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# DIPLOMARBEIT

Titel der Diplomarbeit

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

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# VORWORT

Die vorliegende Diplomarbeit wurde als Manuskript zur Begutachtung bei der Zeitschrift *Zoological Systematics and Evolutionary Research* (Wiley-Blackwell) mit dem Titel

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Das Manuskript umfasst in der vorliegenden Arbeit den Bereich von „Introduction" bis inklusive Appendix I (S. 5-47) sowie das Abstract (S. 63, Appendix II). Im Appendix II (S. 48-66) werden weiters zusätzliche Ergebnisse dargestellt sowie Fotos von Vertretern der meisten Gattungen gezeigt.

# INHALTSVERZEICHNIS

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## INTRODUCTION

Lacertid lizards with about 280 (Arnold et al. 2007, the most recent list can be found at the webpage www.lacerta.de) species 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 revision (1989). 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. (1998 a, 1998 b) were the first to use mitochondrial DNA (mtDNA) 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 (Mayer and Pavlicev 2007; Arnold et al. 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. However, 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 the 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, *I. capensis* and *I. squamulosa*, have been included in different studies (Lamb and Bauer 2003; Makokha et al. 2007; Mayer and Pavlicev 2007), although in none of them they were analysed together. 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 the present study we focus on the mainly endemic South African group of Eremiadinae subsequently designated as "South African clade" in this paper 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. Carranza 2004; Harris et al. 1999; 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 colonisation of this group in the context of paleoclimatic data.

## MATERIAL AND METHODS

### *SAMPLING*

The specimens analysed are listed in Table 1 together with their geographic 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 nc protein coding (*c-mos*, *RAG-1*, *RAG-2*, *PRLR*, *EXPH5* and *KIF24*) 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 – 541bp 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). 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). Partition homogeneity (PH) tests were performed with PAUP* (Version 4.0b10, Swofford 1998) to evaluate if the trees based on different genes are consistent.

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 cutoff. Maximum likelihood (ML) and neighbour joining (NJ) trees were calculated with MEGA 5.05. For the Bayesian analysis we employed MrBayes Version 3.2.1 (Ronquist et al. 2012). Bootstrap analyses were carried out with 1000 replicates. For Bayesian inference (BI) we estimated the optimal evolutionary model for each gene with the software jModelTest (Version 0.1.1, Posada 2008). The models selected under the Akaike Information Criterion (AIC) which were used for the different gene sections are listed in Table 3. BI analyses were done by MCMC sampling starting with random trees and ran for five million generations (samplefreq=100; nchains=4). The first 25% of the Bayesian trees were discarded as burn-in and a majority rule consensus tree was calculated out of 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 a likelihood ratio method to check if the rates are homogenous and if a molecular clock could be used. Although there is no reliable fossil calibration and plausible estimate of the evolutionary rate, we performed a relaxed uncorrelated lognormal clock analysis with the program BEAST (Version 1.7.2., Drummond et al. 2012) which estimates simultaneously divergence times and topology. Our aim was to evaluate whether the analysis fits in general to a plausible phylogeographic scenario assuming that the radiation into five lineages started in the Early Late Miocene dry period at 9.7 – 7.7 mya (Diekmann et al. 2003). Therefore, this time range was selected for the node defining the common ancestor of the "South African clade". The analysis consisted of $10^7$ generations with a random starting tree, applying the HKY+G model and assuming a Yule speciation process.

## RESULTS

We calculated separate NJ, ML and BI trees for each of the nine gene segments (not shown), for combined nc and mt data sets, 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, however, 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*. However, a BI tree excluding the *RAG-1* gene did not show any differences in topology compared to 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 Figure 2. The results of the different algorithms (NJ, ML, BI) 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 (PP) support for the main differentiation of "East" and "South African clades". Bootstrap support (BS) values in the ML and NJ analyses 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, however, 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 the *Meroles* clade being the sister group of *M. suborbitalis* and *M. knoxii*. Thus, in this tree both genera are paraphyletic. While the close relationship between *I. squamulos*a and *Meroles* is evident and highly supported, it should be noted that there is no maximum support for the node uniting *I. squamulos*a with *M. suborbitalis* and *M. knoxii.* Therefore, we consider the relationships between *M. suborbitalis* + *M. knoxii*, *I. squamulos*a and *M. cuneirostris* 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 pentatomy of five lineages: (1)

*Meroles* + *Ichnotropis*, (2) *Tropidosaura*, (3) *A. rupicola,* (4) *A. australis* and (5) *Pedioplanis.*

To further test the monophyly of the genera *Meroles*, *Ichnotropis* and *Australolacerta* Shimodaira-Hasegawa (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 (1) monophyletic *Australolacerta*, (2) clustering of *Australolacerta* (monophyletic or paraphyletic) with the *Meroles* / *Ichnotropis* clade or with (3) *Tropidosaura*. 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.

