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[1]*Central Research Laboratories, Museum of Natural History Vienna, Vienna Austria;* [2]*Department of Intagrative Zoology, University of Vienna, Vienna Austria;* [3]*Museum für Naturkunde – Leibniz Institute for Research on Evolution and Biodiversity, Berlin Germany*

Multiple nuclear and mitochondrial DNA sequences provide new insights into the phylogeny of South African Lacertids (Lacertidae, Eremiadinae)

Anja Engleder[1,2], Elisabeth Haring[1,2], Sebastian Kirchhof[3] and Werner Mayer[1]

Abstract

Eremiadinae, one of three subfamilies of Lacertidae, are distributed throughout Asia and Africa. Previous phylogenetic studies suggested that one of the main groups of Eremiadinae (the Ethiopian clade) consist of two clades with predominately East-African and South-African distribution. Yet, especially the latter one, which includes the genera *Pedioplanis, Meroles, Ichnotropis, Tropidosaura* and *Australolacerta*, was not well supported in the molecular phylogenetic analysis. In this study, we analysed the phylogenetic relationships among the genera of the 'South African clade' to assess whether this group actually forms a highly supported clade and to address questions concerning the monophyly of the genera. We sequenced sections of the widely used mitochondrial genes coding for *16S rRNA*, *12S rRNA* and *cytochrome b* (altogether 2045 bp) as well as the nuclear genes *c-mos, RAG-1*, *PRLR*, *KIF24*, *EXPH5* and *RAG-2* (altogether 4473 bp). The combined data set increased the support values for several nodes considerably. Yet, the relationships among five major lineages within the 'South African clade' are not clearly resolved even with this large data set. We interpret this as a 'hard polytomy' due to fast radiation within the South African lacertids. The combined tree based on nine marker genes provides strong support for the 'South African Clade' and its sister group relationship with the 'East African Clade'. Our results confirm the genus *Tropidosaura* as a monophylum, while *Ichnotropis* is paraphyletic in our trees: *Ichnotropis squamulosa* appears more closely related to *Meroles* than to *Ichnotropis capensis*. Furthermore, the monophyly of *Meroles* is questionable as well. Based on our results, *I. squamulosa* should be transferred from *Ichnotropis* into the genus *Meroles.* Also, the two species of *Australolacerta* (*A. australis* and *A. rupicola*) are very distantly related and the genus is perhaps paraphyletic, too. Finally we propose a phylogeographical scenario in the context of palaeoclimatic data and compare it with a previously postulated hypothesis.

Key words: Lacertidae – Eremiadinae – 'South African clade' – phylogeny – palaeoclimate – taxonomy

Introduction

Lacertid lizards with more than 280 species (Arnold et al. 2007, the most recent list can be found at the webpage www.lacerta.de) represent one of the most prominent reptile groups in the Mediterranean region as well as in some regions of Africa and Asia. Boulenger's (1920, 1921) systematics of this family based on morphological traits remained nearly unchanged until Arnold's (1989) revision. More recently, investigations of molecular features led to a better insight into the phylogeny of Lacertidae. After albumin-immunological studies (Mayer and Benyr 1994 and references therein) Harris et al. (1998a,b) were the first to use mitochondrial DNA sequences to establish the phylogeny of lacertid lizards. At that time, still at the onset of the era of molecular systematics, marker sequences were short and usually were used to investigate rough phylogenetic relationships. In recent years, with better developed methods, more detailed phylogenetic studies addressed the relationships within single genera (e.g. Lamb and Bauer 2003; Maca-Meyer et al. 2003; Makokha et al. 2007) as well as the complete phylogeny of the family Lacertidae (Fu 1998, 2000; Mayer and Pavlicev 2007).

Currently, three monophyletic groups within the family can be distinguished (Arnold et al. 2007; Mayer and Pavlicev 2007): Gallotiinae, Lacertinae, Eremiadinae. The subfamily Eremiadinae (or tribus Eremiadini sensu Arnold et al. 2007) is widely distributed in the Palearctic and Afrotropic ecozones, especially in xeric regions. Southern Africa seems to be a diversity hotspot within Sub-Saharan Africa (Makokha et al. 2007). About 30 lacertid species of seven genera are endemic to the subcontinent (Branch 1998). The study of Mayer and Pavlicev (2007) based on segments of two nuclear (nc) genes revealed a clade consisting exclusively of taxa from Africa south of the Saharan Desert, which was designated the Ethiopian clade. It comprised two Afrotropical groups of genera, a highly supported clade with a predominately East African distribution (*Pseuderemias*, *Heliobolus*, *Latastia*, *Philochortus* and *Nucras*) and another one with a mainly South African distribution (*Tropidosaura*, *Pedioplanis*, *Meroles* and *Ichnotropis*). The latter group was highly supported in the Bayesian analysis, but obtained only weak bootstrap support (BS) in the Maximum Parsimony analysis, and thus its monophyly required further verification. Furthermore, the taxonomy and the position of the genus *Australolacerta* are questionable. It was established by Arnold (1989) who united two species (*australis* and *rupicola*) within this genus. Both species had been formerly included in the Palearctic genus *Lacerta*, but are endemics in the southwest and extreme northeast of the Republic of South Africa. Yet, this classification appears artificial as the characters unifying the two species seem to be predominately plesiomorphic. Therefore, their phylogenetic relationships are still unclear. Salvi et al. (2011) tried to elucidate the phylogenetic position of *Australolacerta australis* using three mt marker genes. They placed it as sister group of *Tropidosaura*, but did not include *Australolacerta rupicola* in their study.

While the relationships within the two genera *Meroles* (Lamb and Bauer 2003) and *Pedioplanis* (Makokha et al. 2007; Conradie et al. 2012) have been analysed in detail recently, the intergeneric relationships are still unknown. Also the relationships among the species of the genus *Ichnotropis* are not clarified. This might be partly due to the fact that it is difficult to obtain material of these taxa: For decades there have been no records for four of the seven nominal species and the distribution ranges of five species are hardly accessible. Nonetheless, two species, *Ichnotropis capensis* and *Ichnotropis squamulosa*, have been included in different studies (Lamb and Bauer 2003; Makokha

Corresponding author: Werner Mayer (werner.mayer@nhm-wien.ac.at)
Contributing authors: Anja Engleder (anja.engleder@nhm-wien.ac.at), Elisabeth Haring (elisabeth.haring@nhm-wien.ac.at), Sebastian Kirchhof (Sebastian.Kirchhof@mfn-berlin.de)

et al. 2007; Mayer and Pavlicev 2007). Comparisons of the available GenBank sequences suggested that these two *Ichnotropis* species are only distantly related, casting doubts on the monophyly of the genus.

In this study, we focus on the mainly endemic South African group of Eremiadinae subsequently designated as 'South African clade' in this article and their relationships to the 'East African clade'. We address the following questions: (1) Is the 'South African clade' indeed a monophylum? (2) What is the phylogenetic position of the two species of the nominal genus *Australolacerta*? (3) Is *Ichnotropis* monophyletic? (4) What are the phylogenetic relationships of *Tropidosaura* within the 'South African clade' as well as within the genus itself?

