. ellioti*, *T. hoehnelii*, *T. bitaeniatus*, *T. narraoica*, *T. wernerii*, *T. jacksonii*, *T. deremensis* and *T. johnstoni*. *Kinyongia uthmoelleri*, *Chamaeleo dilepis*, *Uromastix acanthinura* and *Leiolepis belliana* were used as outgroups. All specimens included in this study are listed in Tab. 1 and sequences were submitted to GenBank (accession numbers AY927239-AY927276). DNA was extracted using the Chelex method (Walsh et al. 1991). In this study a 5% Chelex solution (2,5 mg Chelating Resin [Iminodiacetic Acid; Sigma Aldrich] in 50ml ddH<sub>2</sub>O) was used (Schmitz A 2003). Two mitochondrial genes which encode for the 16S und 12S subunits of the ribosomes (16S and 12S rRNA genes) were chosen for molecular analysis. Primers 16SA (light chain 5' - CGC CTG TTT ATC AAA AAC AT -3') and 16SB (heavy chain 5' - CCG GTC TGA ACT CAG ATC ACG T) of Palumbi et al. 1991 were used to amplify a section of the 16S rRNA gene. The primers 12SA-L (light chain; 5' - AAA CTG GGA TTA GAT ACC CCA CTA T - 3') and 12SB-H (heavy chain; 5' - GAG GGT GAC GGG CGG TGT GT - 3') of Kocher et al. (1989) were used to amplify a section of the 12S rRNA gene. PCR cycling procedure for both gene-fragments was as follows: Initial denaturation step: 90s at 94°C, 33 cycles: denaturation: 45s at 94°C, primer annealing for 45s at 55°C, extension for 90s at 72°C.

Purification of the PCR products was conducted following standard methods using QIAquick PCR Purification Kit (Qiagen), sequencing reactions of the obtained amplified DNA including cycle sequencing (standard procedures) were conducted by MWG-Biotech and Max Planck Institut für Züchtungsforschung (MPI) in Cologne using ABI Prism DNA automatic sequencer.

Sequences were aligned using the computer program ClustalX (default parameters, Thompson et a. 1997). The alignment was subsequently adjusted manually using the computer program BioEdit (Hall 1999).

33 sequences (combined 16S and 12S rRNA genes) could be obtained, each comprising 861 bp, referring to the aligned sequences including gaps (16S: 479 bp; 12S: 382 bp). The alignment gaps were treated as missing character. The entire 16S+12S alignment was included in the analyses. As with two specimens (ZFMK 63223, 70752) only 12S sequences could be obtained, we conducted a separate analysis using the 12S dataset only.



**Fig. 2.** Sulcal (left) and asulcal (right) view of the hemipenis of *Trioceros hanangensis* n. sp. from Mt. Hanang, Tanzania.

Data were analysed using maximum likelihood and parsimony optimality criteria in PAUP 4.0b10 (Swofford 2002), further we used Bayesian inference as implemented in MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003). For maximum likelihood, a heuristic search was conducted with 10 replicates of random stepwise addition and tree bisection-reconnection (TBR) branch swapping. Relative branch support in phylogenetic analysis was evaluated with 100 bootstrap pseudoreplicates. Parsimony analyses used heuristic searches with 100 random additions of taxa and tree bisection-recombination branch swapping. When more than a single most parsimonious tree was found, a strict consensus tree was generated. Confidence in the nodes was assessed by 2000 bootstrap pseudoreplicates (Felsenstein 1985) with random addition of taxa. Only bootstrap supports of 70 % and higher were considered as reliable, as such values were found to indicate 95 % probability of correct topology (Hillis & Bull 1993). For Bayesian analyses a GTR+I+G model was chosen according to the optimal model and parameter-values based on the AIC criteria determined in MrModeltest 2.2 (Nylander 2004). Two MCMC analyses for  $10^6$  generations each were conducted. The topologies of the sampled trees were used to generate a strict consensus tree. Only clades with a posterior probability of 95% or greater were considered to be significantly supported.

## RESULTS

### *Trioceros hanangensis* n. sp.

**Holotype.** ZFMK 82369, adult male with everted hemipenis. Locality: Tanzania: Mt. Hanang Forest Reserve near the village Jorodom ( $4^{\circ} 25'$  to  $4^{\circ} 35'$  S and  $35^{\circ} 20'$  to  $35^{\circ} 25'$  E) at 2800 m, collected by P. Krause, March 2002.

**Paratypes.** ZFMK 82368, subadult male, same data as the holotype; ZSM 111/1981/1- ZSM 111/1981/20 collected by L. Uthmoeller (1937) from the same locality as the holotype.

