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A new species of chameleon (Sauria: Chamaeleonidae) from the highlands of northwest Kenya

JAN STIPALA[1,5], NICOLA LUTZMÁNN[2], PATRICK K. MALONZA[3], LUCA BORGHESIO[4], PAUL WILKINSON[1], BRENDAN GODLEY[1], MATTHEW R. EVANS[1]

[1]*School of Biosciences, University of Exeter, Tremough Campus, Penryn, Cornwall, TR10 9EZ, UK.*
E-mail: janstipala@hotmail.com; m.r.evans@exeter.ac.uk; b.j.godley@exeter.ac.uk; p.a.wilkinson@exeter.ac.uk
[2]*Seitzstrasse 19, 69120 Heidelberg, Germany. E-mail: langstrasse@web.de*
[3]*Herpetology section, National Museums of Kenya, Museum Hill, Nairobi, Kenya. E-mail: kmalonza@museums.or.ke*
[4]*Department of Biological Sciences, University of Illinois at Chicago, 60607 Chicago USA. E-mail: borghesio@gmail.com*
[5]*Corresponding author. E-mail: janstipala@hotmail.com*

Abstract

A new species of chameleon, *Trioceros nyirit* **sp. nov.**, is described from the northwest highlands of Kenya. It is morphologically similar to *T. hoehnelii* and *T. narraioca*, possessing a short rostral appendage, but differs from them by having a straight or weakly curved parietal crest and forward-pointing rostral projection. A phylogeny based on mitochondrial DNA shows that the proposed new taxon is a distinct clade within the *bitaeniatus*-group and a sister lineage to *T. schubotzi*. Its distribution appears to be restricted to the Cherangani Hills and adjacent Mtelo massif to the north. It is associated with afromontane forest edge, afroalpine ericaceous vegetation and also occurs in agricultural landscapes.

Key words: afroalpine, afromontane, East Africa, *nyirit*, phylogenetics, taxonomy, *Trioceros*

Introduction

East Africa has a diverse chameleon fauna with over 50 species described to date (Tilbury 2010). The majority of these species are regional endemics and are restricted to highlands areas, adapted to cooler, higher rainfall environments. Phylogeographic studies of the chameleon fauna of East Africa suggest that their diversification has been driven by climatic fluctuations during the Miocene and Plio-Pleistocene thought to have caused repeated range expansion and fragmentation, mountains acting as refugia and speciation occurring through divergence in isolation (Matthee *et al*. 2004, Mariaux & Tilbury 2006). While we understand something about the processes that have generated chameleon diversity in the region, the full extent of species diversity is likely to be significantly higher than is currently recognised and highlighted by the continued discovery of new taxa (Mariaux & Tilbury 2006; Mariaux *et al.* 2008; Necas 2009; Necas *et al.* 2009; Menegon *et al.* 2009; Krause & Böhme 2010; Lutzmann *et al.* 2010).

The classification of chameleon diversity in East Africa has a complex history. Numerous species were described by taxonomists in the 19th and early 20th centuries but many were reduced to synonyms or were designated as subspecies of widespread and morphologically variable species (Werner 1911; Mertens 1966; Loveridge 1957; Klaver & Böhme 1997). Recent molecular studies have shed light on some of these morphologically diverse and taxonomically problematic groups and revealed deep phylogenetic splits within species and the presence of numerous cryptic species (Matthee *et al.* 2004; Mariaux & Tilbury 2006; Mariaux *et al.* 2008; Krause & Böhme 2010).

One group of chameleons in East Africa that requires further detailed investigation, from morphological and molecular perspectives, is the *bitaeniatus*-group (sensu Rand 1963), a sub-lineage within the genus *Trioceros* (Tilbury & Tolley 2009) that are distributed throughout the highlands of the East and Central Africa. Morphologically they are quite distinctive with heterogenous body scalation, well-developed dorsal and gular crests, absence

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of occipital lobes and prominent lateral and parietal crests that form a triangular casque. The *bitaeniatus*-group has been subject to many taxonomic changes and early taxonomists described numerous species, many of which were later reduced to synonyms of what was considered to be a single morphologically variable species, *Chamaeleo* (= *Trioceros*) *bitaeniatus* FISCHER 1884 (Tornier 1896; Werner 1911; Loveridge 1957; Mertens 1966). A detailed study by Rand (1963) identified several morphologically distinct groups, some with parapatric or sympatric distributions and *T. bitaeniatus* was split into six species. Rand's (1963) classifcation continues to be recognised (Klaver & Böhme 1997; Spawls *et al*. 2002; Tilbury 2010) and molecular studies have confirmed the specific status of these taxa as distinct evolutionary lineages (Townsend & Larson 2002; Tilbury & Tolley 2009).

The number of species in the *bitaeniatus*-group continues to grow through the discovery of novel specimens in museum collections (Tilbury 1991) and the exploration of remote mountain ranges that have hitherto received little attention from biologists (Böhme & Klaver 1980; Tilbury 1998; Necas *et al*. 2003; Necas *et al*. 2005; Necas *et al*. 2009). Molecular data have also revealed the presence of cryptic species in the group (Krause & Böhme 2010; Stipala *et al*. in prep) and highlighted the potential for further discoveries. In this paper we describe an additional species that is morphologically and genetically distinct from other members of the *bitaeniatus*-group. The new species is apparently restricted to two separate but spatially proximate mountain ranges located in north-west Kenya.

Material and methods

Field survey methods.

We surveyed extensive areas of the central and western highlands of Kenya between March 2006 and March 2007 and in September 2008. Surveys were mostly restricted to afromontane forest habitats but also included afroalpine vegetation on higher massifs and agricultural land adjacent to the forest edge. We conducted opportunistic searches in the morning and evening hours and at night using torches. Night searches were most successful because chameleons typically become very pale at night and contrast against the darker surrounding foliage. All preserved specimens are deposited in the herpetology collection at the National Museums of Kenya, Nairobi.

Morphological analysis.

In addition to the specimens collected during field surveys we examined preserved material of the 14 described species in the *bitaeniatus*-group from the following collections: National Museums of Kenya (NMK), Natural History Museum, London (BMNH) and Zoological Research Museum A. Koenig in Bonn (ZFMK). Numbers of specimens examined in parentheses: *T. bitaeniatus* (14), *T. conirostratus* TILBURY 1991 (1), *T. ellioti* GÜNTHER 1895 (37), *T. hanangensis* KRAUSE & BÖHME 2010 (2), *T. hoehnelii* STEINDACHNER 1891 (74), *T. kinetensis* SCHMIDT 1943 (4), *T. narraioca* NECAS, MODRY & SLAPETA 2003 (14), *T. ntunte* NECAS, MODRY & SLAPETA 2005 (2), *T. nyirit* **sp. nov.** (25), *T. rudis* BOULENGER 1906 (10), *T. schoutendeni* LAURENT 1952 (drawings and descriptions only), *T. schubotzi* STERNFELD 1912 (19), *T. sternfeldi* RAND 1963 (5). The proposed new taxon was previously considered to be a meta-population of *T. hoehnelii* (Spawls & Rotich 1997, Spawls *et al*. 2002), the latter species showing considerable intra-specific morphological variation (Loveridge 1935, Rand 1963). Therefore we decided to examine a large number of specimens of *T. hoehnelii* from numerous localities across its range to assess intra-specific variation and the distinctiveness of the specimens from Cherangani/ Mtelo. All material examined is listed in Appendix I.

