



## A re-appraisal of the systematics of the African genus *Chamaeleo* (Reptilia: Chamaeleonidae)

COLIN R. TILBURY<sup>1</sup> & KRISTAL A. TOLLEY<sup>2,3</sup>

<sup>1</sup>P.O.Box 347, Nottingham Road, KZN 3280 South Africa & Evolutionary Genomics Group, Dept of Botany and Zoology, University of Stellenbosch, Private Bag XI, Stellenbosch, South Africa. E-mail: sacoltil@mweb.co.za

<sup>2</sup>Applied Biodiversity Research, South African National Biodiversity Institute, Private Bag X7, Claremont, 7735, Cape Town, South Africa. E-mail: toley@sanbi.org

<sup>3</sup>Corresponding Author

### Abstract

The genus *Chamaeleo*, currently subdivided into two sub-genera, *Chamaeleo (Chamaeleo)* and *Chamaeleo (Trioceros)* (Klaver & Böhme 1986), is reviewed from both a morphological and genetic basis and it is concluded that the two sub-genera are sufficiently distinct as to warrant their formal elevation to separate and distinct genera. Evaluation of the soft anatomy and several other characters provide sufficient basis for making this distinction. The proposed change is supported by the demonstration of monophyletic groupings (based on two mitochondrial and one nuclear gene) consistent with distinct genera.

**Key words:** *Chamaeleo*, *Trioceros*, Taxonomic review, Taxonomy, Reptilia

### Introduction

Klaver and Böhme (1986) in their landmark study on the comparative anatomy of the Chamaeleonidae, were guided by a detailed analysis of both hemipenial and lung morphology, supplemented by data where available on karyology and cranial structure. They elected to divide the family into six genera, one of which was further sub-divided into two sub-genera viz: *Chamaeleo (Chamaeleo)* and *Chamaeleo (Trioceros)*.

In the intervening years since then, apart from descriptions of the hemipenes of several new species of chameleons, no further work has been done on soft anatomy. The advent of phylogenetics as an adjunct to comparative anatomy has led to an increase in the appreciation of the complexity of evolutionary relationships and in turn has led to several recent taxonomic rearrangements of the African chameleons at the tertiary level (Matthee et al 2004, Tilbury et al 2006). Whilst the taxonomic landscape of the African pygmy chameleons and the enigmatic “*Bradypodion* group” (sensu lato) have been resolved, the genus *Chamaeleo* bears a re-look from a phylogenetic perspective due to the heterogeneous nature of its component sub-genera.

Recent work on the phylogenetics of the Chamaeleonidae cast doubt on the relationship between these two sub-genera at the genus level (Townsend and Larson 2002). In the current paper, the comparative anatomy of the genus *Chamaeleo* is reviewed and additional genetic evidence is presented that allow us to propose that there is no relationship between the two sub-genera of *Chamaeleo* beyond that at a level higher than currently thought.

**Hemipenial Morphology.** The importance of hemipenial morphology as a tool in primary and higher level systematics was demonstrated by Böhme (1988), Böhme & Klaver (1980) and Klaver & Böhme (1986). Several derived hemipenial morphologies have been described, but the plesiomorphic condition seen in members of five of the nine currently described genera consists of a basal pedicel, followed by a truncal stalk with or without truncal calyces. The stalk is topped by an apical section, adorned with a quadruple

arrangement consisting of two pairs of denticulated disc-like, semi-circular or even sickle shaped structures named rotulae. One pair of rotulae is situated on either side of the distal end of the spermatic sulcus and one pair even further distal to this on the asulcal aspect of the apex. This basic plesiomorphic hemipenial structure is seen in two of the three species of the Genus *Rieppeleon*, most species of *Bradypodion*, *Calumma*, *Kinyongia*, *Chamaeleo (Trioceros)* and in two of the thirteen species of *Chamaeleo (Chamaeleo)*. In twelve of the fourteen species of the sub-genus *Chamaeleo (Chamaeleo)*, the plesiomorphic quadruple apical structures are instead replaced by a more complex derived state where up to five pairs of rotulae may be seen on the apex. This multi-rotulae condition was considered by Klaver & Böhme (1986) to be a synapomorphy for the sub-genus *Chamaeleo (Chamaeleo)*.

**Karyotype.** The discontinuous karyotype  $2n=36=12M+24m$  is widely distributed over several families of lizards including the Agamidae, Iguanidae, Amphisbaenids, Gerrhosauridea and the Chamaeleonidae, (Matthey & van Brink 1960, Gorman 1973). It is felt that this karyotype reflects the primitive symplesiomorphic condition (Gorman 1973, Bourgat 1973, Klaver & Böhme 1986). Interestingly, chameleons with this karyotype are found both in Africa and Madagascar and include members of the genera *Chamaeleo (Trioceros)*, *Kinyongia*, *Brookesia* and *Calumma*. Chromosomal analysis (on less than one third of the known species) has shown wide variation in the number of chromosomes ranging from  $2n = 36$  to  $2n = 20$  (Matthey & van Brink 1956, Matthey 1957, Klaver & Böhme 1986). At least fifteen different chromosomal configurations of macro and micro chromosomes have so far been identified – spread broadly across the family (Klaver & Böhme op cit.). The phylogenetic significance of this variation is not easily apparent. However within the six African species of the sub-genus *Chamaeleo (Chamaeleo)* so far characterised, they all group together with the unique derived karyotype  $2n=24=12M+12m$  – also regarded as a synapomorphy for this sub-genus (Klaver & Böhme 1986).

**Skull morphology.** In all the species where the cranial anatomy has been investigated, species of both *Chamaeleo (Chamaeleo)* and *Chamaeleo (Trioceros)*, *Kinyongia*, *Furcifer* and *Calumma* share the plesiomorph condition where the posterior skull table is reduced with the parietal bone present as a smallish roughly triangular bone with a narrow sagittal spur (straight or convexly curved) extending posteriorly. The ascending processes of the two squamosals rise to make contact with the posterior-most tip of the parietal spur or with each other, to completely enclose the upper temporal fossa (Rieppel 1981, 1987, Rieppel & Crumley 1997). As such the anatomy of the cranial parietal complex is not phylogenetically informative for this group of chameleons.

