THE ROYAL SOCIETY

Received 20 February 2004
Accepted 17 May 2004
Published online 18 August 2004

A phylogenetic review of the African leaf chameleons: genus *Rhampholeon* (Chamaeleonidae): the role of vicariance and climate change in speciation

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The phylogenetic associations among 13 currently recognized African leaf chameleon species were investigated by making use of mitochondrial and nuclear DNA sequence data (44 taxa and 4145 characters). The gene tree indicates two divergent clades within *Rhampholeon*; this finding is congruent with previous morphological suggestions. The first clade (I) comprises three taxa (*R. kerstenii*, *R. brevicaudatus* and *R. brachyurus*) and is widely distributed in lowland forest and or non-forest biomes. The second clade (II) comprises the remaining *Rhampholeon* species and can be subdivided into three subclades. By contrast, most taxa belonging to clade II are confined to relict montane forest biotopes. Based on geographical, morphological and molecular evidence, it is suggested that the taxonomy of *Rhampholeon* be revised to include two genera (*Rieppeleon* and *Rhampholeon*) and three subgenera (*Rhampholeon*, *Bicuspis* and *Rhinodigitum*). There is a close correlation between geographical distribution and phylogenetic relatedness among *Rhampholeon* taxa, indicating that vicariance and climate change were possibly the most influential factors driving speciation in the group. A relaxed Bayesian clock suggests that speciation times coincided both with the northern movement of Africa, which caused the constriction of the pan African forest, and to rifting in east Africa *ca*. 20 Myr ago. Subsequent speciation among taxa was probably the result of gradual desiccation of forests between 20 and 5 Myr ago.

Keywords: Chamaeleonidae; mtDNA; RAG1; *Rieppeleon*; *Rhampholeon*; *Rhinodigitum*

1. INTRODUCTION

The genus *Rhampholeon* (African leaf chameleons) comprises 14 described extant species. Most of the taxa are restricted to isolated forest patches, predominantly in east Africa, with one species (*R. spectrum*) occurring on the western side of the continent (figure 1). The genus is one of six currently recognized genera within the monophyletic family Chamaeleonidae (Klaver & Böhme 1986; Raxworthy *et al.* 2002) but its relationship with the remaining chameleon genera (*Brookesia*, *Bradypodion*, *Calumma*, *Chamaeleo* and *Furcifer*) is uncertain (Hillenius 1986; Rieppel 1987; Hofman *et al.* 1991; Bauer 1997; Rieppel & Crumly 1997; Townsend & Larson 2002). Previous morphological studies have suggested that *Brookesia* was the first to diverge from the ancestral chameleon lineage, followed by *Rhampholeon* (Klaver & Böhme 1986; Rieppel & Crumly 1997). Recently, Raxworthy *et al.* (2002) included mtDNA, tRNA and ND4 genetic data and proposed a similar association among the genera. However, when the genetic data used by these authors are analysed separately from the morphological data, no statistical support is found for the monophyly or the second most basal position of *Rhampholeon* (see Townsend & Larson 2002).

Comparisons of a suite of characters from the skull and jaw adductor musculature led Rieppel (1987) to conclude that there were two distinct clades within the genus (Types I and II). Type I *Rhampholeon* comprised two taxa (*R. kerstenii* and *R. brachyurus*), whereas the remaining eight taxa (*R. brevicaudatus*, *R. spectrum*, *R. temporalis*, *R. marshalli*, *R. gorongosae*, *R. boulengeri*, *R. platyceps*, *R. nchisiensis*) comprised the Type II *Rhampholeon*. Subsequently, Tilbury (1992) included *R. brevicaudatus* in the Type I group on external morphological grounds. In addition, several new species have been described (Tilbury 1992; Tilbury & Emmrich 1996; Menegon *et al.* 2002) and more are in the process of being assigned. Furthermore, an East African chameleon (*Bradypodion spinosum*) was recently reassigned to *Rhampholeon* (Tilbury & Mariaux 2004).

Morphological characters can be evolutionarily quite labile within chameleons (Glaw *et al.* 1999; Bickel & Losos 2002; Menegon *et al.* 2002) and in many cases their phylogenetic usefulness may be limited (Rieppel & Crumly 1997). For example, a recent genetic investigation of the southern African dwarf chameleons, genus *Bradypodion*, indicated that evolutionary clades are distributed over distinct geographical regions, and phenotypic groupings are not necessarily indicative of evolutionary relationships (Tolley *et al.* 2004).