In our analyses we used sections of two already well established nc genes: the *recombination activating gene 1* (*RAG-1*) and oocyte maturation factor (*c-mos*) previously used for Lacertids (e.g. Harris et al. 1999; Carranza et al. 2004; Mayer and Pavlicev 2007). The previously used data set of 1593 bp of Mayer and Pavlicev (2007) was not informative enough to resolve the tree sufficiently. Therefore, we added three commonly used mt genes coding for *12S rRNA* (*12S*), *16S rRNA* (*16S*) and *cytochrome b* (*cyt b*). Furthermore, we implemented four nc genes some of which were only recently introduced in molecular systematic studies of reptiles: the *recombination activating gene 2* (*RAG-2*), *exophilin 5* (*EXPH5*), *kinesin family member 24* (*KIF24*) and *prolactin receptor* (*PRLR*). Altogether these marker genes add up to a sequence information of 4473 bp for nc genes and 2045 bp for mt genes. Besides the task to acquire data sets of sufficiently long DNA sequences to clarify the above mentioned questions on the 'South African clade' of Lacertidae, we were also interested to assess the suitability of the marker genes. We asked whether the various genes are equally appropriate to arrive at well-supported topologies and whether the nc genes resolve deeper nodes better than mt genes. Thus, a comparison of evolutionary rates of the various genes should be performed. Finally, we tried to interpret our results on the phylogeny of the 'South African clade' with respect to the dispersal and colonization of this group in the context of palaeoclimatic data.

Materials and methods

Sampling

The specimens analysed are listed in Table 1 together with their geographical origin, lab codes, and GenBank accession numbers. The study comprises 19 species of lacertid lizards (18 representing Eremiadinae and as outgroup *Lacerta agilis*, a member of Lacertinae). The specimens (altogether 24 samples) were selected to represent all genera of the 'South African clade' and included also individuals from which some of the marker sequences were analysed previously (Mayer and Pavlicev 2007; Pavlicev and Mayer 2009). These sequences are indicated in Table 1. Sample localities are also shown in Fig. 1.

Genetic analysis

Total genomic DNA was extracted from frozen or ethanol preserved tissue samples (tails, tongues or liver) using the GEN-IAL First-DNA *All*-tissue DNA-Kit (Troisdrof, Germany) according to the standard procedure as provided by the manufacturers' instructions.

For the phylogenetic analyses sequences of six protein coding nc genes (*c-mos*, *RAG-1*, *RAG-2*, *PRLR*, *EXPH5* and *KIF24*; comprising only exon sequences) and three mt (*12S*, *16S* and *cyt b*) genes were PCR amplified and sequenced. Various primers were used (taken from the literature, partially modified or designed in the course of this study) in different combinations to generate the complete nine marker sequences. Primer sequences used for amplification and sequencing as well as annealing temperatures are listed in Table 2. Only in *I. capensis* the complete *PRLR* sequence could not be obtained. We reconstructed internal primers to amplify a shorter sequence (length 440 bp; positions 1–52 bp and 493–541 bp in the alignment are missing).

PCR amplifications were performed on a Mastercycler gradient thermocycler (Eppendorf, Hamburg, Germany) in 25 µl with 0.5 units DyNAzyme II DNA polymerase (Finnzymes, Vantaa, Finnland), 1 µM of each primer and 0.2 mM of each dNTP (Roche, Mannheim, Germany). PCR conditions included an initial denaturation step of 2 min at 94°C, followed by 35 cycles of 15 s at 94°C, 20 s at annealing temperature, 60 s at 72°C, and a final extension step of 5 min at 72°C. For detecting any contaminated reagents negative controls for all DNA extractions (without sample) and for PCR reactions (with distilled water instead of template DNA) were included. For direct sequencing of PCR products, they were purified with the QIAquick PCR Purification Kit (Qiagen, Venlo, The Netherlands). In some cases, especially with the *cytochrome b* amplicons (1143 bp), PCR products had to be cloned to guarantee exact reads of the ends. The PCR products were also cloned when the PCR repeatedly performed poorly and yielded only faint bands. For this purpose, gel-purified PCR products (QIAquick Gel Extraction Kit; Qiagen) were cloned using the TOPO TA Cloning Kit (Life Technologies, Carlsbad, CA, USA). Sequencing (both strands) was performed by LGC Genomics (Berlin, Germany) using the primers listed in Table 2 and for cloned fragments, using universal M13 primers.

Data analysis

The protein coding nc and mt sequences were edited and aligned manually with the program BioEdit (Version 7.0.9, Hall 1999), while sequences of mt rRNA genes were first aligned with clustalx 2.1 (Larkin et al. 2007) and further adjusted manually in BioEdit. Ambiguous positions in nc sequences, were coded according the IUPAC code. Altogether the sections of the nc genes analysed sum up to an alignment of 4473 bp (*c-mos*: 581 bp, *RAG-1*: 1012 bp, *RAG-2*: 943 bp, *EXPH5*: 906 bp, *KIF24*: 490 bp, *PRLR*: 541 bp). For the mt genes the length of the complete data set was 2146 bp (*12S*: 477 bp, *16S*: 526 bp, *cyt b*: 1143 bp). After exclusion of highly variable sections of ambiguous alignment in the *12S* and *16S* genes the mt alignment measured 2045 bp (*12S*: 429 bp, *16S*: 473 bp, *cyt b*: 1143 bp; Alignments can be obtained from the authors on request).

For calculation of p-distances the software mega 5.05 (Version 5, Tamura et al. 2011) was used with the 'partial deletion' option and a 95% site coverage cut-off. dambe (Xia 2001; Xia and Xie 2001) was used to test substitution saturation. Optimal evolutionary models (for genes and partitions) were determined with the software jModelTest (Version 0.1.1, Posada 2008). Maximum likelihood (ML) trees were calculated with the software RAxML (Stamatakis et al. 2008). For the Bayesian analysis we employed MrBayes Version 3.2.1 (Ronquist et al. 2012). A Bayes Factor analysis was performed to determine the most appropriate partition scheme. The following three schemes were tested: (1) unpartitioned (model: TIM2 + I + G), (2) by-gene, that is, each gene representing a distinct partition with its own evolutionary model (see table 3) and (3) three partitions, that is, nc protein coding (GTR + G), mt protein coding (TPM3uf + I + G) and mt rRNA genes (TIM2 + I + G). For each scheme, Bayesian inference (BI) analyses were run for 5 million generations with every 100th tree sampled; 25% of all sampled trees were used as burn in and discarded. Subsequently, the harmonic means of the likelihood scores were used to calculate Bayes factors (Brown and Lemmon 2007). Bayes factors suggested the 'three partitions scheme' as the best. For the *cyt b* gene, we tested whether exclusion of third codon positions would improve the resolution of the tree. As, yet, in that analysis no node obtained a higher support compared with the analysis including all positions (most nodes proved to be worse) complete *cyt b* data were used for all tree calculations. ML analyses were done with the Web-Server of RAxML. Bootstrap analyses were carried out with 100 replicates. BI analyses were done by Markov chain Monte Carlo (MCMC) sampling starting with random trees and ran for 5 million generations (samplefreq = 100; nchains = 4). Convergence of runs was assessed by visual inspection of plotted log-likelihood values using the software tracer v.1.4 (Rambaut and Drummond 2007). In a conservative approach, 25% of sampled trees were discarded, although the plateau of

Table 1. List of analysed specimens, their geographical origin and GenBank accession numbers for all partial gene sequences. Numbers in parentheses refer to numbers in the tree (Fig. 2). Sequences published previously are marked with an black dot