**Lung morphology.** Within the family Chamaeleonidae, two basic lung morphologies have been identified, depending on either the absence or presence of septae that project into the luminal space of the lung. Of those chameleons with septae, the number, form, origin, attachments and development of these, further sub-divide chameleons into five sub-groups viz: B, C, D, E and F (Klaver 1973, 1977, 1979, 1981). The four identified lung types with large internally ending septae (Types C, D, E and F) were presumed to have evolved from lungs with small partial septae that did not penetrate deeply into the lumen of the lung (Type B – Klaver 1981). Klaver & Böhme (1986) grouped all species which had been demonstrated to have lung types with large internal septae that ran lengthwise through the lumen of the lung (viz: Types CDEF – which they regarded as a relative synapomorphy) into the two sub-genera *Chamaeleo (Chamaeleo)* and *Chamaeleo (Trioceros)*. The principal feature differentiating these two sub-genera focuses on the origin and attachments of the lung septae. In *Chamaeleo (Chamaeleo)* - Lung type C, two septae arise from the hilum of the lung and extend posteriorly for variable distances dividing at least the anterior part of the lung into three incomplete “chambers” viz: dorsal, medial and ventral (Klaver 1973, 1977, 1981). In *Chamaeleo (Trioceros)* – Lung types D,E, & F, the septae (between one and three in number), are connected to the ventral, medial and lateral walls and end freely within the lung dividing it into two to four chambers viz: anterior, medial and posterior. Above the terminal ends of the septae, the various chambers communicate via a dorsal space (Klaver 1973, 1977, 1981).

It is not possible to speculate here as to the mechanisms of the evolution of these septae, but the notion that the character “large lung septae” unites all chameleons with this character as a shared derived condition is probably an oversimplification that refutes the possibility that the different forms of lung septation (C vs DEF) may have evolved separately and *de novo*. Indeed if these two sub-groups were related at the generic level, it

would also be likely that they might share other apomorph characters that unite them within a genus. There are apparently none. Rather, the two groups form well-defined and separate clusters of species that should intuitively be recognized as separate genera as had originally been considered by Charles Klaver (1981).

In order to take this notion to the next level, it was decided to proceed with a phylogenetic analysis of the two groups using two mitochondrial and one nuclear marker to determine to what degree genetic divergence has evolved and if whether or not this was within the accepted definition for differentiation at the genus level.

## Materials and methods

### Molecular Analysis

To determine the taxonomic placement of *Chamaeleo* (*Trioceros*), a phylogenetic analysis of 78 chameleons plus two outgroup taxa (Table 1) was run. Four species of *Chamaeleo* (*Chamaeleo*) and sixteen species of *Chamaeleo* (*Trioceros*) were included, covering approximately half of the species described in the sub-genus. Other genera have previously been shown to be monophyletic (Tilbury et al. 2006, Tolley et al. 2004, Townsend and Larson 2002), so the analysis included only a few representatives of each genus (Table 1). Sequences from 39 of these individuals have been published previously (Table 1). DNA extraction, PCR amplification, and cycle sequencing of two mitochondrial gene fragments were carried out following standard procedures formerly outlined in Tolley *et al.* (2004) using the following primers for ND2: L4437b (Macey *et al.* 1997a) and H5934 (Macey *et al.* 1997b), and 16S: L2510 and H3080 (Palumbi 1996). An 821 bp portion of the nuclear gene RAG1 was amplified and sequenced using primers F118 and R1067 (Matthee *et al.* 2004). Standard PCR and sequencing were followed for this gene fragment, with PCR annealing temperature at 57°C. All new sequences have been deposited in GenBank (**Table 1**). Matching voucher specimens are as listed in Table 1.

A Bayesian analysis for a total of 2167 characters from the three markers was run. Bayesian inference was used to investigate optimal tree space using MrBayes 3.1.0 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). MrBayes was run specifying six rate categories with uniform priors for all parameters. This decision was based on a preliminary examination of the dataset using Modeltest 3.6 (Posada & Crandall 1998), whereby both the AIC and LRT test specified the most complex model (GTR+I+G) for the combined dataset. Therefore, the model used included a single data partition for 16S (although 44 bases were removed due to poor alignment) with independent partitions for each codon of the two coding genes (ND2 and RAG1). To confirm that this model was not over-parameterised, an additional MCMC was run with only 3 partitions (one for each gene). To ensure the results converged on the same topology, each MCMC was run twice in parallel for 10 million generations each, with trees sampled every 1000 generations. For all the runs, the first 5 million generations (5000 trees) were removed as burn-in, after examination of the average standard deviation of split frequencies ( $\leq 0.005$ ), the convergence diagnostic (PSRF values  $\sim 1.0$ ) as well as the log-probabilities and the values of each parameter for stabilisation. The remaining 5000 trees were used to construct a 50% majority rule tree and nodes with  $\geq 0.95$  posterior probability were considered supported.

A parsimony analysis was also run in PAUP\*4.0b10 (Swofford 2002) using the same data set as in the Bayesian analysis, although the analysis was limited to only individuals which had sequences for all three genes (16 individuals excluded). A heuristic search was run with 1000 random replicates and 100 trees saved each replicate. One thousand bootstrap replicates were run to evaluate confidence in the nodes (50 random addition replicates, saving 50 trees per replicate). In addition, two competing phylogenetic hypotheses were tested by comparing the tree length of the phylogeny obtained in this study with the enforced monophyly of the *Trioceros*+*Chamaeleo*, using MacClade (Maddison & Maddison 2000). The two trees were compared using the Shimodaira–Hasegawa test (S–H test) for maximum likelihood (1000 replicates) in PAUP\*4.0b10 (Swofford 2002).

**TABLE 1.** Sample numbers, collecting localities, voucher accession numbers (PEM=Port Elizabeth Museum, CAS = California Academy of Sciences, MTSN = Museo Tridentino di Scienze Naturali, ZMB=National Museum Zimbabwe, ZMFK= Forschungsmuseum Koenig), and GenBank accession numbers (ND2, 16S, RAG1) for chameleons used in this study. (T) = Topotype, (P) = Paratype. N/A = data, specimen, or information not available. PEM N/A = specimens deposited, but accession numbers not yet allocated.

