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The measure of success: geographic isolation promotes diversification in *Pachydactylus* geckos

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Abstract

Background: Geckos of the genus *Pachydactylus* and their close relatives comprise the most species-rich clade of lizards in sub-Saharan Africa. Many explanations have been offered to explain species richness patterns of clades. In the *Pachydactylus* group, one possible explanation is a history of diversification via geographic isolation. If geographic isolation has played a key role in facilitating diversification, then we expect species in more species-rich subclades to have smaller ranges than species in less diverse subclades. We also expect traits promoting geographic isolation to be correlated with small geographic ranges. In order to test these expectations, we performed phylogenetic analyses and tested for correlations among body size, habitat choice, range sizes, and diversification rates in the *Pachydactylus* group.

Results: Both body size and habitat use are inferred to have shifted multiple times across the phylogeny of the *Pachydactylus* group, with large size and generalist habitat use being ancestral for the group. Geographic range size is correlated with both of these traits. Small-bodied species have more restricted ranges than large-bodied species, and rock-dwelling species have more restricted ranges than either terrestrial or generalist species. Rock-dwelling and small body size are also associated with higher rates of diversification, and subclades retaining ancestral conditions for these traits are less species rich than subclades in which shifts to small body size and rocky habitat use have occurred. The phylogeny also illustrates inadequacies of the current taxonomy of the group.

Conclusions: The results are consistent with a model in which lineages more likely to become geographically isolated diversify to a greater extent, although some patterns also resemble those expected of an adaptive radiation in which ecological divergence acts as a driver of speciation. Therefore, the *Pachydactylus* group may represent an intermediate between clades in which radiation is adaptive versus those in which it is non-adaptive.

Keywords: Biogeography, Systematics, Timetree, Allopatry, Radiation, Cladogenesis, Ancestral reconstruction, Phylogenetic comparative methods

Background

Discrete geographic regions, both continentally and on islands, often have biotas dominated by a relatively small number of species-rich lineages. The most obvious of these dominant groups are adaptive radiations in which a single ancestral species has given rise to descendants filling numerous niches, examples of which include Galapagos Finches (14 sp., 58% of breeding songbird species in the archipelago), Lake Victoria cichlids (169 sp., 67% of ray-finned fishes in the lake), and West Indies *Anolis* lizards (168 sp., 38% of lizards native to the islands) [1–3]. However, there are many other less-known examples of regionally prominent radiations. Among lizards, one of the most striking are geckos of the genus *Pachydactylus* (56 species) and its close relatives *Chondrodactylus* (6 species), *Colopus* (2 species), and *Elasmodactylus* (2 species). By species number, these geckos are the most successful radiation of lizards in southern Africa. Sixty four of 66 species occur in the southern African subcontinent, defined as that part of Africa south of the Zambezi

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and Kunene rivers, and most are endemic to this region. These species occupy all major habitat types in southern Africa, and many species in the group display morphological novelties such as loss of adhesive toe pads or the evolution of interdigital webbing [4, 5]. Numerous other gecko genera are found in southern Africa, most of which are endemic or reach their peak diversity there, including *Afroedura*, *Afrogecko*, *Cryptactites*, *Goggia*, *Homopholis*, *Narudasia*, *Ramigekko*, and *Rhoptropus*, but *Pachydactylus* group species often dominate the gekkonid fauna, comprising, for example, 13 of 18 species in the Richtersveld of South Africa [6]. Likewise, none of these other genera approach *Pachydactylus* in its diversity of morphological or ecological variation.

Numerous possible causative factors have been posited or shown to explain the relative success of species-rich organismal groups. In classic adaptive radiations, the rapid evolution of morphological disparity may be the key process in spurring lineage accumulation [7, 8]. In other cases, the evolution of a novel trait may allow organisms possessing that trait to access underutilized resources, with utilization promoting ecological diversification and lineage accumulation. Examples include the evolution of antifreeze proteins in Antarctic icefishes and evolution of the pharyngeal jaw structures in parrotfishes [9, 10]. Sexual selection may also promote lineage accumulation, especially when this selection is for traits that serve as prezygotic isolating mechanisms such as male advertisement calls or color patterns serving as visual mate recognition systems [11, 12]. Finally, in some cases species-rich organismal groups may not actually exhibit a high diversification rate at all, but instead have a longer history of occupancy in a geographic region [13].

In the case of *Pachydactylus* and its relatives, none of these potential explanations are likely to fully account for the observed species diversity. *Pachydactylus* is divided into eight well-defined species groups [14, 15], each of which is believed to be monophyletic, and each of which is morphologically conservative. The degree of morphological disparity between these groups is not of a magnitude expected in a classic adaptive radiation [8]. This is not to say that there is no morphological variation within the genus. Some morphological novelties have evolved, including the previously mentioned foot characteristics as well as variation in scalation. However, such morphological novelties do not appear to be strong drivers of speciation in the group. For example, the most species-rich radiation of *Pachydactylus* geckos that has lost toe pads contains only three species, one of which still has toe pads [4].

Many geckos do have visual display systems or other traits which could theoretically promote diversification via divergent sexual selection. Examples include the

semaphore geckos (*Pristurus*), the dwarf geckos (*Sphaerodactylus*), and various day geckos (including *Cnemaspis*, *Lygodactylus*, and *Phelsuma*) [16–18]. In these genera, males are boldly patterned and use signaling behaviors to defend territories or attract mates. *Pachydactylus* and its relatives are strictly nocturnal, however, and typically have a drab pattern. Nor are any other prezygotic isolating mechanisms evident that could plausibly be hypothesized to be under sexual selection. Finally, the relative age of *Pachydactylus* and its relatives likely does not account for its diversity, either – the next closest relative of the *Pachydactylus* group is *Rhoptropus* [5], which also occurs mainly in southern Africa but includes only nine species.

Given that none of these explanations can fully account for the species diversity observed in *Pachydactylus* and its relatives, we hypothesize that geographic isolation leading to allopatric divergence plays a key role in lineage accumulation in *Pachydactylus* and its relatives. Populations in allopatry, if isolated for a sufficient period of time, can naturally speciate via genetic drift without requiring significant contributions from natural selection acting on divergent morphological traits or sexual selection promoting differentiation of mating systems among species [19, 20]. If geographic isolation does play a key role in diversification of *Pachydactylus* and its relatives, then traits promoting the formation of geographic isolation should affect both species' range sizes and diversification rate. The heritability of range size has been a matter of debate, but increasing numbers of studies demonstrate its heritability [21–25]. In at least some clades, including lizards, this heritability is associated with variable morphological or ecological traits [26, 27]. Likewise, numerous studies have reported trait-associated variation in diversification rates, especially since the development of BiSSE (binary-state speciation and extinction) and related models [28].

For *Pachydactylus* and its relatives, two variable traits of interest that may promote geographic isolation are body size and habitat preference. There is substantial body size variation in *Pachydactylus* and related genera, with the largest and smallest species having adult snout–vent lengths of 35 and 113 mm, respectively [29]. In many groups body size has been shown to be positively correlated with range size [30]. Habitat preference within *Pachydactylus* varies, with species showing preferences ranging from sand dunes to rocky cliffs to houses. In southern Africa, the periodic advance and retreat of Kalahari and Namib sands over geological time is linked to climatic variation [31–33]; this process has likely allowed intermittent connections to form between adjacent rocky habitats, but the prevailing pattern is that terrestrial habitats are relatively continuous while rocky habitats are more discontinuous. As a result, a

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preference for rocky habitats may be expected to be associated with geographic isolation and smaller range sizes. Such substrate specialization has been suggested to facilitate speciation in the *Pachydactylus* group [14, 34], but has never been explicitly tested.

We test whether body size or habitat preference is associated with the formation of geographic isolation in the *Pachydactylus* group in a phylogenetic context. We have generated a comprehensive time-calibrated multi-locus phylogeny of the group, and obtained body size and habitat preference trait data for all ingroup species. Geographic range size estimates are produced for all species, and the association between trait data and range size is quantified. We also estimate patterns of lineage accumulation through time and trait-associated estimates of diversification. Our data show that both body size and habitat preference affect range size, and that variation in these traits is also correlated with variation in diversification rate, suggesting that allopatric divergence following isolation has played a key role in speciation in the *Pachydactylus* group.

Methods

Phylogeny estimation

Previous studies have confirmed that the *Pachydactylus* is part of a monophyletic assemblage of morphologically similar geckos, also including genera *Chondrodactylus*, *Colopus*, and *Elasmodactylus* [4, 14, 35]. We sought to estimate a comprehensive phylogeny for this group, and obtained genetic samples from individuals of 55 of 56 *Pachydactylus* species, 6 of 6 *Chondrodactylus*, both *Colopus* species, and both *Elasmodactylus* species. These genera are part of a larger clade of geckos mainly distributed in Africa and Madagascar, and within this larger grouping they are most closely related to the genus *Rhoptropus* [5, 36]. As such, we included exemplars of 9 of 9 *Rhoptropus* species to serve as a near outgroup. An additional 18 gekkotan and 4 non-gekkotan taxa (*Anolis*, *Gallus*, *Python*, *Trachylepis*) were included as more distant outgroups, with outgroup species choice partially determined based on utility for molecular clock calibration. Nearly all ingroup sequences are associated with vouchered museum specimens. Sequences for four species (*Elasmdactylus tuberculosus*, *Pachydactylus namaquensis*, *P. tsodiloensis*, *P. visseri*) are exceptions, with sequences derived from genetic material obtained from captive-bred individuals; in these cases the live specimens were viewed by the authors to confirm identification and associated genetic material has been deposited in the Cryogenic Collection at the Museum of Comparative Zoology, Harvard University.