According to the Likelihood ratio test, the hypothesis of rate homogeneity, and hence a molecular clock, was rejected throughout the tree ($p < 0.001$). A relaxed clock analysis (not shown) resulted – as suggested already by the likelihood ratio test – in a tree with very large and widely overlapping confidence intervals. For example, the confidence intervals for the nodes defining the five major lineages within the "South African clade" cover a broad range of several million years (between ~10 and ~5 mya). Thus, this analysis did not provide meaningful results.

***COMPARISON OF MARKER GENES***

Our analyses are based on nine different genes comprising 6518 bp (lengths of alignments: nc - 4473 bp, mt - 2045 bp). All six nc genes are protein coding. Comparing the various single-gene trees with combined trees reveals that the addition of sequences increases support values considerably. Several of the highly supported nodes in the comprehensive tree are also found in trees based on single genes, though with mostly poor support. E.g., the node uniting *Ichnotropis* and *Meroles* obtained a BS value of 37% in the NJ tree based on *RAG-2* and 94% in the tree based on all nc genes. The "South African clade" is supported by a BS value of 18% in the *c-mos* tree and by 100% in the tree based on combined nc sequences.

Pairwise distances of all marker sequences are compiled in the supplementary material (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 to 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. E.g., the *EXPH5* sequences range from 861 bp to 897 bp, the *KIF24* sequences from 454 bp to 484 bp, and *PRLR* sequences from 529 bp 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. However, this might be due to sequence saturation of the mt marker genes. 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. S1). The nine curves exemplify several findings (1) 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;* (2) The actual relations between rates as deduced from the comparisons of smaller distances (~ the lowest 25 distances which we considered as not markedly saturated) indicates that, e.g., the rate of *cyt b* is three times that of *16S*. The mean and maximum distances calculated only from the section "prior to sequence saturation" are a better approximate of the actual relationships among rates (Table 4). (3) The two slowly evolving rRNA coding mt genes are close to the fastest nc genes.

## DISCUSSION

The present phylogenetic analyses could clearly answer one of the main questions by recovering the "South African clade" as a strongly supported group that reaches maximum (BI) support and very high BS values (in the ML and NJ analyses) 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, however, 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.

### *RELATIONSHIPS BETWEEN ICHNOTROPIS AND MEROLES*

Makokha et al. (2007) presented a tree containing representatives of the "East African clade" (*Heliobolus*, *Nucras*) and 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. However, 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 this tree. The clade consisting of *Meroles* and *Ichnotropis* was highly supported in the present study. Moreover, our doubts on the monophyly of *Ichnotropis* were also confirmed. The Shimodaira-Hasegawa test unambiguously rejected the monophyly of *Ichnotropis* (*I. capensis* and *I. squamulosa*) ($p < 0.001$). It did, however, not prefer a specific position of *I. squamulosa* with respect to *Meroles*.

Beside the *Ichnotropis* species analysed in the present 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 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. However, these features occur sporadically in different other lacertid groups, though 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, and therefore it seems unlikely that it evolved two times independently.

Morphological features 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. However, *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. 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 to 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). However, 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. The number of both dorsal and ventral scale rows is intermediate. Additionally, *I. squamulosa* follows an unusual reproductive period more similar to that of *Meroles* from April to November (Jacobsen 1987; Goldberg 2008) in contrast to the reproductive cycle of the rest of the clade typical for temperate species. Furthermore, *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 and explain the missing features of strong adaptation to Aeolian sands in *I. squamulosa*. 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.

### *AUSTRALOLACERTA*

The position of *Australolacerta* within the "South African clade" was clearly confirmed (Salvi et al. 2011). However, the monophyly of the genus *Australolacerta* as well as the phylogenetic position of its two species remain controversial. In the BI tree both cluster (poorly supported) with *Tropidosaura*, while in the ML tree they are located on different branches and in the NJ tree they are sister groups clustering with *Tropidosaura*. Yet, the low support values for these various branching patterns suggest that the genus might be paraphyletic. Based on 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 emphasised 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 post-ocular part of head (not shorter), hind foot distinctly longer than head (not as long as), nostril pierced between the nasal, two post-nasals 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 monophyly of *Australolacerta*. Nevertheless, given the current state of knowledge we propose to leave both species in one genus.

***TROPIDOSAURA***

This study was the first one including all four *Tropidosaura* species and the monophyly of the genus could be confirmed clearly. An interesting outcome of the analysis are the high intraspecific distances found within *T. montana*. Even considering the geographic distances between sample localities (~1000 km) distances of 7.7% are high compared to 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 phylogeographic 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. Maca-Meyer et al. 2003 for mt sequences) is not reasonable. This is also underlined by the extremely high confidence intervals obtained in the BEAST analysis showing that this analysis provides meaningless results in this case. Nevertheless, we attempted to establish a plausible hypothesis considering the phylogenetic tree with respect to palaeoclimatic factors.