**Diagnosis.** A medium sized, stocky chameleon resembling a typical member of the *Trioceros bitaeniatus* complex (Rand 1963). The maximum total length is 138 mm; the tail comprises an average of 45 %, males with longer tails. Adult males and females have a similar snout-vent-length (SVL) the females being slightly larger. Body scalation is heterogeneous, with strongly enlarged denticulate scales in two distinct rows on the sides of the body. The gular crest is weak to medium, consisting of scales 1.5 times the length of their maximum width. A ventral crest is clearly visible in bigger specimens to almost indistinct in smaller ones. No rostral appendix is visible like in other members of the *bitaeniatus* complex (*Trioceros narraiocha* Nečas, Modry & Slapeta, 2003; *Trioceros hoehnelii* Steindachner, 1891).

This chameleon bears a close morphological resemblance to *Trioceros sternfeldi* from northern Tanzania (Mts. Kilimanjaro, Meru and Ngorongoro Crater) but shows a distinct colour pattern, which differs remarkably from *T. sternfeldi* (Sternfeld 1912a, Figures 3, 5, 6). Further, it possesses scattered melanophores on the sulcus of the hemipenis which are unique as compared to *T. sternfeldi*.

Despite a close genetic relationship with *T. ellioti*, the new form can clearly be distinguished by absence of the distinct longitudinal grooves on the gular pouch, a more heterogeneous scalation and a stouter body as compared with *T. ellioti*, which is rather slim (Günther 1895; Spawls et al. 2002).

The only known population of this species is found on Mt. Hanang above 2800 m in montane bushland.

**Description of holotype.** Head short and broad, distance from tip of snout to end of parietal crest less than twice greatest width between the temporal ridges. Parietal crest not swollen posteriorly, being almost straight in profile. No rostral projection at the point of fusion of canthi rostrales. Occipital flaps absent. Ventral crest moderately developed, composed of cone-shaped, subequal scales, longest on throat, about 3/4 of eye diameter. Throat homogeneously covered with small scales, no indication of longitudinal grooves. Head length (from tip of snout to posterior margin of casque) 21 mm. Height of casque (from angle of mouth to top of casque) 16 mm, slightly less than length of mandible (20 mm). Caudal side of casque homogeneously covered with fine granular scales. Parietal crest consists of 15 enlarged elongate scales. Two other diagonal (paraparietal) crests visible on each side of





**Fig. 3.** Holotype of *Trioceros hanangensis* n. sp. (male, ZFMK 82369) from Mt. Hanang, in life.

head, consisting of 4 enlarged tubercular scales. These originate in the immediate vicinity of the parietal crest and run rostrolaterally towards the orbit. Lateral crests composed of 4 enlarged, prominent pointed scales, extending to the supraorbital crest (11 pointed scales) and to canthi rostrales (5 scales).

Body rather stout. Dorsal crest well developed, composed of scales in groups of three anteriorly and four posteriorly. Increasing posteriorly in size within the group, giving the back a serrate appearance. Dorsal crest extends to the tail and becomes indistinct within its terminal third. The

first scales of the series of three just behind the head are longest, reaching 1.7–2 mm length. Ventral crest moderate to weak, best developed on the throat; posteriorly scales are shorter but still visible up to posterior belly.

Scalation heterogeneous. Sides of body, tail and upper surfaces of limbs covered with irregularly sized, convex, small scales interspersed with larger convex scales. Largest of these form a narrow row running from neck just behind temporal crests to tail. A second row of slightly smaller scales runs from shoulder to hind leg. This row is much less distinct and more irregular. Vent, inner side



**Fig. 4.** *Trioceros hanangensis* n. sp. (subadult male, ZFMK 82368) from Mt. Hanang.



**Fig. 5.** *Trioceros sternfeldi* (male) Ngorongoro Crater.



Fig. 6. *Trioceros sternfeldi* (male) Mt. Kilimanjaro.

of legs, soles and underside of the tail almost homogeneously scaled. No tarsal spurs.

**Hemipenis.** Smooth margined calyces cover the asulcal side of the hemipenis, extending as far as the sulcal lips. Apical tip characterised by two pairs of finely serrate rotulae of roughly semicircular shape, positioned towards the sulcal side and extending around lateral side of hemipenis. Scattered melanophores could be observed in the sulcus (Figure 2).

**Colouration and pattern.** Vivid green with lateral yellow bar following the row of enlarged scales running from neck to the base of tail. Head light blue except gular region. Eyeballs bright blue to turquoise.

**Variation of the paratypes.** Analysis of morphometric variation between male and female specimens of *Trioceros* n. sp. from Mt. Hanang resulted in males having significantly broader heads than females ( $x = 13,09$  mm vs.  $x = 12,59$  mm;  $p = 0,030$ ) and a tendency to increased rugosity in adult males. There is no apparent statistical difference in snout-vent-length (SVL) comparing both sexes.



Fig. 7. Mt. Hanang from Katesh, SSW side of the mountain.

es. (males: SVL = 72,30 mm, N = 9 / females: SVL = 75,52 mm, N = 14). No significant differences in relative tail length (TL) between sexes could be observed (males: TL = 66,22 mm / females: TL = 62,71 mm).

