Cryptic diversity in pygmy chameleons (Chamaeleonidae: *Rhampholeon*) of the Eastern Arc Mountains of Tanzania, with description of six new species.

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SUPPLEMENTARY MATERIAL

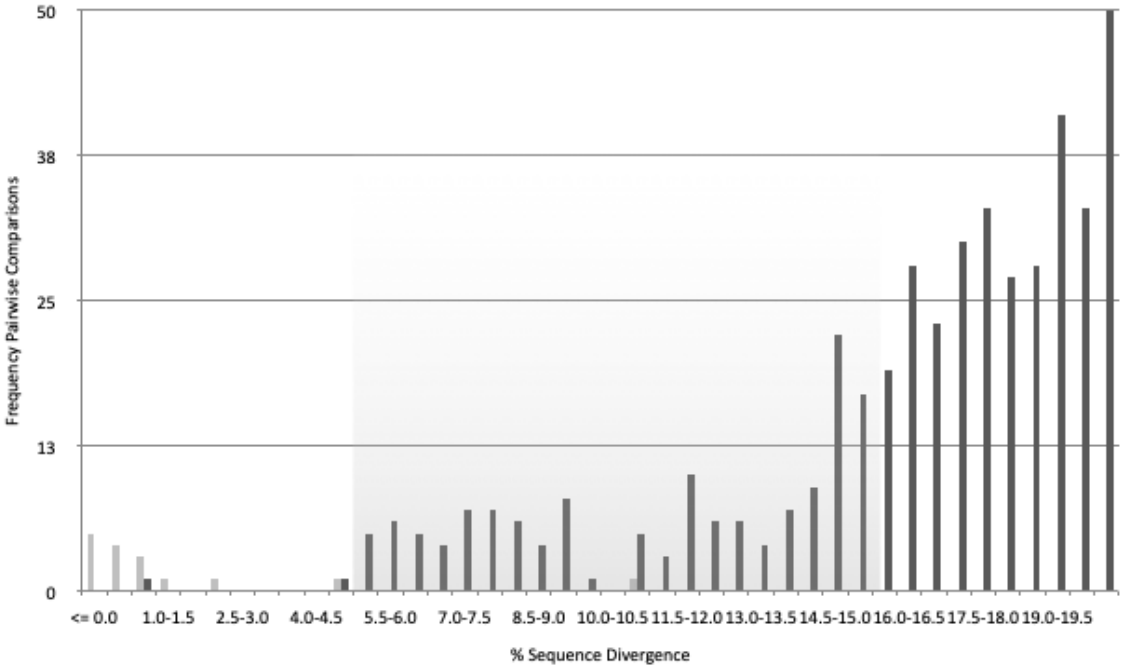

Figure S1. Frequencies of pairwise comparisons for inter- and intra-specific sequence divergences for the genus *Rhampholeon*. Light grey bars represent intra-specific values, and dark grey bars represent inter-specific values. The grey shading shows the range of values among candidate species in the present study.

Table S1. Species name, field ID numbers or voucher accession numbers (CAS = California Academy of Sciences, CT = Colin Tilbury, EBG = Eli Greenbaum, MHNG = Muséum d'histoire naturelle de la Ville de Genève, MTSN = MUSE - Science Museum of Trento, PEM = Port Elizabeth Museum, ZMB = Universität Humboldt, Zoologisches Museum), locality, and GenBank accession numbers for chameleons used in this study. NA = data, specimen, or information not available. * indicates new sequence data {GenBank numbers to be provided upon acceptance}.