We made an initial comparison of the external morphology between the Cherangani Hills/ Mtelo specimens and other species in the *bitaeniatus*-group and recorded the following characters: presence/ absence of rostral horn, rostral horn shape and orientation, parietal crest shape, length of gular crest, length of dorsal crest, body scale heterogeneity, presence/absence of lateral rows of tubercles and their size.

Molecular analysis.

Source material. Tissue samples were taken from leg muscle of freshly killed specimens and stored in 98% ethanol. Figure 1 shows the collecting localities of specimens used in the molecular analysis, including the pro-

posed new taxon, *T. nyirit* **sp. nov.**, described in this paper. *Chamaeleo dilepis* was used as the outgroup. We were unable to obtain tissue samples of *T. narraioca* and only a single 450bp sequence of the 16S gene was available on GenBank (accession no. DQ397298) for inclusion in the phylogenetic analyses.

Laboratory methods. DNA was extracted from the muscle tissue using a standard protocol of Proteinase K digestion and salt extraction (Palumbi *et al*. 1991) and was visualised by running samples across a 1% agarose gel.

We amplified two portions of the mitochondrial genome using the Polymerase Chain Reaction (PCR): partial NADH subunit 4 (ND4) and adjacent tRNA-His, tRNA-Ser and tRNA-Leu regions; and partial 16S ribosomal RNA. For the ND4 gene we used the primers ND4 5'-TGACTACCAAAAGCTCATGTAGAAGC-3' and LEU 5'-TRCTTTTACTTGGATTTGCACCA-3' (Forstner *et al*.1995) and for 16S L1921 5'-CCCGAAACCAAA CGAG-CAA-3' (Fu 2000), L2206 5'-GGCCTAAAAGCAGCCACCTGTAAAGACAGCGT-3' and H3056 5'-CTCCG-GTCTGAACTCAGATCACGTAGG-3' (Honda *et al*. 2003).

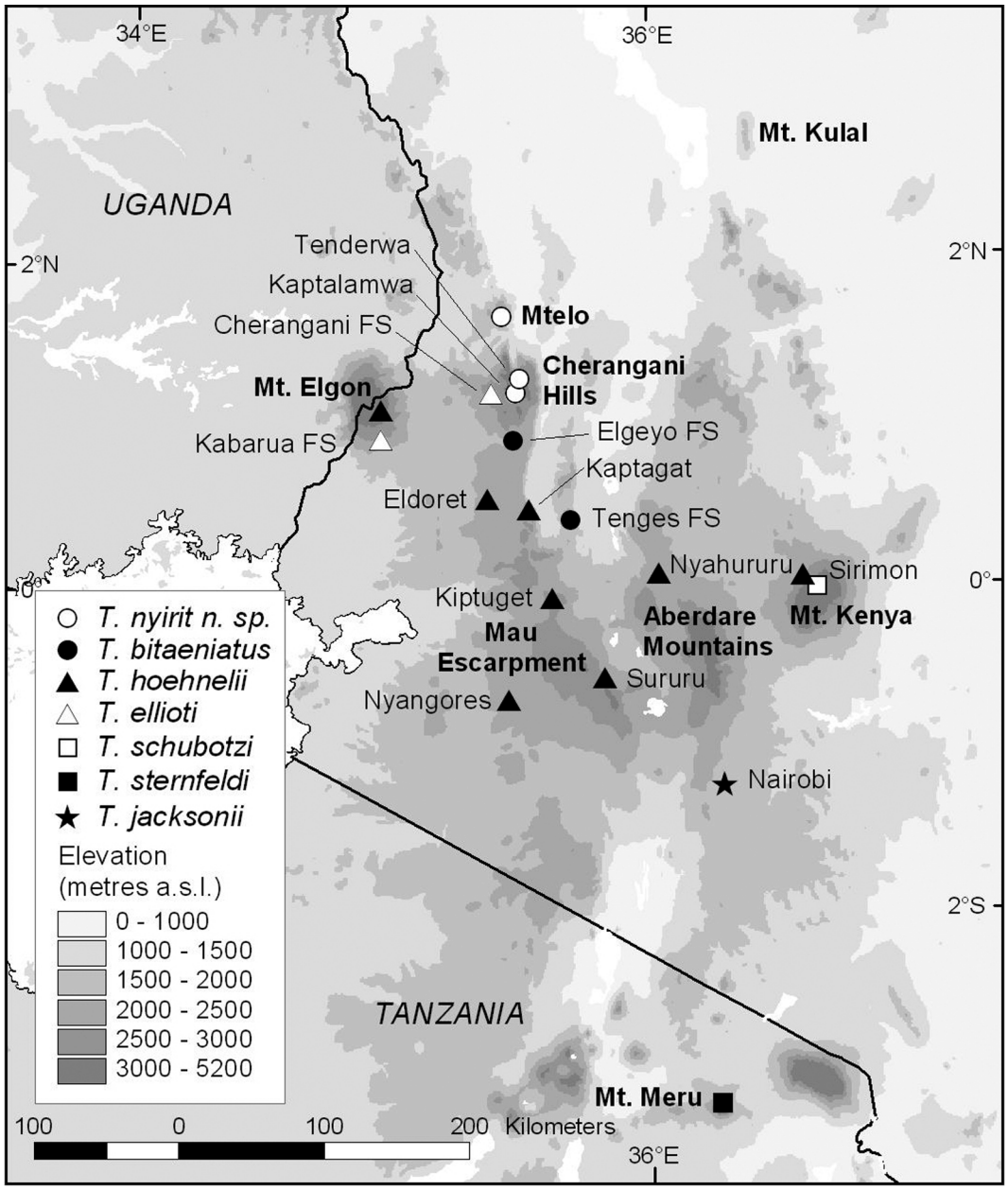

FIGURE 1. Topology of the central and western highlands of Kenya and collecting localities of specimens used in the molecular analysis.

Approximately 10–40ng of total genomic DNA was used as template for the PCR in a final volume of 20µl containing: a thermophilic buffer (50mM KCl, 10mM Tris–HCl, pH 9.0), 2mM $MgCl_2$, 0.5µM of each primer, 0.4mM dNTPs, and 1.25 Units of Taq polymerase.

The cycle profile included an initial denaturing step at 95 ºC for 2 min, followed by 42 cycles of 95 ºC for 45s, 58 ºC (ND4) or 56 ºC (16S) for 45s and 72 ºC for 2 min, with a final extension of 72 ºC for 5 min. PCR products were checked on a 1% agarose gel, then purified with EZNA Cycle Pure PCR Clean-up kits. The concentration of the purified PCR product was determined for optimal sequencing using a SpecroMax spectrophotometer.

PCR fragments were directly sequenced for both strands using the BigDye cycle sequencing kit (Applied Biosystems), and an ABI 377 automated sequencer. Chromatograms were read using Geneious Pro 4.0 and a consensus sequence generated from the forward and reverse primer sequences.

The amplified fragments corresponded approximately to the nucleotide positions 1293–1592 (16S) and 10,712-11,643 (ND4 and adjacent tRNAs) on the mitochondrial genome of *Trioceros melleri*, GenBank accession number AF23758. Unique mitochondrial DNA sequences are deposited in GenBank (Table 4).

Sequence alignment. Sequences were aligned with ClustalW (Thompson *et al*. 1994) as a plug-in to Geneious Pro v4.6, using the default settings. The protein-coding gene ND4 was translated into amino-acid sequences using ORF Finder (http://www.bioinformatics.org/sms2/orf_find.html) (Stothard 2000) to check for unexpected stop codons and no indels were present, either of which would indicate pseudogene sequences (Zhang & Hewitt 1996). Insertions and deletions made homology determination difficult in one region of the tRNAs and seven nucleotides, after base position 738, were excluded from the final alignment and subsequent phylogenetic analyses (Gatesy *et al*. 1993).