The evolution of *Rhampholeon* taxa has great potential to provide insights into the biogeographic history of east Africa because rifting has changed a small, relatively homogeneous geographical area, into a diverse habitat characterized by extreme disjunctions in geology, relief and

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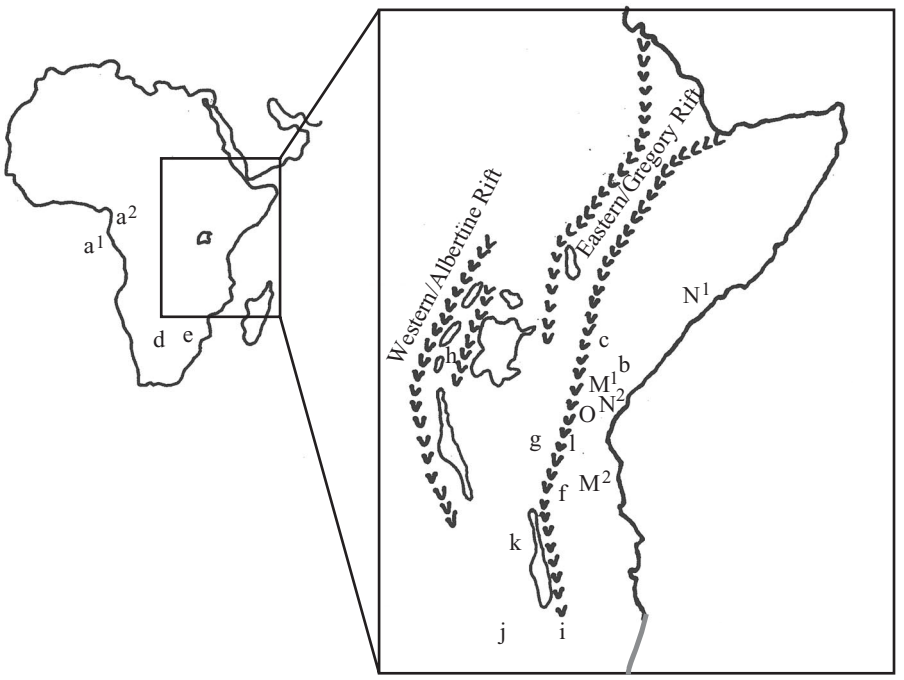

Figure 1. Approximate location of sampling localities and the position of the eastern and western rift valleys in east Africa. The lower case alphabetical characters correspond to *Rhampholeon* taxa whereas the upper case characters represent *Rieppeleon*. Where more than one locality was sampled, superscript letters correspond to the localities given in parentheses. a: *R. spectrum* (Bioko Island[1]; Mekambo[2]); b: *R. temporalis* (East Usambara Mountains); c: *R.* sp. novum (South Pare Mountains); d: *R. marshalli* (Vumba Mountains); e: *R. gorongosae* (Gorongosa Mountain); f: *R. moyeri* (Udzungwa Mountains); g: *R.* sp. novum (Rubeho Mountains); h: *R. boulengeri* (Bwindi Forest); I: *R. platyceps* (Mount Mlanje); j: *R. chapmanorum* (Malawi hills); k: *R. nchisiensis* (Nchisi Mountain, Nyika Plateau); l: *R. uluguruensis* (Uluguru Mountains); M: *R. brevicaudatus* (East Usambara[1]; Udzungwa Mountains[2]); N: *R. kerstenii* (Kilifi[1]; Mount Handeni[2]); O: *R. brachyurus* (Tamota).

vegetation. These changes have been implicated several times as a source of vicariance driving speciation in the region (Freitag & Robinson 1993; Girman *et al.* 1993; Matthee & Robinson 1997; Arctander *et al.* 1999; Pitra *et al.* 2002). In addition, east Africa is a relatively dry region, and the extant forests are generally small and restricted to uplands high enough to attract orographic rainfall (Spawls *et al.* 2002). Rifting and periodic cyclic changes in global temperature during the past 40 Myr resulted in repeated changes in precipitation patterns, which in turn resulted in the expansion and contraction of isolated forests (Lovett 1993; Partridge *et al.* 1995; Burgess *et al.* 1998; Zachos *et al.* 2001). Pertinent to the evolution of *Rhampholeon*, Burgess *et al.* (1998) proposed a biodiversity hypothesis for east Africa and argued that because of the climate history and/or vicariant species evolution, the coastal lowland forests of the region are dominated by relatively few older endemic species (owing to a more stable habitat), whereas the Eastern Arc Mountains are characterized by more endemic taxa with generally more recent origins.

The aims of the present study are therefore: first, to provide a robust molecular phylogeny for the genus *Rhampholeon*; second, to assess the congruence of this molecular hypothesis with current morphological interpretations; and finally, to provide an evolutionary framework for the group that will allow inferences on the abiotic factors driving evolution in Africa.

2. MATERIAL AND METHODS

(a) *Sampling*

Thirty-eight *Rhampholeon* specimens are included in the present investigation (electronic Appendix A). Apart from the recently reassigned *R. spinosus* (Tilbury & Mariaux 2004), all the recognized species are included. In addition, one morphologically distinct unassigned taxon from the South Pare Mountains in Tanzania (electronic Appendix A) and a single specimen from a population in the Rubeho Mountains (morphologically close to *R. uluguruensis/moyeri*) were also included. One representative of each of the five remaining chameleon genera along with the more distantly related agamid *Calotes* were included as outgroups.