Species / Lab code	Geographical origin	GenBank Accession numbers								
		c-mos	*RAG-1*	*RAG-2*	*EXPH5*	*KIF24*	*PRLR*	*cyt b*	*12S*	*16S*
Meroles suborbitalis (Peters, 1869)										
ABJ-25 (1)	NAM; Trekopje; 22°17′S / 15°04′E	JX962912	JX963019	JX963033	JX962947	JX962971	JX962995	JX962926	JX962871	JX962892
ABJ-39 (2)	NAM; Rosh Pinah 27°32′S / 16°42′E	EF632273•	EF632230•	JX963034	JX962948	JX962972	JX962996	JX962927	JX962872	JX962893
Meroles knoxii (Milne-Edwards, 1829)										
ABM-15	NAM; Luderitz, Griffith Bay; 26°39′S / 15°05′E	JX962913	JX963020	JX963035	JX962949	JX962973	JX962997	JX962928	JX962873	JX962894
Meroles cuneirostris (Strauch, 1867)										
ABL-18	NAM; Luderitz, Grasplatz; 26°43′S / 15°17′E	JX962914	JX963021	JX963036	JX962950	JX962974	JX962998	JX962929	JX962874	JX962895
Ichnotropis squamulosa (Peters, 1854)										
ABH-3 (1)	MOC; locality unknown	EF632266•	EF632221•	JX963037	JX962951	JX962975	JX962999	JX962930	JX962875	JX962896
ABH-9 (2)	EAT; Laela; 8°45′S / 32°11′E	JX962915	JX963022	JX963038	JX962952	JX962976	JX963000	JX962931	JX962876	JX962897
Ichnotropis capensis (Smith, 1838)										
ABC-2	NAM; 36 km sw. Katima Mulilo; 17°42′S / 24°00′E	JX962916	JX963023	JX963039	JX962953	JX962977	JX963001	JX962932	JX962877	JX962898
Tropidosaura gularis (Hewitt, 1927)										
ABT-1 (1)	ZA; Western Cape, Jonaskop; 33°58′S / 19°30′E	EF632291•	EF632248•	JX963040	JX962954	JX962978	JX963002	JX962933	JX962878	JX962899
ABT-3 (2)	ZA; Western Cape, Engelseberg; 33°52′S / 22°08′E	JX962917	JX963024	JX963041	JX962955	JX962979	JX963003	JX962934	JX962879	JX962900
Tropidosaura montana (Gray, 1831)										
ABY-2 (1)	ZA; Western Cape, Grootberg; 33°52′S / 22°08′E	JX962918	JX963025	JX963042	JX962956	JX962980	JX963004	JX962935	JX962880	JX962901
ABY-3 (2)	ZA; Western Cape, Turrek Peak; 32°52′S / 19°11′E	JX962919	JX963026	JX963043	JX962957	JX962981	JX963005	JX962936	JX962881	JX962902
ABY-4 (3)	ZA; KwaZulu-Natal, Game Pass; 29°22′S / 29°38′E	JX962920	JX963027	JX963044	JX962958	JX962982	JX963006	JX962937	JX962882	JX962903
Tropidosaura essexi (Hewitt, 1927)										
ACK-1	LS; Metjhatjhaneng; 28°39′S / 28°41′E	JX962921	JX963028	JX963045	JX962959	JX962983	JX963007	JX962938	JX962883	JX962904
Tropidosaura cottrelli (Hewitt, 1925)										
ACJ-1	ZA; Eastern Cape, BenMcDhui; 30°38′S / 27°55′E	JX962922	JX963029	JX963046	JX962960	JX962984	JX963008	JX962939	JX962884	JX962905
Australolacerta australis (Hewitt, 1926)										
ABU-5	ZA; Western Cape, Groot Winterhoek; 33°00′S / 19°03′E	JX962923	JX963030	JX963047	JX962961	JX962985	JX963009	JX962940	JX962885	JX962906
Australolacerta rupicola (Fitzsimons, 1933)										
ADW-5	ZA; Limpopo, Soutpansberg, Lajouma; 23°01′S / 29°26′E	JX962924	JX963031	JX963048	JX962962	JX962986	JX963010	JX962941	JX962886	JX962907
Pedioplanis undata (Smith, 1838)										
ABE-423	NAM; Nauchas; 23°37′S / 16°21′E	EF632280•	EF632237•	JX963049	JX962963	JX962987	JX963011	JX962942	JX962887	DQ871115•
Pedioplanis lineoocellata (Duméril and Bibron, 1839)										

Table 1. (continued)

Species / Lab code	Geographical origin	GenBank Accession numbers								
ABA-18	NAM; Haruchas; 24°21′S / 16°24′E	JX962925	JX963032	JX963050	JX962964	JX962988	JX963012	JX962943	JX962888	JX962908
Nucras lalandii (Milne-Edwards, 1829)										
NUL-1	ZA; Western Cape, Stellenbosch; ca. 34°S / 19°E	EF632276•	EF632233•	JX963051	JX962965	JX962989	JX963013	JX962944	JX962889	JX962909
Heliobolus lugubris (Smith, 1838)										
ABB-20	NAM; Haruchas; 24°21′S / 16°24′E	EF632261•	EF632216•	JX963052	JX962966	JX962990	JX963014	JX962945	JX962890	JX962910
Latastia longicaudata (Reuss, 1834)										
ATA-13	ER; Nakfa; ca. 16°40′N / 38°30′E	EF632272•	EF632229•	JX963053	JX962967	JX962991	JX963015	JX962946	JX962891	JX962911
Ohisops elegans (Ménétriés, 1832)										
OJ-1	GR; Evros, Jianuli; ca. 41°10′N / 26°10′E	EF632278•	EF632235•	JX963054	JX962968	JX962992	JX963016	GQ142116•	GQ142069•	GQ142092•
Atlantolacerta andreanskyi (Werner, 1929)										
LN-4	MA; Djebel Toupkal; ca. 31°N / 8°W	GQ142144•	GQ142154•	JX963055	JX962969	JX962993	JX963017	GQ142117•	GQ142070•	GQ142093•
Lacerta agilis (Linné, 1758)										
WT-1	A; Lower Austria, Weitra; 48°42′N / 14°53′E	EF632267•	EF632222•	JX963056	JX962970	JX962994	JX963018	GQ142118•	AF149947•	AF149963•

likelihood values had been reached before. A majority rule consensus tree was calculated with the remaining trees.

Alternative tree topologies were tested with the Shimodaira–Hasegawa (SH) Test performed with TREE PUZZLE (Version 5.2, Schmidt et al. 2002). The site-log-likelihood values of the various trees were then imported into the program CONSEL (Shimodaira and Hasegawa 2001) to calculate p-values of the different topologies.

The molecular clock was tested in MEGA 5.05 using the likelihood ratio method to check if the rates are homogenous. According to the likelihood ratio test, the hypothesis of rate homogeneity, and hence a molecular clock, was rejected ($p < 0.001$). Furthermore, as a reliable fossil calibration or a plausible estimate of the evolutionary rate is missing, a molecular clock analysis was not performed.

Results

We calculated separate BI and ML trees for each of the nine gene segments (not shown), for combined nc and mt data sets (Figs. S1 and S2), as well as for the complete data set. In the trees based on single genes the clustering of *I. squamulosa* with *Meroles,* the monophyly of *Tropidosaura* as well as the 'East African clade' are well supported in all nine trees. The positions of *Pedioplanis*, of the two species of *Australolacerta*, and of *I. capensis* vary among trees. These positions are, yet, poorly supported in all analyses (see below). The PH test did not detect any conflict between genes in the combined data set. It also showed no conflict when testing the mt data separately, but testing only the nc data revealed a conflicting signal. By performing the test in pairwise comparisons of the nc genes the conflicting signal was found to be due to *RAG-1*. Yet, a BI tree excluding the *RAG-1* gene did not show any differences in topology compared with the tree based on the combined data set. There was only slight variation in some support values. As there is obviously no strong influence of the *RAG-1* sequence on the topology, it was not excluded from the calculations of the combined data set including all marker sequences. The BI tree based on this complete data set (mt plus nc) is shown in Fig. 2. The results of the different algorithms (BI, ML) were generally in accordance and the support values were mostly concordant.

The BI tree calculated with the complete data set shows maximum (1.0) posterior probability support for the main differentiation of 'East' and 'South African clades'. BS values in the ML analysis are high as well. Within the 'South African clade' the monophyly of *Tropidosaura* is confirmed and the clustering of the two representatives of *Pedioplanis* obtained maximum support values. The relationships among the remaining genera, yet, are not resolved unambiguously.