Phylogenetic analysis. Phylogenetic analyses were conducted using Maximum Parsimony (MP) and Bayesian Inference (BI) methods. Distance matrices were computed for with MEGA 2.1 (Kumar *et al*. 2001). For MP we used PAUP*4.0b10 (Swofford 2002). Prior to conducting a MP analysis we concatenated 16S and ND4 genes and conducted a partition homogeneity test, as implemented in PAUP*4.0b10 (Swofford 2002), to assess any for conflicting phylogenetic signals. The MP analysis was performed as an unweighted heuristic search with TBR branch swapping and 1000 random addition sequence replicates. Gaps were treated as fifth character as they have been shown to contain useful phylogenetic signal at lower taxonomic levels (Kawakita *et al*. 2003). Support for internal nodes was estimated using non-parametric bootstrap searches (Felsenstein 1985) with 1000 pseudo-replicates, 25 random addition sequence replicates each and SPR branch-swapping. Nodes with at least 70% bootstrap support were considered to be significantly resolved with a 95% probability of the clade being correct (Hillis & Bull 1993). BI was carried out using Markov Chain Monte Carlo (MCMC) randomization in MrBayes 3.1 (Ronquist & Huelsenbeck 2003). We ran two independent analyses consisting of four Markov chains that ran for $10x10^6$ generations, sampled every 10,000 generations, with a maximum likelihood starting tree, default priors, except with the option ‘‘prset ratepr’’ set as ‘‘variable’’. The programme Tracer v1.4 (Rambut & Drummond 2007) was used to determine ‘burn-in’ and the first 250 trees were discarded, the remaining trees used to generate a 50% majority rule consensus tree.

Results

Morphological analysis

The results of the morphological analysis are shown in Table 1. *T. nyirit* **sp. nov.** differs from most other members of the *bitaeniatus*-group by having a prominent, scale-covered rostral projection that projects beyond the upper anterior edge of the lip, a character shared by only *T. hoehnelii* and *T. narraioca*. *T. nyirit* **sp. nov.** differs from the latter two species in possessing an anteriorly-orientated rostral projection and straight or moderately curved parietal crest that rises gradually posteriorly. Both *T. hoehnelii* and *T. narraioca* have a dorsally-orientated rostral projection and strongly curved parietal crest that rises steeply anteriorly. Figure 2 illustrates the difference in male head shape of proposed new taxon, *T. nyirit* **sp. nov.** and other morphologically similar species from the *bitaeniatus*-group. *T. schubotzi* has been included but because the molecular analysis reveals it is the sister lineage to *T. nyirit* **sp. nov.**

Other characters were very variable among individuals of *T. nyirit* **sp. nov.** and were not distinct from other species. For example, the scales in the gular crest in some individuals were short, similar to most other species in the *bitaeniatus*-group, and long in others, comparable in length the long gular crest in *T. hoehnelii*. The dorsal crest scales ranged from low (cf. *T. bitaeniatus*, *T. rudis*, *T. sternfeldi*) to high (cf. *T. hoehnelii*). Background body scala-

tion was typically heterogeneous, similar in pattern to most other species in the *bitaeniatus* group. The two lateral row of tubercles are present and range from moderately larger than the surrounding flank tubercle to large flat plates, similar to *T. schubotzi* and some individuals of *T. hoehnelii*. Figure 3 shows the range of variation in scale morphology within and between three species, *T. nyirit* **sp. nov.**, *T. hoehnelii* and *T. schubotzi*.

TABLE 1. Main morphological characters that differentiate *T. nyirit* **sp. nov.** from other species in the *bitaeniatus*-group.

Species	Parietal shape	Rostral projection	Horn orientation	Gular crest length
nyirit **sp. nov.**	straight to moderately curved	prominent/ scale-covered	anterior	short to long
hoehnelii	strongly curved	prominent/ scale-covered	dorsal	long
narraioca	strongly curved	prominent/ scale-covered	dorsal	short
schubotzi	straight	absent or v short	n/a	short
sternfeldi	straight	absent	n/a	short
hanangensis	straight	absent	n/a	short
ellioti	straight	absent	n/a	short
rudis	straight	absent or v short	n/a	short to moderate
bitaeniatus	straight	absent	n/a	short
conirostratu	straight	single long conical scale	n/a	short
kinetensis	straight	absent	n/a	short
schoutedeni	straight	absent	n/a	short
jacksonii	straight to moderately curved	very long annular (+ 2 preorbital)	anterior	absent
ntunte	straight	absent	n/a	short
marsabitensis	straight	prominent/ annular	anterior	short

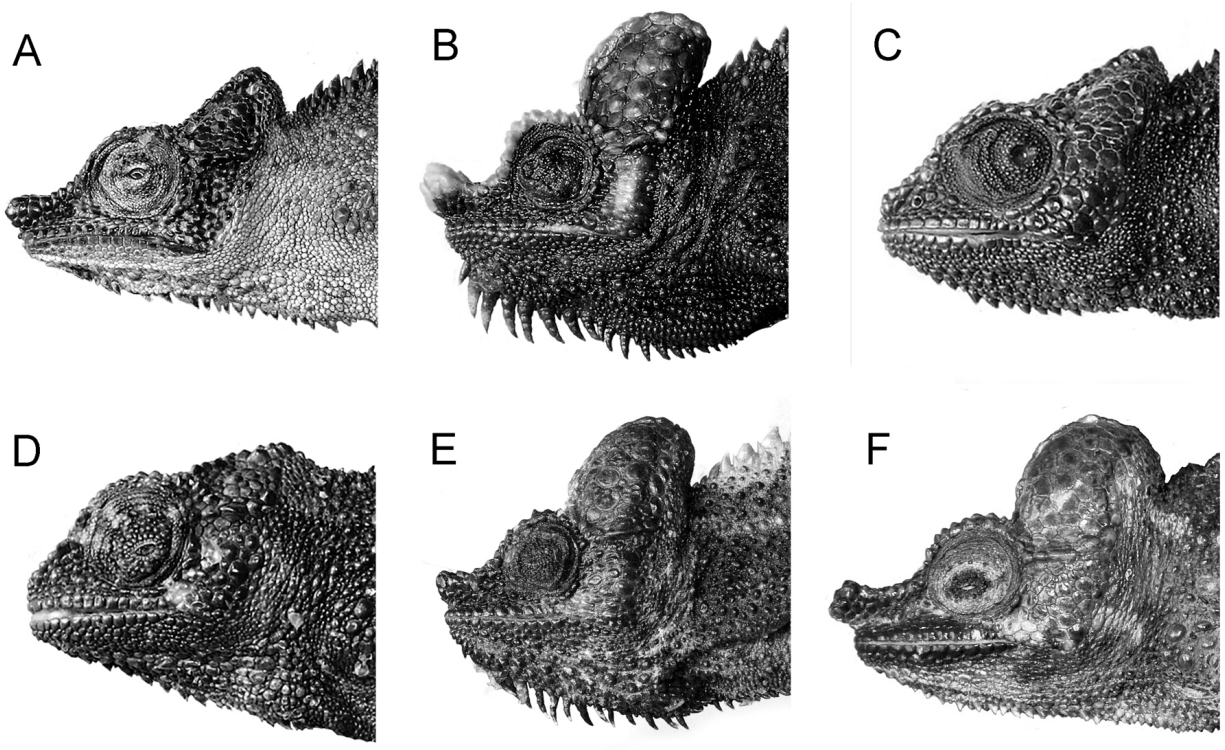

FIGURE 2. Typical head shape of male *T. nyirit* **sp. nov.** and comparison with other morphologically similar or genetically closely related species from the *bitaeniatus group*. Two specimens of *T. hoehnelii* are included to show intra-specific variation. A—*T. nyirit* **sp. nov.**, holotype, Mtelo massif, B—*T. hoehnelii*, Elgeyo Escarpment; C—*T. sternfeldi*, Mt. Meru; D—*T. schubotzi*, Mt. Kenya; E—*T. hoehnelii*, Mt. Elgon; F—*T. narraioca*, Mt. Kulal.