Genus	species	sample ID	Location	Voucher	16S	ND2	RAG1
<i>Brachypodion</i>	<i>damaranum</i>	KTH145	South Africa, Western Cape	N/A	AY756653	AY756703	DQ996646
<i>Brachypodion</i>	<i>melanocephalum</i>	CT016	South Africa, KwaZulu-Natal	PEM R5693	AY289813	AY289869	DQ996647
<i>Brachypodion</i>	<i>pumilum</i>	KT062	South Africa, Western Cape	N/A	AY756639	AY756689	DQ996648
<i>Brachypodion</i>	<i>ventrale</i>	KTH153	South Africa, Eastern Cape	N/A	AY756654	AY756704	DQ996649
<i>Brookesia</i>	<i>brygooi</i>	N/A	N/A	N/A	AF121953	AF448774	N/A
<i>Brookesia</i>	<i>peyrierasi</i>	N/A	N/A	N/A	AF121954	AF448777	N/A
<i>Brookesia</i>	<i>thieli</i>	N/A	Madagascar	N/A	N/A	AF448780	AY662577
<i>Chamaeleo</i>	<i>dilepis</i>	PEM DNA224	Kenya, Muamba	N/A	DQ923815	EF014299	DQ996654
<i>Chamaeleo</i>	<i>dilepis roperi</i>	CT112	Tanzania, Mt. Meru	PEM R16579	FJ17750*	N/A	N/A
<i>Chamaeleo</i>	<i>gracilis</i>	CT088	Guinea, Mamou	PEM N/A	FJ17748*	FJ17798*	FJ746587*
<i>Chamaeleo</i>	<i>gracilis</i>	PEM R2304	Ivory Coast, Haute Dodo Forest Reserve	PEM R2304	DQ923819	EF014303	DQ996658
<i>Chamaeleo</i>	<i>dilepis quilensis</i>	CT024	South Africa, Mpumalanga	PEM R5890	DQ923817	EF014301	DQ996656
<i>Chamaeleo</i>	<i>dilepis quilensis</i>	CT025	Botswana, Kanye	PEM R5891	DQ923818	EF014302	DQ996657
<i>Chamaeleo</i>	<i>dilepis quilensis</i>	CT058	Botswana, Gabarone	PEM N/A	FJ17749*	FJ17795*	FJ746588*
<i>Chamaeleo</i>	<i>senegalensis</i>	CT086	Guinea, Kan Kan	PEM R16626	FJ17751*	FJ17796*	FJ746589*
<i>Chamaeleo</i>	<i>senegalensis</i>	CT087	Guinea, Kan Kan	PEM R16627	FJ17752*	FJ17797*	FJ746590*
<i>Furcifer</i>	<i>antimena</i>	M38	Madagascar, Tulear District	N/A	FJ17753*	N/A	FJ746591*
<i>Furcifer</i>	<i>antimena</i>	M39	Madagascar, Tulear District	N/A	FJ17754*	FJ17814*	FJ746592*
<i>Furcifer</i>	<i>labordi</i>	N/A	Madagascar	N/A	AF215264	AY448767	N/A
<i>Furcifer</i>	<i>verrucosus</i>	M01	Madagascar, Tulear District	N/A	FJ17755*	FJ17813*	FJ746593*
<i>Kinyongia</i>	<i>adolffriderici</i>	CAS201593	Uganda, Bwindi Impenetrable National Park	CAS201593	DQ923820	EF014304	DQ996659
<i>Kinyongia</i>	<i>carpenteri</i>	CT345	Uganda, Rwenzori Mountains. (T)	PEM R16572	DQ923821	EF014305	DQ996660
<i>Kinyongia</i>	<i>excubitor</i>	CT209	Kenya, Mount Kenya, Meru Forest. (T)	PEM R16571	DQ923823	EF014307	DQ996661
<i>Kinyongia</i>	<i>multituberculata</i>	CT201	Tanzania, West Usambara Mountains	PEM R16559	DQ923827	EF014311	DQ996664
<i>Kinyongia</i>	<i>oxyrhina</i>	CT192	Tanzania, Uluguru Mountains	PEM R16569	DQ923831	EF014315	DQ996669
<i>Kinyongia</i>	<i>tavetana</i>	CT113	Tanzania, Mount Kilimanjaro, Marangu. (T)	PEM R5736	DQ991233	FJ17801*	DQ996671
<i>Kinyongia</i>	<i>tavetana</i>	CT207	Tanzania, Mount Meru, Irikon Camp	PEM R16563	DQ923833	EF014317	DQ996672
<i>Kinyongia</i>	<i>tenuis</i>	CAS 168917	Tanzania, East Usambara, Tanga Region. (T)	CAS 168917	EF014318	DQ923834	N/A
<i>Kinyongia</i>	<i>tenuis</i>	CT103	Tanzania, East Usambara (T)	PEM R5731	DQ923835	EF014319	DQ996673

TABLE 1 (continued)

Genus	species	sample ID	Location	Voucher	16S	ND2	RAG1
<i>Kinyongia</i>	<i>xenorhina</i>	CT350	Uganda, E. Rwenzori Mountains (T)	PEM R16570	DQ923838	EF014322	DQ996676
<i>Nadzikambia</i>	<i>mlanjensis</i>	CT055	Malawi, Mt. Mulanje (T)	PEM R5746	AY289860	AY289918	DQ996681
<i>Nadzikambia</i>	<i>mlanjensis</i>	PEM R16294	Malawi, Mt. Mulanje	PEMR 16294	DQ923841	EF014325	DQ996679
<i>Nadzikambia</i>	<i>mlanjensis</i>	PEM R16315	Malawi, Mt. Mulanje	PEM R16315	DQ923842	EF014326	DQ996680
<i>Rhampholeon</i>	<i>boulengeri</i>	Rboulen24	Uganda, Bwindi Forest	CAS 201682	AY524877	AY524915	N/A
<i>Rhampholeon</i>	<i>marshalli</i>	Rmarshalli15	Zimbabwe, Vumba Mountains	PEMR 16244	AY524871	AY524909	AY524947
<i>Rhampholeon</i>	<i>moyeri</i>	Rmoyeri29	Tanzania, Udzungwa Mountains	MTSN001TA	AY524876	AY524914	AY524952
<i>Rhampholeon</i>	<i>platyceps</i>	Rplatyceps11	Malawi, Mt. Mlanje	PEMR 16251	AY524879	AY524917	AY524954
<i>Rhampholeon</i>	<i>spectrum</i>	Rspectrum20	Equatorial Guinea, Bioko Island	CAS 207683	AY524863	AY524900	AY524938
<i>Rhampholeon</i>	<i>temporalis</i>	Rtemporalis3	Tanzania, East Usambara Mountains	PEMR 16255	AY524867	AY524905	AY524943
<i>Rhampholeon</i>	<i>uluguruensis</i>	Ruluguruensis31	Tanzania, Uluguru Mountains	ZMB 48421	AY524896	AY524934	N/A
<i>Rieppeleon</i>	<i>brachyurus</i>	Rbrachyurus5	Tanzania, Tamota	PEMR 16264	AY524899	AY524937	N/A
<i>Rieppeleon</i>	<i>brevicaudatus</i>	Rbrevicaudatus4	Tanzania, East Usambara Mountains	PEMR 16257	AY524888	AY524926	AY524963
<i>Rieppeleon</i>	<i>kerstenii</i>	Rkerstenii23	Kenya, Kilifi	CAS 169939	AY524890	AY524928	AY524965
<i>Trioceros</i>	<i>affinis</i>	CT020	Ethiopia, Goba	ZFMK 63063-65	FJ17756*	FJ17787*	FJ746594*
<i>Trioceros</i>	<i>affinis</i>	CT021	Ethiopia, Addis Ababa	ZFMK 63063-65	FJ17757*	FJ17788*	FJ746595*
<i>Trioceros</i>	<i>balebicornutus</i>	CT022	Ethiopia, Bale Mountains	ZFMK 63050-58	FJ17758*	FJ17789*	FJ746596*
<i>Trioceros</i>	<i>bitaeniatus</i>	CT337	Tanzania, Oldonyo Sambu	PEM N/A	FJ17759*	FJ17810*	FJ746597*
<i>Trioceros</i>	<i>bitaeniatus</i>	CT338	Tanzania, Oldonyo Sambu	PEM N/A	FJ17760*	N/A	N/A
<i>Trioceros</i>	<i>deremensis</i>	CT106	Tanzania, East Usambara Mountains	PEM N/A	FJ17761*	FJ17799*	FJ746598*
<i>Trioceros</i>	<i>deremensis</i>	CT107	Tanzania, East Usambara Mountains	PEM N/A	FJ17762*	FJ17800*	FJ746599*
<i>Trioceros</i>	<i>elliotti</i>	CT214	Sotik, Kenya	PEM N/A	FJ17763*	N/A	FJ746600*
<i>Trioceros</i>	<i>elliotti</i>	CT348	Masaka, Uganda	PEM N/A	FJ17764*	N/A	N/A
<i>Trioceros</i>	<i>elliotti</i>	CT354	Mt. Rwenzori, Uganda (T)	PEM N/A	FJ17765*	FJ17812*	N/A
<i>Trioceros</i>	<i>elliotti</i>	UG05-CH05	Uganda	N/A	FJ17766*	N/A	FJ746601*
<i>Trioceros</i>	<i>feae</i>	CAS 207681	Equatorial Guinea, Bioko Island	CAS 207681	FJ17767*	N/A	AF448749
<i>Trioceros</i>	<i>goetzei</i>	CT050	Malawi, Nyika Plateau	PEM N/A	FJ17768*	FJ17791*	FJ746603*
<i>Trioceros</i>	<i>goetzei</i>	CT051	Malawi, Nyika Plateau	PEM N/A	FJ17769*	FJ17792*	FJ746604*
<i>Trioceros</i>	<i>goetzei</i>	CT052	Malawi, Nyika Plateau	PEM N/A	FJ17770*	FJ17793*	FJ746605*
<i>Trioceros</i>	<i>goetzei</i>	KTH06-17	Zambia, Nyika Plateau	N/A	FJ17771*	N/A	N/A
<i>Trioceros</i>	<i>harennae</i>	CT023	Ethiopia, Bale Mountains	ZFMK 63059-62	FJ17772*	FJ17790*	FJ746606*