**Distribution.** Only known from Mt. Hanang Catchment Forest Reserve (4° 25' to 4° 35' S and 35° 20' to 35° 25' E) situated about 4 km from the district capital Katesh and 70 km from the town Babati in the administrative region of Arusha. The new species is named after the type-locality Mount Hanang.

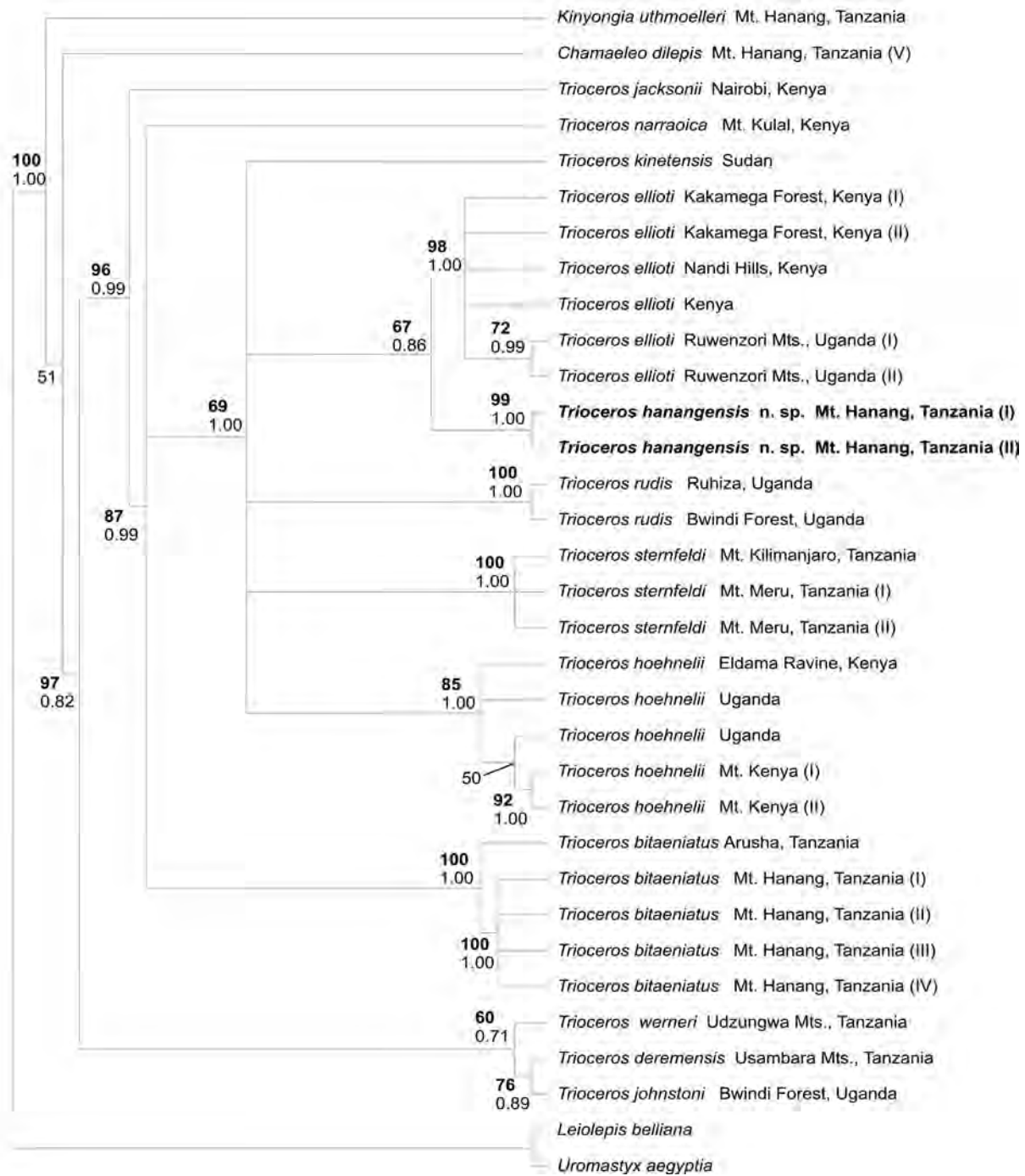
**Ecological remarks and habitat preference.:** *T. hanangensis* n. sp. was found in upland moorland at 2800 m on the moister north-eastern side of Mt. Hanang. Despite a prevailing semi-arid climate with 750–1500 mm annual rainfall, a pronounced mist effect on the eastern and north-eastern slopes is leading to a maximum of 2000 mm precipitation per year at the higher regions of the mountain. The sampled specimens were observed during daytime in bushy highland vegetation (with *Hypericum* sp., *Myrsine africana*, *Protea* sp.) near the source of the river Him-it, roosting on twigs at a height ranging from 100–150 cm from the ground.