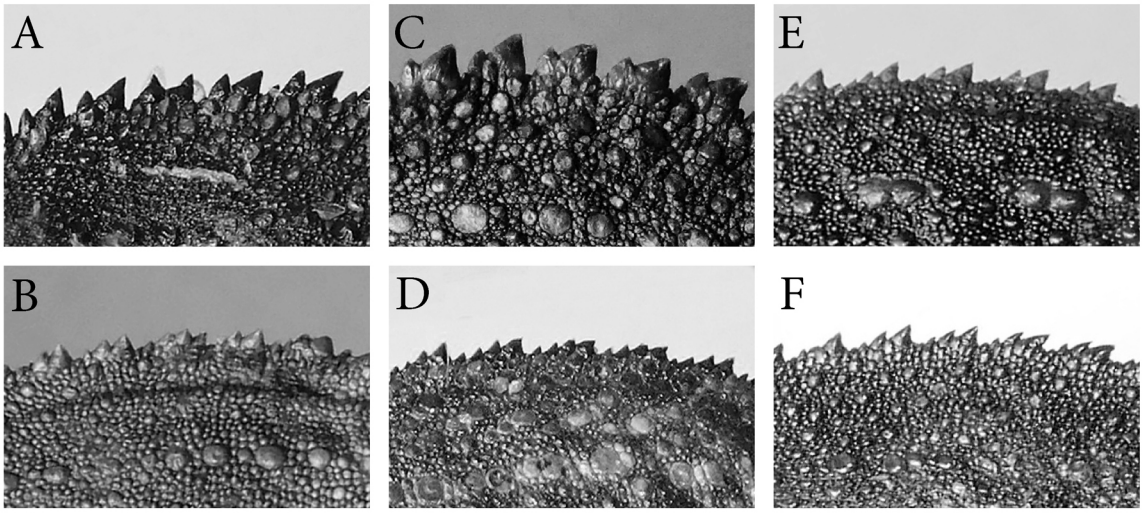

FIGURE 3. Dorsal crest and body scale variation in *T. nyirit* **sp. nov.** (A & B), *T. hoehnelii* (C & D) and *T. schubotzi* (E & F).

Molecular analysis

The final sequence alignment consisted of 732 base pairs of the 16S rRNA marker and 832 base pairs of the ND4 marker. No indels were present in the ND4 protein coding region and the translated amino-acid sequences revealed no unexpected stop codons, either of which might indicate the presence of pseudogenes.

The partition homogeneity test failed to reject the null hypothesis of congruence between genes (p = 0.80) and the two genes were concatenated and analysed together in both the MP and Bayesian analyses. Of a total of 1564 characters, 223 were variable and 187 were parsimony informative, including the out-group.

MP and Bayesian analyses produced identical topologies with high bootstrap support values and posterior probabilities for most nodes, with the exception of *T. ellioti*, which in the Bayesian tree was placed as a basal lineage to the clade containing *T. schubotzi* and *T. nyirit* **sp. nov.** The phylogeny shows that specimens from the proposed new taxon form a monophyletic clade within the *bitaeniatus*-group that is a sister lineage to *T. schubotzi*. The sister relationship to *T. schubotzi* is contrary to expectations based on external morphology as *T. nyirit* **sp. nov.** is morphologically more similar to *T. hoehnelii*,.

A phylogeny including *T. narraioca* was restricted to 450bp of the 16S gene. Both MP and BI analyses produced trees with low bootstrap support values and Bayesian posterior probabilities for most nodes. Nevertheless, *T. narraioca* was placed as a basal linage in the *bitaeniatus* clade (not including *T. jacksonii*), which agrees with another phylogenetic analysis of the *bitaeniatus* group based on combined 12S and 16S sequences (Krause & Böhme 2010).

The average genetic distances (uncorrected p-distances) between *T, nyirit* **sp. nov.** and other species in the *bitaeniatus group* (shown in Table 2) was 3.7%±0.4 for 16S and 7.5%±0.5 for ND4. These values are within the range of genetic distances between the other recognised species in the *bitaeniatus* group (2.2 –5.9% for 16S; 5.5-10.2% for ND4). A Z-test confirms that *T. nyirit* does not differ from the average among other species (16S, p = 0.12; ND4, p = 0.25). Studies of other lineages within the Chamaeleonidae have recorded similar inter-specific distances for 16S (Tolley *et al.* 2004, Ullenbruch *et al.* 2007, Mariaux *et al.* 2008, Barej *et al.* 2010). There are currently no published inter-specific distances for ND4.

The phylogeny in Figure 3 shows that there are two divergent haplotypes within *T. nyirit* **sp. nov.**, both occurring in the Cherangani Hills and the Mtelo samples forming a sub-clade within one of those clades. This phylogeographic pattern is interesting given that Mtelo and Cherangani populations appear to be currently geographically isolated from each other by a valley (elevation, <1400m) that almost certainly prevents dispersal between the massifs. This suggests that isolation between the populations on the two massifs has been broken in the recent past by episodes of dispersal and geneflow between the two populations. It is possible that more extensive sampling would reveal the presence of the second Cherangani haplotype on the Mtelo massif, missed in our study because of our small sample size (n=2).

TABLE 2. Average genetic distances (uncorrected p-distances) among species in the *bitaeniatus*-group abd *C. dilepis*. Lower-left numbers = ND4, upper-right numbers = 16S.

SPECIES	*nyirit* **sp. nov.**	*hoehnelii*	*bitaeniatus*	*dilepis*	*ellioti*	*jacksonii*	*schubotzi*	*sternfeldi*
nyirit **sp. nov.**	-	0.035	0.041	0.127	0.033	0.053	0.023	0.035
hoehnelii	0.067	-	0.044	0.124	0.029	0.049	0.038	0.031
bitaeniatus	0.088	0.081	-	0.114	0.042	0.059	0.050	0.041
dilepis	0.195	0.182	0.196	-	0.121	0.121	0.129	0.117
ellioti	0.078	0.075	0.086	0.186	-	0.050	0.035	0.022
jacksonii	0.091	0.080	0.095	0.182	0.096	-	0.057	0.052
schubotzi	0.067	0.082	0.097	0.205	0.091	0.102	-	0.040
sternfeldi	0.061	0.055	0.084	0.186	0.058	0.084	0.070	-