TABLE 1 (continued)

Genus	species	sample ID	Location	Voucher	16S	ND2	Genus
<i>Trioceros</i>	<i>hoehemelli</i>	CT210	Kenya, Mount Kenya	PEM N/A	FJ717773*	FJ717805*	FJ746607*
<i>Trioceros</i>	<i>jacksonii jacksonii</i>	CAS 199070	Kenya, Nairobi	CAS 199070	FJ717774*	AF448753	FJ746608*
<i>Trioceros</i>	<i>jacksonii xantholophus</i>	CT208	Kenya, Mount Kenya	PEM N/A	FJ717775*	FJ717804*	FJ746609*
<i>Trioceros</i>	<i>johnstoni</i>	CAS 201596	Uganda, Kabale District	CAS 201596	DQ923812	EF014298	DQ996650
<i>Trioceros</i>	<i>johnstoni</i>	CT353	Uganda, E. Rwenzori Mountains	PEM N/A	FJ717776*	FJ717811*	FJ746610*
<i>Trioceros</i>	<i>melleri</i>	CT056	Malawi, Zomba	N/A	FJ717777*	FJ717794*	FJ746611*
<i>Trioceros</i>	<i>melleri</i>	PEM R13428	Mozambique, Malema River	PEM R13428	DQ923813	N/A	DQ996651
<i>Trioceros</i>	<i>melleri</i>	PEM R12204	Malawi, Mulanje District	PEM R12204	DQ923814	N/A	DQ996652
<i>Trioceros</i>	<i>rudis</i>	CAS 201716	Uganda, Kabale District	CAS 201716	DQ923811	EF014297	DQ996653
<i>Trioceros</i>	<i>sternfeldi</i>	CT114	Tanzania, Ngorongoro crater rim	PEM N/A	FJ717778*	FJ717802*	FJ746612*
<i>Trioceros</i>	<i>sternfeldi</i>	CT115	Tanzania, Ngorongoro crater rim	PEM N/A	FJ717779*	FJ717803*	FJ746613*
<i>Trioceros</i>	<i>sternfeldi</i>	CT326	Tanzania, Mt. Meru	PEM N/A	FJ717780*	FJ717808*	FJ746614*
<i>Trioceros</i>	<i>sternfeldi</i>	CT327	Tanzania, Mt. Hanang	PEM N/A	FJ717781*	FJ717809*	FJ746615*
<i>Trioceros</i>	<i>schubotzi</i>	CT211	Kenya, Mt. Kenya	PEM N/A	FJ717782*	FJ717806*	FJ746616*
<i>Trioceros</i>	<i>schubotzi</i>	CT212	Kenya, Mt. Kenya	PEM N/A	FJ717783*	FJ717807*	FJ746617*
<i>Trioceros</i>	<i>schubotzi</i>	SCHBZ	Kenya, Mt. Kenya	N/A	FJ717784*	FJ717815*	FJ746618*
<i>Trioceros</i>	<i>tempeli</i>	CT187	Tanzania, Owembe Forest, Njombe	PEM N/A	FJ717785*	N/A	FJ746619*
<i>Trioceros</i>	<i>tempeli</i>	CT340	Tanzania, Udzungwa Mountains	PEM N/A	FJ717786*	N/A	N/A
<b>Outgroup</b>							
<i>Calotes</i>	<i>versicolor</i>	N/A	N/A	N/A	AB031981	AF128489	AY662584
<i>Leiopelis</i>	<i>belliana</i>	N/A	N/A	N/A	AF378379	LBU82689	AY662587

\*Sequenced for this study.

## Results

### Molecular analysis

The phylogenetic analysis showed that members of the sub-genus *Trioceros* form a well-supported clade (Fig. 1). The *Trioceros* clade is neither within, nor sister to, the clade representing the genus *Chamaeleo*. Bayesian posterior probabilities for supported nodes and tree topologies were nearly identical for the two models, with the 3 partition model performing marginally better. The parsimony analysis (tree not shown) produced 6 equally parsimonious trees (5176 steps, CI 0.39, RI 0.55) that differed only in terminal branch swapping. The parsimony trees had the same overall topology and supported nodes ( $\geq 70\%$  bootstrap) as the Bayesian analysis, although the Bayesian analysis performed better and showed support for several additional nodes not supported by parsimony. Monophyly of *Trioceros*+*Chamaeleo* was not supported by the S-H test ( $p < 0.01$ ), showing a significantly worse fit than the topology obtained in the present study.

### Discussion

On the basis of the combined anatomical and genetic data as presented above, it is here proposed to elevate the sub-genus *Chamaeleo* (*Trioceros*) from the genus *Chamaeleo* to effectively instate both *Chamaeleo* and *Trioceros* as full genera. A re-definition of both genera follows.