**Phylogenetic affinities.** In order to infer the phylogenetic relationships between the different members of the *bitaeniatus* group on the basis of molecular data maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses (PP) were conducted. Maximum likelihood analysis was conducted with a reduced dataset, due to computational constraints. Sequences obtained from the combined 16S+12S rRNA mitochondrial genes were analysed together with gene-fragments of *Trioceros sternfeldi*, *T. rudis*, *T. ellioti*, *T. hoehnelii*, *T. bitaeniatus*, *T. narraoica*, *T. weneri*, *T. jacksonii*, *T. deremensis* and *T. johnstoni*. The two included specimens of *T. hanangensis* n. sp. were found in a well supported clade in the MP and Bayesian trees (MP: 99; PP: 1.00), separating them

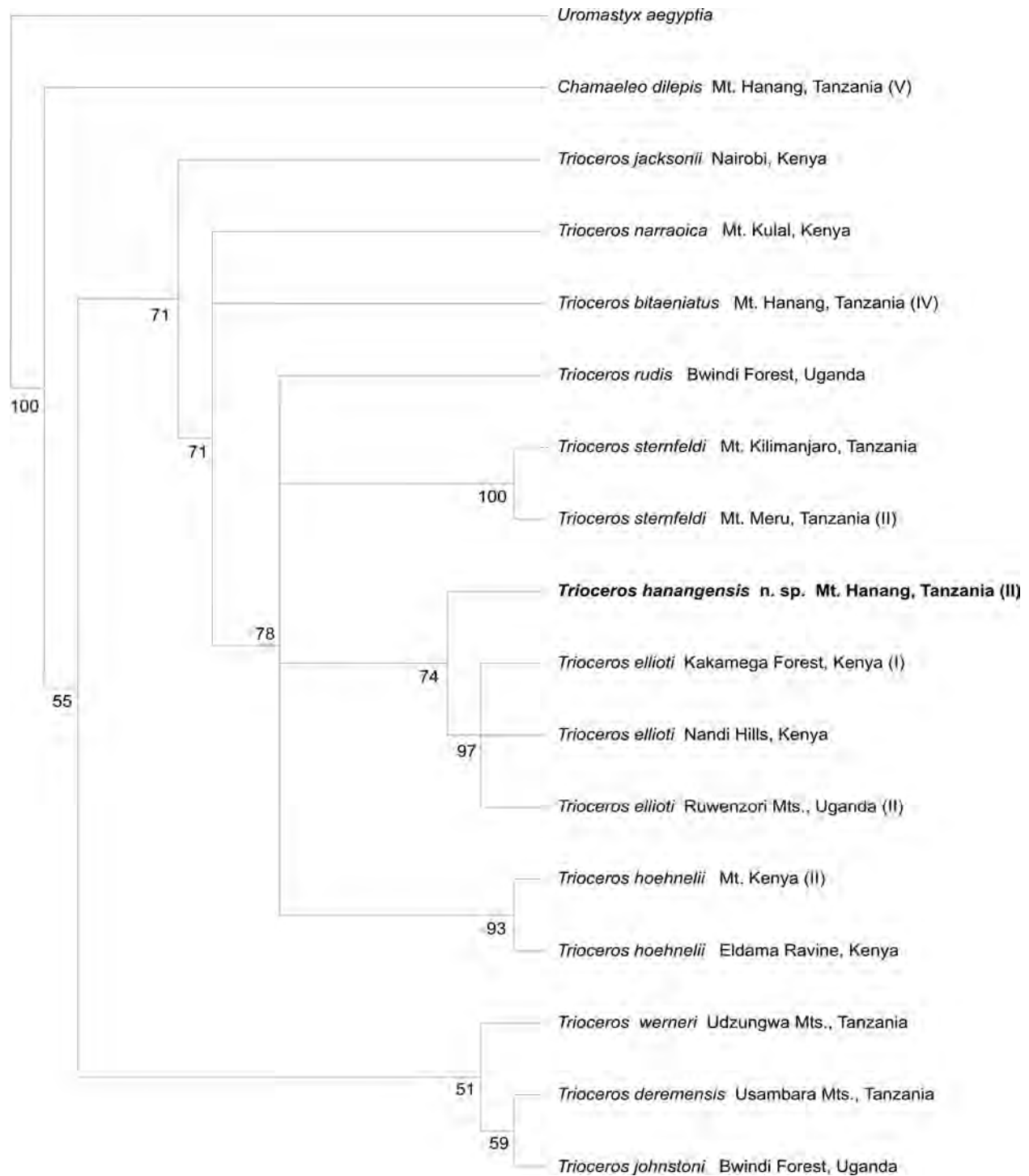


Fig. 8. Type locality of *Trioceros* at 2800 m altitude.