We constructed a sequence data set of nuclear and mitochondrial genes that evolve in a relatively clocklike fashion and have proven useful for determining relationships among species within gekkonid genera [37, 38]. The combined data set is 3443 bp (base pairs), including portions of the nuclear genes RAG1 (recombination activating gene 1; 1053 bp), KIF24 (kinesin family member 24; 592 bp) and PDC (phosducin; 395 bp), along with the complete mitochondrial ND2 gene (NADH dehydrogenase subunit 2; 1041 bp) and several adjacent tRNA genes (transfer RNA; 361 bp) (Table 1). All newly generated sequences were deposited in GenBank (accession numbers KY224166–KY224347).

For new sequences generated in this study, DNA was obtained from frozen or ethanol-preserved tissue samples using Qiagen DNeasy tissue kits under the manufacturer's protocol. PCR (polymerase chain reaction) amplification of fragments was performed in 25 μL reactions, under standard reaction conditions [39]. ND2, tRNA, RAG1, and PDC primers used in PCR and sequencing were the same as those used in [37]; KIF24 primers were derived from [40]. PCR purification was performed using AMPure magnetic beads, followed by cycle sequencing and purification using CleanSeq magnetic beads. Capillary electrophoresis was performed on an Applied Biosystems 3730xl sequencer. Sequence assembly was performed using BioEdit [41] or Geneious 5.1 [42], with alignment using Clustal [43]. Alignments of the protein-coding genes were edited manually to preserve reading frame and checked to ensure absence of premature stop codons, while those of the tRNAs were edited manually to preserve secondary structural features estimated in ARWEN [44].

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian (BI) optimality criteria. For each analysis, model and partition choices were separately identified under the Bayesian Information Criterion using PartitionFinder [45]. In each case considered models of evolution were limited to those models that can be implemented by the programs used for phylogeny estimation. Greedy search schemes were employed and thirteen potential data blocks were considered: twelve data blocks corresponding to the three codon positions for each of the four protein-coding genes and the tRNA data comprising the thirteenth data block.

The ML analysis was performed using RAxML 8.2.4 [46]. One hundred independent searches were implemented on the original data set to identify the best tree, followed by 1,000 non-parametric bootstrap replicates to assess branch support. Based on the PartitionFinder results, the data were divided into eight partitions, each using one of two models: ND2 codon position 1, ND2 codon position 2, tRNAs, and (PDC position 1 + 2 + RAG1 position 1 + 2) used the GTR (general time reversible) + I + Γ model, while ND2 position 3, (PDC position 3 + RAG1 position 3), (KIF24 position 1 + 2) and KIF24 position 3 used the GTR + Γ model.

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Table 1 Specimens and GenBank accession numbers of specimens used in this study

Species	ID Number	ND2	Rag1	PDC	KIF24
Anolis carolinensis	n/a	EU747728	AAWZ 02015549	AAWZ 02013979	NW_003338919
Chondrodactylus angulifer	MCZ R-184985	KY224209	KY224307	KY224257	
Chondrodactylus bibronii	CAS 201841	JN543886	JN543930	KY224258	KY224166
Chondrodactylus fitzsimonsi	MCZ R-185712	JN393945	KY224308	KY224259	KY224167
Chondrodactylus laevigatus	MCZ R-184819	KY224211	KY224310	KY224260	KY224168
Chondrodactylus pulitzerae	CAS 193828	KY224210	KY224309		
Chondrodactylus turneri	MCZ R-184410	KY224249	KM073525	KM073612	KM073800
Coleonyx variegatus	MVZ 161445	AB114446			
Coleonyx variegatus	CAS 205334		EF534777	EF534817	
Colopus kochii	CAS 214803	KY224212	KY224311	KY224261	KY224169
Colopus wahlbergii	NMZB 16974	JN569158	JN569191	JQ945366	
Correlophus ciliatus	AMS R-146595	JX024438	EF534778	EF534818	KU157544
Elasmodactylus tetensis	PEM R-5540	KY224213	KY224312	KY224262	KY224170
Elasmodactylus tuberculosus	MCZ:Cryo 3006	KY224214	KY224313	KY224263	KY224171
Euleptes europaea	no number	JN393941	EF534806	EF534848	KU157420
Gallus gallus	n/a	KT626857	NM_001031188	XM_004943303	NC_006127
Goggia braacki	PEM R-11911	KM073689	KM073528	KM073614	KM073802
Nephrurus levis	AMS 140561		GU459544	GU459746	KU157421
Nephrurus levis	SAMA R-19968	AY369018			
Oedura marmorata	SAMA R-34209	AY369015			
Oedura marmorata	AMS 143861		EF534779	EF534819	KU157428
Ophidiocephalus taeniatus	SAM R-44653	AY134601	HQ426303	HQ426214	KU157422
Pachydactylus acuminatus	MCZ R-185739	KY224215	KY224314	KY224264	KY224172
Pachydactylus affinis	PEM R-17545	KY224216	KY224315	KY224265	
Pachydactylus amoenus	AMB 8670	JN569163			
Pachydactylus angolensis	CAS 254887	KY224217	KY224316		
Pachydactylus atorquatus	MCZ R-184811	KY224218	KY224317	KY224266	
Pachydactylus austeni	LSUMZ H1629	KY224250	JQ945321	JQ945389	KY224173
Pachydactylus barnardi	MCZ R-184749	KY224219	KY224318	KY224267	
Pachydactylus bicolor	NMNW (AMB 7631)	JN543870+ KY224220	JN543911	KY224268	
Pachydactylus boehmei	MCZ R-184883	JN543906	JN543947	KY224270	KY224174
Pachydactylus capensis	MCZ R-184499	HQ165962	HQ165992	HQ165977	KY224175
Pachydactylus caraculicus	MCZ R-185767	JN543889	JN543933	KY224271	
Pachydactylus carinatus	LSUMZ 57293	KY224221	KY224319	KY224272	
Pachydactylus etultra	MCZ R-184978	HQ165959	HQ165989	HQ165974	KY224176
Pachydactylus fasciatus	MCZ R-185759	HQ165949	HQ165978	HQ165963	
Pachydactylus formosus	CAS 206715	KY224222	KY224320	KY224273	
Pachydactylus gaiasensis	MCZ R-184169	JN543891	KM073533	KM073615	KY224177
Pachydactylus geitje	PEM R-11226	JN543887	JN543931	KY224274	KY224178
Pachydactylus goodi	MCZ R-184783	KY224223	KY224321	KY224275	KY224179
Pachydactylus griffini	MCZ R-185741	KY224224	KY224322	KY224276	KY224180
Pachydactylus haackei	CAS 186341	KY224225	KY224323	KY224277	
Pachydactylus kladaroderma	PEM R-1253	KY224251	JQ945323	JQ945391	
Pachydactylus kobosensis	CAS 223904	KY224226	KY224324	KY224278	KY224181

Table 1 Specimens and GenBank accession numbers of specimens used in this study *(Continued)*

Pachydactylus labialis	MCZ R-184758	KY224227	KY224325	KY224279	KY224182
Pachydactylus latirostris	PEM R-16720	JN569141	JN569173	KY224280	KY224183
Pachydactylus macrolepis	PEM R-17668	JN569139	JN569170	KY224281	KY224184
Pachydactylus maculatus	CAS 186380	KY224228	KY224326	KY224282	KY224185
Pachydactylus maraisi	NMNW (JV 1856)	JN543871	JN543912	KY224269	
Pachydactylus mariquensis	NMB R10936	JN569157	JN569190	KY224283	KY224186
Pachydactylus mclachlani	MCZ R-185094	HQ165950	HQ165980	HQ165965	KY224187
Pachydactylus monicae	CAS 193418	HQ165952	HQ165982	HQ165967	KY224188
Pachydactylus montanus	MCZ R-184243	KY224229	KY224327	KY224284	KY224189
Pachydactylus namaquensis	MBUR 01770	KY224230			
Pachydactylus namaquensis	MCZ:Cryo 3007		KY224328	KY224285	KY224190
Pachydactylus oculatus	PEM R-1284	KY224231	KY224329	KY224286	
Pachydactylus oreophilus	MCZ R-185769	JN543892	JN543936	KY224287	KY224191
Pachydactylus oshaughnessyi	NMZB (DGB 611)	KY224232	KY224330	KY224288	KY224192
Pachydactylus otaviensis	MCZ R-184867	JN543893	JN543937	KY224289	KY224193
Pachydactylus parascutatus	CAS 214750	JN543894	JN543938	KY224290	KY224194
Pachydactylus punctatus	PEM R-12461	KY224233	KY224331	KY224291	
Pachydactylus purcelli	PEM R-16895	HQ165954	HQ165984	HQ165969	
Pachydactylus purcelli	MCZ R-184796				KY224195
Pachydactylus rangei	MCZ R-183725	JN543907	JN543948	JQ945392	
Pachydactylus reconditus	MCZ R-184856	KY224234	KY224332	KY224292	
Pachydactylus robertsi	NMNW R6697	KY224235	KY224333	KY224293	
Pachydactylus rugosus	CAS 201905	KY224252	JQ945325	JQ945393	
Pachydactylus sansteynae	CAS 214589	JN543898	KY224334	KY224294	
Pachydactylus scherzi	MCZ R-184938	KY224236	KY224335	KY224295	
Pachydactylus scutatus	MCZ Z37843	JN543901	JN543943	KY224296	KY224196
Pachydactylus serval	MCZ R-185989	HQ165956	HQ165986	HQ165986	KY224197
Pachydactylus tigrinus	NMB R10936	KY224237	KY224336	KY224297	KY224198
Pachydactylus tsodiloensis	MCZ:Cryo 3008	KY224238	KY224337	KY224298	KY224199
Pachydactylus vansoni	MCZ R-184434	KY224239		KY224299	KY224200
Pachydactylus vanzyli	NMNW (JV1761)	KY224253	JQ945326	JQ945394	KY224201
Pachydactylus visseri	MCZ:Cryo 3009	KY224240	KY224338	KY224300	KY224202
Pachydactylus waterbergensis	MCZ R-184751	KY224241	KY224339	KY224301	
Pachydactylus weberi	PEM R-12449	HQ165960	HQ165990	HQ165975	KY224203
Pachydactylus werneri	MCZ R-184960	KY224242	KY224340	KY224302	KY224204
Phelsuma inexpectata	JB 56	JN393939	JN393983	JN394016	
Phelsuma rosagularis	n/a	EU423292			
Phelsuma rosagularis	JB 109		HQ426306	HQ426217	
Phyllopezus pollicaris	MZUSP 92491	JX041417	EU293635		
Phyllopezus pollicaris	CENPAT12084				JQ827509
Phyllopezus pollicaris	JFBM 15822			HQ426225	
Pygopus nigriceps	ERP R29509	AY134604			
Pygopus nigriceps	SAMA R-23908		FJ571628		
Pygopus nigriceps	MVZ 197233			EF534823	
Python bivittatus	n/a		AEQU 010344888	AEQU 01027927	NW_006537073