### Genus *Chamaeleo* Laurenti 1768

Type species: *Chamaeleo chamaeleon* (Linnaeus 1758)

Generic synonyms:

*Phumanola* Gray 1864. Type species *Chamaeleo namaquensis* Smith 1831

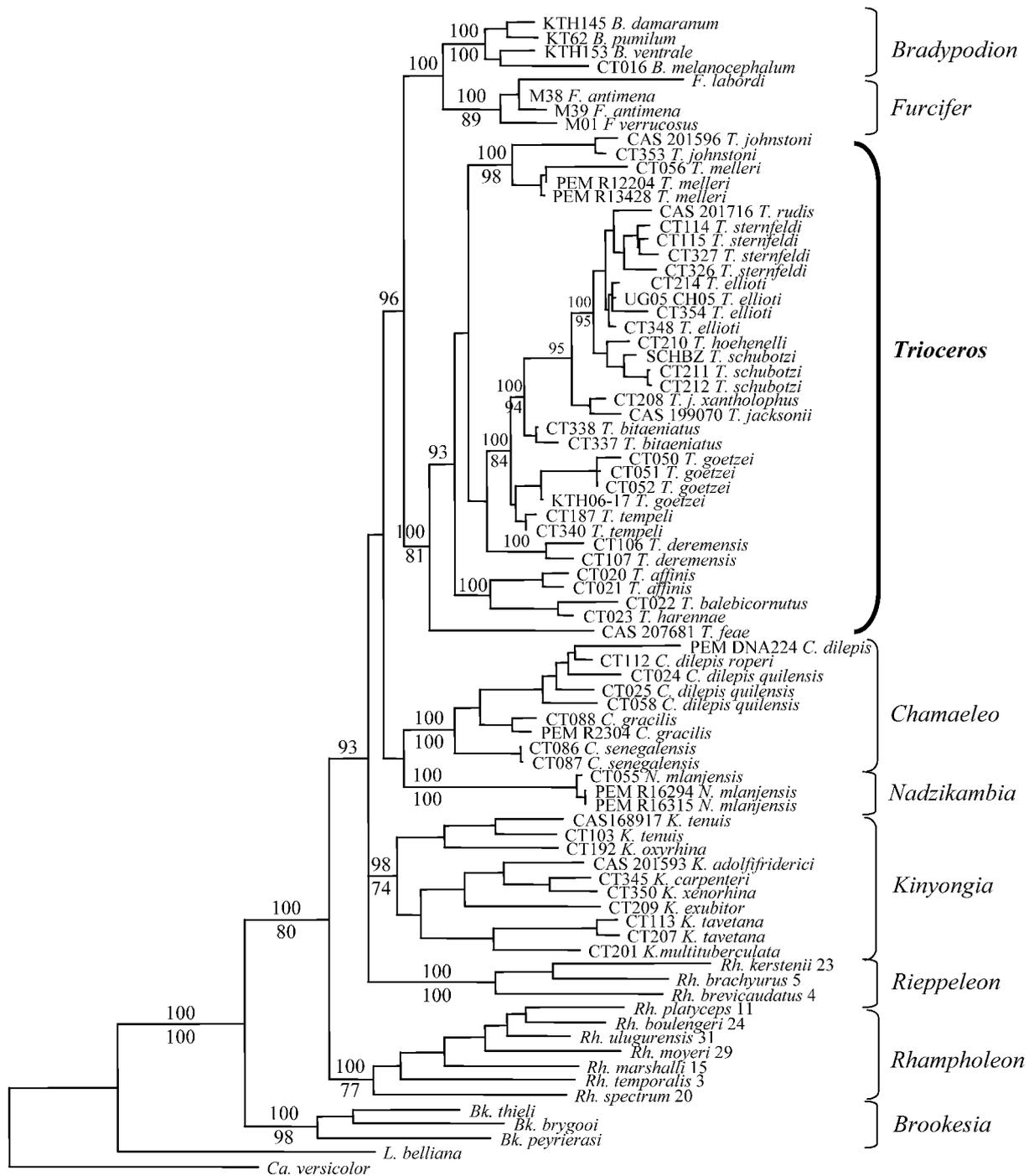
*Calyptosaura* Gray 1864. Type species *Chamaeleo calyptratus* Dumeril & Bibron 1851

*Erizia* Gray 1864. Type species *Chamaeleo senegalensis* Daudin 1802.

**Species content:** *africanus*, *anchietae*, *arabicus*, *calcaricarens*, *calyptratus*, *chamaeleon*, *dilepis*, *gracilis*, *laevigatus*, *monachus*, *namaquensis*, *necasi*, *senegalensis*, *zeylanicus*.

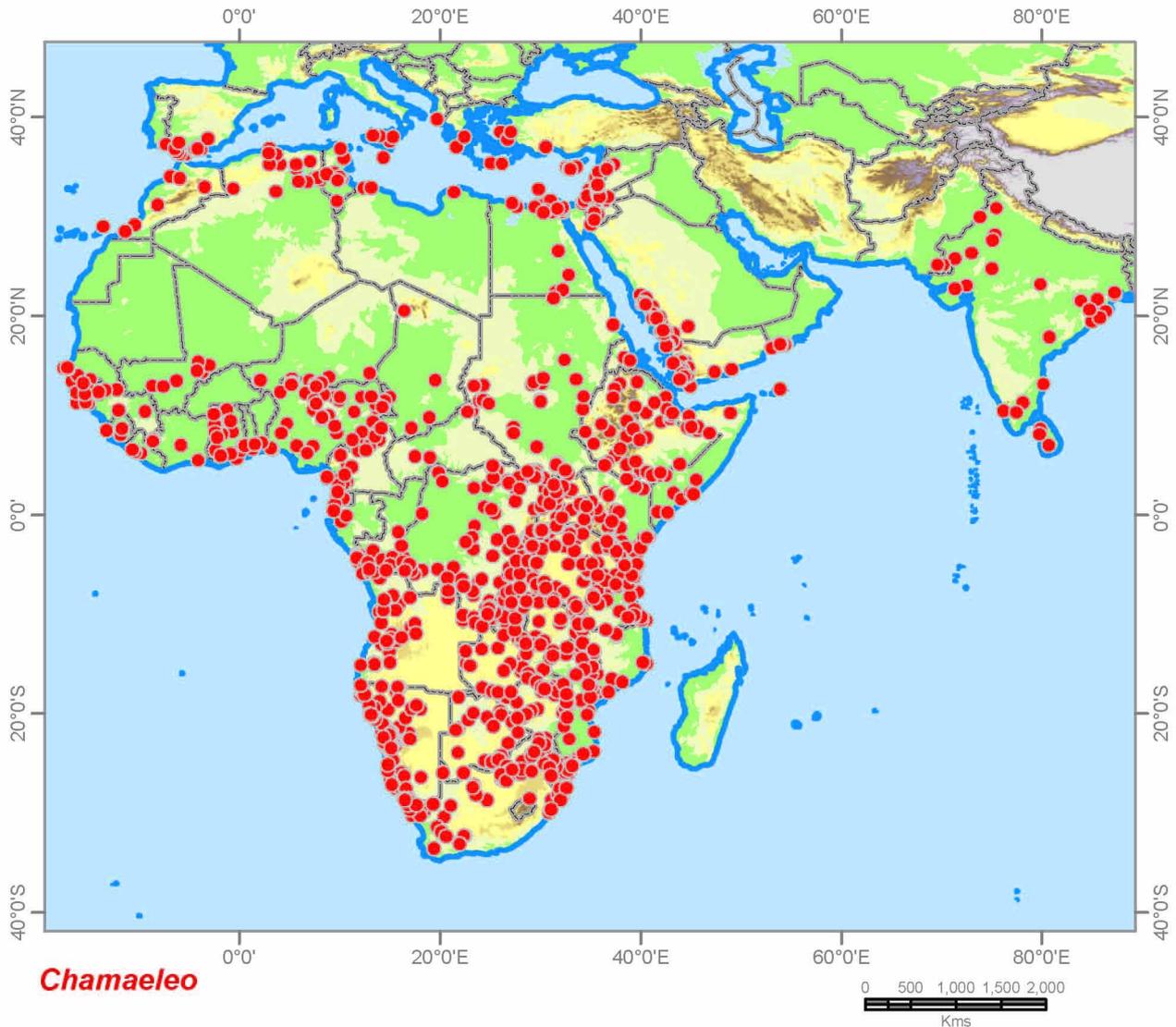
This genus has a wide ranging pan African distribution extending into Europe, the Middle East, Arabia and the Indian sub-continent (**Fig. 2**). One species is confined to the island of Socotra. The distribution areas of the mainland species tend to be large and continuous except for *Ch. anchietae* which appears to have population pockets restricted to highland plateaux. Although some species may penetrate into lowland forest, or high altitude grasslands, the species of the genus generally occupy moist and dry woodland savannahs, thorn scrub, semi desert and in one species, true desert.