In the highly supported clade comprising *Meroles* and *Ichnotropis,* the two representatives of the latter genus are quite distantly related and *I. squamulos*a clusters (with maximum support) with *Meroles* being the sister group of *M. suborbitalis* and *M. knoxii*. Thus, in this tree both genera are paraphyletic. Yet, it should be noted that, while the close relationship between *I. squamulos*a and *Meroles* is evident and highly supported, there is no maximum support for the node uniting *I. squamulos*a with *M. suborbitalis* and *M. knoxii.* Therefore, we consider the relationship for these three taxa as an unresolved trichotomy. The two species of *Australolacerta* do not cluster and are very distantly related. They branch off from the lineage leading to *Tropidosaura*, but the respective nodes obtained only low support.

Within the 'South African clade' some basal branches have short lengths and quite low support values. Thus, there is an unresolved polytomy of five lineages: (1) *Meroles* + *Ichnotropis*, (2) *Tropidosaura*, (3) *A. rupicola,* (4) *A. australis* and (5) *Pedioplanis.*

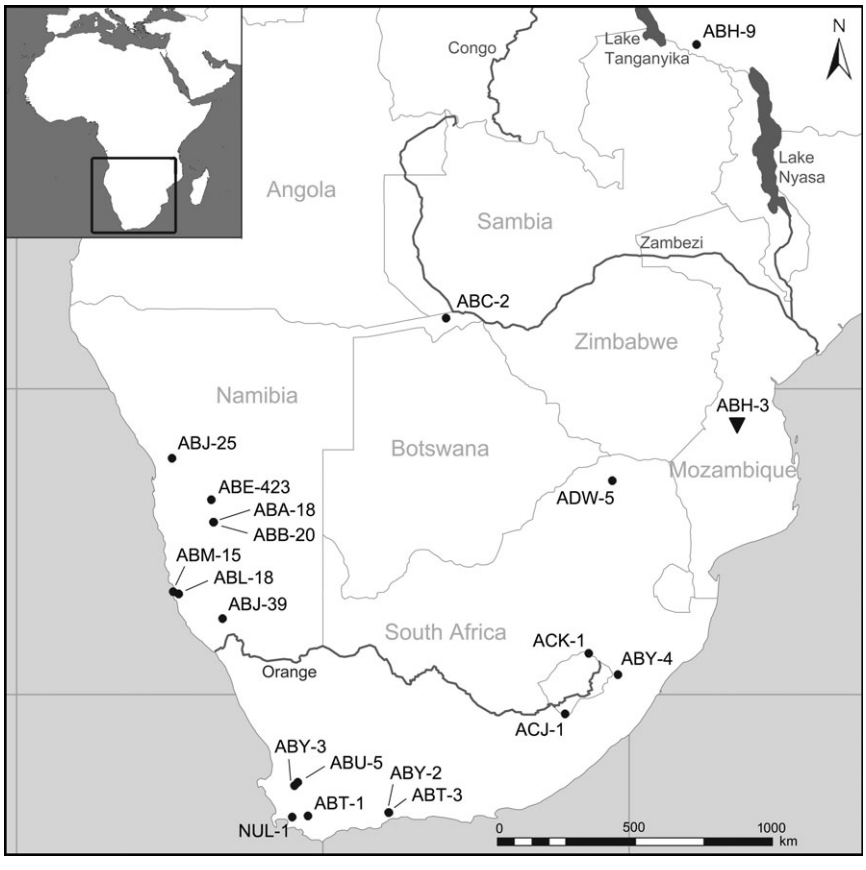

Fig. 1. Map of southern Africa. Sample localities of individuals analysed are marked and labelled with the lab code (see Table 1); the exact locality of ABH-3 (triangle) is unknown

To further test the monophyly of the genera *Meroles*, *Ichnotropis* and *Australolacerta* SH tests were performed. While the monophyly of *Ichnotropis* was clearly rejected ($p < 0.001$), the monophyly of *Meroles* was not ($p > 0.8$). Concerning *Australolacerta* we tested its clustering with (1) the *Meroles*/*Ichnotropis* clade or with (2) *Tropidosaura* as well as (3) the monophyletic *Australolacerta* as an independent lineage. The tests did not reject any of these topologies (all p values >0.5). To summarize, among the three genera only the monophyly of *Ichnotropis* is clearly rejected, while for *Meroles* and *Australolacerta* neither monophyly nor paraphyly can be rejected.

Comparison of marker genes

Our analyses are based on nine different genes comprising 6518 bp (lengths of alignments: six protein coding nc genes – 4473 bp; one protein coding and two rRNA mt genes – 2045 bp). Comparing the various single gene and combined trees revealed that the addition of sequences increased support values considerably. Several of the highly supported nodes in the combined tree were also found in trees based on single genes, though with mostly poor support.

Pairwise distances of all marker sequences are compiled in the Supporting information (Table S1). Maximum and mean distances for each marker sequence (Table 4) show that three of the six nc genes evolve quite slow (*RAG-1, RAG-2*, *c-mos*) in comparison with the other three nc genes (*EXPH5*, *KIF24*, *PRLR*). These 'faster' nc genes also differ from the other ones by displaying various length polymorphisms, while *RAG-1, RAG-2* and *c-mos* do not have any insertions/deletions (indels) of amino acid codons. For example, the *EXPH5* sequences range from 861 to 897 bp, the *KIF24* sequences from 454 to 484 bp and *PRLR* sequences from 529 to 541 bp.

Not surprisingly, the mt genes analysed (*12S*, *16S* and *cyt b*) are faster evolving than the nc genes, *cyt b* being the fastest. This is better visible in the mean p-distances, whereas the maximum value of *KIF24* is the same as the maximum value among *16S* distances. Yet, this might be due to sequence saturation of the mt marker genes. Although saturation tests indicated that none of the genes shows significant saturation in our data set, it should be emphasized that saturation is a continuous phenomenon and any limit defining when saturation is reached is somehow arbitrary. In our data set especially the *cyt b* curve flattened noticeably with higher distances indicating an increasing proportion of multiple substitutions. To compare the different evolutionary rates of marker genes in more detail, pairwise distances were plotted for each gene. As the statistical spread for each comparison is quite high, we illustrate the relations in a summarizing plot for which the pairwise distances of each gene were plotted in an ascending order and the resulting curves were combined into one figure (Fig. S3). For this comparison we used only the lowest 25% of comparisons to ensure that saturation effects are minimal in these comparisons. The nine curves exemplify that the order of evolutionary rates among the marker genes is (in ascending order): *RAG-2*, *RAG-1*, *c-mos*, *KIF24*, *EXPH5*, *PRLR*, *16S*, *12S* and *cyt b.* The mean and maximum distances calculated only from the lowest 25% of comparisons ('with minimal saturation') are a better approximate of the actual relationships among rates (Table 4). These comparisons show that the two slowly evolving rRNA coding mt genes are close to the fastest nc genes and that, for example, the rate of *cyt b* is approximately three times that of *16S*.