Two different time frames have been proposed for the colonization of Africa by Lacertidae and the subsequent radiation and diversification (Mayer and Pavlicev 2007; Hipsley et al. 2009) (Fig. S2). 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 date 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 (as defined 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. S2). The pivotal climatic incident which led to the explosive radiation within the "South African clade" can then be allocated to a later arid period between 9.7 and 7.7 mya (Diekmann et al. 2003). In this period a permanent ice cap formed on the whole Antarctic continent (Lockwood 1979; Deacon 1983) and the cold upwelling within the Benguela current system was initiated. The Antarctic glaciation is strongly linked with the desiccation of the Namib Desert (Lockwood 1979; Partridge 1993; Zachos 2001; Bobe 2006) and intensified significantly after about 10 mya (Diester-Haass et al. 2002). The first-time emergence of the still prevailing desert conditions in the Namib at that time after a prolonged tropical period (Partridge 1993) would explain the divergence into the highly xerophylic lineage *Meroles*, the desert and semi-desert taxa belonging to *Pedioplanis*, and *Ichnotropis*, and the mesic lineages *Tropidosaura*, *Australolacerta australis* and *A. rupicola.* The latter 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 northeast to the Cape Fold Mountains in the southwest.

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. S2) 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). It should be mentioned, however, that no support values were provided for the nodes in their molecular tree (mt: *12S, 16S, cyt b* and nc: *RAG-1, c-mos*) and that the sister group relationship between *Timon* and *Dalmatolacerta* appears highly improbable. In the analysis of Pavlicev and Mayer (2009) these genera appear as two quite distantly related lineages, a result also obtained by Arnold et al. (2007). Arguments based on morphological characters suggesting a sister group relationship between the two genera are missing too and thus it appears not meaningful to use them as a calibration point.

Hipsley et al. (2009) proposed that major radiation events of the Eremiadinae would have happened after the Early Eocene Climatic Optimum (52-50 mya), which was followed by a gradual cooling trend lasting 17 my (Zachos et al. 2001). For the split of Ethiopian and Saharo-Eurasian clades Hipsley et al. (2009) propose a period around ~43 (37.6-48.8) mya, for the split between "East African" and "South African clades" ~38 (33.3-43.5) mya, and for the first diversification of the South African genera (*Pedioplanis*, *Tropidosaura*) ~27.5 (22.3-32.7) mya. The divergence of the xeric *Meroles* spp. from the lineage of the (recently) more mesic and semi-desert *I. squamulosa* should have occurred at ~18.5 (13.6-23.4) mya.

From a paleoclimatic point of view both hypothetical scenarios might appear plausible within the proposed time frames, although there is, e.g., a difference of ~20 my between the two estimates for the node defining the "South African clade". However, 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 in a humid period around the Mid Miocene Climatic Optimum (Fig. S2). 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 the first 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 tremendously 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., Salvi et al. 2011; Lamb and Bauer 2003; Makokha et al. 2007; 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 (e.g., especially the node of *Ichnotropis* and *Meroles* increased strongly from 37 to 94). 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 the present 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. The present 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. Based on "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 (present study; Makokha et al. 2007; Salvi et al. 2011) revealed intraspecific p-distances of up to 4.0%. However, compared to other representatives of the "South African clade", this value is in a range quite common for intraspecific variation. *16S* sequence data of *M. suborbitalis* (present study; Harris et al. 1998 b; 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% (present study; Lamb and Bauer 2003; Makokha et al. 2007). In *Tropidosaura* intraspecific distances range around 4.5% (*T. gularis*: present study; Harris et al. 1998 b; Fu 2000) and 7.7% (*T. montana*: present 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. Comprehensive phylogeographic analyses using both mt and nc sequences 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 southwest and extreme northeast, 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 implicate that *I. squamulosa* must be transferred from *Ichnotropis* to the genus *Meroles*.

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# TABLES

**Table 1.** List of analyzed specimens, their geographical origin and GenBank Accession numbers for all partial gene sequences. Numbers in parentheses refer to numbers in the tree.

| Species / Lab code | Geographical origin | c-mos | RAG-1 | RAG-2 | EXPH5 | KIF24 | PRLR | cyt b | 12S | 16S |
|---|---|---|---|---|---|---|---|---|---|---|
| **Authorities of scientific names** | **GenBank Accession numbers** | | | | | | | | | |
| *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)** | | | | | | | | | |
| 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 |

• Previously used sequences are marked with a black spot.