Genus	Species	ID Number	16S	ND2	Locality	Year
Rhampholeon	*moyeri*	MTSN5374	NA	ON571556	Kihanga, Tanzania	1998
Rhampholeon	*moyeri*	MTSN5378	ON563705	ON571557	Kihanga, Tanzania	1998
Rhampholeon	*moyeri*	MTSN5376	ON563704	NA	Kihanga, Tanzania	1998
Rhampholeon	*princeeai* sp. nov.	MTSN5537	JX301704	NA	Nguru, Tanzania	2008
Rhampholeon	*princeeai* sp. nov.	MTSN5538	JX301705	NA	Nguru, Tanzania	2008
Rhampholeon	*waynelotteri* sp. nov.	MTSN8539	NA	ON571561	Kanga, Tanzania	2004
Rhampholeon	*waynelotteri* sp. nov.	MTSN8542	NA	ON571562	Kanga, Tanzania	2004
Rhampholeon	*colemani* sp. nov.	MTSN5379-5380	ON563703 (MTSN5379)	ON571555 (MTSN5380)	Kitolomero, Tanzania	1999
Rhampholeon	*sabini* sp. nov.	MTSN5092	ON563706	ON604685	Nguu, Tanzania	2002
Rhampholeon	*sabini* sp. nov.	MTSN5193	NA	ON571560	Nguu, Tanzania	2002
Rhampholeon	*rubeho* sp. nov.	MTSN5014	ON563707	ON571559	Rubeho, Tanzania	
Rhampholeon	*nicolai* sp. nov.	MTSN5592	NA	ON571558	Ukaguru, Tanzania	2004
Rhampholeon	*acuminatus*	CT153	HF570459	NA	Nguru, Tanzania	
Rhampholeon	*beraduccii*	MHNG 2655.019	AM055667	NA	Morogoro, Tanzania	2001
Rhampholeon	*boulengeri*	EBG 1702	KM589411	KM589406	Itombwe Plateau, DRC	

Genus	Species	ID Number	16S	ND2	Locality	Year
Rhampholeon	*bruessoworum*	PEM R18429	HG798975	HG798989	Mount Inago, Mozambique	2009
Rhampholeon	*bruessoworum*	PEM R18430	HG798976	HG798990	Mount Inago, Mozambique	2009
Rhampholeon	*chapmanorum*	PEM R16245	AY524881	AY524919	Malawi Hill, Malawi	1998
Rhampholeon	*gorongosae*	PEM R16252	AY524873	AY524911	Mount Gornogosa, Mozambique	1998
Rhampholeon	*gorongosae*	PEM R16253	AY524874	AY524912	Mount Gornogosa, Mozambique	1998
Rhampholeon	*hattinghi*	PEM R19196	KM589414	KM589408	Mount Nzawa, DRC	2010
Rhampholeon	*hattinghi*	PEM R19197	KM589415	KM589409	Mount Nzawa, DRC	2010
Rhampholeon	*marshalli*	PEM R16243	AY524870	AY524908	Vumba Mountains, Zimbabawe	1998
Rhampholeon	*marshalli*	PEM R16244	AY524871	AY524909	Vumba Mountains, Zimbabawe	1998
Rhampholeon	*maspictus*	PEM R17911	HG798971	HG798984	Mount Mabu, Mozambique	2009
Rhampholeon	*maspictus*	PEM R17912	HG798972	HG798985	Mount Mabu, Mozambique	2009
Rhampholeon	*nchisiensis*	PEM R16241	AY524882	AY524920	Nchisi Mountain, Malawi	1997
Rhampholeon	*nchisiensis*	PEM R16247	AY524885	AY524923	Nchisi Mountain, Malawi	1998
Rhampholeon	*nebulauctor*	PEM R17281	HG798974	HG798988	Mount Chiperone, Mozambique	2008
Rhampholeon	*nebulauctor*	PEM R 17280	HG798973	HG798987	Mount Chiperone, Mozambique	2008
Rhampholeon	*platyceps*	PEM R17136	HG798964	HG798977	Mount Mulanje, Malawi	2006
Rhampholeon	*platyceps*	PEM R17137	HG798965	HG798978	Mount Mulanje, Malawi	2006