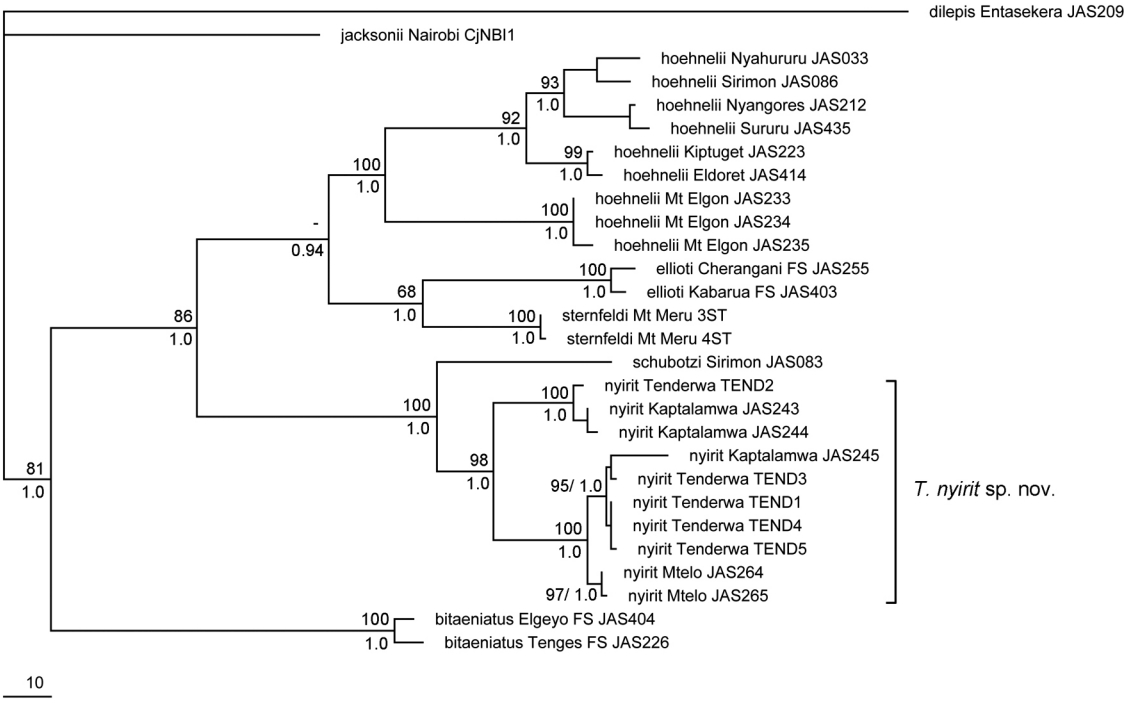

FIGURE 4. Phylogeny generated from Maximum Parsimony analysis, based on partial 16S and ND4 mtDNA markers. Above branches = MP bootstrap support values. Below branches = Bayesian posterior probabilities. Scale bar indicates the number of nucleotide substitutions.

Taxonomy

Trioceros nyirit sp. nov.

(Figures 2B and 5A & B)

Holotype. NMK-L3166/1, adult male, Gatau Pass (2500m), Mtelo massif, northwest Kenya, collected by Jan Stipala and Joash Nyamache on 21st July 2006.

Paratypes. 4 males and 4 females. NMK-L3166/2, male, NMK-L3166/ 3-4, females, southern slopes of Mtelo massif (2200-3100m); NMK-L2990/1 & 5, males, NMK-L2990/ 2-3, females, Gatau Pass, Mtelo massif (2200-2500m).

Non-type material. NMK-L2998/1-5, males, NMK-L2998/6-11, females, Kaptalamwa and Kapiego (2900-3000m), Cherangani Hills; BMNH 1969.2588-9, BMNH 1969.2591, BMNH 1969.2595-6, Sondang (3050m), Cherangani Hills.

Diagnosis. A medium sized chameleon (largest male 191mm TL, largest female 180.5mm TL) with heterogeneous body scalation; short, forward-pointing rostral process covered in enlarged scales; straight to weakly curved parietal crest, rising gradually posteriorly to meet the lateral crests to form a prominent, moderately steep triangular casque; This combination of characters differentiates *T. nyirit* **sp. nov.** from other species in the *bitaeniatus*-group.

Description of holotype. Total length 165mm (SVL 80mm, tail 85mm). The scalation is heterogenous with rounded or oval convex tubercles of different sizes scattered on the body and legs and tail. There are two clearly defined horizontal rows of enlarged tubercles on each flank, larger than those elsewhere on the body, the lower row extending between the front and hind legs, the second row higher on the flank. The scales on the belly are fine and relatively homogenous. The dorsal crest is well-developed along the entire length of the dorsal keel and extends onto the proximal two thirds of the tail, decreasing in size posteriorly. It is composed of enlarged conical tubercles, formed into sequential groups of three or four scales, the scales within each group increasing in size posteriorly, the most posterior scale much larger than the one before it. The ventral crest consists of short conical tubercles and is contiguous with the gular crest.

The head has prominent tubercular crests. The supra-orbital crests are continuous with the lateral crests, which rise posteriorly to meet the parietal crest forming a distinct, raised triangular casque. The parietal crest is straight and rises gradually posteriorly. It is forked anteriorly, the branches extending anterio-laterally to contact the supra-orbital crests. The scales on the casque are large, flat and polygonal. The scales between the supra-orbital crests are smaller than on top of the casque and moderately convex. A row of five strongly convex scales form a median ridge between the orbits and is distinct from the flat scales around it. The supra-orbital crests are contiguous with the canthi rostrales and merge anteriorly to form a short, forward-pointing rostral projection, which is covered in enlarged scales and extends beyond the the edge of the snout by 1mm. The sides of the head are covered in moderately enlarged convex tubercles. The scales on the eyelids are fine and weakly heterogenous. The gular region has several longitudinal rows of strongly convex tubercles that border a distinct gular crest. The gular crest is composed of conical scales, short anteriorly, becoming longer under the posterior half of the head, the longest scales about equal in length to the diameter of the eye opening.

The hemipenis is structurally similar to that of *T. hoehnelii* (Böhme & Klaver 1986) but differs, the two pairs of cogwheels being more strongy serrated. The parietal peritoneum shows only light pigmentation and is not extensive as seen in other members of the *bitaeniatus* group (Bauer 1997).

Colour in preservation. The holotype is uniform black.

Variation in paratypes and non-type material. The following snout-vent length (SVL) and tail length measurements were taken from living specimens, some of which were preserved and deposited in the NMK. Mean SVL: males = 76.4mm ± 8.5 (n = 27). Females = 72.9mm ±7.1 (n = 35). Largest male: 191mm total length (SVL = 91mm, tail = 100mm. Largest female: 160mm total length (SVL = 85mm, tail 75mm). Morphometric measurements and inter-orbital scale count of type and non-type specimens are given in Table 3.

Several morphological characters were quite variable. Body scales in some individuals are fine, granular and almost homogenous except for the two rows of tubercles, which in some individuals can be indistinct, especially the lower row. Gular crest development is variable in both Mtelo and Cherangani populations, being very short in some individuals (see Figure 4B) and long in others, equivalent in length to some specimens of *T. hoehnelii*. The parietal crest is straight or weakly curved, however it never reaches the extent of strong curvature nor rises steeply anteriorly as seen in *T. hoehnelii* and *T. narraioca*.

Colour in life. Holotype was uniform bright green with an indistinct wash of turquoise bands on the body, legs and tail. The eye is bright green with a dark horizontal line passing through it. The dorsal crest and adjacent scales on the dorsal keel, the head crests and rostral projection are dark red. The toes and soles of the feet are bright yellow.

Other males were also typically uniform bright green, the lower flanks, gular region, legs and tail a lighter turquoise-green. The head crests, rostral projection, dorsal crest and adjacent scales on the dorsal keel vary from red to black. When stressed, excited or basking, in males a darker pattern of broad vertical bars appears on the flanks, the rows of flank tubercles remaining light green. Bright yellow toes and soles of the feet were only seen in Mtelo males.

Females were more variable in colour than males. Some females were uniform green, while others were shades of light and dark brown, the body with several broad bands, the flank tubercles and throat cream-white or yellow. The head and dorsal crests and rostral projection were red in most individuals.