Discussion

The present phylogenetic analyses could clearly answer one of the main questions by recovering the 'South African clade' as a

Table 2. Primers used. Ranges of annealing temperatures [T (°C)] indicate that various temperatures were used for different species

Gene	Primer name	Sequence	Direction	Purpose	T (°C)	Source
c-mos	L-1zmos	5′-CTAGCTTGGTGTTCTATAGACTGG-3′	Fwd	PCR	55	Whiting et al. (2003)
	Hcmos3	5′-GGTGATGGCAAATGAGTAGAT-3′	Rev	PCR	55	Mayer and Pavlicev (2007)
	CMS-77L	5′-CTACGTACCATGGAGCTAC-3′	Fwd	Sequencing	–	Mayer and Pavlicev (2007)
	CMS-482H	5′-TTGGGAACATCCAAAGTCTC-3′	Rev	Sequencing	–	Mayer and Pavlicev (2007)
RAG-1	RAG-fo	5′-GAAAAGGGCTACATCCTGG-3′	Fwd	PCR	52	Mayer and Pavlicev (2007)
	RAG-R1	5′-AAAATCTGCCTTCCTGTTATTG-3′	Rev	PCR	52	Mayer and Pavlicev (2007)
	RGS-380L	5′-CTCAGTACCAAGATCCTTGC-3′	Fwd	Sequencing	–	Mayer and Pavlicev (2007)
	RGS-587H	5′-AGCCAAACTGTTGAGGATAC-3′	Rev	Sequencing	–	Mayer and Pavlicev (2007)
RAG-2	rag2_lung35_fw	5′-GGCCAAAGAGRTCYTGTCCIACTGG-3′	fwd	PCR	50	Chiari et al. (2004) and Hoegg et al. (2004)
	rag2_H1306_rv	5′-GHGAAYTCCTCTGARTCTTC-3′	Rev	PCR	50	Vidal and Hedges (2005)
	rag2_L562L_fw	5′-CCTGAAGCYAGATATGGCCATAC-3′	Fwd	Sequencing	–	Modified after Vidal and Hedges (2005)
	rag2_lung320L_rv	5′-ATTTCCCATATCRCTCCCAAACC-3′	Rev	Sequencing	–	Modified after Hoegg et al. (2004)
	rag2_Lac 1fw	5′-CCTTCTTGATTTCAAAAAGGAAGA-3′	Fwd	PCR/Sequencing	50	This study
	rag2_Lac 3fw	5′-GAACTCAAACTGAAGCCGACA-3′	Fwd	PCR/Sequencing	50	This study
EXPH5	EXPH5 F1	5′-AATAAACTKGCAGCTATGTACAAAACAAGTC-3′	Fwd	PCR/Sequencing	54	Portik et al. (2010)
	EXPH5 R1	5′-AAYGCCCTTCTGTGAGTGACCTCT-3′	Rev	PCR/Sequencing	54	Portik et al. (2010)
	EXPH5 a	5′-AATAAACTGGCAGCTATGTACAAAACAAGTC-3′	Fwd	PCR/Sequencing	54	Modified after Portik et al. (2010)
	EXPH5 b	5′-AACGCCCTTCTGTGAGTGACCTCT-3′	Rev	PCR/Sequencing	54	Modified after Portik et al. (2010)
	EXPH Lac1fw	5′-GTCGAAAGTTTTCCAGCAAG-3′	Fwd	PCR/Sequencing	54	This study
	EXPH Lac2rv	5′-CCTCTTTATGTTATCCAAGAAAGC-3′	Rev	PCR/Sequencing	54	This study
KIF24	KIF24 F1	5′-SAAACGTRTCTCCMAAACGCATCC-3′	Fwd	PCR/Sequencing	57	Portik et al. (2010)
	KIF24 R1	5′-WGGCTGCTGRAYTGCTGGTG-3′	Rev	PCR/Sequencing	57	Portik et al. (2010)
	KIF24 a	5′-AAACGTGTCTCCCCAACGCATCC-3′	Fwd	PCR/Sequencing	57	Modified after Portik et al. (2010)
	KIF24 b	5′-GGCTGCTGGACTGCTGGTG-3′	Rev	PCR/Sequencing	57	Modified after Portik et al. (2010)
	KIF24 Lac1fw	5′-CATGCAAGGTGGGGAAAAGAGC-3′	Fwd	PCR/Sequencing	57	This study
	KIF24 Lac2 rv	5′-CTGGTGGTAAAGGCGGAGGT-3′	Rev	PCR/Sequencing	57	This study
PRLR	PRLR_f1	5′-GACARYGARGACCAGCAACTRATGCC-3′	Fwd	PCR/Sequencing	50–57	Townsend et al. (2008)
	PRLR_r3	5′-GACYTTGTGRACTTCYACRTAATCCAT-3′	Rev	PCR/Sequencing	50–57	Townsend et al. (2008)
	PRLR a	5′-GACAGCGAGGACCAGCAACTGATGCC-3′	Fwd	PCR/Sequencing	50–57	Modified after Townsend et al. (2008)
	PRLR b	5′-GACCTTGTGGACTTCCACGTAATCCAT-3′	Rev	PCR/Sequencing	50–57	Modified after Townsend et al. (2008)
	PRLR Lac1fw	5′-GACCAGCAACTRATGCCAAACACYG-3′	Fwd	PCR/Sequencing	50–57	This study
	PRLR Lac2rv	5′-ACTTCYACRTAATCCATGGGYTTTG-3′	Rev	PCR/Sequencing	50–57	This study
	PRLR Lac3fw	5′-GACARYGARGACCAGCAACTRAT-3′	Fwd	PCR/Sequencing	50–57	This study
	PRLR Lac4rv	5′-GACYTTGTGRACTTCYACRTAAT-3′	Rev	PCR/Sequencing	50–57	This study
	PRLR_int_fwJ	5′-AAGCTGGTGCACCAGGAA-3′	Fwd	PCR/Sequencing	50–57	This study
	PRLR_rvJ	5′-TTGACTTTGTGGACTTCTACATA-3′	Rev	PCR/Sequencing	50–57	This study
12S rRNA	L01091	5′-AAACTGGGATTAGATACCCCACTAT-3′	Fwd	PCR/Sequencing	50	Pavlicev and Mayer (2009)
	H1557	5′-GTACACTTTACCTTGTTACGACTT-3′	Rev	PCR/Sequencing	50	Pavlicev and Mayer (2009)
	t-Phe_Lac	5′-AAAGCACGGCACTGAAGATG-3′	Fwd	PCR/Sequencing	50	Present study
16S rRNA	LE02190	5′-GTAGGCCTCAAAGCAGCCAC-3′	Fwd	PCR	50	Pavlicev and Mayer (2009)
	H03056	5′-CCGGTCTGAACTCAGATCACG-3′	Rev	PCR	50	Pavlicev and Mayer (2009)
	LE2493	5′-CCAACTGTTTACCAAAAACATAG-3′	Fwd	Sequencing	–	Pavlicev and Mayer (2009)
cty b	LgluLK	5′-AACCGCTGTTGTCTTCAACTA-3′	Fwd	PCR	50	Pavlicev and Mayer (2009)
	t-Glu2	5′-CGACTCGAAAAACCGCCGTTG-3′	Fwd	PCR	50	This study
	LGlu-cons	5′-GAAAAACCACCGTTGTATTCAACTA-3′	Fwd	PCR	50	This study
	NTheH	5′-GGTTTACAAGACCAGTGCTTT-3′	Rev	PCR	50	Pavlicev and Mayer (2009)

Table 3. Alignment lengths, range of sequence lengths (in parentheses) and evolutionary models of jModelTest used for the Bayesian analyses

Gene	Alignment length (Sequence length) (bp)	Models (AIC) used for Bayesian inference
c-mos	581	HKY + G; nst = 2, rates = gamma
RAG1	1012	TiM + I + G; nst = 6, rates = invgamma
RAG2	943	HKY + G; nst = 2, rates = gamma
EXPH5	906 (861–903)	TiM + G; nst = 6, rates = gamma
KIF24	490 (454–484)	TPM3 uf + G; nst = 6, rates = gamma
PRLR	541 (440–541)	TrN + G; nst = 6, rates = gamma
12S	477/429 (455–464)	GTR + I + G; nst = 6, rates = invgamma
16S	526/473 (489–506)	TiM2 + I + G; nst = 6, rates = invgamma
cyt b	1143	TPM3 uf + I + G; nst = 6, rates = invgamma

Except *cyt b*, which is the complete gene sequence, all other marker sequences are partial genes. For *12S* and *16S,* the two lengths of alignments indicate before/after exclusion of ambiguous regions.

strongly supported group that reaches maximum BI support and very high BS values (in the ML analysis) in the trees based on the complete marker set. Furthermore, the sister group relationship between the 'East African clade' and the 'South African clade' obtained maximum support. This result is in accordance with the tree of Salvi et al. (2011) in which, yet, the 'South African clade' was represented by a much smaller set of taxa. Within the 'South African clade' there are some uncertainties. The two species assigned to *Australolacerta* switch position in the tree depending on the calculation method used. Although the relationship between the genera *Ichnotropis* and *Meroles* remains ambiguous, *Ichnotropis* is clearly paraphyletic. *Ichnotropis squamulosa* clusters within the *Meroles* group, whereas *I. capensis* is the sister group of this clade.