**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 | present study |
| rag2_Lac 3fw | 5´-GAACTCAAACTGAAGCCGACA-3' | fwd | PCR/Sequencing | 50 | present 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 | present study |
| EXPH Lac2rv | 5‘-CCTCTTTATGTTATCCAAGAAAGC-3‘ | rev | PCR/Sequencing | 54 | present 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 | present study |
| KIF24 Lac2rv | 5’-CTGGTGGTAAAGGCGGAGGT-3’ | rev | PCR/Sequencing | 57 | present 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 | present study |
| PRLR Lac2rv | 5’-ACTTCYACRTAATCCATGGGYTTTG-3’ | rev | PCR/Sequencing | 50-57 | present study |
| PRLR Lac3fw | 5’-GACARYGARGACCAGCAACTRAT-3’ | fwd | PCR/Sequencing | 50-57 | present study |
| PRLR Lac4rv | 5’-GACYTTGTGRACTTCYACRTAAT-3’ | rev | PCR/Sequencing | 50-57 | present study |
| PRLR_int_fwJ | 5’-AAGCTGGTGCACCAGGAA-3’ | fwd | PCR/Sequencing | 50-57 | present study |
| PRLR_rvJ | 5’-TTGACTTTGTGGACTTCTACATA-3’ | rev | PCR/Sequencing | 50-57 | present 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 |
| *cyt b* | | | | | |
| LgluLK | 5'-AACCGCTGTTGTCTTCAACTA-3' | fwd | PCR | 50 | Pavlicev and Mayer 2009 |
| t-Glu2 | 5'-CGACTCGAAAAACCGCCGTTG-3' | fwd | PCR | 50 | present study |
| LGlu-cons | 5'-GAAAAACCACCGTTGTATTCAACTA-3' | fwd | PCR | 50 | present 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<br>Sequence length (bp) | Models (AIC) used for<br>Bayesian inference |
|---|---|---|
| *c-mos* | 581 bp | HKY+G; nst=2, rates=gamma |
| *RAG-1* | 1012 bp | TiM+I+G; nst=6, rates=invgamma |
| *RAG-2* | 943 bp | HKY+G; nst=2, rates=gamma |
| *EXPH5* | 906 bp<br>(861-903 bp) | TiM+G; nst=6, rates=gamma |
| *KIF24* | 490 bp<br>(454-484 bp) | TPM3 uf+G; nst=6, rates=gamma |
| *PRLR* | 541 bp<br>(440-541 bp) | TrN+G; nst=6, rates=gamma |
| *12S* | 477 bp / 429 bp<br>(455-464 bp) | GTR+I+G; nst=6, rates= invgamma |
| *16S* | 526 bp / 473 bp<br>(489-506 bp) | TiM2+I+G; nst=6, rates=invgamma |
| *cyt b* | 1143 bp | TPM3uf+I+G; nst=6, rates=invgamma |

Note - 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.

**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 |

## FIGURES


**Figure 1.** Map of Southern Africa with sample localities of individuals analysed (lab codes). The locality of ABH-3 (triangle) is unknown.