TABLE 3. *Trioceros nyirit* **sp. nov.** morphometric measurements and scale counts of type and non-type material. Key to codes. SVL (Snout-Vent Length – mouth tip to opening of vent); Tail (tail tip to vent); Total length (snout tip to tail tip); RTL (Relative Tail Length—tail as a percentage of Total Length); MHL (Maximum Head Length—tip of mouth to occiput); HW (Head Width—distance between lateral crests at angle); MHL/ HW (ratio of MHL/ HW); SL (Snout Length - tip of mouth to orbit); OD (Orbit Diameter—vertical diameter of orbit); IOD (Inter-Orbital Distance—distance between supra-orbital ridges above orbits); IOSC (Inter-Orbital Scale Count—number of scales between supra-orbital crests, including crest scales); CH (Casque Height—distance from angle in lateral crest to occiput); LCL (Lateral crest Length—distance from angle in lateral crest to orbit).

Status	Museum number	Massif	Sex	SVL	Tail	Total length	RTL	MHL	HW	MHL/ HW	SL	OD	IOD	IOSC	CH	LCL
holoptype	NMK-L2990/5	Mtelo	male	64	59	123	48	23.0	11.0	2.1	6.5	6.9	7.2	15	6.3	5.5
paratype	NMK-L2990/1	"	"	67	65	132	49	23.6	10.9	2.2	6.5	6.8	7.6	15	6.6	5.5
"	NMK-L3166/1	"	"	80	85	165	52	25.7	12.0	2.1	7.2	7.1	7.7	14	7.5	6.4
"	NMK-L3166/2	"	"	88	98	186	53	27.4	13.4	2.0	7.4	8.4	8.9	14	8.6	6.7
"	NMK-L2990/2	"	female	67	78	145	54	23.8	11.3	2.1	6.4	6.6	8.1	14	6.8	5.7
"	NMK-L2990/3	"	"	62	71	133	53	23.5	10.7	2.2	6.4	7.0	8.6	17	6.1	5.6
"	NMK-L3166/3	"	"	70	65	135	48	23.7	11.3	2.1	6.7	6.7	7.6	13	5.8	5.4
"	NMK-L3166/4	"	"	75	67	142	47	21.9	11.4	1.9	6.6	7.0	6.9	14	5.5	5.3
non-type	NMK-L2998/1	Cherangani	male	81	74	155	48	29.1	13.2	2.2	5.3	7.1	6.5	12	7.1	6.3
"	NMK-L2998/2	"	"	81	87	168	52	28.9	14.4	2.0	4.7	8.2	9.2	14	8.3	7.0
"	NMK-L2998/3	"	"	88	96	184	52	26.2	12.2	2.1	4.5	7.6	8.5	15	9.2	5.3
"	NMK-L2998/4	"	"	88	59	147	40	28.4	14.8	1.9	5.0	8.6	8.3	13	8.6	6.6
"	NMK-L2998/6	"	female	81	67	148	45	24.6	11.4	2.2	6.1	7.6	8.0	14	7.5	5.0
"	NMK-L2998/8	"	"	78	65	143	45	24.4	12.3	2.0	6.8	7.6	8.1	15	7.1	5.6
"	NMK-L2998/9	"	"	69	65	134	49	23.0	11.2	2.1	4.3	7.0	8.0	14	7.2	5.8
"	NMK-L2998/10	"	"	69	65	134	49	21.4	10.2	2.1	3.7	6.4	8.0	15	5.9	5.3
"	NMK-L2988/11	"	"	87	76	163	47	22.5	11.2	2.0	3.8	6.9	7.6	15	6.5	6.3

Etymology. Named after the Pokot word for chameleon, *nyirit*. The distribution of *T. nyirit* **sp. nov.** occurs mainly within the Pokot tribal area.

Distribution. *Trioceros nyirit* **sp. nov.** was recorded between 2900-3150m on the Cherangani Hills and between 2276-3121m on the Mtelo massif. At lower elevations other chameleon species (*T. ellioti* and *T. conirostratus*) were abundant, suggesting that *T. nyirit* **sp. nov.** is restricted to higher elevations. In the Cherangani Hills *T. nyirit* **sp. nov.** appears to be widespread as specimens were collected from Sondang (3000m) in the north and Cheptongoi Hills (3000m) to the south of the range. We did not sample between 2300m and 2900m in the Cherangani Hills but *T. nyirit* **sp. nov.** is likely to occur below 2900m given its elevation range on Mtelo. We estimated the distribution of *T. nyirit* **sp. nov.**using maximum and minimum elevation values from a GPS recorded at collecting localities and using Google Earth. The predicted range size of *T. nyirit* **sp. nov.** in the Cherangani Hills (assuming that all sites >2900m are potential habitat) is 754km^2 and on the Mtelo massif is 93km^2 (>2276m).

FIGURE 5. A. Adult male, holotype, *T. nyirit* **sp. nov.**, Gatau Pass, Mtelo massif. B. Adult female, same locality.

FIGURE 6. Habitat of *T. nyirit* **sp. nov.** on the Mtelo massif. Specimens were found in the ericaceous zone (foreground) at 3000m and in shrubs adjacent to the forest at 2800m (middle ground).

Ecology. On the Mtelo massif, *T. nyirit* **sp. nov.** was found on shrubs at the edges of cleared afromontane forest and also in the ericaceous zone above the forest. In the Cherangani Hills, specimens were found on shrubs and small trees at the edge of fields, on hedges and roadside vegetation.

Conservation status. As well as occurring in ericaceous afroalpine vegetation and shrubs at the forest edge, *T. nyirit* **sp. nov.** were also collected in disturbed habitats in agricultural landscapes and appears to be relatively abundant in these areas. This suggests that despite its limited distribution it does not seem to be threatened by anthropogenic activities. However, deforestation and conversion of natural habitats to agriculture on the Mtelo massif continues to reduce forest cover (John Yoposiwa, pers. comm.) and rates of habitat change are believed to be high also in the Cherangani (Wass 1995; Akotsi & Gachanja 2004).

TABLE 4. Specimens used in the phylogenetic analyses: species name, collecting locality, field tag ID, voucher specimen ID and GenBank accession numbers for 16S and ND4 sequences. ** indicates specimen not collected or held in a museum collection.