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Table 1 Specimens and GenBank accession numbers of specimens used in this study *(Continued)*

Python regius	n/a	AB177878		
Rhoptropus afer	MCZ R-183711	KY224254	KM073535	KM073616	KM073806
Rhoptropus barnardi	CAS 214658	KY224243	KY224341	KY224303	KY224205
Rhoptropus benguelensis	ANG_WC1834	KY224246	KY224346		
Rhoptropus biporosus	CAS 224030	KY224244	KY224342	KY224304	KY224206
Rhoptropus boultoni	CAS 214713	KY224256	EF534810	EF534852	KY224207
Rhoptropus bradfieldi	NMNW (to be accessioned)	KY224245	KY224343	KY224305	
Rhoptropus diporus	MCZ R-183737	KY224255	KY224344	KY224306	KY224208
Rhoptropus montanus	CAS 254867	KY224247	KY224345		
Rhoptropus taeniostictus	CAS 254908	KY224248	KY224347		
Sphaerodactylus nicholsi	CAS 198444	KU158020	EF534786	EF534826	KU157415
Sphaerodactylus roosevelti	CAS 198428	JN393943	EF534785	EF534825	
Sphaerodactylus torrei	JB 34	JX440519	EF534788	EF534829	KU157416
Teratoscincus microlepis	JFBM 15				KU157417
Teratoscincus microlepis	TG 00074	JX041451	EF534800	EF534842	
Teratoscincus roborowskii	CAS 171203	AF114252			
Teratoscincus roborowskii	TG 00070		EF534799	EF534841	
Teratoscincus roborowskii	JFBM 14				KU157418
Teratoscincus scincus	CAS 228808				KU157419
Teratoscincus scincus	JFBM 14252	JX041454	EF534801	EF534843	
Trachylepis varia	MCZ-R 184873		GU931671		
Trachylepis varia	TNHC 68769	GU931603			GU931534
Trachylepis varia	ZFMK 68413			KC345241	
Woodworthia maculata	RAH 292	GU459852	GU459449	GU459651	
Woodworthia maculata	RAH 92				KU157432

Specimen ID codes are as follows: AMB (Aaron M. Bauer field collection); AMS (Australian Museum, Sydney); ANG_WC (Werner Conradie field collection); CAS (California Acedemy of Sciences); CENPAT (Centro Nacional Patagónico, Puerto Madryn); DGB (Donald G. Broadley field collection); ERP (Eric R. Pianka field collection); JB (Jon Boone tissue collection); JFBM (James Ford Bell Museum of Natural History, University of Minnesota); JV (Jens Vindum field collection); LSUMZ (Louisiana State University Museum of Zoology); MBUR (Marius Burger field collection); MCZ (Museum of Comparative Zoology, Harvard University); MVZ (Museum of Vertebrate Zoology, University of California); MZUSP (Museum of Zoology, University of Sao Paulo); NMB (National Museum, Bloemfontein); NMNW (National Museum of Namibia, Windhoek); NMZB (National Museum of Zimbabwe, Bulawayo); PEM (Port Elizabeth Museum); RAH (Rod A. Hitchmough tissue collection); SAM/SAMA (South Australian Museum); TG (Tony Gamble tissue collection); TNHC (Texas Natural History Collection); ZFMK (Zoologisches Forschungsmuseum Alexander Koenig)

The BI analysis was implemented in BEAST 1.8.2 [47], using a Yule tree prior and uncorrelated lognormal relaxed clock. Based on the PartitionFinder results, the data were divided into ten partitions employing six distinct models: ND2 position 1, ND2 position 2, and tRNAs used the GTR + I + Γ model. ND2 position 3 used the GTR + Γ model. RAG1 position 1 + 2 used the TrN (Tamura-Nei) + I + Γ model. RAG1 position 3 and KIF24 position 1 + 2 used the HKY (Hasegawa-Kishino-Yano) + Γ model. PDC position 3 and KIF24 position 3 used the K80 (Kishino 1980) + Γ model. PDC position 1 + 2 used the TrNef + I + Γ model. Four replicate analyses were run for 50 million generations, sampled every 1000 generations. The first 5 million generations were discarded as burn-in. Effective sample sizes were estimated in Tracer 1.5 (>300 for all parameters in each run) to confirm the chain length was adequate.

BEAST 1.8.2 was also used to estimate divergence times simultaneously with phylogenetic relationships. The root prior (Lepidosauria-Archosauria divergence) was given a normal distribution (mean = 275 Ma [million years ago], SD = 15) encompassing the range of estimates for this divergence [48, 49]. Five constraints were also applied to internal nodes: most recent common ancestor (MRCA) of *Phelsuma rosagularis* and *P. inexpectata* (uniform prior; 0–8 Ma; [37]). MRCA of sampled *Sphaerodactylus* – *S. ocoae*, *S. roosevelti*, and *S. torrei* (exponential prior; mean = 3, offset = 15 Ma; [50, 51]). MRCA of *Woodworthia maculata* and *Oedura marmorata* (exponential prior; mean = 17, offset = 16; [52]). MRCA of *Ophidocephalus taeniatus* and *Pygopus nigriceps* (exponential prior; mean = 10, offset = 20; [53, 54]). MRCA of *Teratoscincus roborowskii* and *T. scincus* (exponential prior; mean = 3, offset = 10; [55]).

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Trait data

Body size and habitat preference data were assigned to each species based on the authors' observations of specimens in the wild (62 of 66 ingroup species have been observed in-situ by the authors), supplemented by examination of vouchered museum specimens and information obtained from the literature [29, 56–58]. Maximum body size was treated in two ways depending on analysis. When possible, SVL (snout-vent length) was treated as a continuous character and the log-transformed maximum SVL was used. However, when treatment of size as a continuous character was not computationally feasible we instead treated size as a binary character. In *Pachydactylus* and related genera SVL is bimodal (Fig. 1). Those species with a maximum snout–vent length (SVL) < 70 mm comprised the "small" category, and those with a maximum SVL >75 mm comprised the "large" category. Habitat preference was divided into three categories. Those species that primarily shelter in burrows or under surface debris (logs, loose stones, aloe leaves, etc.), and forage actively on the ground, were classified as "terrestrial." Species that primarily shelter in rock cracks and forage on cliff faces or boulders were classified as "rupicolous." Finally, unspecialized species that both shelter and forage on a variety of surfaces (rock faces, tree trunks, buildings, etc.) were classified as "generalist climbers."
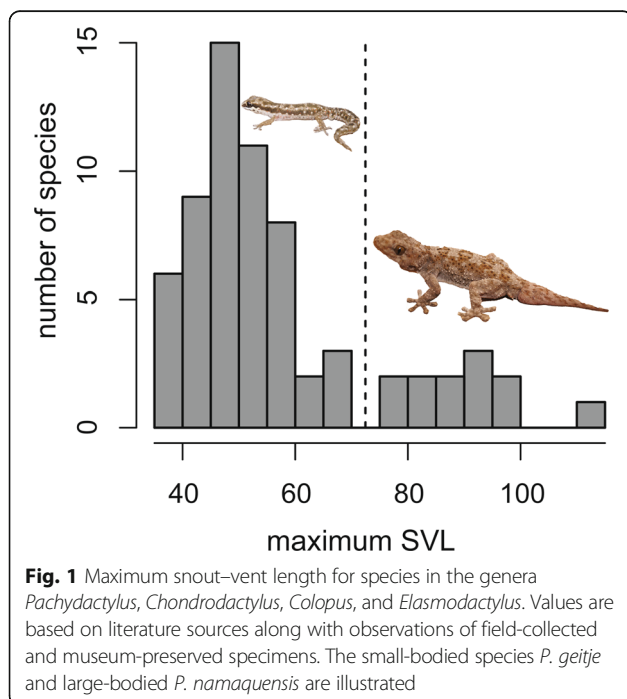
Range size estimates

Extent of occurrence (EOO) and area of occupancy (AOO) were defined as per the current International Union for the Conservation of Nature (IUCN) standards [1]. EOO was calculated as the area of the minimum convex polygon enclosing distribution records for each respective taxon. AOO was initially calculated as the sum of the total area of the quarter degree grid squares within which at least one record occurs. The final AOO was adjusted to an estimate of the actual suitable habitat within the occupied quarter degree squares based on the literature and the authors' field knowledge of each species. For all endemic South African species and for most species with a portion of their distribution occurring in South Africa, EOO and AOO values were previously estimated as part of the red list evaluation carried out in association with the Atlas and Red List of the Reptiles of South Africa, Lesotho and Swaziland [58]. Calculated EOO usually provides the broadest possible interpretation of the space used by a species, whereas the AOO represents a quite conservative estimate. However, for taxa known from single localities or several localities that are very close to one another, AOO as calculated above may yield a greater area than EOO. We used EOO or AOO, which ever was the greater, as our estimates of species' ranges. These values were log transformed when used in analyses.