Table 4. Maximum and mean p-distances (partial deletion) of single genes and combined nc as well as combined mt genes

Gene	Maximum distance	Mean distance
c-mos	8.1	4.4
RAG-1	9.1	4.3
RAG-2	6.8	3.5
EXPH5	12.8	6.9
KIF24	16.9	8.6
PRLR	14.9	8.5
nuclear	9.7	5.6
12S	18.7	12.2
16S	16.9	11.5
cyt b	25.8	21.8
mitochondrial	21.7	17.5

Relationships between Ichnotropis and Meroles

Makokha et al. (2007) presented a tree containing representatives of the 'East African clade' (*Heliobolus*, *Nucras*) and its sister group, the 'South African clade' (*Pedioplanis*, *Meroles*, *Ichnotropis*). While the analysis was dedicated specifically to the genus *Pedioplanis* including all species known at that time, it revealed also a poorly supported clade uniting *I. capensis* and *Meroles*, which is in accordance with our results. Yet, it has to be mentioned that in Makokha et al. (2007) the outgroup choice (*Australolacerta*) was unsuitable. As our results clearly show, *Australolacerta* belongs to the 'South African clade' and thus should not be used to root a tree comprising both the 'East African clade' and the 'South African clade'. In our study, the clade consisting of *Meroles* and *Ichnotropis* was highly supported. Furthermore, our doubts on the monophyly of *Ichnotropis* were also confirmed. The SH test unambiguously rejected the monophyly of *Ichnotropis* (*I. capensis* and *I. squamulosa*) ($p < 0.001$). It

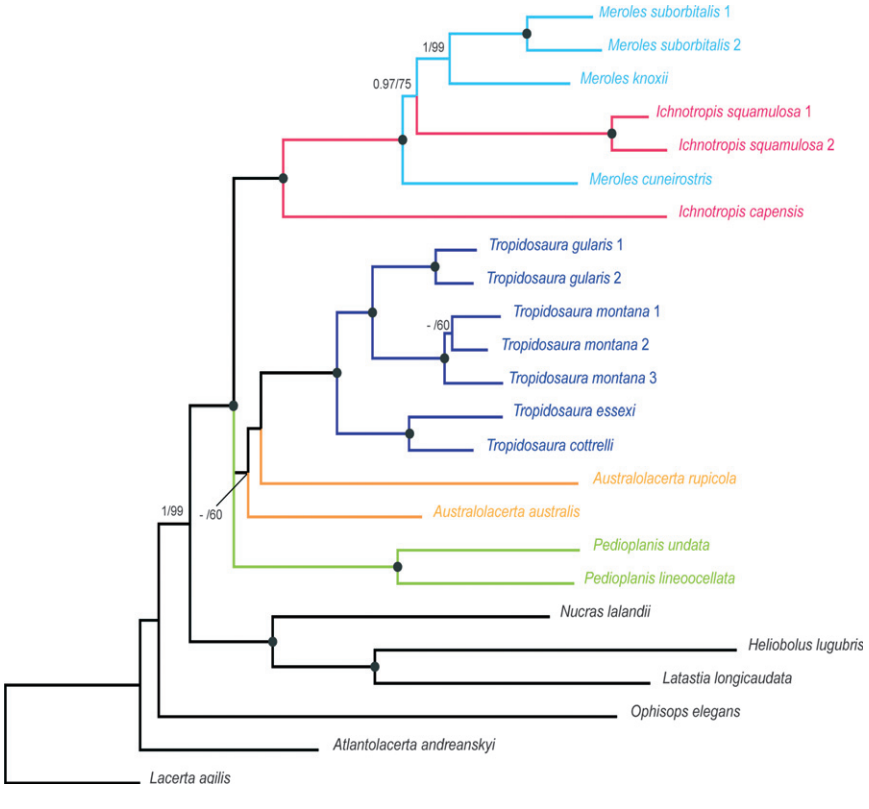

Fig. 2. Phylogenetic BI tree based on the combined nc and mt gene sequences. Nodes with maximum support values from BI/Maximum likelihood (ML) are marked with a black spot. Support values below 0.95 (BI) and 50% (ML) are not shown

did, yet, not prefer a specific position of *I. squamulosa* with respect to *Meroles*.

Beside the *Ichnotropis* species analysed in this study (*I. squamulosa*, *I. capensis*), there are currently five other described species (*I. bivittata*, *I. chapini*, *I. grandiceps*, *I. microlepidota*, *I. tanganicana*). They all are more similar to *I. capensis* (species typica) than to *I. squamulosa* in terms of scalation, habitus and – as far as known – colouration (data collected from Boulenger 1917, 1921; Schmidt et al. 1919; Marx 1956; Broadley 1967). Hence, in the following they will be referred to as *Ichnotropis* sensu stricto (s.str.) in contrast to *Ichnotropis* sensu lato (s.l.), which also includes *I. squamulosa*.

Three meristic traits are characteristic for *Ichnotropis* s.l.: dorsal scales large, rhombic or lanceolate, strongly keeled and imbricate; pileus shields keeled or striated; collar absent. Yet, these features occur sporadically in different other lacertid groups, although never in this combination. When examined in more detail, the dorsals can also be smaller (or less large) (e.g. in *I. squamulosa*, *I. microlepidota*, *I. grandiceps*), and the pileus shields can be weakly striated (e.g. in *I. grandiceps*, *I. tanganicana*). Ecologically, *I. squamulosa* and *I. capensis* are both short-lived annual species, a trait probably unique among lacertids, which appears unlikely to have developed multiple times in different genera.

Meristic traits characteristic for the genus *Meroles* and considered unique in the 'South African clade' include the occurrence of lobed or completely covered ear openings and fringed toes. Yet, *Meroles* includes species endemic to the Namib Desert with long digital fringes and wedge-shaped snouts as strong adaptations to aeolian sands (*M. anchietae*, *M. cuneirostris*, *M. ctenodactylus*, *M. micropholidotus*) as well as intermediate and generalist species such as *M. reticulatus* and the more widely distributed *M. suborbitalis* and *M. knoxii* with 'normal' head shape and feebler fringes. All species of *Meroles* contain a subocular scale above the labials, a trait, which is only shared by *I. squamulosa* within the 'South African clade'. Ecologically, *M. anchietae* and *M. suborbitalis* are capable of continuous reproductive activity throughout the year typical for tropical species (Goldberg and Robinson 1979; Goldberg 2006) in contrast with all other species of the 'South African clade' (October to March) (Branch 1998).