(b) *DNA extraction and PCR*

Total genomic DNA was extracted from ethanol-preserved tissue using phenol/chlorophorm/isoamyl alcohol procedures or the QIAamp DNA purification kit (Qiagen Ltd). Two mtDNA gene regions (ND2 and 16S rRNA) and one nuclear DNA protein-coding gene (RAG1) were targeted for amplification using published primers (ND2: L4437—Macey *et al.* 1997*a*; L4882—Macey *et al.* 2000; H5934—Macey *et al.* 1997*b*; 16S: L2510 and H3080—Palumbi 1996; RAG1: R13—Groth & Barrowclough 1999; RAG1B—Frippiat *et al.* 2001). In addition to these, custom-made forward and reverse primers were used (electronic Appendix B). PCR cycling details for the mtDNA amplification followed those described in Tolley *et al.* (2004) whereas the RAG1 amplification followed Groth & Barrowclough (1999). Some individuals failed to amplify for the RAG1 gene fragment; this was mainly because of the degraded nature of the DNA available to us. Automated sequencing was performed using BigDye (v. 3.1; Applied Biosystems) and the products were analysed on a 3100 AB sequencer. All data are deposited in GenBank (electronic Appendix A).

Table 1. Characteristics of the gene segments used in this investigation.
(The number of taxa used in each analysis is presented and the values for each segment include: the total number of characters (including alignment gaps), the number of parsimony informative and variable characters. The number of equally parsimonious (EP) trees, the retention index (RI) value, and the number of trees in the 95% posterior probability analyses are given for each dataset.)

gene	total number of taxa	total number of characters	parsimony informative characters	variable characters	MP tree length	RI value	number of EP trees	number of trees in 0.95 posterior intervals
16S rRNA	43	432	138	180	502	0.74	40	5893
ND2	44	914	560	674	2568	0.72	4	2169
RAG1	37	2799	517	911	1369	0.86	72	409
mtDNA	44	1346	698	854	3091	0.72	6	740
all data	44	4145	1216	1773	4496	0.75	4	126

(c) *Sequence alignment*

Protein-coding sequences were translated to amino acids and then aligned manually. Two small regions of the 16S rRNA sequence could not be aligned unambiguously (17 bp starting at position 256 and 18 bp starting at position 286). These were excluded from all analyses. The remaining 21 alignment gaps all restricted to the 16S rRNA gene were treated as missing characters.

(d) *Phylogenetic approach*

Data were analysed using parsimony (Pars) and maximum-likelihood (ML) optimality criteria in PAUP v. 4.0b10 (Swofford 2002), and Bayesian inference (BI) as implemented in MrBayes v. 3.0b3 (Ronquist & Huelsenbeck 2003). Pars analyses used heuristic searches with 100 random additions of taxa and tree bisection–recombination branch swapping. One thousand parsimony bootstrap replicates were performed. ML analyses were performed specifying the optimal model and parameter values based on the AIC criteria determined in Modeltest v. 3.06 (Posada & Crandall 1998). Owing to time constraints, a reduced dataset was analysed for ML and 100 bootstrap replicates were performed. In this latter instance only the two most divergent representatives of each species were included (a maximum of 25 taxa together with two outgroups: *Brookesia* and *Calotes*). Posterior probabilities for the BI analysis were determined following standard procedures (Tolley *et al.* 2004). Datasets containing more than one gene fragment were analysed in a partitioned manner to allow for different regional optimizations and all analyses were performed twice.

Data were analysed using the following partitions: (i) each gene separately, (ii) a combined mtDNA dataset and (iii) a total dataset including all data. We compared the phylogenetic utility of each partition (i and ii) with that of the total topology (iii). To investigate possible conflict among the different gene fragments we used a parsimony approach (incongruence length difference (ILD) as implemented in PAUP v. 4.0b10; Farris *et al.* 1995; Baker & De Salle 1997) and statistical support ($p < 0.05$) for incongruence was estimated using 1000 repartitions. Competing phylogenetic hypotheses were tested for significance using the Shimodaira–Hasegawa test under ML as well as the Templeton–Wilcoxon test under parsimony. Both these tests were performed in PAUP v. 4.0b10. Constraint topologies were constructed using MacClade v. 4.0, which was also used to determine the number of additional steps for alternative hypotheses.