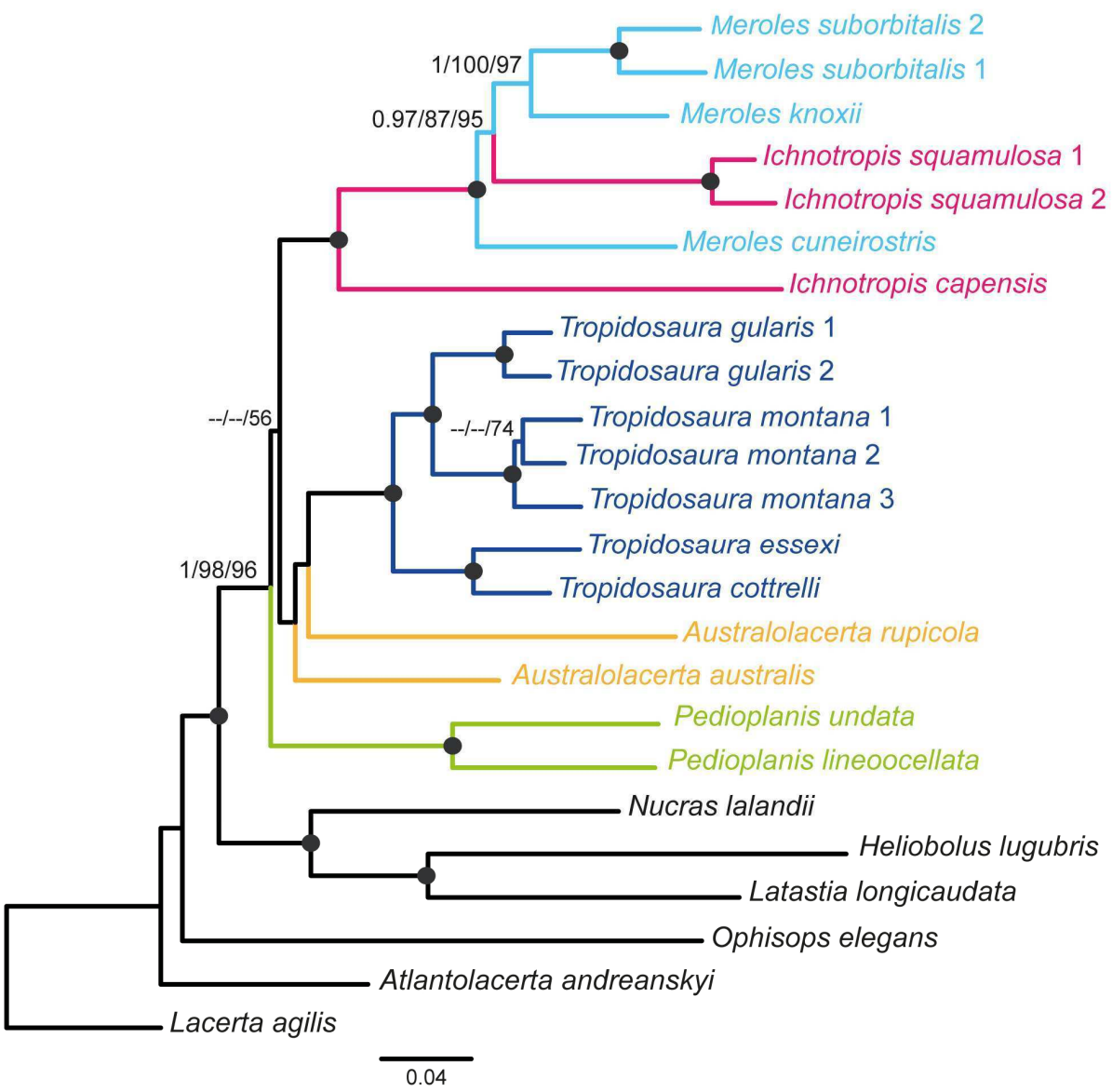


**Figure 2.** Phylogenetic BI tree based on the concatenated nc and mt gene sequences. Nodes with maximum support values from BI/ML/NJ are marked with a black spot. Support values under 0.95 (BI) and 50 % (ML, NJ) are not shown.

# APPENDIX I

## TABLES

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

| ***c-mos* p-distance** | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 0.69 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 2.41 | 2.24 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 2.24 | 1.89 | 2.75 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 2.24 | 1.89 | 3.10 | 2.75 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 2.93 | 2.58 | 3.44 | 3.10 | 0.86 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 4.13 | 3.96 | 4.48 | 3.61 | 4.82 | 5.16 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 4.13 | 3.96 | 4.48 | 3.61 | 4.48 | 4.82 | 3.79 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 4.30 | 4.13 | 4.65 | 3.79 | 4.65 | 4.99 | 3.96 | 0.17 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 5.16 | 4.99 | 5.16 | 4.65 | 5.51 | 5.85 | 4.65 | 1.38 | 1.55 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 5.16 | 4.99 | 5.34 | 4.65 | 5.51 | 5.85 | 4.65 | 1.55 | 1.72 | 0.69 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 4.99 | 4.82 | 5.16 | 4.48 | 5.34 | 5.68 | 4.48 | 1.20 | 1.38 | 0.69 | 0.69 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 4.82 | 4.65 | 5.16 | 4.30 | 5.16 | 5.51 | 4.13 | 1.72 | 1.89 | 2.58 | 2.93 | 2.58 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 4.30 | 4.13 | 4.65 | 3.79 | 4.65 | 4.99 | 3.96 | 1.20 | 1.38 | 2.07 | 2.41 | 2.07 | 0.86 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 4.13 | 3.96 | 4.48 | 3.61 | 4.82 | 5.16 | 3.61 | 2.24 | 2.41 | 3.27 | 3.44 | 3.10 | 2.93 | 2.41 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 4.99 | 4.82 | 5.34 | 4.65 | 4.99 | 5.51 | 4.82 | 3.10 | 3.27 | 4.13 | 4.30 | 3.96 | 3.79 | 3.27 | 3.61 | | | | | | | | |
| (17) *P. undata* ABE-423 | 4.82 | 4.65 | 4.99 | 4.48 | 5.51 | 6.20 | 4.82 | 2.75 | 2.93 | 3.96 | 4.13 | 3.79 | 3.79 | 3.27 | 3.61 | 4.65 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 6.02 | 5.51 | 6.37 | 5.68 | 6.20 | 6.88 | 6.37 | 4.65 | 4.82 | 5.51 | 5.68 | 5.34 | 5.34 | 4.82 | 4.82 | 5.68 | 3.61 | | | | | | |
| (19) *N. lalandii* NUL-1 | 6.02 | 5.85 | 6.37 | 5.51 | 6.37 | 6.71 | 4.82 | 3.61 | 3.79 | 4.30 | 4.48 | 4.13 | 3.96 | 3.44 | 4.13 | 4.82 | 4.99 | 6.54 | | | | | |
| (20) *H. lugubris* ABB-20 | 7.24 | 7.07 | 7.41 | 6.72 | 7.76 | 8.10 | 6.03 | 5.34 | 5.52 | 6.03 | 6.21 | 5.86 | 5.69 | 5.17 | 5.00 | 6.55 | 6.72 | 7.76 | 4.83 | | | | |
| (21) *L. longicaudata* ATA-13 | 6.54 | 6.37 | 6.71 | 5.68 | 7.06 | 7.40 | 5.34 | 4.30 | 4.48 | 4.99 | 5.16 | 4.82 | 4.99 | 4.48 | 4.48 | 5.34 | 5.68 | 7.06 | 3.79 | 3.10 | | | |
| (22) *O. elegans* OJ-1 | 6.88 | 6.71 | 7.06 | 6.37 | 7.57 | 7.92 | 6.20 | 4.30 | 4.48 | 4.65 | 4.82 | 4.82 | 4.99 | 4.48 | 5.16 | 5.34 | 5.68 | 7.06 | 5.16 | 6.90 | 6.02 | | |
| (23) *A. andreanskyi* LN-4 | 4.48 | 4.30 | 4.82 | 3.96 | 4.48 | 4.82 | 3.61 | 2.75 | 2.93 | 3.44 | 3.61 | 3.27 | 3.44 | 2.93 | 2.75 | 4.13 | 4.13 | 4.99 | 3.27 | 5.00 | 4.13 | 4.99 | |
| (24) *L. agilis* WT-1 | 4.48 | 4.30 | 4.82 | 4.30 | 4.82 | 5.16 | 3.79 | 2.41 | 2.58 | 3.10 | 3.27 | 2.93 | 3.10 | 2.58 | 3.10 | 4.13 | 4.13 | 5.68 | 3.79 | 5.52 | 4.48 | 4.99 | 2.75 |