Apart from occipital lobes in some species and prominent parietal crests in others, they have little other head ornamentation. None of them possess horns or any form of rostrum-nasal or pre-orbital projections. A gular-ventral crest of single cones is found in all species being more or less developed in the various forms from very prominent in *Ch. calyptratus* to almost indiscernible in *Ch. namaquensis*. None of the species of this genus demonstrate a temporal crest. The casque is edged in a lateral parietal crest originating as a posterior continuation of the supra-orbital ridge which delineates the posterior ramus of the squamosal bone. The temporal zone is undivided. The background scalation of the flanks is generally composed of relatively homogeneous to finely heterogeneous closely packed granular tubercles. The tail of all species within this group is smooth. The plantar surfaces are smooth and claws simple. This is the only genus where the presence of tarsal spurs is seen in several of the species (*Ch. arabicus*, *Ch. monachus*, *Ch. chamaeleon*, *Ch. necasi*, *Ch. zeylanicus*, *Ch. dilepis*, *Ch. gracilis*, *Ch. calyptratus*, *Ch. africanus*). These tend to be best developed in males and usually absent or much reduced in females. Tarsal spurs may be a synapomorphy for the genus *Chamaeleo*.



**FIGURE 1.** Bayesian consensus phylogram for the Chamaeleonidae (not including genus *Calumma*). Bayesian posterior probabilities are given above each node, while parsimony bootstrap values are given below each node. Although some interior nodes (within genera) were supported, only some representative values within the *Trioceros* are shown.

The basic internal lung morphology consists of two large septae arising from the region of the hilum of the lung which end freely within the lung, dividing it into three chambers viz a small dorsal, a large middle and a small ventral chamber. All species possess a gular pouch and in the lung - a membrano-fibrous diaphragm that partially separates off a small dorso-cranial compartment. Many species also have several small partial septae that arise from the dorsal wall of the lung near the cranial end. The lungs are invariably adorned with varying numbers of diverticulae that trail from the inferior and posterior margins of the lung. The diverticulae vary in length and number and may be branched (Klaver 1973, 1977, 1981).



**FIGURE 2.** Map showing the recorded distributions of the combined species within the genus *Chamaeleo* demonstrating its pan-African, European, Arabian and Asian spread.

Hemipenes are calyculate, with a multi rotulae arrangement of between three to five pairs of denticulated rotulae except for *Ch. arabicus* and *Ch. namaquensis* which have retained the plesiomorphic four rotulae (two pairs) configuration (Klaver & Böhme 1986).

The genus is oviparous with a cyclic reproductive strategy – usually a single brood but up to three clutches of eggs per year in some species in ideal conditions. These species tend to have relative longevity. Females are usually sexually mature within one year and over the next few years will produce at least one clutch of eggs annually. The parietal peritoneum is unpigmented.

The documented karyotype of the several species so far examined (Matthey 1931, 1957, Matthey & van Brink 1956) is  $2n=24=12M+12m$  and appears to be restricted to this genus as a synapomorphic character (Klaver & Böhme 1986)