(e) *Molecular clock analyses*

To date the divergences among *Rhampholeon* lineages, a relaxed Bayesian method with multilocus data was applied using the Multidivergence software package (Thorne *et al.* 1998; Thorne & Kishino 2002). Divergence dates can be influenced by missing data (Matthee *et al.* 2004) and for consistency among genes we only included full data for one representative from each species (22 taxa in total). The most parsimonious cladogram of the combined data was specified and used in PAML v. 3.14 (Yang 1997) to create the output files needed for the Multidivergence package developed by Thorne *et al.* (1998) and Kishino *et al.* (2001). The rank correlation coefficient for each pair of genes and the approximate *p*-value for evaluating the null hypothesis that the pair of genes change rate independently were also recorded. The Markov Chain Monte Carlo analyses followed the default settings of the software and as priors, we adopted 100 Myr (s.d. = 100 Myr) between the tip and the root and 0.01 (s.d. = 0.01) substitutions per site per million years for the rate at the root node. *Calotes* was used as outgroup and, we imposed time constraints taken from the fossil record (18 Myr ago for the lower time limit representing the origins of *Rhampholeon* taxa and 26 Myr ago representing the lower limit for the origin of *Chamaeleo* taxa; Rieppel *et al.* 1992).

3. RESULTS

(a) *Sequence characteristics*

A total of 4145 bp (*ca*. 33% mitochondrial and 66% nuclear) were included in the total dataset (table 1). As expected, the more rapidly evolving mtDNA fragments contribute most of the parsimony-informative characters but also show the highest levels of homoplasy (see RI values in table 1). The GTR + I + G model was significantly better for all gene fragments and thus selected for all ML and Bayesian analyses.

(b) *Individual versus combined gene analyses*

A fairly large number of equally parsimonious trees were recovered when the 16S rRNA and the RAG1 data were each analysed separately and the alternative topologies mainly involve nodes with low bootstrap/posterior probability support (table 1). Combining the two mtDNA fragments and combining the mtDNA with the nuclear DNA fragments is supported by the ILD tests (16S rRNA and ND 2: $p = 0.097$; mtDNA and RAG1: $p = 0.08$) and improved the phylogenetic resolution in both instances (measured by a decrease in the number of trees in the 95% probability interval and the statistical support for nodes; table 2). For comparison purposes we chose the topology derived from all data combined as representing the most accurate reflection of the phylogenetic associations among *Rhampholeon* species. The choice was supported by the fact that in the latter analyses 11 of the 12 internal nodes relating

the different species (labelled a–j) were consistently recovered across methods, and these nodes were also supported by high bootstrap and significant posterior probabilities (table 2).

(c) Rhampholeon *phylogeny*

All our analyses failed to recover a monophyletic *Rhampholeon*. Two distinct clades were present (*Rhampholeon* Types I and II; figure 2) and the monophyly of each of these were strongly supported by the conservative 16S rRNA and RAG1 sequences (nodes a and b, figure 2; table 2). When all taxa characterized by missing RAG1 data were excluded from the divergence analyses, the average total number of substitutions per site among members belonging to the two *Rhampholeon* clades was estimated to be 0.14 ± 0.001, a value well within the range detected among recognized genera (from 0.08 between *Chamaeleo* and *Bradypodion* to 0.16 between *Brookesia* and *Rhampholeon*). Unfortunately, despite the inclusion of representatives of all the chameleon genera, the phylogenetic placement of these two clades within the family Chamaeleonidae could not be resolved unequivocally. The only intergeneric association that was consistently retrieved by the combined analyses is the placement of *Brookesia* as the sister taxon of the remaining chameleons (figure 2). Saturation of the ND2 data at deeper levels could be partly responsible for this lack of intergeneric resolution (Townsend & Larson 2002). However, the evolution of both the16S rRNA and RAG1 genes are more slowly evolving, and the lack of phylogenetic resolution rather suggests a rapid cladogenesis early in the history of the Chamaeleonidae.

The first *Rhampholeon* clade (I) comprises three taxa (*R. kerstenii*, *R. brevicaudatus* and *R. brachyurus*) and corresponds exactly to the 'Type I' assemblage previously identified by morphological characters (Rieppel 1987; Tilbury 1992). Within this clade, the mtDNA fragments and the combined analyses strongly suggest a sister taxon relation between *R. kerstenii* and *R. brachyurus* (node l; table 2; figure 2) whereas the RAG1 nuclear data rather suggest a sister taxon relationship between *R. brevicaudatus* and *R. brachyurus*. The latter nuclear association was supported by a single synapomorphic character and when the total dataset was constrained to test these conflicting hypotheses, the clustering of *R. brevicaudatus* with *R. brachyurus* resulted in the addition of 25 steps, and was significantly rejected using both the SH test (total topology $= -\ln$ 28 702.94; nuclear topology $= -\ln$ 28 719.46; $p = 0.03$) and the Templeton–Wilcoxon test ($p = 0.01$).