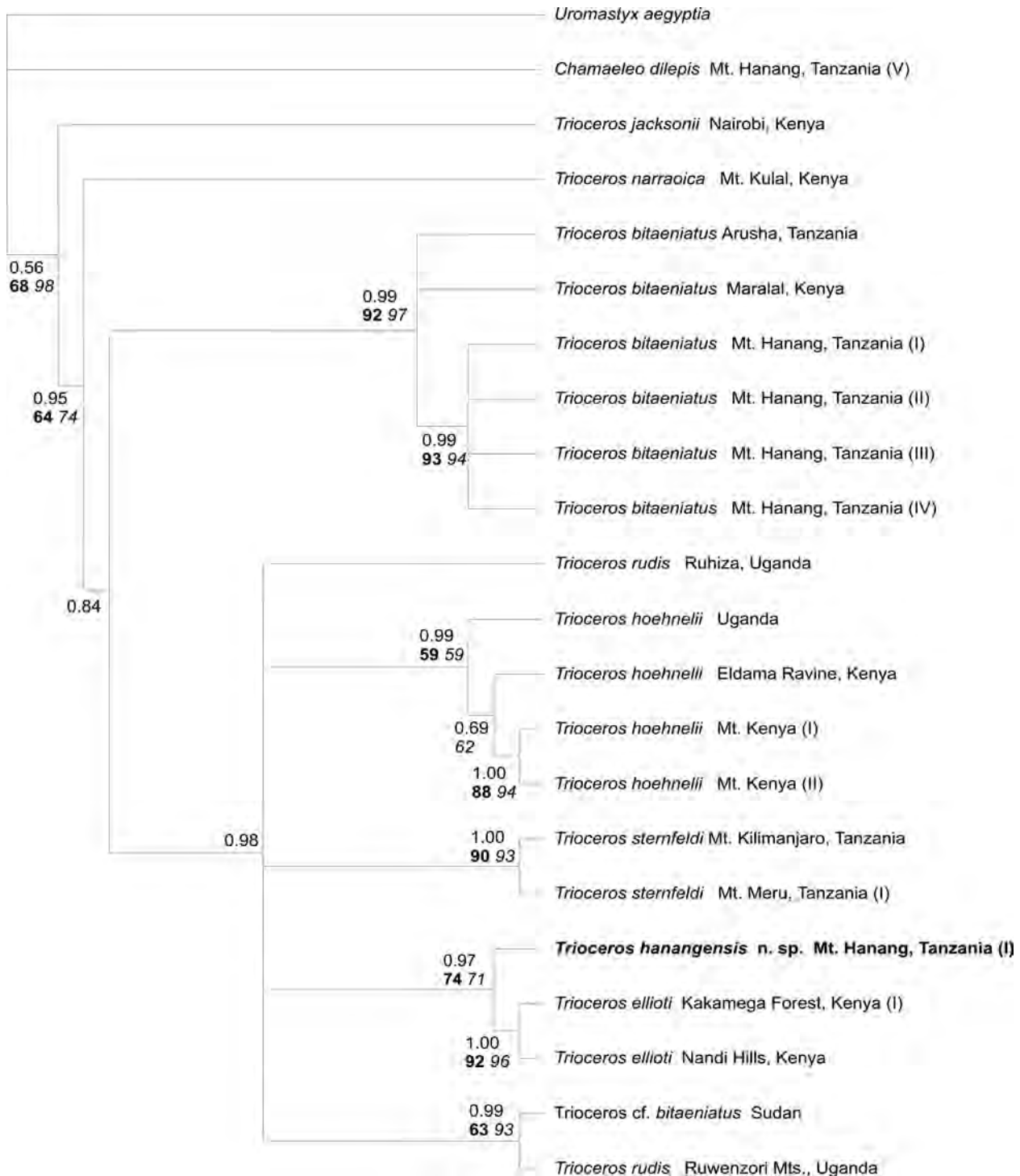




**Fig. 9.** Strict consensus parsimony tree derived from the combined fragment of the mitochondrial 12S and 16S rRNA genes. Parsimony bootstrap values are given above the nodes (bold), lower values are posterior probabilities produced by Bayesian inference. Nodes with less than 50 % bootstrap support are not labelled.



**Fig. 10.** ML tree derived from the combined fragment of the mitochondrial 12S and 16S rRNA genes. Bootstrap values are given above the nodes, nodes with less than 50 % bootstrap support are not labelled.



**Fig. 11.** Consensus tree derived from Bayesian inference using the mitochondrial 12S gene. Posterior probabilities are given above, ML bootstrap values (bold) and MP bootstrap values (inverse) below. Nodes with less than 50 % bootstrap support are not labelled.