## *RAG*-1 p-distance

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 0.70 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 1.70 | 1.79 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 2.29 | 2.58 | 2.59 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 2.71 | 2.69 | 2.21 | 3.00 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 2.99 | 3.08 | 2.59 | 3.28 | 1.20 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 4.59 | 4.67 | 4.10 | 4.78 | 4.60 | 5.07 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 3.68 | 3.57 | 3.19 | 3.77 | 3.59 | 3.97 | 4.08 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 3.58 | 3.47 | 3.09 | 3.67 | 3.49 | 3.87 | 3.98 | 0.30 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 2.99 | 3.37 | 2.99 | 3.37 | 3.49 | 4.07 | 4.17 | 1.98 | 1.88 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 3.18 | 3.37 | 2.99 | 3.37 | 3.49 | 3.87 | 3.98 | 1.78 | 1.68 | 0.59 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 3.38 | 3.77 | 3.19 | 3.47 | 3.69 | 4.46 | 4.08 | 2.28 | 2.18 | 0.89 | 0.89 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 3.48 | 3.57 | 2.89 | 3.37 | 2.99 | 3.67 | 3.88 | 2.08 | 2.08 | 1.98 | 1.78 | 2.08 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 3.78 | 3.87 | 3.29 | 3.67 | 3.49 | 3.97 | 3.98 | 1.78 | 1.78 | 1.88 | 1.68 | 1.98 | 0.89 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 3.78 | 4.06 | 3.49 | 3.67 | 3.49 | 4.07 | 4.47 | 2.58 | 2.48 | 2.87 | 2.87 | 3.17 | 2.87 | 2.97 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 4.18 | 4.06 | 3.89 | 4.07 | 3.99 | 4.17 | 4.67 | 3.47 | 3.37 | 3.47 | 3.07 | 3.77 | 3.27 | 3.57 | 3.37 | | | | | | | | |
| (17) *P. undata* ABE-423 | 5.27 | 5.55 | 4.89 | 5.36 | 5.49 | 6.05 | 4.77 | 4.26 | 4.26 | 4.36 | 4.36 | 4.66 | 3.96 | 4.26 | 4.56 | 5.45 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 5.12 | 5.20 | 4.63 | 5.01 | 5.14 | 5.71 | 4.81 | 3.80 | 3.70 | 4.20 | 4.00 | 4.30 | 4.20 | 4.10 | 4.20 | 4.90 | 3.40 | | | | | | |
| (19) *N. lalandii* NUL-1 | 4.58 | 4.66 | 4.09 | 4.77 | 4.70 | 5.06 | 4.98 | 4.07 | 3.97 | 3.87 | 3.87 | 4.37 | 3.87 | 3.97 | 4.17 | 4.37 | 5.26 | 5.11 | | | | | |
| (20) *H. lugubris* ABB-20 | 7.16 | 7.23 | 6.38 | 6.94 | 7.19 | 7.34 | 7.16 | 6.44 | 6.34 | 6.64 | 6.64 | 6.74 | 6.05 | 6.34 | 6.24 | 6.94 | 7.23 | 6.80 | 6.35 | | | | |
| (21) *L. longicaudata* ATA-13 | 5.48 | 5.56 | 5.19 | 5.46 | 5.89 | 6.26 | 5.57 | 5.06 | 4.96 | 5.06 | 5.06 | 5.16 | 4.56 | 5.06 | 4.96 | 5.26 | 5.95 | 5.91 | 4.57 | 5.95 | | | |
| (22) *O. elegans* OJ-1 | 5.77 | 5.65 | 5.58 | 6.25 | 5.99 | 6.15 | 6.66 | 6.05 | 6.05 | 6.14 | 5.75 | 6.34 | 6.05 | 6.05 | 6.44 | 6.54 | 7.23 | 7.30 | 6.75 | 9.12 | 7.54 | | |
| (23) *A. andreanskyi* LN-4 | 3.98 | 4.06 | 3.39 | 4.27 | 4.19 | 4.27 | 4.47 | 3.67 | 3.57 | 3.67 | 3.27 | 3.96 | 3.47 | 3.57 | 3.96 | 3.77 | 4.66 | 4.70 | 4.07 | 6.44 | 5.16 | 5.15 | |
| (24) *L. agilis* WT-1 | 4.58 | 4.66 | 4.49 | 4.76 | 4.99 | 5.16 | 5.57 | 4.36 | 4.26 | 4.36 | 4.36 | 4.46 | 4.36 | 4.26 | 4.66 | 5.05 | 5.35 | 5.60 | 5.16 | 6.64 | 5.95 | 6.05 | 3.07 |