The second clade (II) contains the remaining *Rhampholeon* species and corresponds to the 'Type II' morphological grouping. Within this assemblage there is good evidence for the existence of three subclades (i–iii; figure 2). The mtDNA and nuclear topologies were largely congruent using the different methods of analysis, and fairly high bootstrap and posterior probabilities were also found for relationships among species within the subclades (table 2). A single conflicting node was found (node d). The mtDNA data place subclade i and ii as sister taxa, whereas the nuclear and combined data support the clustering of subclade ii with subclade iii. The difference between these two topologies in the total dataset resulted in a single step addition and both are equally likely when the SH test was used (mtDNA topology $= -\ln$ 29 594.32; nuclear topology $= -\ln$ 29 579.45; $p = 0.12$). A similar non-significant difference was suggested by the Templeton–Wilcoxon test ($p = 0.16$).

(d) *Molecular clock analyses*

The relaxed Bayesian analyses indicate that the diversification of the chameleon genera started *ca*. 46.55 Myr ago when the *Brookesia* lineage diverged from the ancestor to the remaining chameleons (figure 2). There were positive correlations in substitution rates among genes, but none of these were significant.

4. DISCUSSION

(a) *Molecular phylogeny*

The combined molecular topology provided strong support for the recognition of the morphologically distinguishable Type I and II *Rhampholeon* lineages (Rieppel 1987; Tilbury 1992; figure 2; electronic Appendix C). The two *Rhampholeon* Types are also distinguished by habitat preference and geographical distribution. Apart from *R. spectrum* and *R. boulengeri*, all the Type II *Rhampholeon* species have restricted allopatric distributions centred on the mountainous slopes of the Albertine rift and relict forests of the east African Afro-montane archipelago (Menegon *et al.* 2002). The three species comprising the *Rhampholeon* Type I clade have much larger distributions (sometimes overlapping), with more general habitat requirements (found predominantly in lowland coastal areas including forests, grassland and semi-arid habitats; Tilbury & Emmrich 1996).

(b) *Resolution among taxa within clades*

Within the *Rhampholeon* Type I clade the associations among the three species are not consistent when the mtDNA gene tree (which groups *R. kerstenii* with *R. brachyurus*) is compared with the nuclear phylogeny (which groups *R. brevicaudatus* and *R. brachyurus*). Interestingly, the morphological evidence is also equivocal on these relationships. For example, all three species have a thin lateral flank ridge of tubercles extending caudally from the temporal crest of the head. In *R. brevicaudatus* and *R. brachyurus*, these tubercles extend to the dorsal keel over the sacrum, whereas the tubercles extend to the base of the tail in *R. kerstenii* (thus supporting the nuclear tree). By contrast, the orientation of the dorsal process of the squamosal and the hemipenal apical ornamentation supports the mtDNA topology (Rieppel 1987).

Within the *Rhampholeon* Type II clade all the genetic data support the existence of three subclades/evolutionary lineages (i–iii, figure 2; table 2). Within subclade i (node c; figure 2), a total of three taxa/species were found (*R. spectrum*, *R. temporalis* and *R. sp. nova*). These species are the only *Rhampholeon* that possess calyces on the hemipenal truncus and possess a more complex apical ornamentation than the other Type II species (Klaver & Böhme 1986). Unlike the other Type II subclades, which are restricted to relatively small areas of east Africa, this group has a pan-African distribution. *Rhampholeon temporalis* and *R. sp. nova* are found in east Africa (united by having simple claws) whereas *R. spectrum* is found on the western side of the continent.

Subclade ii comprises *R. marshalli* and *R. gorongosae*. The former taxon has been previously considered unique

Table 2. Summary of the phylogenetic analyses performed on each gene partition.
(The bootstrap support and posterior probability values are indicated for selected nodes recovered by the combined data. When nodes were not found by any particular analyses these are indicated by n.f.; the dashes represent results from nodes in the RAG analyses due to missing taxa. Values in the % found and % support rows represent the percentage of times a certain node was present (% found) and supported by more than 69% bootstrap and more than 0.94 posterior probability (% support). The values in the % found and % support columns represent the percentage of nodes recovered by each data partition and method (% found) and how many of these nodes had bootstrap or posterior probability support (% support).)

gene	node method	a	b	c	d	e	f	g	h	i	j	k	l	% found	% support
16S	Pars	87	79	< 50	< 50	< 50	88	64	n.f.	n.f.	61	80	70	83	42
	ML	55	91	n.f.	n.f.	n.f.	< 50	91	n.f.	n.f.	< 50	70	n.f.	50	25
	BI	100	99	n.f.	n.f.	84	n.f.	95	n.f.	n.f.	61	99	94	58	33
ND2	Pars	62	100	63	n.f.	77	< 50	100	58	52	57	94	98	92	42
	ML	66	100	68	< 50	98	57	99	83	63	< 50	96	98	100	50
	BI	0.64	1.00	0.99	n.f.	1.00	0.62	0.98	0.74	0.71	n.f.	1.00	1.00	83	50
mtDNA	Pars	98	100	82	n.f.	92	88	100	73	57	81	98	99	92	83
	ML	95	100	78	n.f.	99	94	100	92	< 50	81	99	99	92	83
	BI	1.00	1.00	1.00	n.f.	1.00	1.00	1.00	1.00	0.72	0.84	1.00	1.00	92	75
RAG	Pars	97	100	61	91	100	100	—	—	100	70	97	n.f.	90	80
	ML	97	100	62	88	100	100	—	—	100	73	97	n.f.	90	80
	BI	1.00	1.00	0.98	1.00	1.00	1.00	—	—	1.00	0.98	1.00	n.f.	90	90
total	Pars	100	100	85	78	100	98	100	86	64	78	100	98	100	92
	ML	100	100	84	72	100	99	100	94	58	81	99	95	100	92
	BI	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	0.92	1.00	1.00	100	92
% found		100	100	87	53	93	93	100	75	80	93	100	73		
% support		73	100	53	40	80	67	92	58	27	47	100	67		