### **Genus *Trioceros* Swainson 1839**

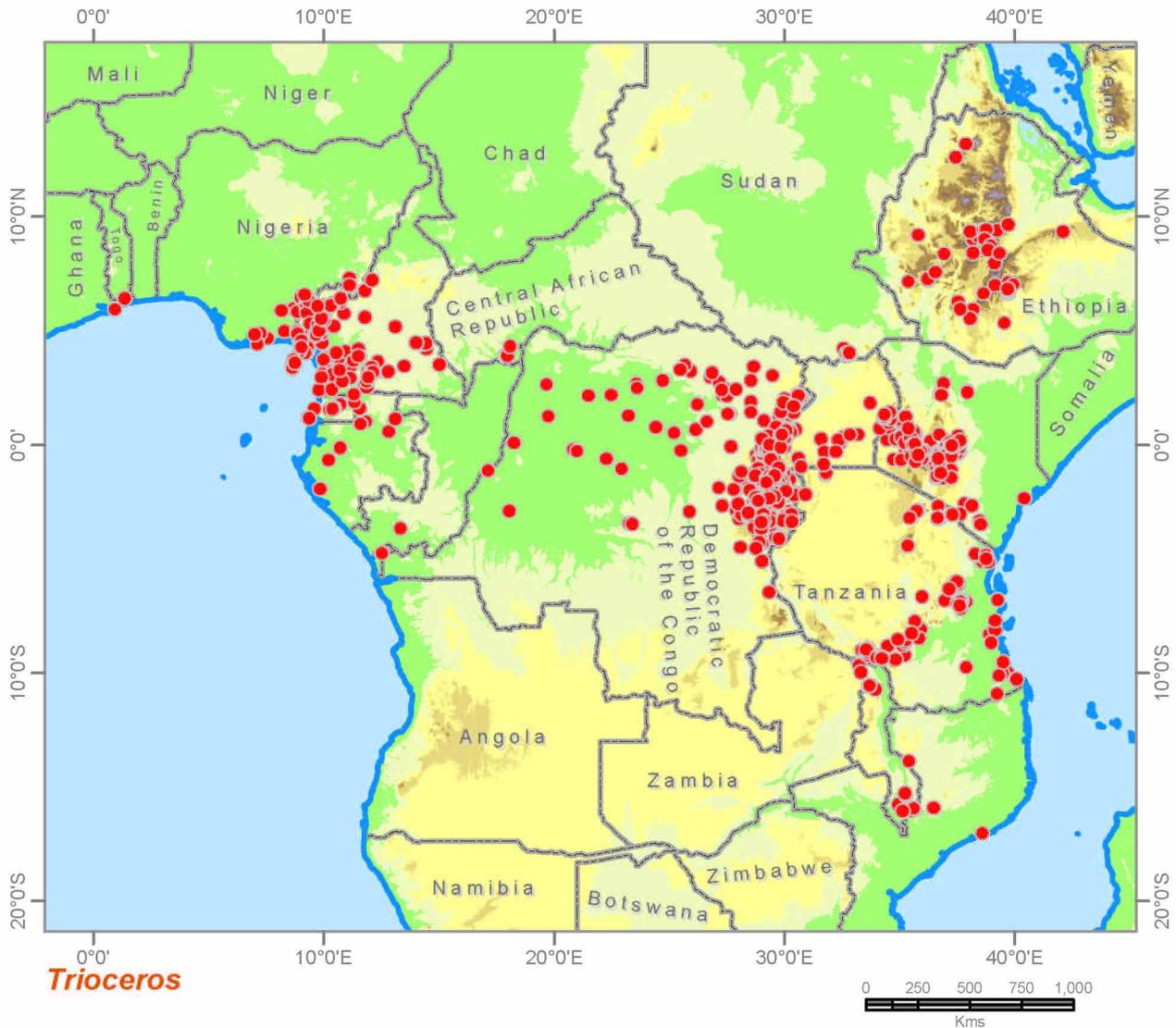
Type species: *Trioceros oweni* Gray 1831

Generic synonyms:

*Triceras* Fitzinger 1843. Type species *Chameleon oweni* Gray 1831.

*Pterosaurus* Gray 1864. Type species *Chameleo cristatus* Stutchbury 1837.  
*Ensirostris* Gray 1864. Type species *Ensirostris melleri* Gray 1865.

**Content:** *affinis*, *balebicornutus*, *bitaeniatus*, *camerunensis*, *chapini*, *conirostratus*, *cristatus*, *deremensis*, *eisentrauti*, *elliotti*, *feae*, *fuelleborni*, *goetzei*, *harennae*, *hoehnelii*, *incornutus*, *ituriensis*, *jacksonii*, *johnstoni*, *kinetensis*, *laterispinis*, *marsabitensis*, *melleri*, *montium*, *narraioaca*, *ntunte*, *oweni*, *pfefferi*, *quadricornis*, *rudis*, *schoutedeni*, *schubotzi*, *sternfeldi*, *tempeli*, *weneri*, *wiedersheimi*.



**FIGURE 3.** Map showing the recorded distributions of the combined species within the genus *Trioceros* demonstrating its tropical African spread.

The genus has a tropical pan-African distribution extending from east to west, with the most southerly species occurring in Mozambique and Malawi (*T. melleri*) and the most northerly in Ethiopia (*T. affinis*). (Fig. 3). Most of the species within *Trioceros* are confined to wet evergreen forest biotopes or their peripheries with only a few species found out of evergreen forest proper (*T. melleri*, *T. bitaeniatus*, *T. goetzei*, *T. schubotzi*, *T. rudis*).

This is the only genus where the development of cylindrical annulated bony horns is seen. This character does not occur in all species of this genus but is found in representatives across the various sub-groups within the genus. These structures may be considered to be a synapomorphy for the genus *Trioceros* (Klaver & Böhme 1986). Other head ornamentation may include such features as occipital lobes and dual gular crests

although these are not only found within this genus. The scalation is variable from sub-homogeneous and granular to markedly heterogeneous. The plantar surfaces are smooth and the claws simple.

The lungs of this genus are characterized by having between one to three large septae that are attached to and possibly arise from the ventral, medial and lateral walls of the lung (Klaver 1973, 1977, 1981). The origin and the nature of these septae is regarded as a synapomorph character for the genus (Klaver & Böhme 1986). The septae sub-divide the lung into two to four chambers. The chambers are arranged from anterior to posterior. The septae do not reach the dorsal wall allowing all chambers to communicate with each other via a common space in the dorsal zone. A membrano-fibrous diaphragm partially delimits a small dorso-cranial compartment at the anterior end of the lung. Varying numbers of small partial septae arise from the antero-dorsal and antero-ventral walls of the lung. In all species examined so far, the inferior and posterior surfaces of the lungs are festooned with diverticulae of varying length, structure and numbers.

The hemipenes are calyculate with a plesiomorphic four rotulae apical ornamentation (Klaver & Böhme 1986). Several subsequent species descriptions in the genus validate this as a general statement (Tilbury 1998, Necas et al 2003, 2005). The species differ in the finer detail of calyceal structure, rotulae size, orientation and number and site of apical papillae.

The karyotype of representative species of two of the groups differs from  $2n=36=12M+24m$  in the *cristatus* group (Matthey 1957) to  $2n=24=20M+4m$  in the *bitaeniatus* group (Matthey & van Brink 1956, Klaver & Böhme 1986).

This African genus comprises a somewhat heterogeneous collection of chameleons which encompasses at least four species complexes (*affinis cristatus*, *bitaeniatus*, *weneri* – Hillenius 1959, Klaver 1981, Koreny 2006) which are currently loosely named “groups” as well as a single species that does not fit into any of the other groups viz: *T. melleri*. Three of the five sub-groups within this genus have probably independently developed a viviparous reproductive strategy (*affinis*, *bitaeniatus* and *weneri* groups), whilst the fourth group (*cristatus*) and *T. melleri* have retained oviparous modes of reproduction. The viviparous groups have a dense melanotic infiltration of the parietal pigmentation – a condition usually associated with viviparity in the Chamaeleonidae.

## Acknowledgements

The authors would like to acknowledge the South African National Biodiversity Institute for funding the laboratory component of this study. We are grateful to the California Academy of Sciences and the Port Elizabeth Museum (Bayworld) for access to their collections. Some of the analyses were run on the freely available Bioportal at the University of Oslo, Norway ([www.biportal.uio.no](http://www.biportal.uio.no)). Thanks also go to Keshni Gopal, John Measey, Joe Beraducci, Bill Branch and Simon van Noort for assistance with this study, and to Neil Ayres for providing the maps.