## *RAG-2* p-distance

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 1.06 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 2.34 | 2.12 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 2.67 | 2.46 | 3.10 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 2.98 | 2.98 | 3.40 | 2.57 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 2.87 | 2.87 | 3.08 | 2.46 | 0.32 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 4.35 | 4.14 | 4.35 | 3.95 | 4.26 | 4.04 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 3.40 | 3.19 | 3.83 | 2.89 | 3.41 | 3.09 | 3.93 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 3.29 | 3.08 | 3.72 | 2.67 | 3.30 | 2.98 | 3.72 | 0.21 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 3.72 | 3.50 | 4.14 | 3.10 | 3.72 | 3.40 | 4.14 | 1.38 | 1.17 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 3.61 | 3.61 | 4.25 | 3.21 | 3.72 | 3.40 | 4.25 | 1.38 | 1.17 | 0.85 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 3.40 | 3.19 | 3.83 | 2.78 | 3.41 | 3.09 | 3.83 | 0.96 | 0.74 | 0.64 | 0.85 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 2.56 | 2.35 | 3.20 | 1.93 | 2.67 | 2.56 | 3.42 | 1.39 | 1.17 | 1.49 | 1.71 | 1.28 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 2.76 | 2.44 | 3.40 | 2.14 | 2.77 | 2.66 | 3.40 | 1.49 | 1.28 | 1.59 | 1.81 | 1.38 | 0.00 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 3.29 | 3.08 | 3.93 | 3.10 | 3.83 | 3.72 | 4.03 | 2.76 | 2.55 | 2.97 | 3.08 | 2.66 | 2.03 | 2.13 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 3.82 | 3.61 | 4.25 | 3.31 | 3.83 | 3.72 | 4.46 | 3.51 | 3.29 | 3.72 | 3.82 | 3.40 | 2.45 | 2.55 | 3.18 | | | | | | | | |
| (17) *P. undata* ABE-423 | 3.76 | 3.55 | 4.41 | 3.25 | 3.99 | 3.88 | 4.19 | 3.55 | 3.33 | 3.76 | 3.87 | 3.44 | 2.70 | 2.80 | 3.33 | 3.87 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 3.54 | 3.33 | 4.29 | 3.02 | 3.98 | 3.87 | 4.51 | 3.44 | 3.22 | 3.65 | 3.76 | 3.44 | 2.59 | 2.69 | 3.11 | 3.65 | 1.52 | | | | | | |
| (19) *N. lalandii* NUL-1 | 4.04 | 3.83 | 4.78 | 3.42 | 4.37 | 4.26 | 4.68 | 3.62 | 3.40 | 3.61 | 3.72 | 3.30 | 2.99 | 3.09 | 3.72 | 4.36 | 4.31 | 4.08 | | | | | |
| (20) *H. lugubris* ABB-20 | 6.50 | 6.28 | 6.71 | 6.11 | 6.19 | 6.08 | 6.60 | 5.97 | 5.86 | 6.07 | 6.18 | 5.76 | 5.46 | 5.54 | 6.28 | 6.82 | 6.47 | 6.46 | 5.44 | | | | |
| (21) *L. longicaudata* ATA-13 | 5.53 | 5.31 | 6.16 | 5.24 | 5.32 | 5.32 | 5.53 | 5.21 | 4.99 | 5.10 | 5.42 | 4.79 | 4.38 | 4.36 | 5.10 | 5.63 | 5.49 | 5.26 | 4.57 | 5.65 | | | |
| (22) *O. elegans* OJ-1 | 4.46 | 4.25 | 5.31 | 4.27 | 4.36 | 4.36 | 5.52 | 3.72 | 3.50 | 3.93 | 4.03 | 3.61 | 3.31 | 3.40 | 4.14 | 4.88 | 4.62 | 4.51 | 4.57 | 6.71 | 6.06 | | |
| (23) *A. andreanskyi* LN-4 | 2.35 | 2.13 | 2.99 | 2.04 | 2.78 | 2.67 | 3.20 | 2.03 | 1.81 | 2.24 | 2.35 | 1.92 | 1.18 | 1.28 | 2.13 | 2.56 | 2.49 | 2.37 | 2.67 | 4.93 | 4.06 | 2.45 | |
| (24) *L. agilis* WT-1 | 3.73 | 3.51 | 4.37 | 3.32 | 3.95 | 3.84 | 4.15 | 2.88 | 2.66 | 3.19 | 3.30 | 2.88 | 2.36 | 2.45 | 3.30 | 3.94 | 3.88 | 3.44 | 3.84 | 5.77 | 5.12 | 3.62 | 1.71 |