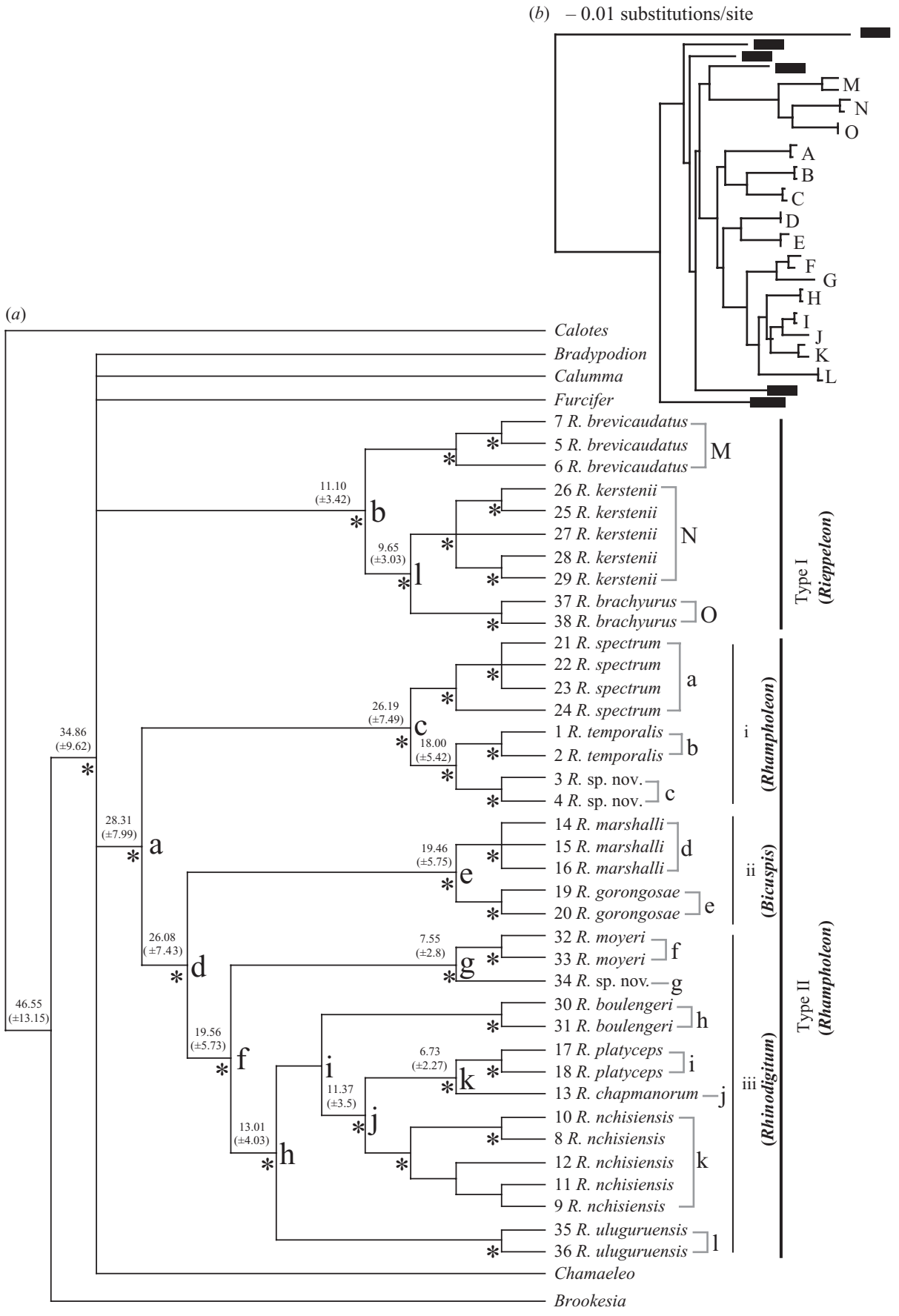

Figure 2. (*Caption opposite.*)