**Table 1.** Specimens included in this study.

Species	Locality	Collection number	Accession number 16S/12S	
<i>Kinyongia uthmoelleri</i>	Mt. Hanang, Tanzania	ZFMK 82191	DQ397231	DQ397245
<i>Chamaeleo dilepis</i>	Mt. Hanang, Tanzania (V)	ZFMK 82381	AY927263	AY927244
<i>Trioceros ellioti</i>	Kakamega Forest, Kenya (I)	ZFMK 82058	DQ397278	DQ397268
<i>Trioceros ellioti</i>	Kakamega Forest, Kenya (II)	ZFMK 30835	DQ397279	DQ397259
<i>Trioceros ellioti</i>	Nandi Hills, Kenya	ZFMK 82057	DQ397225	DQ397239
<i>Trioceros ellioti</i>	Kenya	ZFMK 70818	DQ397280	DQ397260
<i>Trioceros ellioti</i>	Ruwenzori Mts., Uganda (I)	ZFMK 63287	DQ397281	DQ397266
<i>Trioceros ellioti</i>	Ruwenzori Mts., Uganda (II)	ZFMK 63227	DQ397282	DQ397267
<i>Trioceros hanangensis</i> s.p. nov.	Mt. Hanang, Tanzania (I)	ZFMK 82368	DQ397283	DQ397264
<i>Trioceros hanangensis</i> s.p. nov.	Mt. Hanang, Tanzania (II)	ZFMK 82369	DQ397284	DQ397265
<i>Trioceros rudis</i>	Ruhiza, Uganda	CAS 201781	DQ397223	DQ397223
<i>Trioceros rudis</i>	Bwindi Forest, Uganda	CAS 201711	DQ397285	DQ397252
<i>Trioceros rudis</i>	Uganda	ZFMK63223	-	DQ397277
<i>Trioceros kinetensis</i>	S-Sudan	ZFMK 29714	DQ397286	DQ397253
<i>Trioceros sternfeldi</i>	Mt. Kilimanjaro, Tanzania	ZFMK 68629	DQ397287	DQ397261
<i>Trioceros sternfeldi</i>	Mt. Meru, Tanzania (I)	T068 (prov. ID)	DQ397288	DQ397263
<i>Trioceros sternfeldi</i>	Mt. Meru, Tanzania (II)	ZFMK 82250	DQ397289	DQ397262
<i>Trioceros hoehnelii</i>	Mt. Kenya (I)	ZFMK 70678	DQ397290	DQ397265
<i>Trioceros hoehnelii</i>	Uganda	voucher not collected	DQ397291	DQ397266
<i>Trioceros hoehnelii</i>	Mt. Kenya (II)	ZFMK 66578	DQ397292	DQ397264
<i>Trioceros hoehnelii</i>	Eldama Ravine, Kenya	ZFMK 82088	DQ397224	DQ397238
<i>Trioceros hoehnelii</i>	Uganda	voucher not collected	DQ397293	DQ397267
<i>Trioceros bitaeniatus</i>	Mt. Hanang, Tanzania (I)	ZFMK 74949	DQ397222	DQ397237
<i>Trioceros bitaeniatus</i>	Mt. Hanang, Tanzania (II)	ZFMK 74952	DQ397294	DQ397269
<i>Trioceros bitaeniatus</i>	Mt. Hanang, Tanzania (III)	ZFMK 82365	DQ397295	DQ397270
<i>Trioceros bitaeniatus</i>	Mt. Hanang, Tanzania (IV)	ZFMK 82366	DQ397296	DQ397271
<i>Trioceros bitaeniatus</i>	Arusha, Tanzania	ZFMK 82251	DQ397297	DQ397272
<i>Trioceros bitaeniatus</i>	Maralal, Kenya	ZFMK 70752	-	DQ397276
<i>Trioceros narraoica</i>	Mt. Kulal, Kenya	ZFMK 73958	DQ397298	DQ397268
<i>Trioceros jacksonii</i>	Nairobi, Kenya	CAS 199070	DQ397226	DQ397240
<i>Trioceros wemeri</i>	Udzungwa Mts., Tanzania	ZFMK 70452	DQ397299	DQ397274
<i>Trioceros deremensis</i>	East Usambara Mts., Tanzania	CAS 168888	DQ397300	DQ397273
<i>Trioceros johnstoni</i>	Bwindi Forest, Uganda	CAS 201597	DQ397301	DQ397275
<i>Leiolopis belliana</i>	Egypt	GenBank	AB031986	AB031989
<i>Uromastyx aegyptia</i>	Thailand	GenBank	AB031994	AB031977

from all other included taxa. In the tree resulting from the maximum likelihood analysis, the single included specimen of *T. hanangensis* n. sp. clustered together with *T. ellioti* (ML: 74). Genetic distances to all other included taxa of the *bitaeniatus* group were relatively high (2,2–4 %) and were within the range which could be found between well defined species. This confirms the independent specific status of *Trioceros hanangensis* n. sp.

Phylogenetic inference of the combined 16S+12S rRNA dataset revealed a sistergroup relationship with *T. ellioti*, which was weakly to moderately supported with all methods (MP: 67; PP: 0.86; ML: 74). A genetic distance of 2,05–2,18 % could be observed between *Trioceros hanangensis* n. sp and the included specimens of *T. ellioti*. Using only the 12S fragment for phylogenetic inference, a much higher support for this sistergroup relationship could be observed (MP: 89; PP: 0.99; ML: 81). Nevertheless, the relationships of *T. hanangensis* n. sp. towards the other taxa of the *bitaeniatus* group, especially towards *T. sternfeldi* could not be finer resolved with the available limited genetic dataset, resulting in a large polytomy in all trees.