Which morphological and ecological characters support the topology revealed in our tree? Generally, the entire family Lacertidae has quite consistent general morphology and the degree of homoplasy is very high, especially with respect to external features (Borsuk-Bialynicka et al. 1999). Furthermore, our results reveal one common ancestor of five different clades comprising a variety of morphological traits that are distributed rather inconsistently among the different species (e.g. absence of collar in all *Tropidosaura*, in one *Meroles* and in *Ichnotropis*). This complicates the use of morphological characters in phylogenetic analyses of Lacertidae in general and of the 'South African clade' in particular. Consequently, *I. squamulosa* shares meristic, mensural and ecological features with both *Ichnotropis* s.str. as well as *Meroles* (Table S2). Yet, considering the highly supported paraphyly of *Ichnotropis* revealed in this study placing *I. squamulosa* within *Meroles*, any features characteristic for a genus *Ichnotropis* s.l. and differentiating it from *Meroles* must be regarded as convergences. Consequently, the synapomorphy of the genus *Meroles* and *I. squamulosa* that distinguishes this clade from all other species of the South African radiation (although present in other clades of Lacertidae) is the presence of the subocular scale separated from the lip by a labial shield. *I. squamulosa* is also more similar to *Meroles* in mostly lacking the occipital scale and having a longitudinally bisected frontonasal scale. The number of both dorsal and ventral scale rows is intermediate. In addition, *I. squamulosa* follows an unusual reproductive period more similar to that of *Meroles* from April to November (Jacobsen 1987; Goldberg 2008) in contrast with the reproductive cycle of the rest of the clade typical for temperate species. In regard of the annual life span of *I. capensis* and *I. squamulosa*, the revealed topology implies either that this trait was developed twice in both *Meroles* and *Ichnotropis* or that it must have been secondarily lost in *Meroles*. An explanation of the missing features of strong adaptation to aeolian sands in *I. squamulosa* might be that *I. squamulosa* constantly clusters with the members of the generalist group analysed in this study (*M. suborbitalis*, *M. knoxii*). This might be taken as a hint for a closer relationship between them as opposed to the group of highly specialized psammophilous taxa (*M. anchietae*, *M. cuneirostris*, *M. reticulatus*, *M. ctenodactylus*, *M. micropholidotus*) represented here by *M. cuneirostris*. Nevertheless, this assumption should be taken with caution as the node combining these species did not obtain maximum support in our trees. Future studies with complete samples sets of all *Meroles* taxa should reveal a clearer picture. To summarize, even if the phylogenetic relationships within *Meroles* and the relationships to *I. squamulosa* are not clearly resolved, the paraphyly of *Ichnotropis* should result in taxonomic consequences. We propose the most conservative and parsimonious change, the inclusion of *I. squamulosa* into the genus *Meroles*.

Australolacerta

The position of *Australolacerta* within the 'South African clade' was clearly confirmed (Salvi et al. 2011). Yet, the monophyly of the genus *Australolacerta* as well as the phylogenetic position of its two species remain controversial. In both the BI and the ML tree they cluster (poorly supported) with *Tropidosaura*, but without being sister groups. Although the support for this branching pattern is very poor, the tree suggests that the genus might be paraphyletic. On the basis of analyses of *12S* and *16S rRNA* genes, Salvi et al. (2011) reported *A. australis* as the sister group to *Tropidosaura* (represented by *T. gularis*). Although our comprehensive tree seems to support this hypothesis, it should be emphasized that this topology obtained very low support. Testing the different tree topologies concerning the position of *Australolacerta* resulted in ambiguity. Likelihood values for placing *Australolacerta* as the sister group to *Tropidosaura* are similar to those placing it as the sister group of the *Meroles/Ichnotropis* group. Thus, although there seems to be a trend for placing *Australolacerta* close to *Tropidosaura*, neither this hypothesis nor the monophyly of the genus could be clearly confirmed or rejected. The two species of *Australolacerta* are quite distinct. *A. rupicola* differs from *A. australis* in the following features: head and body strongly compressed (not somewhat depressed), snout longer than postocular part of head (not shorter), hind foot distinctly longer than head (not as long as), nostril pierced between the nasal, two postnasals and the first upper labial (not separated from the labial), parietal foramen present (not absent), five upper labials anterior to the subocular (not four), dorsal scales hexagonal, sometimes keeled and subimbricate (not granular and smooth), collar serrated (not even-edged). Finally, they largely differ in colouration. To summarize, neither the genetic data nor morphological characters indicate a closer relationship of the two species or provide support for the artificial genus *Australolacerta*. Nevertheless, given the current state of knowledge we propose to leave both species in one genus.

Tropidosaura

This study was the first including all four *Tropidosaura* species, and the monophyly of the genus could be confirmed clearly. An interesting outcome of the analysis is the high intraspecific distances found within *T. montana*. Even considering the geographical distances between sample localities (~1000 km), distances of 7.7% are high compared with other lacertid species (see below). One individual (ABY4) collected in KwaZulu–Natal is slightly more distant to the other two *T. montana* and belongs to the subspecies *T. montana natalensis*. Whether there is a clear phylogeographical structure differentiating the three described subspecies remains to be analysed in more detail.

Radiation of the 'South African clade'

Our results indicate that the 'South African clade' consists of five distinct lineages, but their relationships cannot be resolved unambiguously, not even with this large data set of 6518 bp. Therefore, we consider this pentatomy comprising (1) *Meroles* + *Ichnotropis*, (2) *Tropidosaura*, (3) *A. rupicola*, (4) *A. australis* and (5) *Pedioplanis* as a hard polytomy assuming that a fast 'explosive' diversification must have happened in the southern regions of Africa in connection with an incisive climatic event in the past.

Despite the considerable length of the sequence, a molecular clock analysis appears problematic for the following reasons. (1) The big problem dating the diversification of African lacertid lizards is the complete lack of fossil records. (2) The rejection of the molecular clock assumption indicates that even the application of an empirically determined rate from the literature (e.g. for mt sequences: Maca-Meyer et al. 2003) is not reasonable. Despite the fact that a molecular clock analyses was not performed because of the above mentioned reasons, we attempted to establish a plausible phylogeographical hypothesis considering the phylogenetic tree with respect to palaeoclimatic factors and compared it with a scenario previously proposed by Hipsley et al. (2009).

Two different time frames were proposed for the colonization of Africa by Lacertidae and the subsequent radiation and diversification (Mayer and Pavlicev 2007; Hipsley et al. 2009) (Fig. S4). Mayer and Pavlicev (2007) assumed that lacertids colonized Africa in the Early Miocene about 17 million years ago (mya) via the Arabian land bridge (Rögl and Steininger 1983). In that period, Southern Africa was vastly covered with tropical rain forests and woodlands (Lockwood 1979; Hendey 1983). Assuming that this dating is correct, all further divisions into clades consisting of mesic and xeric taxa might have been caused by stepwise cooling and drying after the Mid Miocene Climatic Optimum (Flower and Kennett 1994) as revealed through sediment analyses by Diekmann et al. (2003). Following this scenario, the split between the Ethiopian and Saharo-Eurasian clades (designated by Arnold 1989 and confirmed by Mayer and Pavlicev 2007), which probably did not occur in Southern Africa, fits to the Mid Miocene climate transition around 14–12 mya, in a long-lasting global cooling period (16 mya to present). The subsequent split between 'East African' and 'South African clades' can be attributed to the arid period from 11.6 to 10.7 mya (Fig. S4). The pivotal climatic incident leading to the explosive radiation within the 'South African clade' can be assigned to the period between 9.7 and 7.7 mya (Diekmann et al. 2003). In that period, a permanent ice cap was formed on the whole Antarctic continent (Lockwood 1979; Deacon 1983) and the cold upwelling within the Benguela current system was initiated. The Antarctic glaciation, which increased significantly after about 10 mya (Diester-Haass et al. 2002) was strongly linked with the desiccation of the Namib Desert (Lockwood 1979; Partridge 1993; Zachos et al. 2001; Bobe 2006). The emergence of new habitats after a prolonged tropical period (Partridge 1993) would explain the colonization and radiation into the various lineages: the highly xerophilous *Meroles*, the desert and semidesert taxa belonging to *Pedioplanis* and *Ichnotropis*, and the mesic lineages *Tropidosaura*, *A. australis* and *A. rupicola.* The latter three lineages survived the aridification of the South African inland in humid refugial areas on the mountain slopes of South Africa (Hendey 1983), particularly at the escarpment from the Soutpansberg in the north-east to the Cape Fold Mountains in the south-west.