## References

- Böhme, W. (1988) Zur Genitalmorphologie der Sauria: Funktionelle und stammesgeschichtliche Aspekte. *Bonner Zoologische Monographien*, 27, 1–165.
- Böhme, W. & Klaver, C. (1980) The systematic status of *Chamaeleo kinetensis* Schmidt, 1943, from the Imatong mountains, Sudan, with comments on lung and hemipenal morphology within the *Chamaeleo bitaeniatus* group. *Amphibia - Reptilia*, 1, 3–17.
- Bourgat, R.M. (1973) Cytogénétique des chaméléons de Madagascar. Incidences taxonomiques, biogéographiques et phylogénétiques. *Bulletin Societe Zoologique, France*, 98(1), 81–90.
- Gorman, G.C. (1973) The chromosomes of Reptilia, a cytotoxic interpretation. In: Chiarelli A.B. & Capama E. (Eds.), *Cytotaxonomy and vertebrate evolution*. Academic Press, New York, pp. 347 – 424.
- Hillenius, D. (1959) The differentiation within the genus *Chamaeleo* Laurenti 1768. *Beaufortia*, 8(89), 1–92.

- Huelsenbeck, J.P. & Ronquist, F. (2001) MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754–755.
- Klaver, C.J.J. (1973) Lung anatomy: aid in chameleon taxonomy. *Beaufortia*, 20(269), 155–177.
- Klaver, C.J.J. (1977) Comparative lung morphology in the genus *Chamaeleo* Laurenti, 1768 (Sauria: Chamaeleonidae) with a discussion of taxonomic and zoogeographic implications. *Beaufortia* 25(327), 167–199.
- Klaver, C.J.J. (1979) A review of *Brookesia* systematics with special reference to lung morphology. *Bonner zoologische Beiträge*, 30, 163–175.
- Klaver, C.J.J. (1981) Lung morphology in the Chamaeleonidae (Sauria) and its bearing on phylogeny, systematics and zoogeography. *Zeitschrift fuer zoologische Systematik Evolutionsforsch*, 19, 36–58.
- Klaver, C.J.J. & Bohme, W. (1986) Phylogeny and classification of the Chamaeleonidae (Sauria) with special reference to hemipenis morphology. *Bonner Zoologische Monographien*, 22, 1–64.
- Koreny, L. (2006) Phylogeny of East-African chameleons. MSc Thesis, Faculty of Biological Sciences, University of South Bohemia in Ceske Budejovice, April 2006.
- Macey, J.R., Larson, A., Ananjeva, N.B. & Papenfuss, T.J. (1997a) Evolutionary shifts in three major structural features of the mitochondrial genome among iguanian lizards. *Journal of Molecular Evolution*, 44, 660–674.
- Macey, J.R., Larson, A., Ananjeva, N.B., Fang, Z.L. & Papenfuss, T.J. (1997b) Two novel gene orders and the role of light-strand replication in rearrangement of the vertebrate mitochondrial genome. *Molecular Biology and Evolution*, 14, 91–104.
- Maddison, D.R. & Maddison, W.P. (2000) *MacClade 4: Analysis of Phylogeny and Character Evolution*, 4.0. Sinauer Associates, Sunderland, MA.
- Matthee, C.A., Tilbury, C.R. & Townsend, T. (2004) A phylogenetic Review of the African leaf chameleons genus *Rhampholeon* (Chamaeleonidae): The role of vicariance and climate change in speciation. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 1967–1975.
- Matthey, R. (1931) Chromosomes de reptiles sauriens, ophiidiens, chelonians. L'évolution de la formule chromosomiale chez les sauriens. *Revue Suisse de Zoologie* 38(9), 146–147.
- Matthey, R. (1957) Cytologie comparée et taxonomie des Chamaeleontidae (Reptilia - Lacertilia). *Revue Suisse de Zoologie* 64, 709–732.
- Matthey, R. & van Brink, J.M. (1956) Note préliminaire sur la cytologie chromosomique comparée des caméléons. *Revue Suisse de Zoologie* 63, 241–246.
- Matthey, R. & van Brink, J.M. (1960) Nouvelle contribution a la cytologie compare des Chamaeleontidae (Reptilia – Lacertilia). *Bulletin de la Societe Vaudoise des Sciences Naturelles*, 67, 333–348.
- Necas, P., Modry, D. & Slapeta, J.R. (2003) *Chamaeleo (Trioceros) narraioca* n.sp. (Reptilia: Chamaeleonidae) a new species from a relict montane forest of Mount Kulal, northern Kenya. *Tropical Zoology*, 16, 1–12.
- Necas, P., Modry, D. & Slapeta, J.R. (2005) *Chamaeleo (Trioceros) ntunte* n.sp. (Reptilia: Chamaeleonidae) a new chameleon species from Mt. Nyiro, northern Kenya. *Herpetozoa* 18(3/4), 125–132.
- Palumbi, S. (1996) Nucleic acids II. The polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), *Molecular Systematics*, second edition, Sinauer Associates, Sunderland, MA., pp. 205 – 247.
- Posada, D. & Crandall, K.A. (1998) Modeltest: testing the model of DNA substitution *Bioinformatics*, 14, 817–818.
- Rieppel, O. (1981) The skull and jaw adductor musculature in chameleons. *Revue Suisse de Zoologie*, 88, 433–445.
- Rieppel, O. (1987) The phylogenetic relationships within the Chamaeleonidae, with comments on some aspects of cladistic analysis. *Zoological Journal of the Linnaen Society*, 89, 41–62.
- Rieppel, O. & Crumly, C. (1997) Paedomorphosis and skull structure in Malagasy chameleons (Reptilia: Chamaeleoninae). *Journal Zoological Society London*, 243, 351–380.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19, 1572–1574.
- Swofford, D.L. (2002) *PAUP\* Phylogeny Analysis using Parsimony (\*and other methods). Version 4.0b10*. Sinauer Associates, Sunderland, MA.
- Tilbury, C.R., Tolley, K.A. & Branch, W.R. (2006) A review of the systematics of the genus *Bradypodion* (Sauria: Chamaeleonidae), with the description of two new genera. *Zootaxa*, 1363, 23–38.
- Tilbury, C.R. (1998) Two new chameleons (Sauria: Chamaeleonidae) from isolated Afromontane forests in Sudan and Ethiopia. *Bonner zoologische Beiträge*, 47(3–4), 293–299.
- Tolley, K.A., Tilbury C.R., Branch W.R. & Matthee C.A. (2004) Phylogenetics of the Southern African dwarf chameleons, *Bradypodion* (Squamata: Chamaeleonidae). *Molecular Phylogenetics and Evolution*, 30, 354–365.
- Townsend, T. & Larson, A. (2002) Molecular Phylogenetics and Mitochondrial Genomic Evolution in the Chamaeleonidae (Reptilia, Squamata). *Molecular Phylogenetics and Evolution*, 23(1), 22–36.