Figure 2. (*a*) Strict consensus parsimony tree derived from the combined mitochondrial and nuclear DNA data. Nodes labelled by asterisks are significantly supported by all three methods of phylogenetic inference (bootstrap more than 70% and posterior probability $\geqslant 0.95$) and the node letters correspond to those in table 2. Approximate time of divergence and standard deviations are indicated above relevant nodes. Numbers associated with the taxon names correspond to those in electronic Appendix A, and the alphabetical character following the name matches the locality designation in figure 1. The two *Rhampholeon* morphotypes (Type I and Type II) and the three *Rhampholeon* subclades (i–iii) are indicated, together with the suggested new names for these assemblages. The inset (*b*) represents a phylogram derived from ML, the black boxes represent outgroups and alphabetical characters correspond to figure 1 and the main topology.

enough to warrant subgroup status based on morphology (Loveridge 1956). The synapomorphies relevant to this subclade are the presence of independently evolved parietal peritoneal pigmentation and dual bifid hemipenal apical horns (Klaver & Böhme 1986). These two taxa occur in southern Africa (Zimbabwe and Mozambique) to the south of the rift systems and are thus also geographically separated from the remaining east African taxa.

Subclade iii consists entirely of species restricted to the slopes of the Rift regions and the Afro-montane archipelago. All of these taxa possess acalyculate hemipenes with dual apical horn ornamentation (Klaver & Böhme 1986). All these species have very truncated tails averaging *ca*. 25% of total length in males and 20% in females. Within this subclade evolutionary relationships among the taxa are once again geographically correlated. For example, *R. platyceps* and *R. chapmanorum* are shallowly divergent sister taxa that together cluster with *R. nchisiensis* (figure 2, node j). All three taxa occur in the southern regions of the *Rhampholeon* distribution generally associated with the Malawi rift system (figure 1: i, j, k). There is some genetic evidence suggesting that *R. boulengeri*, which is found in isolated forests northwards on the Albertine rift (figure 1: h), is the sister taxon of this clade (node i; figure 2). The remaining two most basal taxa in subclade iii (*R. uluguruensis* and *R. moyeri*; figure 1: f, l) are both restricted to isolated forests that cloak the central and south-eastern mountains of the Tanzanian eastern arc complex and would be expected to group with other as yet unassigned populations of *Rhampholeon* in the south/central arc (Rubeho, Ukaguru, Nguru, Nguu).

(c) *Revised taxonomy of the genus* Rhampholeon

It has been noted that there is more phenotypic similarity specifically between the Type I *Rhampholeon* species and the Madagascan *Brookesia* (in particular the *B. nasus* and *B. lolotany* group) than between the mainland African Type I and the Type II species (Klaver 1979). Based on the strong correlation between the genetic and the morphological data together with the magnitude of sequence difference between *Rhampholeon* Types I and II, it is proposed that two African genera are recognized. The name *Evoluticauda* was created by Angel (1942) for all the African *Rhampholeon* and some species of Madagascan *Brookesia*. However, no type species was designated. By subsequent designation, *Brookesia tuberculata* Moquard was nominated as the type species (Guibé 1954). There is thus no available genus name for any of the species of the Type I group and it is here proposed that the species comprising Type I be removed from the genus *Rhampholeon* and assigned to a new genus. It is also our proposition that the reduced *Rhampholeon* genus (Type II) be subdivided into three sub-genera:

Rieppeleon, new genus
Type species: *Rhampholeon kerstenii* (Peters 1868)
Content: *Rieppeleon kerstenii* (Peters 1868), *R. brevicaudatus* (Matschie 1892), *R. brachyurus* (Gunther 1893).
Characterization and diagnosis: electronic Appendix C(i)
Etymology: The name is a patronym in honour of the contribution to the tertiary systematics of the family Chamaeleonidae in general and the genus *Rhampholeon* in particular by Dr Olivier Rieppel. The generic name is masculine.

Genus ***Rhampholeon*** Gunther 1874
Characterization and diagnosis: electronic Appendix C(i)

Subgenus (***Rhampholeon***) Gunther 1874
Type species (by monotypy): *Rhampholeon spectrum* Buchholz 1874
Content: *Rhampholeon* (*Rhampholeon*) *spectrum* (Bucholz 1874), *R.* (*R.*) *temporalis* (Matschie 1892) and other as yet unassigned taxa in the north and south Pare Mountains.
Characterization and diagnosis: electronic Appendix C(ii)

Subgenus (***Bicuspis***) Loveridge 1956
Type species (by monotypy): *Rhampholeon marshalli* Boulenger 1906
Content: *Rhampholeon* (*Bicuspis*) *marshalli* Boulenger 1906, *R.* (*B.*) *gorongosae* Broadley 1971.
Characterization and diagnosis: electronic Appendix C(ii)

Rhinodigitum, new subgenus
Type species: *Rhampholeon platyceps* Gunther 1893
Content: *Rhampholeon* (*Rhinodigitum*) *platyceps* Gunther 1893, *R.* (*Rd*) *boulengeri* Steindachner 1911, *R.* (*Rd*) *nchisiensis* (Loveridge 1953), *R.* (*Rd*) *chapmanorum* Tilbury 1992, *R.* (*Rd*) *uluguruensis* Tilbury & Emmrich 1996, *R.* (*Rd*) *moyeri* Menegon *et al*. 2002.
Characterization and diagnosis: electronic Appendix C(ii)