				GenBank accession no.	
Species	Locality	Field tag ID	Museum ID	ND4	16S
T. bitaeniatus	Elgeyo FS, Elgeyo escarpement	JAS404	L3041/1	JN161102	JN165387
T. bitaeniatus	Tenges FS, Tugen Hills	JAS226	L3035	JN161103	JN165388
C. dilepis	Entasekera, Nguruman Escarp.	JAS209	L2982/1	JN161104	JN165389
T. ellioti	Cherangani FS, Cherangani Hills	JAS255	L2991	JN161105	JN165390
T. ellioti	Kabarua FS, Mt. Elgon	JAS403	L3040/2	JN161106	JN165391
T. hoehnelii	Eldoret	JAS414	L3047	JN161107	JN165392
T. hoehnelii	Kiptuget FS, Mt. Londiani	JAS223	L2984/2	JN161108	JN165393
T. hoehnelii	roadhead, Mt. Elgon N. P.	JAS233	L2987/1	JN161109	JN165394
T. hoehnelii	roadhead, Mt. Elgon N. P.	JAS234	L2987/2	JN161110	JN165395
T. hoehnelii	roadhead, Mt. Elgon N. P.	JAS235	L2987/3	JN161111	JN165396
T. hoehnelii	Nyahururu	JAS033	L2962/2	JN161112	JN165397
T. hoehnelii	Nyangores FS, Mau Escarpment	JAS212	L2994/1	JN161113	JN165398
T. hoehnelii	Sirimon route, Mt. Kenya	JAS086	L2972/2	JN161114	JN165399
T. hoehnelii	Sururu FS, Mau Escarpment	JAS435	L3044/3	JN161115	JN165400
T. jacksonii	Nairobi	CjNBI1	L2976	JN161116	JN165401
T. nyirit **sp. nov.**	Kaptalamwa, Cherangani Hills	JAS243	L2988/1	JN161117	JN165402
T. nyirit **sp. nov.**	Kaptalamwa, Cherangani Hills	JAS244	L2988/2	JN161118	JN165403
T. nyirit **sp. nov.**	Kaptalamwa, Cherangani Hills	JAS245	L2988/3	JN161119	JN165404
T. nyirit **sp. nov.**	Mtelo massif	JAS264	L2990/5	JN161120	JN165405
T. nyirit **sp. nov.**	Mtelo massif	JAS265	L2990/1	JN161121	JN165406
T. nyirit **sp. nov.**	Tenderwa, Cherangani Hills	TEND1	**	JN161122	JN165407
T. nyirit **sp. nov.**	Tenderwa, Cherangani Hills	TEND2	**	JN161123	JN165408
T. nyirit **sp. nov.**	Tenderwa, Cherangani Hills	TEND3	**	JN161124	JN165409
T. nyirit **sp. nov.**	Tenderwa, Cherangani Hills	TEND4	**	JN161125	JN165410
T. nyirit **sp. nov.**	Tenderwa, Cherangani Hills	TEND5	**	JN161126	JN165411
T. schubotzi	Sirimon route, Mt. Kenya	JAS083	L2971/2	JN161127	JN165412
T. sternfeldi	Mt. Meru, Tanzania	3ST	**	JN161128	JN165413
T. sternfeldi	Mt. Meru, Tanzania	4ST	**	JN161129	JN165414

Discussion

The number of described chameleon taxa in East Africa continues to rise steadily (Mariaux & Tilbry 2006, Mariaux *et al.* 2008; Menegon *et al.* 2009; Necas 2009; Necas *et al.* 2009; Krause & Böhme 2010; Lutzmann *et al.*

2010) and no doubt further discoveries will be made, particularly on massifs that have been poorly surveyed by biologists. However, the presence of an unrecorded species in the Cherangani Hills is somewhat suprising given that it is not a particularly inaccessible or remote massif. It seems probable that *T. nyirit* **sp. nov.** was mis-identified as *T. hoehnelii*, which is morphologically similar and has been reported from the Cherangani Hills (Spawls & Rotich 1997, Spawls *et al*. 2002). There are numerous museum specimens of taxonomic uncertainty reported from the East African highlands and also intra-specific geographic variation that may represent further cryptic species diversity (Rand 1963, Eason *et al.* 1988, Tilbury 2010). This study also highlights the importance of the combined use of morphological and molecular data in assessing cryptic species diversity within the Chameleonidae (Tolley *et al.* 2004, Tilbury & Mariaux 2006, Mariaux *et al.* 2008).

An unexpected result of the molecular analysis is the sister relationship between *T. nyirit* **sp. nov.** and the morphologically dissimilar *T. schubotzi* and not the morphologically more similar *T. hoehnelii*. Incongruence between morphological and molecular characters (i.e. when morphologically similar species are not necessarily phylogenetically close relatives) may be the result of substantial adaptive divergence between closely related species that have been exposed to divergent natural selection (Schluter 2000). When these episodes of divergent selection are replicated across multiple pairs of sister species, it is expected the evolution of multiple convergences among non-phylogenetically related species that resemble each other, as traditionally observed in *Anolis* lizards (Losos 2009), and even in chameleons (Bickel & Losos 2002). Both *T. nyirit* **sp. nov.** and *T. hoehnelii* occupy similar ecological niches in afromontane forest and ericaceous vegetation in the lower afroalpine zone, which in turn differs from *T. schubotzi*, an endemic of the afroalpine zone. Similarities in body size between *T. nyirit* **sp. nov.** and *T. hoehnelii* may be explained as the result of convergent natural selection arising from similar environmental factors such as food availability and thermal constraints (Sears & Angilletta 2004). On the other hand, phenotypic convergences in traits potentially subjected to sexual selection may occur when sexual selection dynamics are mediated by similar ecological conditions experienced by unrelated species (Losos *et al.* 2003). This scenario appears to be plausible in sexually dimorphic traits such as the rostral horn and raised casque, which is more likely to have been driven by sexual selection (Stuart-Fox & Ord 2004) The functional role of horns and casques in chameleons has only been investigated in a few species but evidence suggests that these traits are used in social signaling (Karsten *et al*. 2009, Stuart-Fox & Moussalli 2007, Stuart-Fox & Moussalli 2008) although horns have also been observed to be employed during male-male contests (Spawls *et al.* 2002, Tilbury 2010). Environmental conditions are thought to influence female mate-choice and, therefore, maximising signaling efficacy may drive the evolution of male morphological traits (Schluter & Price 1993, Stuart-Fox & Ord 2004, Stuart-Fox & Moussalli 2007, Measey *et al.* 2009, Hopkins & Tolley 2011) resulting in the convergent evolution in chameleon species employing similar signaling strategies and exploiting similar habitats. Species subjected to this form of convergent signal evolution do not need to be closely related, but simply ecologically equivalent.

An alternative view is provided by the Reproductive Character Displacement Hypothesis, which predicts that divergence between sympatric species results from reciprocal divergence in sexually selected traits as an adaptive strategy to minimize heterospecific crossings, which in turn reinforces reproductive isolation (Dobzansky 1940, Mayr 1942, Coyne & Orr 2004). Reproductive character displacement at contact zones between closely related parapatric species have been cited as evidence in support of this hypothesis (Saetre *et al.* 1997, Martin *et al*. 2009). The functional role of head shape in chameleons in inter-species recognition has rarely been investigated experimentally (Parcher 1974) and discussed only briefly (Rand 1961, Rand 1963, Wild 1993) despite the amazing array of head ornamentation seen in the Chamaeleonidae. The differences in horn orientation and casque curvature between *T. nyrit* **sp. nov.** and *T. hoehnelii* may be the result of character displacement due to their general similarity in head morphology. Specimens of *T. hoehnelii* from the Elgeyo Escarpment, geographically proximate to the Cherangani Hills, have relatively larger rostral horns and steeper casques than those from other areas of its distribution and may represent further evidence of character displacement, although collecting locality records suggests that *T. hoehnelii* and *T. nyirit* **sp. nov.** are currently allopatric.

The two sister taxa, *T. nyirit* **sp. nov.** and *T. schubotzi* have a disjunct distribution. This pattern is consistent with the hypothesis that historical climatic fluctuation during the Pleio-pleistocene and Miocene caused range expansion and fragmentation in several montane chameleon lineages and generated the relatively high species diversity and endemism seen in the region (Matthee *et al.* 2004, Mariaux & Tilbury 2006, Mariaux *et al.* 2008). Several other mountain ranges occur between Mt. Kenya and the Cherangani Hills and we might expect to see other lineages closely related to these two taxa on some of these mountain ranges, although currently none are

known. Although no published data exists on the phylogenetic position of *T. ntunte*, this taxon is morphologically similar to *T. schubotzi* and may be indicative of a historically widespread lineage that now survives as relict, geographically isolated populations in montane refugia across the East African highlands.