**Conservation status.** The forested area on Mt. Hanang is classified as a Catchment Forest Reserve covering approx. 40 km<sup>2</sup> which is relatively small compared to other reserves in Tanzania. The genuine forest of Mt. Hanang has been drawn back to the very base of the mountain and is under increasing threat by illegal logging, fires and drought. Due to the small size of the reserve, even minor disturbances are likely to affect the habitat of forest dependant species like *T. hanangensis* n. sp. Along with the well-known mountains in northern Tanzania like Meru, Kilimanjaro, Ngorongoro and the Eastern Arc, Mt. Hanang is a further example of a remnant mountain forest with quite unique climatic and ecological conditions. For this reason the protection and management of the remaining forests on Mt. Hanang should receive urgent priority.

## DISCUSSION

*Trioceros hanangensis* n. sp. is a member of a species complex which is closely associated with the forest covering many of the East African mountains. These

chameleons represent a subgroup of the *bitaeniatus*-group (the “*rudis* group” sensu Rand 1963). In contrast to other species within the *bitaeniatus* group, populations of the *rudis* group are restricted to mountain forests with widely disjunct distribution, each population bearing specific morphological peculiarities distinguishing it from the others as first observed by Rand (1963).

Like the *bitaeniatus* group as a whole, the different forms of the *rudis* group can most easily be understood as relics of a period equating with the last pluvial period of the Pleistocene when the rainfall in East Africa was higher and the forest was much more extensive than today. Drier periods following the pluvial maxima resulted in a retreat of the forests from the lower elevations to their present positions on the higher mountains. As a consequence the chameleon populations living in these forests (Wasser & Lovett 1993) were also fragmented. Different selection pressures formed the various taxa (*T. schubotzi*, *T. sternfeldi* or *T. hanangensis* n. sp.) which are today restricted to relatively small areas or certain types of habitat. In contrast to the mountains of the Eastern Arc, most volcanoes in northern Tanzania are relatively young and result from volcanic activity persisting from the Oligocene (37 myr ago) to the Quaternary. As a consequence, the origin of the different *rudis*-forms cannot lie very far in the past which makes it a rewarding subject for the investigation of beginning speciation processes.

Despite a morphological similarity with *Trioceros sternfeldi* (Mts. Meru, Ngorongoro and Kilimanjaro) *T. hanangensis* n. sp. can be regarded as valid species, chief indicators being a distinct colour-pattern, presence of scattered melanophores in the sulcus of the hemipenis (not present in *T. sternfeldi*) and the high degree of genetic differentiation towards *T. sternfeldi* and the other members of the group. Molecular analysis of the combined 16S+12S dataset shows a genetic differentiation between 2.2–4 %, which is clearly above the intraspecific level and confirms the independent specific status of *T. hanangensis* n. sp.

A weakly supported sister-group relationship towards *T. ellioti* is indicated by all the given trees (MP: 67; PP: 0.86; ML: 74). In contrast to *T. ellioti* *T. hanangensis* n. sp. does not possess the distinct longitudinal grooves on the gular pouch as present in *T. ellioti*, it has a more heterogeneous scalation and a stouter body than *T. ellioti* (Günther 1895; Spawls et al. 2002). Further, *T. ellioti* has a wide distribution ranging from western Kenya, the northern shore of Lake Victoria in Uganda, western Uganda, Rwanda and Burundi and occurs in open bushland and grassland.

Limited by the available genetic dataset, phylogenetic relationships towards the other members of the *bitaeniatus*

group could not be sufficiently resolved. Phylogenetic affinities of *T. hanangensis* n. sp. and *T. sternfeldi* can only be assumed by means of morphological data: specimens of *T. sternfeldi* from Mt. Kilimanjaro have dark eyeballs, in contrast to *T. sternfeldi* from Ngorongoro and *T. hanangensis* n. sp. which both have light blue eyeballs and are more alike in general colouration. The parietal knob observed by Rand (1963) associated with specimens from Mt. Meru and Mt. Kilimanjaro could not be observed in specimens from Mt. Hanang respectively Ngorongoro. These observations support the assumption that *T. hanangensis* n. sp. is more closely related to *T. sternfeldi* from Ngorongoro than with the *sternfeldi*-populations from Meru and Kilimanjaro. The geography of the region would have allowed gene flow between the populations of Hanang and Ngorongoro in the past: particularly to the north-west of Mt. Hanang a continuous forest could have existed during moister periods linking the habitat of *T. hanangensis* n. sp. with the Nou- and Marang forests and further north with Oldeani (3188 m) and Ngorongoro (3000 m). Today, a dry and hot valley separates the mountain from the rift-wall and forms an impenetrable barrier for forest dependent species.