Phylogenetic comparative methods

We performed a variety of comparative analyses to investigate the relationship among phylogeny, divergence times, trait data, and range sizes. All comparative analyses were completed in replicate on both the BEAST maximum clade-credibility tree and on 1000 post-burnin trees randomly sampled from the BEAST posterior distribution. These trees were pruned to remove outgroups (for which we have incomplete taxon sampling and no trait data). The package Phytools [59], implemented in R 3.2.2 [60] was used compute phylogenetic signal of range size using both the K and λ statistics [61, 62]. We also used phytools to plot lineages through time and test for constancy of lineage accumulation through time using the γ statistic of Pybus and Harvey [63].

State dependent diversification of range size on trait data (SVL or habitat) were tested using OUwie [64], which allows for tests of correlation between a multistate vs. continuous trait in a phylogenetic context. Because body size evolution and habitat choice may be coupled [65], we also tested for auto-correlation between these two traits, for a total of three analyses: (1) SVL vs. range size, (2) habitat vs. range size, and (3) SVL vs. habitat. SVL was treated as a multistate (binary) trait in test (1), but was treated as a continuous trait in test (3) to facilitate analysis. In all three cases, we tested the hypothesis that the optimum continuous trait value, θ, differed depending on the identity of the multistate trait value, i.e. whether large- and small-bodied species differ in range

Fig. 1 Maximum snout–vent length for species in the genera *Pachydactylus, Chondrodactylus, Colopus,* and *Elasmodactylus.* Values are based on literature sources along with observations of field-collected and museum-preserved specimens. The small-bodied species *P. geitje* and large-bodied *P. namaquensis* are illustrated

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size (test 1), whether species differing in habitat use differ in range size (test 2), or whether species differing in habitat use differ in body size (test 3). We performed these tests by estimating ancestral states of the multistate character on the phylogeny, and then fitting values of θ (trait optimum), α (pull toward optimum), and σ (rate of change of trait) for the continuous trait under two model regimes. The null Brownian Motion (BM) model regime estimated single values of θ, α, and σ that did not depend on the state of the multistate character. This was tested against a more complex Ornstein-Uhlenbeck (OU) model in which there were multiple θ parameters, one per multistate character state. We used ΔAICc (corrected Akaike Information Criterion) values to identify which model provided a better fit for the data. All three tests were performed on 1000 trees randomly sampled from the post-burnin BEAST posterior distribution, and on each of these 1000 trees we performed 100 ancestral reconstructions of the multistate trait using stochastic character mapping [66] implemented in phytools, resulting in a total of 100,000 model fits per test, each with a unique combination of phylogeny and ancestral state estimate.

We also estimated trait-associated rates of speciation (λ), extinction (μ), and transition rate (q) under a BiSSE [28] model for SVL data or a multiple-state speciation and extinction (MuSSE) [67] model for habitat data implemented in Diversitree. Because hypothesis testing in a BiSSE or MuSSE framework can have a high Type I error rate [68–70] and low statistical power when data sets contain fewer than several hundred terminal taxa [71], we refrain from explicitly testing the statistical significance of character-associated variation in model parameter estimates. Instead, we fit models in which each trait was given individual λ, μ, and q parameters strictly to determine estimates of these model parameters. Model fitting was performed in an Markov chain Monte Carlo (MCMC) framework with runs lasting 1100 generations and the first 100 discarded as burn-in. These estimates were obtained for each of 1000 trees randomly sampled from the BEAST posterior distribution, resulting in each parameter estimate being obtained from 1,000,000 observations.

Results

Phylogeny and divergence times

The phylogenies estimated in both the ML and BI analyses are very similar (Fig. 2), and most branches receive strong support. As expected, the grouping of *Pachydactylus*, *Chondrodactylus*, *Colopus*, and *Elasmodactylus* is monophyletic (BI/ML support values 1.0/97) and these are in turn most closely related to *Rhoptropus* (support values 100/1.0). Within the ingroup, the topology resembles that estimated by Bauer and Lamb [14], which

included 26 fewer ingroup taxa and was estimated from ~1,600 fewer nucleotide sites, but there are some notable differences. Most notably, both the genera *Colopus* and *Elasmodactylus* are recovered as non-monophyletic. One species of *Colopus*, *C. kochi*, is embedded in *Pachydactylus* and is most closely related to the *Pachydactylus mariquensis* group (support values 1.0/93), a set of four species represented by a single taxon in [14]. The other *Colopus* species, *C. wahlbergii*, is also embedded in *Pachydactylus*, but there is not strong support for any set of *Pachydactylus* species being its closest relatives (support values 0.76/54), although there is strong support for its association with *Pachydactylus* to the exclusion of *Chondrodactylus* and *Elasmodactylus* (100/1.0). The two *Elasmodactylus* species are outside a group containing all *Pachydactylus*, *Colopus*, and *Chondrodactylus* species, with *E. tuberculosus* being more closely related, but with poor support (0.37/46). Within *Pachydactylus*, recognized species groups [14, 15, 35, 72–74] are recovered as monophyletic with strong support as are many of the species-level relationships within these groups. However, within the speciose *serval/weberi* and northwestern groups, in which many new taxa have been added, species relationships are more highly modified. In the first of these, the basal division into reciprocally monophyletic *serval* and *weberi* groups is not supported, and the former makes the latter paraphyletic. Relationships among species groups in *Pachydactylus* remain unresolved, with most groups connected by exceptionally short internodes. There are two exceptions. The *serval/weberi* group and *capensis* group are closest relatives, as are the *geitje* and *rugosus* groups.

The divergences between *Rhoptropus* and *Pachydactylus* + *Chondrodactylus* + *Colopus* + *Elasmodactylus* occurred in the early Cenozoic (66–43 Ma). This is a similar pattern as observed in other gekkonids, in which relatively species-rich regional radiations undergo initial diversification in the early Cenozoic (e.g. [5, 37, 75]). The short internodes connecting *Pachydactylus* species groups are indicative of a relatively high diversification rate in the mid-Cenozoic ~30–35 Ma. The lineage through time (LTT) plot shows that the rate of lineage accumulation remains steady or slowly increases to this point after which there is a noticeable decline (Fig. 3). The overall trend is of significantly decreasing lineage accumulation through time (mean γ value = −5.8, p < 1 x 10^{-5} for all 1000 sampled trees).
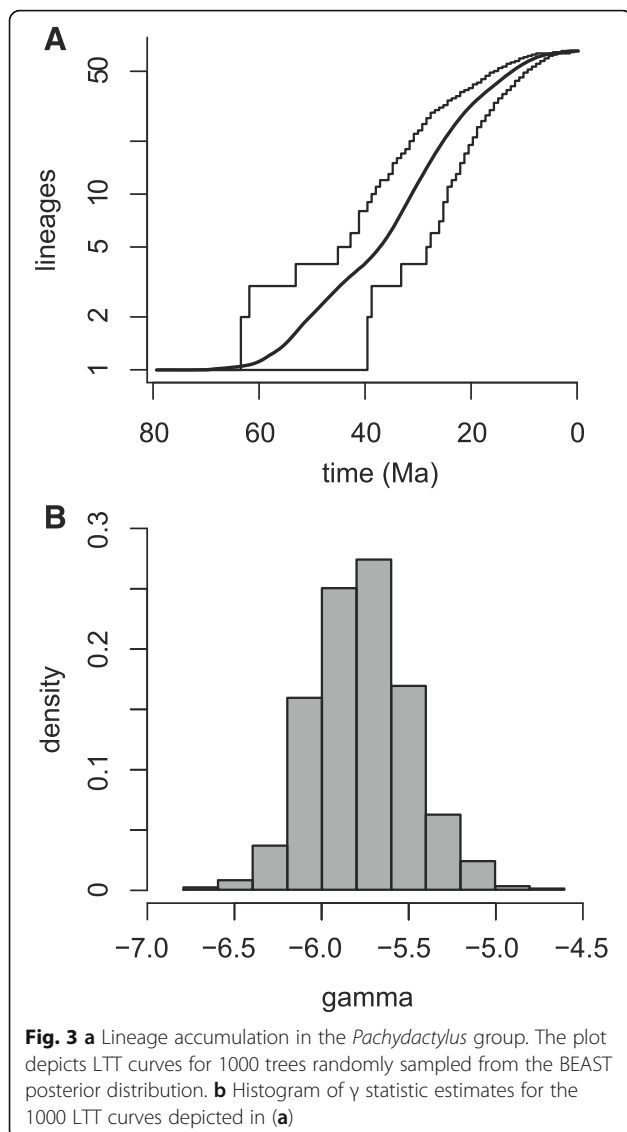
Comparative analyses