(d) *Molecular clock and the biogeographical hypothesis*

Based on our estimates of the divergence dates it seems reasonable to argue that apart from *Brookesia*, which diverged early in the evolutionary history of the group, all the remaining genera included in our study diverged over a fairly short timespan (a maximum period of 4.3 Myr between 30.5 and 34.86 Myr ago—data not shown). At approximately this same point in time (34.86 ± 9.62 Myr ago) *Rhampholeon* also diverged from the newly named *Rieppeleon* (figure 2). The latter taxon occurs exclusively to the east of the eastern (or Gregory) rift system and occurs predominantly in lowland coastal areas whereas *Rhampholeon*, by contrast, is more widespread and generally occurs in relict forests at higher altitude.

The Bayesian clock analyses suggest that a single *Rieppeleon* lineage persisted for at least 20 Myr before it diversified into three distinct lineages roughly between 11.10 and 9.65 Myr ago (figure 2). Within the past 30 Myr most of the lowland habitat occupied by *Rieppeleon* would have been

under the sea at some point and the last major inundation was between 18 and 12 Myr ago (Burgess *et al.* 1998; Zachos *et al.* 2001). These changes might have caused local extinctions of lowland taxa and/or confinement to areas of higher ground. Although sea-level fluctuations could be put forward for the survival of a single lineage (being confined to a refuge), the last major sea-level fluctuations are only barely recent enough to account for divergences between the extant *Rieppeleon* taxa (confined to multiple refugia). The exact biogeographic factors driving the evolution of *Rieppeleon* are thus uncertain but it is noteworthy that in general this study tends to support the thesis that the coastal lowland forests of east Africa are characterized by relatively few and older endemic species (Burgess *et al.* 1998).

The biogeographic scenario for the extant *Rhampholeon* (i.e. Type II) lineages is complex and the interlineage divergence dates suggest that the three subclades (i–iii; figure 2) within *Rhampholeon* diverged from each other *ca*. 26.08–28.31 Myr ago (figure 2). In general, this clade is distributed at higher altitudes and is characterized by a larger number of endemic taxa with generally more recent origins. This once again supports the thesis that climatic history and/or vicariant species evolution has contributed to the diversification of the group (Burgess *et al.* 1998). The divergence estimates among *Rhampholeon* species (figure 2) indicate that since 28 Myr ago there has been a gradual process of isolation among lineages, with major diversifications at 18–19, 11–13 and finally 6–7 Myr ago. Although the first rifting activities date back to *ca*. 70 Myr ago, most of the landscape uplifting and volcanic eruptions took place between 25 and 10 Myr ago (Partridge *et al.* 1995). It has been suggested that by 17 Myr ago, both the pronounced Albertine (western) rift and the less spectacular Gregory (eastern) rift had been formed (Partridge *et al.* 1995). Rifting was also associated with global climate changes and the onset of a considerable drying period in northern Africa. These factors caused the repeated fragmentation of montane forest habitat, especially over the past 10 Myr, when the climate trend has been one of steadily increasing aridity.

Within subclade i, *R. spectrum* and *R. temporalis* occur on opposite sides of the continent and the isolation between these lineages (*ca*. 26.19 ± 7.49 Myr ago) was probably the result of the contraction of the pan-African forests due to the gradual northern movement of the African continent and the closure of the Tethys Ocean (Axelrod & Raven 1978). *Rhampholeon marshalli* and *R. gorongosae* (subclade ii) occur southwest of the eastern (or Gregory) rift system and the remaining taxa (subclade iii) all occur close to, or on the slopes, of the Albertine rift system. The diversification of the *Rhampholeon* lineages thus broadly coincides with landscape uplifts and associated periods of forest desiccation (wetter climatic regimes were detected between 9 and 6.4 Myr ago and 4.6 and 2.4 Myr ago; Lovett 1993). Thus, when combined with the observation that sister taxa within *Rhampholeon* tend to be geographical neighbours inhabiting close but isolated montane forests, these palaeoclimatological data strongly suggest regional aridification and a resulting vicariance as the driving forces behind speciation in *Rhampholeon*.

The authors thank several people who provided either vital logistical help and support for this study and/or genetic material for DNA analysis, including: Donald and Sheila Broadley (Zimbabwe), Michele Menegon and Sebastiano Salvidio (Italy), Bob Drewes and Jens Vindum (CALACAD), Jean Mariaux (Geneva), Joe Beraducci (Tanzania), Dietmar Emmrich (Germany), Alan Channing (Stellenbosch). The genetic research component was sponsored by a Stellenbosch University grant to C. A. Matthee.

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