Genus	Species	ID Number	16S	ND2	Locality	Year
Rhampholeon	*spectrum*	CAS 207683	AY524863	AY524900	Bioko Island, Equatorial Guinea	1998
Rhampholeon	*spectrum*	PEM R16262	AY524865	AY524903	Mekambo, Gabon	2002
Rhampholeon	*spinosus*	PEMR05738 (CT118)	HF570460	HF570510	Usambara Mountains, Tanzania	2001
Rhampholeon	*temporalis*	PEM R16254	AY524866	AY524904	Usambara Mountains, Tanzania	2001
Rhampholeon	*temporalis*	PEM R16255	AY524867	AY524905	Usambara Mountains, Tanzania	2001
Rhampholeon	*tilburyi*	PEM R17132	EF114320	EF114328	Mount Namuli, Mozambique	2006
Rhampholeon	*tilburyi*	PEM R17133	EF114321	EF114329	Mount Namuli, Mozambique	2006
Rhampholeon	*uluguruensis*	ZMB 48421	AY524896	AY524934	Uluguru, Tanzania	
Rhampholeon	*uluguruensis*	ZMB 48431	AY524897	AY524935	Uluguru, Tanzania	
Rhampholeon	*viridis*	PEM R16525 (CT204)	HF570461	HF570511	North Pare Mountains, Tanzania	2004
Rhampholeon	*viridis*	PEM R16260	AY524868	AY524906	South Pare Mountains, Tanzania	2001
Rhampholeon	*viridis*	PEM R16259	AY524869	AY524907	South Pare Mountains, Tanzania	2001
Rieppeleon	*brachyurus*	PEM R16263	AY524898	AY524936	Tamota, Tanzania	No date
Rieppeleon	*brachyurus*	PEM R16264	AY524899	AY524937	Tamota, Tanzania	No date

Genus	Species	ID Number	16S	ND2	Locality	Year
Rieppeleon	*brevicaudatus*	PEM R16256	AY524887	AY524925	East Usambara Mountains, Tanzania	2001
Rieppeleon	*brevicaudatus*	PEM R16257	AY524888	AY524926	East Usambara Mountains, Tanzania	2001
Rieppeleon	*kerstenii*	CAS 169939	AY524890	AY524928	Kilifi, Kenya	1988
Rieppeleon	*kerstenii*	NA	AY524892	AY524930	NA	

Table S2. Pairwise *post hoc* Bonferroni test results for the nine species of *Rhampholeon* from the analysis of variance of principal component 1 (PC1). The column for the mean difference shows the magnitude of the difference on PC1, with the direction of the difference indicated by a negative or a positive number. The first species in the list are A and the second are B, such that a positive number indicates species A is larger, whereas a negative number indicates species B is larger. The significance values for the mean difference are given ($p < 0.05$).

Species pair		Mean difference (A-B)	Sig.
beraducci	colemani	.6823912	1.000
	waynelotteri	-.3990023	1.000
	moyeri	.4739728	1.000
	nicolae	-.9692425	1.000
	princeeae	.5967961	1.000
	rubeho	-.2287248	1.000
	sabini	-1.1958948	1.000
	uluguruensis	.5962510	1.000
colemani	beraducci	-.6823912	1.000
	waynelotteri	-1.0813934	.660
	moyeri	-.2084184	1.000
	nicolae	-1.6516337*	**.004**
	princeeae	-.0855950	1.000
	rubeho	-.9111159	.870
	sabini	-1.8782860*	**.001**
	uluguruensis	-.0861401	1.000
waynelotteri	beraducci	.3990023	1.000
	colemani	1.0813934	.660
	moyeri	.8729750	1.000
	nicolae	-.5702402	1.000
	princeeae	.9957984	1.000
	rubeho	.1702775	1.000
	sabini	-.7968926	1.000
	uluguruensis	.9952533	1.000
moyeri	beraducci	-.4739728	1.000
	colemani	.2084184	1.000
	waynelotteri	-.8729750	1.000
	nicolae	-1.4432153*	**.021**
	princeeae	.1228234	1.000
	rubeho	-.7026975	1.000
	sabini	-1.6698676*	**.003**
	uluguruensis	.1222782	1.000
nicolae	beraducci	.9692425	1.000
	colemani	1.6516337*	**.004**
	waynelotteri	.5702402	1.000
	moyeri	1.4432153*	**.021**
	princeeae	1.5660386*	**.025**
	rubeho	.7405177	1.000