Trioceros nyirit **sp. nov.** appears to have a relatively limited distribution, restricted to cooler, high-rainfall mountain regions like the majority of chameleon species in East Africa (Tilbury 2010). Highland regions also support dense human populations because of the suitable climate and soils for agriculture and the demand for land is intense. Most natural habitats have been cleared at lower elevations and the remaining habitats require strict protection (Akotsi & Gachanja 2004). While researchers in West Africa (Wild 1994; Akani *et al*. 2001; Gonwouo *et al*. 2006) and Madagascar (Jenkins *et al.* 2003; Raselimanana & Rakotomalala 2004) have documented the effects of anthropogenic change on chameleon faunas, the impacts of people on the distribution and abundance on chameleons in East Africa are almost entirely anecdotal (Spawls *et al*. 2002; Tilbury 2010) and there have been no detailed ecological studies. Therefore it is difficult to assess the potential impacts of human activities and current conservation status of *T. nyirit* **sp. nov.**. Studies on chameleons outside East Africa have shown that while some chameleon species are negatively affected by habitat loss, degradation or disturbance, others appear to benefit and may become more abundant (Akani *et al*. 2001). There is a lack of general information on the biology of many chameleon species in East Africa (Spawls *et al*. 2002; Tilbury 2010) and baseline data on their distribution and ecology are needed to assess the direct effects of habitat loss and degradation as well as indirect effects of local climatic change, particularly associated with deforestation (Akotsi & Gachanja 2004). The discovery of a new vertebrate in the East African highlands highlights their biological uniqueness and the need for continued research to document its biological diversity.

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APPENDIX I. Material examined.

Trioceros bitaeniatus: KENYA: Entasekera, Nguruman Escarpment (2000m) (NMK-L3030/1-2); Maralal, Samburu District (1900m) (NMK-L3065/1-3); Elgeyo forest station, Marakwet District (2400m) (NMK-L3041/1-3); Magadi Road, Kajiado District (NMK-L1953/1-4). TANZANIA: Mwanza, Tanzania (NMK-L2154/1-2).

Trioceros conirostratus: SUDAN: Lomoriti, southwest Imatong Mountains, Sudan (3500ft) (NMK-L1949).

Trioceros ellioti: KENYA: Nandi forest, Nandi District (NMK-L2653/1-2); Kabarua forest station, Mt. Elgon District (NMK2488/1-4); Chemisia, North Nandi forest (NMK-L1271/ 1-20); Nyangores forest station, Bomet District (2280m) (NMK-L2995/1-2); Kericho forest station, Kericho District (NMK-L2989/1-2). UGANDA: Ibanda, Ruwenzori Mountains (4500ft) (NMK-L1078-80); Mabai forest (NMK302/1-4).

Trioceros hanangensis: TANZANIA: Mt. Hanang (ZFMK 82368-9).

Trioceros hoehnelii: KENYA: Kaptagat forest, Kericho District (NMK-L2993/1-4); Nyangores forest station (2280m), Bomet District (NMK-L2994/1-2); Nyaru, Elgeyo Escarpment, Koibatek District (NMK-L3022/1-2; Nabkoi forest station, Uasin Gishu District (NMK-L3042/1-8); Sururu forest station, Nakuru District (NMK-L3044/1-8); Ndaragwa forest station, Nyandarua District (NMK-L3045/ 1-8); Eldoret, Uasin Gishu District (NMK-L3047/ 1-5); Ngare Ndare forest, Mt. Kenya (NMK-L3066/1-4); Lokirikiti farms, Mau Hills, Narok District (NMK-L3140/1-3); Limuru, Kiambu District (NMK-L687-9); Kiandogoro Moorland, Aberdare Mountains (NMK-L761-769); Elgeyo forest, Eldoret district (NMK-L749); Mt. Elgon (3500m), Trans-Nzoia District (NMK-L2987/1-9); Kiptuget forest station, Mt. Londiani, Kericho District (NMK-L2984/1-2); Nyahururu, Nyandarua District (NMK-L2962/1-4); Sirimon route, Mt. Kenya, Meru Central District (NMK-L2635/1-2); Naro Moru Met. Station (3000m), Mt. Kenya (NMK2949/1-2);

Trioceros kinetensis: SUDAN: Talanga forest (ZFMK 29712); Imatong Mountains (ZFMK 25670-1, ZFMK 34531).

Trioceros narraioca. KENYA: Mt. Kulal, Marsabit District (NMK-L2521/1-8, ZFMK 73956-62)

Trioceros ntunte. KENYA: Mt. Nyiru (ZFMK 74221, ZFMK 82148)

Trioceros nyirit **sp. nov.**: see description above.

Trioceros rudis. UGANDA: Ruwenzori trail above Ibanda, Uganda (NMK-L1983/1-4); Gorilla reserve, Rwanda (8-10,000ft) (NMK-L1151/1-2); Nyakalengijo, Ruwenzori (ZFMK 63219-23).

Trioceros schubotzi. KENYA: Sirimon, nwest slopes of Mt. Kenya (11.000ft) (NMK-L1599/1-5); Sirimon, Mt. Kenya, Nyeri District (NMK-L2971/1-2); Mt. Kenya moorlands, Meru Central District (3588m) (NMK-L2325); Mt. Kenya camping grounds (11,000ft) (NMK1954/1-5); Old Moses campsite, Meru Central District (NMK-L2637/1-6).

Trioceros sternfeldi. TANZANIA: Mt. Meru crater, Arusha (NMK1300-2); Mt. Meru (ZFMK 82250); Mt. Kilimanjaro (ZFMK 70527-8)

APPENDIX II. Collecting localities.

The specimens collected for this study come from the following localities [locality, mountain, coordinates, altitude]:

Nyahururu Forest Station, Aberdares, 36°22'02"E, 00°02'42"N, 2350m; Sirimon route, Mt. Kenya National Park 37°17'06"E, 00°02'23"S, 3000m; Nyangores Forest Station, Mau Escarpment 35°25'38"E, 00°43'19"S, 2210m; Sururu Forest Station, Mau Escarpment 36°02'13"E, 00°32'53"S, 2470m; Kiptuget Forest Station, Mt. Londiani 35°42'05"E, 00°06'06"S, 2650m; Eldoret 35°16'04"E, 00°30'52"N, 2100m; Roadhead, Mt. Elgon National Park 34°37'44"E, 01°05'25"N, 3500m; Cherangani Forest Station, Cherangnai Hills 35°19'31"E, 01°02'10"N, 2300m; Kabarua Forest Station, Mt. Elgon 34°40'53"E, 00°52"59"N, 2200m; Mt. Meru, Tanzania 36°45'05"E, 03°13'03"S, 2300m; Kaptalamwa/ Kapiego, Cherangani Hills 35°24'57"E, 01°05'21"N, 2900-3000m; Kaptagat forest, Elgeyo Escarpment 35°28'07"E, 00°25'48"N, 2400m; Tenderwa, Cheranagani Hills 35°24'58"E, 01°22'56"N, 3150m; Gatau Pass, Mtelo massif 35°22'57"E, 01°37'28"N, 2176-3121m; Elgeyo Forest Station, Elgeyo Escarpment 35°31'34"E, 00°46'10"N, 2400m; Tenges Forest Station, Tugen Hills 35°48'12"E, 00°18'44"N, 1950m; Sondang, Cherangani Hills 35°23'60"E, 01° 23'60"N, 3000m; Nairobi 36°48'15"E, 01°16'14"S, 1850m.

Additional comparative material is listed in Appendix I.