A similar observation could be made with *Kinyongia uthmoelleri*, an endemic of the highlands of Hanang and Ngorongoro: the two populations show closest morphological and genetic relationships (Krause 2006) which as well supports the above presumed close relationship between *T. hanangensis* n. sp. and *T. sternfeldi* from Ngorongoro.

**Acknowledgements.** The work of the senior author (PK) was supported by a grant of the German Foreign Exchange Service (DAAD). Herpetological researches in Tanzania were authorized by Tanzania Commission for Science and Technology (COSTECH). Many thanks to Mr. Joas Kahembe, Babati, M. Nikundiwe and K. Howell (University of Dar es Salaam) who gave support with a lot of practical advice and assisted obtaining research permits.

## REFERENCES

- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791
- Glaw F, Vences M, Ziegler T, Böhme W, Köhler J (1999) Specific distinctness and biogeography of the dwarf chameleon; *Brookesia minima*, *B. peyrierasi* and *B. tuberculata* (Reptilia: Chamaeleonidae): evidence from hemipenial and external morphology. *Journal of Zoology* 247: 225–238
- Günther A (1895) Notice of Reptiles and Batrachians collected in the eastern half of tropical Africa. *Annals and Magazine of Natural History* (6) 15: 523–529

- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98
- Hillis DM, Bull JJ (1993) An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* 42: 182–192
- Klaver C, Böhme W (1986) Phylogeny and Classification of the Chamaeleonidae (Sauria) with special Reference to Hemipenis Morphology. *Bonner Zoologische Monographien* 22: 1–64
- Klaver C, Böhme W (1997) Chamaeleonidae. *Das Tierreich. Eine Zusammenstellung und Kennzeichnung der rezenten Tierformen*. Walter de Gruyter, Berlin; New York, p. I–XII, 1–85
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, F. X. Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences, USA* 86: 6196–6200
- Krause P (2006) Taxonomic, Phylogenetic and Zoogeographic Studies on the Herpetofaunas of Mts. Hanang and Kwaraha in Tanzania with Special Reference to the Chamaeleonidae (Reptilia: Sauria). Unpubl. PhD thesis, University of Bonn, 267 pp
- Nečas P, Modry D, Slapeta JR (2003) *Chamaeleo (Trioceros) narraioca* n. sp. (Reptilia: Chamaeleonidae), a new chameleon species from a relict montane forest of Mount Kulal, northern Kenya. *Tropical Zoology* 16: 1–12
- Nylander JAA (2004) MrModeltest v2.2. Evolutionary Biology Centre, Uppsala University, Sweden
- Palumbi S, Martin A, Romano S, McMillian WO, STICE L, Grabowski G. (1991) The simple fool's guide to PCR. Department of Zoology and Kewalo Marine Laboratory, Hawaii, Honolulu
- Pilley R (2000) A Revision of the *Bitaeniatus* Chameleon Group (Hillenius 1959) with Reference to Biogeographical Origins and Radiation. Vol. M.Sc. thesis. The Natural History Museum, Department of Zoology. University of London, London
- Rand AS (1963) Notes on the *Chamaeleo bitaeniatus* complex. *Bulletin of the Museum of Comparative Zoology* 130: 1–29
- Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574
- Schmitz A (2003) Taxonomic and phylogenetic studies on scincid lizards (Reptilia: Scincidae). Unpubl. PhD thesis. University of Bonn, 262 pp
- Spawls S, Howell KM, Drewes RC, Ashe J (2002) A Field Guide to the Reptiles of East Africa. Academic Press, San Diego
- Steindachner F (1891) Bericht über die von Herrn Linienschiffsleutenant Ritter von Höhnel während der Graf Samuel Telekis ostafrikanischer Expedition gesammelten Reptilien. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien* 100: 307–313
- Sternfeld R (1912a) Der Formenkreis des *Chamaeleo bitaeniatus*. *Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin* 7: 379–384
- SPSS (2003) SPSS for Windows, Release 12.0. SPSS inc, Chicago, Illinois, USA
- Swofford DL (2002) PAUP\* Phylogenetic Analysis Using Parsimony. (\*and other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts, USA
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25: 4876–4882
- Tilbury C, Tolley K (2009) A re-appraisal of the systematics of the African genus *Chamaeleo* (Reptilia: Chamaeleonidae). *Zootaxa* 2079: 57–68
- Walsh S, Metzger D, Higuchi R. (1991) Chelex 100 as a Medium for Simple Extraction of DNA for PCR-Based Typing from Forensic Material. *BioTechniques* 10: 506–513
- Wasser SK, Lovett JC (1993) Introduction to the biogeography and ecology of the rain forests of eastern Africa. In: *Biogeography and Ecology of the Rain Forests of Eastern Africa*. J. C. Lovett and S. K. Wasser (eds.), Cambridge University Press, Cambridge

Received: 04.12.2009

Accepted: 10.02.2010

Corresponding editor: F. Herder