Ancestral reconstruction of body size in the *Pachydactylus* group suggests that being large-bodied is ancestral for the group (Fig. 4). A shift to small body size occurred once early in the evolutionary history of the group, and there have been only two reversals. Reconstruction of
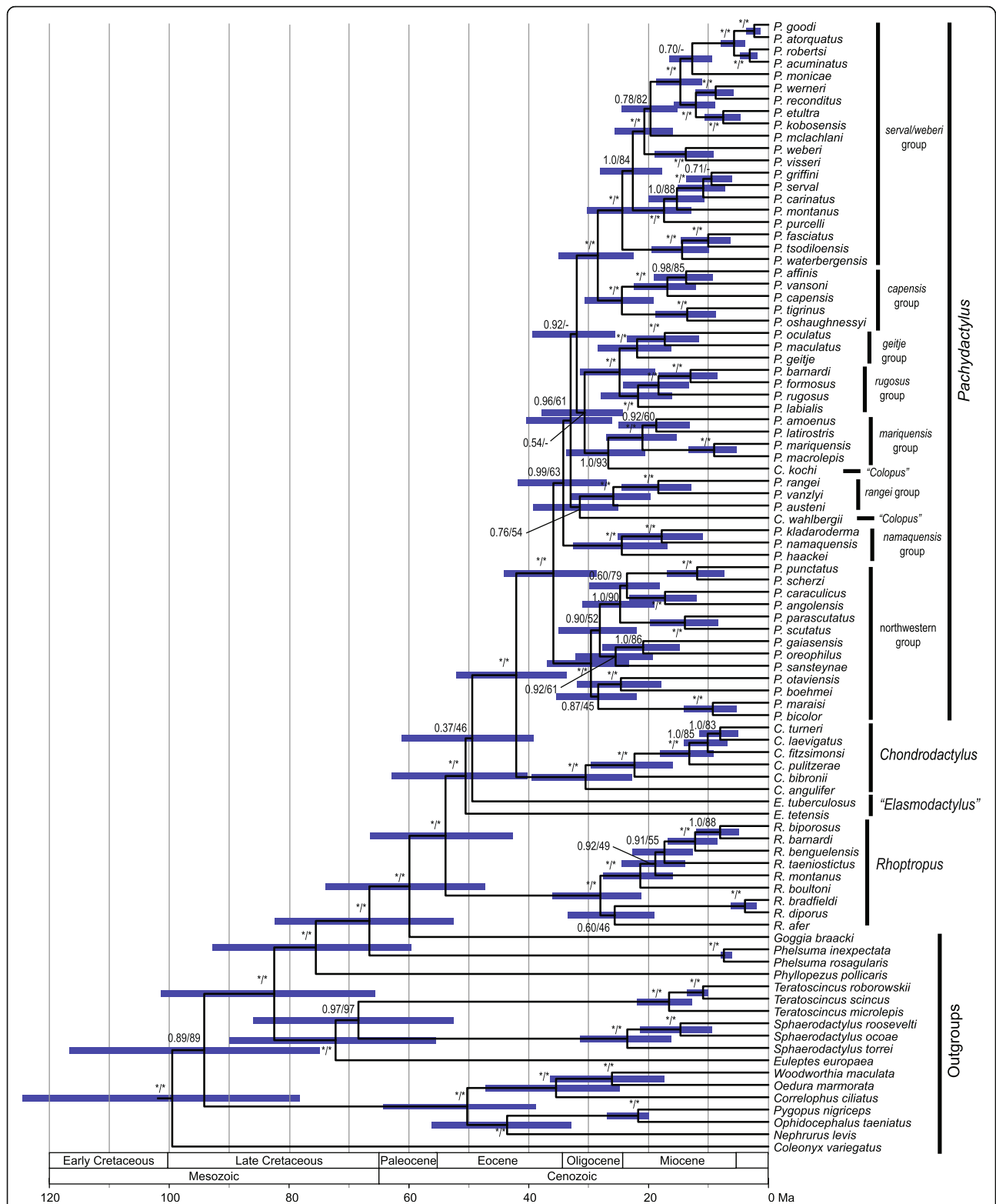
Fig. 2 Time-calibrated phylogeny of *Pachydactylus* and related genera. The topology is the maximum clade credibility tree estimated in BEAST with non-gekkotan outgroups cropped for clarity. Support values (Bayesian posterior probabilities/ML bootstrap) are given at nodes; asterisks indicate nodes with Bayesian support values = 1.0 and ML bootstrap values > 95. Named species groups and genera are given to the right. Geologic epochs and eras are indicated on the timescale; post-Miocene epochs (Pliocene, Pleistocene, Holocene) are not labeled

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Fig. 3 a Lineage accumulation in the *Pachydactylus* group. The plot depicts LTT curves for 1000 trees randomly sampled from the BEAST posterior distribution. **b** Histogram of γ statistic estimates for the 1000 LTT curves depicted in (**a**)

Pagel's λ (λ = 0.46, p = 0.13), but the estimate of K is slightly non-significant (K = 0.57, p = 0.077). The estimated global optimum range size (θ) for small-bodied species is $10^{4.5}$ km^2, approximately one order of magnitude smaller than large-bodied species ($\theta = 10^{5.4}$ km^2). When comparing habitat preference, rock-dwelling species have the smallest estimated global optimum extent of occurrence ($\theta = 10^{4.1}$ km^2), followed by terrestrial species ($\theta = 10^{5.1}$ km^2) with generalized climbers having the largest geographic ranges ($\theta = 10^{5.8}$ km^2). Trait-associated estimates of speciation and extinction rates are less variable (Fig. 6). Small-bodied species are estimated to have slightly higher speciation (mean λ[small-bodied] = 0.055; mean λ[large-bodied] = 0.040) and lower extinction rates, but there is extensive overlap. Habitat-associated estimates of diversification rate also overlap, especially between terrestrial species and generalized climbers, although rock-dwelling species are estimated to have speciated at somewhat higher rates (mean λ[generalized climber] = 0.032; mean λ[terrestrial] = 0.012; mean λ[rock-dwelling] = 0.065).
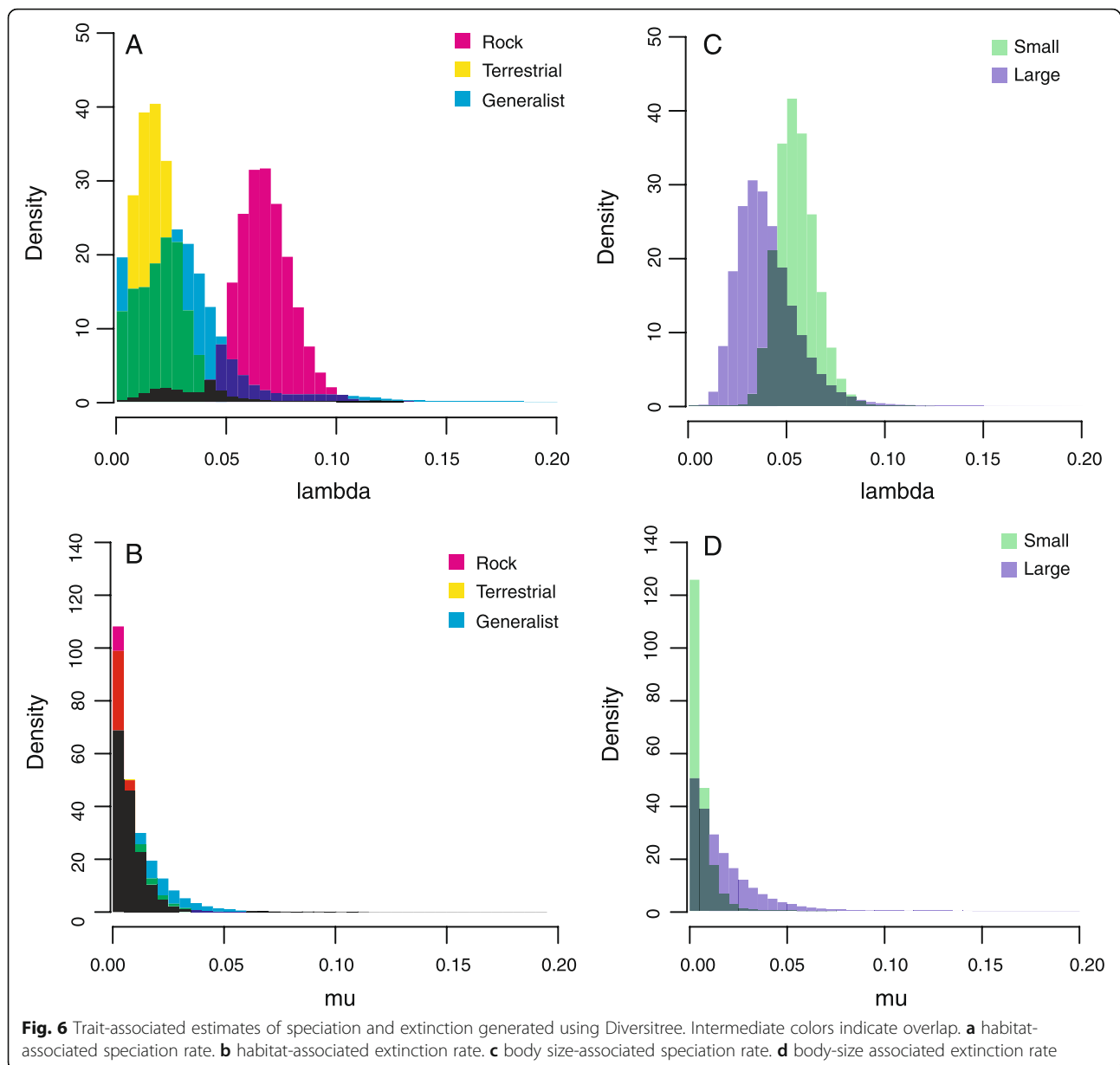
Discussion