	sabini	-.2266523	1.000
	uluguruensis	1.5654935*	**.013**
princeeae	beraducci	-.5967961	1.000
	colemani	.0855950	1.000
	waynelotteri	-.9957984	1.000
	moyeri	-.1228234	1.000
	nicolae	-1.5660386*	**.025**
	rubeho	-.8255209	1.000
	sabini	-1.7926910*	**.005**
	uluguruensis	-.0005451	1.000
rubeho	beraducci	.2287248	1.000
	colemani	.9111159	.870
	waynelotteri	-.1702775	1.000
	moyeri	.7026975	1.000
	nicolae	-.7405177	1.000
	princeeae	.8255209	1.000
	sabini	-.9671701	.495
	uluguruensis	.8249758	1.000
sabini	beraducci	1.1958948	1.000
	colemani	1.8782860*	**.001**
	waynelotteri	.7968926	1.000
	moyeri	1.6698676*	**.003**
	nicolae	.2266523	1.000
	princeeae	1.7926910*	.005
	rubeho	.9671701	.495
	uluguruensis	1.7921459*	.002
uluguruensis	beraducci	-.5962510	1.000
	colemani	.0861401	1.000
	waynelotteri	-.9952533	1.000
	moyeri	-.1222782	1.000
	nicolae	-1.5654935*	**.013**
	princeeae	.0005451	1.000
	rubeho	-.8249758	1.000
	sabini	-1.7921459*	**.002**

Table S3. Variable DNA bases for the new species of *Rhampholeon* and comparative species *R. acuminatus* and *R. uluguruensis* for a) the ND2 gene and b) the 16S gene. Top row indicates the base numbers for that gene. ND2 bases are given as codons with the amino acid code corresponding to the codon. Variable sites are indicated with bold font. Both genes were aligned against the complete mitochondrial genome for *Kinyongia fischeri* (GenBank accession number: NC012465). The hypervariable region for 16S was retained in the alignment. Alignment gaps are indicated with a dash. NA indicates no sequences available.

a)	365–367	421–423	436–438	445–447	454–456	460–462	463–465
R. acuminatus	NA	NA	NA	NA	NA	NA	NA
R. colemani	ACT (threonine)	ATC (isoleucine)	TAC (tyrosine)	ATA (methionine)	AAA (lysine)	ATA (methionine)	**ATC (isoleucine)**
R. moyeri	**GCC (alanine)**	ATT (isoleucine)	TAC (tyrosine)	ATA (methionine)	**CAA (glutamine)**	ATA (methionine)	ATA (methionine)
R. nicolae	ACT (threonine)	ATT (isoleucine)	TAC (tyrosine)	**ATG (methionine)**	**ACA (threonine)**	ATA (methionine)	ATA (methionine)
R. princeeae	NA	NA	NA	NA	NA	NA	NA
R. rubeho	ACC (threonine)	**GTT (valine)**	TAC (tyrosine)	ATA (methionine)	AAA (lysine)	ATA (methionine)	ATA (methionine)
R. sabini	ACC (threonine)	ATC (isoleucine)	TAC (tyrosine)	ATA (methionine)	AAA (lysine)	**GTA (valine)**	ATA (methionine)
R. waynelotteri	ACC (threonine)	ATT (isoleucine)	**CAC (histidine)**	**ACA (threonine)**	AAA (lysine)	ATA (methionine)	ATA (methionine)
R. uluguruensis	ACC (threonine)	ATC (isoleucine)	TAC (tyrosine)	ATA (methionine)	AAA (lysine)	ATA (methionine)	ATA (methionine)