The alternative scenario suggested by Hipsley et al. (2009) assumes that the ancestor of extant African Lacertidae immigrated into north-western Africa from western Europe via a chain of islands during the mid-Eocene (around 47 mya). This hypothesis (illustrated in Fig. S4) was based on three early fossil records of non-lacertid reptiles (228, 113 and 64 mya) as calibration points and, in addition, one fossil placed near the split between the genera *Timon* and *Dalmatolacerta* (5.3 mya). However, as the relationship between the latter two is quite ambiguous (see Arnold et al. 2007; Pavlicev and Mayer 2009), it appears not reasonable to use them as a calibration point. The early fossil records used by Hipsley et al. (2009) to calibrate the molecular clock led to the assumption that the Ethiopian and Saharo-Eurasian clades split already around ~43 (37.6–48.8) mya, the 'East Africa' and 'South African clades' ~38 (33.3–43.5) mya, and the first diversification of the South African genera (*Pedioplanis*, *Tropidosaura*) occurred ~27.5 (22.3–32.7) mya. The divergence of the xeric *Meroles* spp. from the lineage of the (recently) more mesic and semidesert *I. squamulosa* should have occurred at ~18.5 (13.6–23.4) mya.

From a palaeoclimatic point of view, both hypothetical scenarios might appear plausible within the proposed time frames, although there is, for example, a difference of ~20 my between the two estimates for the node defining the 'South African clade'. We currently cannot test the scenarios with a reliably calibrated molecular clock. Nonetheless, we regard the scenario of Hipsley et al. (2009) as less plausible because it assumes that the radiation of the xeric species of *Meroles* took place just in the humid period around the Mid Miocene Climatic Optimum (Fig. S4). In contrast, the formation of the Benguela current, the development of the hyperarid Namib Desert and the alternating cycles of arid and humid episodes in Southern Africa as proposed in our scenario have earlier been shown to be of crucial importance in the evolution of *Pachydactylus* geckos (Bauer 1999), cordylid lizards (Daniels et al. 2004), *Bradypodion* chameleons (Tolley et al. 2008) and *Capensibufo* toads (Tolley et al. 2010).

Marker genes

Although molecular systematics has made tremendous progress throughout the last decade and many new marker sequences were introduced, still many analyses are based on a few mt sequences and, exceptionally, on one or two nc genes only. This is true also for lacertids (e.g. Lamb and Bauer 2003; Makokha et al. 2007; Salvi et al. 2011; Conradie et al. 2012). In our analyses, we employed nine different genes comprising 6518 bp (nc 4473 bp and mt 2045 bp). This high amount of DNA sequence information increased the support values for several nodes considerably. Although fast or slowly evolving genes might influence node support (of basal and distal nodes) differently, it seems that the increased length of sequence in general pushes support values up. Even single genes, each providing low phylogenetic information, together may contribute to increased node support in the

combined calculations. This finding is in line with the concept of hidden support (Gatesy et al. 1999), which has been identified in many phylogenetic studies (summarized in Thompson et al. 2012). However, it should be mentioned that the tree based on nc data alone obtained almost the same high support values as the comprehensive tree (nc plus mt), while the tree based on combined mt data only was less well supported at several nodes. Thus, one could deduce that analysing groups of organisms at a similar level of divergence as in this study (subfamily Eremiadinae), the combination of nc data alone would be sufficient to resolve phylogenetic relationships. Nevertheless, as in many cases, the levels of divergence become apparent only in the course of the analysis itself and investigating and comparing both mt and nc data may reveal different parts of the phylogenetic history of the taxa, the generally accepted strategy to combine data sets from both genomes is most reasonable. This study may serve as a suitable pilot study for the application of previously rarely used nc markers. An interesting observation concerning evolutionary rates is the fact that rates of slowly evolving mt genes are almost in the same range as those of fast nc genes.

Despite the high support of most nodes in our comprehensive tree, there are still poorly supported ones and polytomies which cannot be resolved even with this comprehensive set of data (e.g. position of *Australolacerta*). We interpret this polytomy as 'hard polytomy' due to fast radiation within the South African lacertids. Whether this assumption is true might be revealed by a comprehensive molecular phylogeny of the whole family Lacertidae based on these marker genes.

Genetic distances and species delimitation

Sometimes genetic distances are used to support a separation of species or subspecies. On the basis of 'high' distance values of 3.0–3.4% (uncorrected pairwise distances, *16S* gene) within *A. australis* (Makokha et al. 2007; Salvi et al. 2011), Salvi et al. (2011) assumed that *A. australis* is a polytypic species or even a species complex. Analyses of all *16S* sequences of *A. australis* available so far (this study; Makokha et al. 2007; Salvi et al. 2011) revealed intraspecific p-distances of up to 4.0%. However, compared with other representatives of the 'South African clade', this value is in a range quite common for intraspecific variation. *16S* sequence data of *M. suborbitalis* (this study; Harris et al. 1998b; Lamb and Bauer 2003; Fu 2000; Makokha et al. 2007) show p-distances of up to 7.1%, and in *M. knoxii* up to 4.3% (this study; Lamb and Bauer 2003; Makokha et al. 2007). In *Tropidosaura,* intraspecific distances range around 4.5% (*T. gularis*: this study; Harris et al. 1998b; Fu 2000) and 7.7% (*T. montana*: this study). The argumentation of Salvi et al. (2011) was based on comparisons with species of the genus *Podarcis* (Salvi et al. 2011), but this presumed analogy appears not reasonable for South African Eremiadinae. Otherwise, one had to propose a plethora of cryptic species within this group. We do not want to exclude the possibility that so far unknown species exist within the genus *Australolacerta*, but we refrain from species delimitation based solely on certain distance levels. Such an approach is meaningless applying any of the numerous species concepts, but specifically within the biological species concept which we adhere to. Even the 'conclusion by analogy approach', that is, using within-group distances for comparison to infer species borders (e.g. Fritz et al. 2012; Kindler et al. 2012) might not be reasonable in all cases, as species delimitation of taxa used for comparison of distances might be doubtful, too. Comprehensive phylogeographical analyses using both mt and nc (e.g. microsatellite) markers could provide more detailed insights into the intra- and interspecific classification within the 'South African clade'.

Taxonomical implications

The position of the genus *Australolacerta* still remains questionable, although our results suggest that it might be paraphyletic. The genus was established by Arnold (1989) who quite arbitrarily united the two species (*australis* and *rupicola*) formerly included in the Palearctic genus *Lacerta.* But, the characters unifying the two species seem to be predominately plesiomorphic. Both are endemics in the south-west and extreme north-east, respectively, of the Republic of South Africa and differ considerably in morphology and colouration (see chapter *Australolacerta*). However, in spite of, or because of the unresolved phylogenetic position of *A. australis* and *A. rupicola*, we propose to retain the genus *Australolacerta* in the actual extent.

Concerning the genus *Ichnotropis*, our results are straightforward and implicate that *I. squamulosa* should be transferred from *Ichnotropis* to the genus *Meroles*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogenetic BI tree based on nuclear gene sequences.

Figure S2. Phylogenetic BI tree based on the mitochondrial gene sequences.

Figure S3. Summarizing plot (all nine resulting curves) of pairwise distances of each gene plotted in an ascending order.

Figure S4. Two hypotheses of the radiation of the 'South African clade'.

Table S1. Pairwise distances of all marker genes (*c-mos, RAG-1, RAG-2, EXPH5, KIF24, PRLR, 12S, 16S* and *cyt b*) in percent.

Table S2. Characters relevant for differentiation of *Ichnotropis* s.str. (*I. capensis, I. bivittata*, *I. chapini*, *I. grandiceps*, *I. microlepidota*, *I. tanganicana*), *I. squamulosa* and *Meroles*.