While the heritability of range size has been demonstrated for many lineages, possible mechanistic explanations have varied, and include niche breadth [27], dispersal ability [76, 77], and morphological characteristics [26, 78] of lineages, as well as the geographic limits of biomes, landmasses, or hydrological basins [79]. In many cases, these factors may be interlinked. In this study, we focus on two traits, body size and habitat requirements, that were expected to affect dispersal ability either directly because smaller organisms, including some lizards, may disperse shorter distances [80], or indirectly, because habitat patchiness can restrict dispersal if appropriate dispersal corridors are not available [81]. As expected, within the *Pachydactylus* group the smaller-bodied species occupying more patchily distributed habitats are the species with the smallest geographic ranges. Other studies that have measured dispersal ability directly have shown that reduced dispersal ability does not always lead to reduced range size [77], but in *Pachydactylus* and its relatives our data suggest that dispersal ability and range size are correlated. Traits affecting dispersal ability are likely not the only factors affecting range size, however. Minimally, it is likely that geographic barriers, including major river systems and mountain ranges, also play a significant role in restricting the ranges occupied by individual species. For example, the species *P. austeni* and *P. goodi* are known only from south of the Orange River even though suitable habitats for each of these species also exist to the north [58].

habitat preference is more equivocal, but the common ancestor of the group is most commonly reconstructed as a generalized climber (in 80% of reconstructions). What is clear is that more shifts in habitat preference have occurred than shifts in body size, with approximately 26 transitions indicated in total, most commonly between rock-dwelling and terrestrial habitat preferences. Although both habitat and body size are estimated to have shifted multiple times, including reversals, correlation between the two traits is not particularly strong based on fits of BM and OU models — out of 100,000 model fits, the BM model incorporating only a single global SVL optimum was favored according to the AIC 31% of the time (Fig. 5a).

In contrast, both body size and habitat preference are strongly correlated with range size (Fig. 5b, c). Range size displays significant phylogenetic signal based on

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Fig. 4 Ancestral states for body size and habitat preference, based on 100 stochastic character maps for each trait on the maximum clade credibility tree. Range size values for each species are given to the right of each terminal branch

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Fig. 5 Histogram of AICc values for model fits of Brownian motion (BM) and Ornstein-Uhlenbeck (OU) models of trait diversification estimated in OUwie. **a** habitat preference vs. SVL. **b** habitat preference vs. range size. **c** SVL vs. range size

Taken as a whole, the observed patterns of trait evolution, range size, and diversification are consistent with an evolutionary scenario in which diversification has been dominated by geographic isolation followed by allopatric speciation. Based on our analyses, we suggest that geographic isolation has developed more easily in *Pachydactylus* + *Colopus* than it has in *Elasmodactylus* or *Chondrodactylus*, at least partly as a result of *Pachydactylus* + *Colopus* species being more likely to have traits promoting this isolation. Ancestral species in the *Pachydactylus* group as a whole were most likely large-bodied habitat generalists, and most *Chondrodactylus* and *Elasmodactylus* species have retained these traits to the present. We infer small body size and habitat specialization (for either terrestrial or rock-dwelling lifestyles) to appear in the common ancestor of *Pachydactylus* + *Colopus*, coincident with a brief observed increase in the rate of lineage accumulation in the *Pachydactylus* group, followed by a general decline in diversification rate measured across the *Pachydactylus* group as a whole. Rock-dwelling species especially differ strikingly in range size and diversification rate, having extents of occurrence two orders of magnitude smaller than habitat generalists and estimated rates of diversification 2–4X higher than other species. Allopatric speciation of isolated small-bodied, rock-dwelling lineages therefore can account for much of the observed taxonomic diversity in the *Pachydactylus* group. Not surprisingly, the subclades that have retained ancestral traits (*Chondrodactylus* and *Elasmodactylus*) are much less species-rich than those that have not.

The overall decline in diversification rate through time that we observe in the *Pachydactylus* group is similar to patterns documented in many lineages that are often attributed to reduced ecological opportunity through time as niches are filled (e.g. [82–85]). In the case of the *Pachydactylus* group, a general pattern of morphological conservatism within species groups, exemplified by the small number of shifts in body size (Fig. 2) and digital morphology [4, 5] through time is in line with expectations if ecological opportunity has decreased through time. However, shifts in habitat use are more frequent, and the number of co-occurring *Pachydactylus* group species varies from 1 to 13, suggesting that ecological niche space has not been exhausted. An alternative explanation that may also partly explain the observed rate slowdown is a geographic model as described above. In clades dominated by allopatric speciation, diversification rates may decline as vicariance events affect fewer species as species' geographic ranges decline through time [86, 87]. The relatively low species diversity of *Chondrodactylus*, which includes mostly large-bodied habitat generalists (i.e., species with large geographic ranges),

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Fig. 6 Trait-associated estimates of speciation and extinction generated using Diversitree. Intermediate colors indicate overlap. **a** habitat-associated speciation rate. **b** habitat-associated extinction rate. **c** body size-associated speciation rate. **d** body-size associated extinction rate

compared to *Pachydactylus*, which includes mostly small-bodied habitat specialists, supports this model.

As indicated above, a jump in lineage accumulation coincident with the appearance of habitat-specialist clades in the mid-Cenozoic ~30–35 Ma is contrary to the general pattern of declining diversification rate through time. It is possible that climatic or geomorphic processes active at this time were especially favorable for isolating lineages, resulting in increased speciation. Major periods of tectonic uplift in eastern and southern Africa did not commence until approximately the Oligocene-Miocene boundary (23 Ma) [88–90], making large-scale geomorphological change incompatible with the observed rate

increase. However, a major climatic regime shift did occur at the Eocene-Oligocene boundary: a global cooling associated with Antarctic glaciation [91, 92]. In Africa, this shift resulted in aridification and greater environmental heterogeneity, including reduction in forest cover [93, 94], and would have greatly increased the available habitat for arid-adapted *Pachydactylus* group geckos, potentially facilitating rapid radiation. A similar pattern occurs in forest-adapted chameleons, where rapid radiation is coincident with wide availability of suitable habitat, in the case of chameleons during the Eocene [95].

In performing this study, we have attempted to minimize confounding factors and methological biases.

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For example, we collected data for nearly all target taxa and integrated all comparative analyses across a sample of 1000 credible trees to avoid sampling or phylogenetic biases. Even so, our interpretations should be treated cautiously. The observed relationships between trait data and geographic range extent are based on correlation. While we chose to focus on body size and habitat use specifically because we expected them to affect geographic range, it is possible that one or more other factors co-varying with body size and habitat use are the actual drivers of range size variation among species. Trait-associated measurements of diversification rate utilized the BiSSE model. Even though our ingroup phylogeny was comprehensive, the number of taxa in our data set may not have been large enough to avoid inadequacies of the model [69, 71], which is why we refrain from ascribing statistical significance to these results. Alternate methods of trait-dependant diversification (e.g. [70]) likewise are best suited to larger data sets. One possible way to increase data set size is to incorporate taxa from across the Afro-Malagasy clade of geckos, but the interrelationships of genera within this large radiation are still poorly resolved and relevant trait data are missing for many species. Finally, range size estimates are based on known collection localities and a correct interpretation of species-level taxonomy in the group. Collecting effort varies greatly by country, with, for example, less than 10,000 amphibian and reptile collection records in Angola, 38,000 in Namibia, and >100,000 in South Africa [58, 96]. Some species also vary phenotypically and have named subspecies that further study may reveal to warrant specific status (e.g., *Pachydactylus punctatus*; [97]). However, given that trait-associations with range size varied by orders of magnitude, we do not expect refinement of species' range limits or taxonomy to strongly influence our results.

Beyond interpretation of evolutionary patterns, the results of this study also have significant implications for taxonomy and conservation. The phylogenetic results indicate that *Elasmodactylus* and *Colopus* are not monophyletic, and both species of *Colopus* are nested in *Pachydactylus*. Although we recover *Elasmodactylus* as non-monophyletic, its monophyly cannot be wholly discounted given the poor support for the node joining *E. tuberculosus* with *Chondrodactylus* + *Colopus* + *Pachydactylus*. Performing a Shimodaira-Hasegawa (SH) test also shows that the likelihood of our best-scoring tree (lnL −97035.168404) is not significantly higher than the likelihood of a tree in which *Elasmodactylus* is constrained to be monophyletic (lnL −97036.513963; *p* > 0.05). These species also share morphological traits rare or absent in other *Pachydactylus* group species, including preanal pores and easily broken skin [14]. Thus, we suggest that taxonomic decisions regarding these species

be delayed until each species' phylogenetic position is better established. We refer both *Colopus* species to *Pachydactylus*. *Colopus wahlbergii* is morphologically divergent and in our analyses its position in *Pachydactylus* is equivocal, with moderate support for an association with the *rangei* group. Thus, we refer it to no species group within *Pachydactylus*. In contrast, *Colophs kochi* is deeply nested in *Pachydactylus*, and there is strong support for its placement as closely related to the *P. mariquensis* group. This species was also included in *Pachydactylus* until recently [14]. We therefore advise that *C. kochi* be re-assigned to the *mariquensis* group within *Pachydactylus*.

One important determinant of rarity is range size, and small range size is a key predictor of extinction risk [98, 99]. Thus, species in the *Pachydactylus* group inheriting traits promoting smaller ranges also inherit traits promoting greater rarity. However, our analyses show these same traits to be associated with higher rates of diversification. Given the difficulty in estimating extinction rates from phylogenies [100], it is unclear if this higher diversification rate is observed despite a higher extinction rate, or if extinction rates do not depend on the measured traits in the *Pachydactylus* group. Notwithstanding this difficulty, these results stress the importance of defining a frame of reference when measuring evolutionary "success." In the case of the *Pachydactylus* group, more widespread, common species tend to belong to relatively species-poor subclades.