a) continued	466–468	469–471	475–477	478–480	487–489	577–579	583–585
R. acuminatus	NA	NA	NA	NA	NA	NA	NA
R. colemani	ACC (threonine)	ATC (isoleucine)	ATT (isoleucine)	ATG (methionine)	CTG (leucine)	**GTC (valine)**	**ACC (threonine)**
R. moyeri	GCC (alanine)	ATC (isoleucine)	**GTC (valine)**	**GTC (valine)**	CTG (leucine)	ATC (isoleucine)	ACT (threonine)
R. nicolae	ACC (threonine)	GTT (valine)	ATT (isoleucine)	ATG (methionine)	CTG (leucine)	ATC (isoleucine	ACC (threonine)
R. princeeae	NA	NA	NA	NA	NA	NA	NA
R. rubeho	ACC (threonine)	**CTT (leucine)**	ATT (isoleucine)	ATG (methionine)	CTG (leucine)	ATC (isoleucine	ACC (threonine)
R. sabini	ACC (threonine)	ATC (isoleucine)	ATT (isoleucine)	ATG (methionine)	**CTA (leucine)**	ATC (isoleucine	**CCC (proline)**
R. waynelotteri	GCC (alanine)	GTT (valine)	ATT (isoleucine)	**TTG (leucine)**	CTG (leucine)	ATC (isoleucine	ACC (threonine)
R. uluguruensis	**TTC (phenylalanine)**	ATC (isoleucine)	**ATC (isoleucine)**	ATG (methionine)	**ATT (isoleucine)**	ATC (isoleucine	ACC (threonine)

a) continued	598–600	619–621	643–645	664 - 666	667–669
R. acuminatus	NA	NA	NA	NA	NA
R. colemani	ACT (threonine)	GCC (alanine)	ACA (threonine)	ATG (methionine)	TCA (serine)
R. moyeri	ACT (threonine)	**GCA (alanine)**	ACA (threonine)	ATA (methionine)	TCA (serine)
R. nicolae	GCT (alanine)	GCC (alanine)	ACA (threonine)	ATA (methionine)	TCA (serine)
R. princeeae	NA	NA	NA	NA	NA
R. rubeho	ACT (threonine)	GCC (alanine)	ACA (threonine)	ATA (methionine)	TCA (serine)
R. sabini	ACT (threonine)	**ACC (threonine)**	**ATA (methionine)**	**ACA (threonine)**	**ACA (threonine)**
R. waynelotteri	ACT (threonine)	GCC (alanine)	ACA (threonine)	ATA (methionine)	TCA (serine)
R. uluguruensis	ACT (threonine)	GCC (alanine)	NA	NA	NA

b)	891	896	917	965	980	1008	1021	1075	1080	1123	1125	1126	1135	1171	1227	1234	1269	1271
R. acuminatus	C	A	**A**	A	**A**	A	**T**	A	T	**C**	C	T	**C**	**A**	**T**	**G**	**A**	A
R. beraduccii	T	A	C	**G**	C	G	C	A	T	-	C	A	T	C	C	A	**A**	**G**
R. colemani	C	A	C	A	T	A	T	A	T	-	C	A	T	C	C	A	G	A
R. moyeri	C	A	C	A	T	A	T	A	T	-	C	A	T	C	C	A	G	A
R. nicolae	NA	A	NA	A	NA	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
R. princeeae	**T**	A	C	A	C	**A**	**C**	**G**	**C**	-	A	**C**	T	C	C	A	G	A
R. rubeho	C	A	C	A	C	A	T	A	T	**T**	**T**	A	A	C	C	A	G	A
R. sabini	C	A	C	A	C	A	T	A	T	-	A	A	T	C	C	A	G	A
R. waynelotteri	NA	A	NA	A	NA	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
R. uluguruensis	C	**G**	C	A	T	A	T	A	T	-	C	T	T	C	C	A	G	A