Conclusions

The relationships among morphological and ecological traits, range size, and diversification that we observe in the *Pachydactylus* group points to a history of geographic isolation contributing significantly to the group's species richness compared to other African geckos. Even so, some aspects of diversification in the *Pachydactylus* group, including early evolution of divergent traits within the group, are consistent with patterns observed in classic adaptive radiations. In this sense, the process of diversification of *Pachydactylus* group geckos may be considered intermediate between a true adaptive radiation on one hand and a non-adaptive radiation (as observed in plethodontid salamanders; [101, 102]) on the other. It is likely that many other species-rich groups share this same intermediate pattern.

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speciation and extinction; ND2: NADH dehydrogenase subunit 2; OU: Ornstein-Uhlenbeck; PCR: Polymerase chain reaction; PDC: Phosducin; RAG1: Recombination activating gene 1; SH: Shimodaira-Hasegawa; SVL: Snout-vent length; TrN: Tamura-Nei model; tRNA: Transfer RNA

Availability of data and material
The data sets supporting the results of this article are available in the GenBank repository, [accession numbers KY224166–KY224347 in http://www.ncbi.nlm.nih.gov/genbank/].

Authors' contributions
MPH and AMB designed the project. Data were collected by MPH, TRJ, and AMB. Data analysis was performed by MPH. MPH wrote the paper with input from TRJ and AMB. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
Not applicable.

Ethics approval and consent to participate
Use of animals to obtain tissue samples for genetic analysis was reviewed and approved by the Villanova University Institutional Animal Care and Use Committee. Field work in southern Africa that generated the tissues used in this work was made possible by permits (1987–2014) to AMB from the relevant wildlife authorities of South Africa, Namibia and Zimbabwe.

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References
1. IUCN. The IUCN Red List of Threatened Species. Version 2015–4. http://www.iucnredlist.org. Accessed 20 Mar 2016.
2. Froese R, Pauly D. FishBase, version (10/2015). http://www.fishbase.org. Accessed 20 Mar 2016.
3. Hedges, SB. CaribHerp: West Indian Amphibians and Reptiles. http://www.caribherp.org. Accessed 20 Mar 2016.
4. Lamb T, Bauer AM. Footprints in the sand: independent reduction of subdigital lamellae in the Namib–Kalahari burrowing geckos. Proc R Soc Lond B Biol Sci. 2006;273:855–64.
5. Gamble T, Greenbaum E, Jackman TR, Russell AP, Bauer AM. Repeated origin and loss of adhesive toepads in geckos. PLoS One. 2012;7:e39429.
6. Bauer AM, Branch WR. The herpetofauna of the Richtersveld national park and the adjacent northern Richtersveld, Northern cape Province, Republic of South Africa. Herpetological Nat Hist. 2001;8:111–60.
7. Schluter D. The ecology of adaptive radiation. Oxford: Oxford University Press; 2000.
8. Losos JB, Mahler DL. Adaptive radiation: the interaction of ecological opportunity, adaptation, and speciation. In: Bell MA, Futuyma DJ, Eanes WF, Levinton JS, editors. Evolution since Darwin: the first 150 years. Sunderland: Sinauer; 2010. p. 381–420.
9. Alfaro ME, Brock CD, Banbury BL, Wainwright PC. Does evolutionary innovation in pharyngeal jaws lead to rapid lineage diversification in labrid fishes? BMC Evol Biol. 2009;9:1.
10. Rutschmann S, Matschiner M, Damerau M, Muschick M, Lehmann MF, Hanel R, Salzburger W. Parallel ecological diversification in Antarctic notothenioid fishes as evidence for adaptive radiation. Mol Ecol. 2011;20:4707–21.
11. Deutsch JC. Colour diversification in Malawi cichlids: evidence for adaptation, reinforcement or sexual selection? Biol J Linn Soc. 1997;62:1–4.
12. Masta SE, Maddison WP. Sexual selection driving diversification in jumping spiders. Proc Natl Acad Sci. 2002;99:4442–7.
13. Wiens JJ, Graham CH, Moen DS, Smith SA, Reeder TW. Evolutionary and ecological causes of the latitudinal diversity gradient in hylid frogs: treefrog trees unearth the roots of high tropical diversity. Am Nat. 2006;168:579–96.
14. Bauer AM, Lamb T. Phylogenetic relationships of southern African geckos in the *Pachydactylus* group (Squamata: Gekkonidae). Afr J Herpetol. 2005;54:105–29.
15. Bauer AM, Heinicke MP, Jackman TR, Branch WR. Systematics of the *Pachydactylus mariquensis* group of geckos (Reptilia: Squamata: Gekkonidae): Status of *P. mariquensis latirostris*, *P. m. macrolepis* and *P. amoenus*. Navorsinge van die Nasionale Museum Bloemfontein. 2011;27:85–107.
16. Arnold EN. Historical changes in the ecology and behaviour of semaphore geckos (*Pristurus*, Gekkonidae) and their relatives. J Zool. 1993;229:353–84.
17. Ikeuchi I, Mori A, Hasegawa M. Natural history of *Phelsuma madagascariensis kochi* from a dry forest in Madagascar. Amphibia-Reptilia. 2005;26:475–83.
18. Regalado R. Does dichromatism variation affect sex recognition in dwarf geckos? Ethol Ecol Evol. 2015;27:56–73.
19. Coyne JA. Genetics and speciation. Nature. 1992;355:511–5.
20. Coyne JA, Orr HA. Speciation. Sunderland: Sinauer Associates; 2004.
21. Jablonski D. Heritability at the species level: analysis of geographic ranges of Cretaceous mollusks. Science. 1987;238:360–3.
22. Gaston KJ. Species-range size distributions: products of speciation, extinction and transformation. Philos Transact A Math Phys Eng Sci. 1998;353:219–30.
23. Webb TJ, Gaston KJ. On the heritability of geographic range sizes. Am Nat. 2003;161:553–66.
24. Waldron A. Null models of geographic range size evolution reaffirm its heritability. Am Nat. 2007;170:221–31.
25. Vamosi SM, Vamosi JC. Perspective: Causes and consequences of range size variation: the influence of traits, speciation, and extinction. Front Biogeography. 2012;4:168–77.
26. Lee MS, Skinner A, Camacho A. The relationship between limb reduction, body elongation and geographical range in lizards (*Lerista*, Scincidae). J Biogeogr. 2013;40:1290–7.
27. Slatyer RA, Hirst M, Sexton JP. Niche breadth predicts geographical range size: a general ecological pattern. Ecol Lett. 2013;16:1104–14.
28. Maddison WP, Midford PE, Otto SP. Estimating a binary character's effect on speciation and extinction. Syst Biol. 2007;56:701–10.
29. Branch WR. Field guide to snakes and other reptiles of southern Africa. 3rd ed. Struik: Cape Town; 1998.
30. Gaston KJ, Blackburn TM. Range size-body size relationships: evidence of scale dependence. Oikos. 1996;479–85.
31. Lancaster N. Late Quaternary paleoenvironments in the southwestern Kalahari. Palaeogeogr Palaeoclimatol Palaeoecol. 1989;70:367–76.
32. Partridge TC. The evidence for Cainozoic aridification in southern Africa. Quat Int. 1993;17:105–10.
33. Thomas DS, Shaw PA. The evolution and characteristics of the Kalahari, southern Africa. J Arid Environ. 1993;25:97–108.
34. Bauer AM. Evolutionary scenarios in the *Pachydactylus* group geckos of southern Africa: new hypotheses. Afr J Herpetol. 1999;48:53–62.
35. Lamb T, Bauer AM. Phylogenetic relationships of the large-bodied members of the African lizard genus *Pachydactylus* (Reptilia: Gekkonidae). Copeia. 2002;2002:586–96.
36. Bauer AM. Phylogeny and biogeography of the geckos of southern Africa and the islands of the western Indian Ocean: a preliminary analysis. In: Peters G, Hutterer R, editors. Vertebrates in the Tropics. Bonn: Zoologisches Forschungsinstitut und Museum A. Koenig; 1990. p. 274–84.
37. Heinicke MP, Greenbaum E, Jackman TR, Bauer AM. Phylogeny of a trans-Wallacean radiation (Squamata, Gekkonidae, *Gehyra*) supports a single early colonization of Australia. Zool Scr. 2011;40:584–602.
38. Heinicke MP, Greenbaum E, Jackman TR, Bauer AM. Evolution of gliding in Southeast Asian geckos and other vertebrates is temporally congruent with dipterocarp forest development. Biol Lett. 2012;8:994–7.

Heinicke *et al. BMC Evolutionary Biology* (2017) 17:9

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39. Heinicke MP, Daza JD, Greenbaum E, Jackman TR, Bauer AM. Phylogeny, taxonomy and biogeography of a circum-Indian Ocean clade of leaf-toed geckos (Reptilia: Gekkota), with a description of two new genera. Syst Biodivers. 2014;12:23–42.

40. Portik DM, Bauer AM, Jackman TR. The phylogenetic affinities of *Trachylepis sulcata nigra* and the intraspecific evolution of coastal melanism in the western rock skink. Afr Zool. 2010;45:147–59.

41. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95–8.

42. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28:1647–9.

43. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23:2947–8.

44. Laslett D, Canbäck B. ARWEN, a program to detect tRNA genes in metazoan mitochondrial nucleotide sequences. Bioinformatics. 2008;24:172–5.

45. Lanfear R, Calcott B, Ho SY, Guindon S. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. Mol Biol Evol. 2012;29:1695–701.

46. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3.

47. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012;29:1969–73.

48. Kumar S, Hedges SB. A molecular timescale for vertebrate evolution. Nature. 1998;392:917–20.

49. Reisz RR, Müller J. Molecular timescales and the fossil record: a paleontological perspective. TRENDS Genet. 2004;20:237–41.

50. Kluge AG. Cladistic relationships of sphaerodactyl lizards. Am Mus Novit. 1995;3139:1–23.

51. Iturralde-Vinent MA, MacPhee RD. Age and paleogeographical origin of Dominican amber. Science. 1996;273:1850.

52. Lee MS, Hutchinson MN, Worthy TH, Archer M, Tennyson AJ, Worthy JP, Scofield RP. Miocene skinks and geckos reveal long-term conservatism of New Zealand's lizard fauna. Biol Lett. 2009;5:833–7.

53. Hutchinson MN. The first fossil pygopodid (Squamata, Gekkota) and a review of mandibular variation in living species. Memoirs Queensland Museum. 1997;41:355–66.

54. Lee MS, Oliver PM, Hutchinson MN. Phylogenetic uncertainty and molecular clock calibrations: a case study of legless lizards (Pygopodidae, Gekkota). Mol Phylogenet Evol. 2009;50:661–6.

55. Macey JR, Wang Y, Ananjeva NB, Larson A, Papenfuss TJ. Vicariant patterns of fragmentation among gekkonid lizards of the genus *Teratoscincus* produced by the Indian collision: a molecular phylogenetic perspective and an area cladogram for Central Asia. Mol Phylogenet Evol. 1999;12:320–32.

56. Loveridge A. Revision of the African lizards of the family Gekkonidae. Bull Mus Comp Zool. 1947;98:1–469.

57. Alexander GJ, Marais J. A guide to the reptiles of southern Africa. Cape Town: Struik; 2007.

58. Bates MF, Branch WR, Bauer AM, Burger M, Marais J, Alexander GJ, De Villiers MS (eds). Atlas and red list of the reptiles of South Africa, Lesotho and Swaziland. Pretoria: South African National Biodiversity Institute; 2014.

59. Revell LJ. Phytools: an R package for phylogenetic comparative biology (and other things). Methods Ecol Evol. 2012;3:217–23.

60. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2015.

61. Blomberg SP, Garland T, Ives AR. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution. 2003;57:717–45.

62. Pagel M. Inferring the historical patterns of biological evolution. Nature. 1999;401:877–84.

63. Pybus OG, Harvey PH. Testing macro–evolutionary models using incomplete molecular phylogenies. Proc R Soc Lond B Biol Sci. 2000;267:2267–72.

64. Beaulieu JM, Jhwueng DC, Boettiger C, O'Meara BC. Modeling stabilizing selection: expanding the Ornstein–Uhlenbeck model of adaptive evolution. Evolution. 2012;66:2369–83.

65. Collar DC, Schulte II, James A, Losos JB. Evolution of extreme body size disparity in monitor lizards (*Varanus*). Evolution. 2011;65:2664–80.

66. Huelsenbeck JP, Nielsen R, Bollback JP. Stochastic mapping of morphological characters. Syst Biol. 2003;52:131–58.

67. FitzJohn RG. Quantitative traits and diversification. Syst Biol. 2010;59:619–33.

68. FitzJohn RG. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol Evol. 2012;3:1084–92.

69. Rabosky DL, Goldberg EE. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst Biol. 2015;64:340–55.

70. Rabosky DL, Huang H. A robust semi-parametric test for detecting trait-dependent diversification. Syst Biol. 2016;65:181–93.

71. Davis MP, Midford PE, Maddison W. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. BMC Evol Biol. 2013;13:1.

72. Bauer AM, Lamb T. Phylogenetic relationships among members of the *Pachydactylus capensis* group of southern African geckos. Afr Zool. 2002;37: 209–20.

73. Bauer AM, Lamb T, Branch WR. A revision of the *Pachydactylus serval* and *P. weberi* groups (Reptilia: Gekkota: Gekkonidae) of Southern Africa, and with the description of eight new species. Proc Calif Acad Sci. 2006;57:595–709.

74. Lamb T, Bauer AM. Relationships of the *Pachydactylus rugosus* group of geckos (Reptilia: Squamata: Gekkonidae). Afr Zool. 2000;35:55–67.

75. Wood PL, Heinicke MP, Jackman TR, Bauer AM. Phylogeny of bent-toed geckos (*Cyrtodactylus*) reveals a west to east pattern of diversification. Mol Phylogenet Evol. 2012;65:992–1003.

76. Böhning-Gaese K, Caprano T, van Ewijk K, Veith M. Range size: disentangling current traits and phylogenetic and biogeographic factors. Am Nat. 2006; 167:555–67.

77. Lester SE, Ruttenberg BI, Gaines SD, Kinlan BP. The relationship between dispersal ability and geographic range size. Ecol Lett. 2007;10:745–58.

78. Olifiers N, Vieira MV, Grelle CE. Geographic range and body size in Neotropical marsupials. Glob Ecol Biogeogr. 2004;13:439–44.

79. Machac A, Zrzavý J, Storch D. Range size heritability in Carnivora is driven by geographic constraints. Am Nat. 2011;177:767–79.

80. Sinervo B, Calsbeek R, Comendant T, Both C, Adamopoulou C, Clobert J. Genetic and maternal determinants of effective dispersal: the effect of sire genotype and size at birth in side-blotched lizards. Am Nat. 2006;168:88–99.

81. Beier P, Noss RF. Do habitat corridors provide connectivity? Conserv Biol. 1998;12:1241–52.

82. Rabosky DL, Lovette IJ. Density-dependent diversification in North American wood warblers. Proc R Soc Lond B Biol Sci. 2008;275:2363–71.

83. Gavrilets S, Losos JB. Adaptive radiation: contrasting theory with data. Science. 2009;323:732–7.

84. Burbrink FT, Pyron RA. How does ecological opportunity influence rates of speciation, extinction, and morphological diversification in New World ratsnakes (tribe Lampropeltini)? Evolution. 2010;64:934–43.

85. Mahler DL, Revell LJ, Glor RE, Losos JB. Ecological opportunity and the rate of morphological evolution in the diversification of Greater Antillean anoles. Evolution. 2010;64:2731–45.

86. Pigot AL, Phillimore AB, Owens IP, Orme CD. The shape and temporal dynamics of phylogenetic trees arising from geographic speciation. Syst Biol. 2010;59:660–73.

87. Moen D, Morlon H. Why does diversification slow down? Trends Ecol Evol. 2014;29:190–7.

88. Partridge TC. Of diamonds, dinosaurs and diastrophism: 150 million years of landscape evolution in southern Africa. S Afr J Geol. 1998;101:167–84.

89. Sepulchre P, Ramstein G, Fluteau F, Schuster M, Tiercelin J, Brunet M. Tectonic uplift and eastern Africa aridification. Science. 2006;313:1419–23.

90. Cowling RM, Proches S, Partridge TC. Explaining the uniqueness of the Cape flora: incorporating geomorphic evolution as a factor for explaining its diversification. Mol Phylogenet Evol. 2009;51:64–74.

91. Zachos J, Pagani M, Sloan L, Thomas E, Billups K. Trends, rhythms, and aberrations in global climate 65 Ma to present. Science. 2001;292:686–93.

92. Liu Z, Pagani M, Zinniker D, DeConto R, Huber M, Brinkhuis H, Shah SR, Leckie RM, Pearson A. Global cooling during the Eocene-Oligocene climate transition. Science. 2009;323:1187–90.

93. Davis CC, Bell CD, Fritsch PW, Mathews S. Phylogeny of *Acridocarpus-Brachylophon* (Malpighiaceae): implications for Tertiary tropical floras and Afroasian biogeography. Evolution. 2002;56:2395–405.

94. Kissling WD, Eiserhardt WL, Baker WJ, Borchsenuis F, Couvreur TLP, Balslev H, Svenning J. Cenozoic imprints on the phylogenetic structure of palm species assemblages worldwide. Proc Natl Acad Sci. 2012;109:7379–84.

95. Tolley KA, Townsend TM, Vences M. Large-scale phylogeny of chameleons suggests African origins and Eocene diversification. Proc R Soc B. 2013;280: 20130184.

Heinicke *et al. BMC Evolutionary Biology* (2017) 17:9

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96. JRS Biodiversity Foundation grant. Digitizing southwestern-African herpetological collections. http://jrsbiodiversity.org/grants/university-of-florida/. Accessed 1 Aug 2016.

97. Hewitt J. Some new forms of batrachians and reptiles from South Africa. Rec Albany Mus. 1935;4:283–357.

98. Gaston KJ. Rarity. London: Chapman & Hall; 1994.

99. Harris G, Pimm SL. Range size and extinction risk in forest birds. Conserv Biol. 2008;22:163–71.

100. Rabosky DL. Extinction rates should not be estimated from molecular phylogenies. Evolution. 2010;64:1816–24.

101. Kozak KH, Wiens J. Does niche conservatism promote speciation? A case study in North American salamanders. Evolution. 2006;60:2604–21.

102. Kozak KH, Weisrock DW, Larson A. Rapid lineage accumulation in a non-adaptive radiation: phylogenetic analysis of diversification rates in eastern North American woodland salamanders (Plethodontidae: *Plethodon*). Proc R Soc Lond B Biol Sci. 2006;273:539–46.