## *EXPH5* p-distance

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 1.80 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 2.91 | 2.47 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 4.74 | 4.19 | 3.95 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 5.04 | 4.38 | 4.37 | 5.19 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 5.17 | 4.74 | 4.27 | 5.44 | 1.24 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 8.85 | 8.08 | 7.72 | 8.58 | 8.74 | 9.24 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 7.05 | 6.29 | 5.82 | 7.00 | 6.94 | 7.09 | 7.15 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 6.73 | 6.18 | 5.94 | 7.23 | 6.84 | 7.21 | 7.39 | 1.01 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 7.28 | 6.62 | 5.94 | 7.34 | 7.17 | 7.20 | 7.95 | 2.80 | 3.03 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 6.51 | 5.85 | 5.39 | 6.67 | 6.62 | 6.88 | 7.51 | 2.36 | 2.36 | 0.90 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 7.61 | 6.96 | 6.49 | 7.79 | 7.73 | 7.99 | 8.17 | 3.14 | 3.14 | 1.68 | 1.23 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 7.97 | 7.20 | 6.51 | 7.69 | 7.86 | 7.89 | 8.30 | 3.25 | 3.37 | 4.26 | 3.82 | 4.26 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 6.72 | 6.17 | 5.49 | 7.00 | 7.28 | 7.42 | 7.15 | 3.25 | 3.14 | 3.81 | 3.14 | 3.81 | 2.24 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 6.18 | 5.18 | 4.94 | 5.89 | 6.29 | 6.66 | 6.26 | 3.37 | 3.49 | 3.93 | 3.38 | 4.04 | 4.39 | 3.48 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 6.64 | 6.09 | 5.07 | 6.58 | 6.64 | 6.56 | 6.62 | 4.50 | 4.40 | 4.73 | 4.40 | 5.07 | 5.19 | 4.50 | 3.39 | | | | | | | | |
| (17) *P. undata* ABE-423 | 8.77 | 8.33 | 7.74 | 9.07 | 9.45 | 9.61 | 8.78 | 6.49 | 6.61 | 6.83 | 6.28 | 6.95 | 7.31 | 6.61 | 5.60 | 6.19 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 8.57 | 7.57 | 7.33 | 8.18 | 8.46 | 8.49 | 9.03 | 6.09 | 6.09 | 6.31 | 5.76 | 6.65 | 6.67 | 5.86 | 4.98 | 5.56 | 5.85 | | | | | | |
| (19) *N. lalandii* NUL-1 | 7.84 | 6.85 | 6.49 | 6.77 | 7.73 | 8.10 | 7.95 | 5.82 | 5.49 | 6.38 | 5.84 | 6.72 | 6.40 | 5.60 | 4.49 | 5.07 | 6.83 | 6.31 | | | | | |
| (20) *H. lugubris* ABB-20 | 10.11 | 9.57 | 9.10 | 9.51 | 10.67 | 11.06 | 10.02 | 8.31 | 8.44 | 9.10 | 8.33 | 8.88 | 9.12 | 7.98 | 7.22 | 8.02 | 8.80 | 8.82 | 6.40 | | | | |
| (21) *L. longicaudata* ATA-13 | 12.00 | 11.24 | 10.87 | 11.07 | 12.11 | 12.50 | 11.82 | 9.87 | 9.65 | 10.65 | 10.00 | 10.31 | 10.56 | 9.53 | 8.77 | 9.47 | 10.72 | 10.72 | 7.85 | 7.99 | | | |
| (22) *O. elegans* OJ-1 | 10.92 | 9.90 | 9.99 | 10.19 | 11.03 | 11.55 | 11.07 | 8.25 | 8.37 | 9.18 | 8.50 | 8.83 | 9.20 | 8.36 | 7.58 | 9.11 | 10.40 | 10.18 | 8.59 | 11.77 | 12.79 | | |
| (23) *A. andreanskyi* LN-4 | 7.17 | 6.40 | 5.94 | 6.66 | 7.39 | 7.76 | 7.38 | 5.04 | 5.16 | 5.60 | 5.05 | 5.94 | 6.06 | 5.15 | 3.71 | 4.84 | 6.95 | 6.65 | 5.15 | 7.42 | 9.30 | 8.25 | |
| (24) *L. agilis* WT-1 | 7.84 | 7.07 | 6.61 | 7.67 | 7.61 | 8.21 | 8.29 | 5.15 | 5.04 | 5.94 | 5.05 | 5.82 | 5.95 | 5.04 | 4.61 | 5.63 | 8.20 | 7.44 | 5.60 | 7.98 | 9.87 | 7.55 | 4.70 |

## *KIF24* p-distance

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 1.06 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 1.91 | 2.12 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 1.56 | 1.78 | 0.44 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 7.01 | 6.36 | 6.36 | 4.89 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 6.37 | 5.72 | 5.30 | 4.67 | 2.33 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 12.53 | 12.29 | 12.08 | 11.11 | 15.04 | 13.98 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 7.43 | 7.20 | 6.78 | 6.22 | 9.53 | 8.69 | 11.02 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 7.86 | 7.63 | 7.20 | 6.44 | 9.96 | 9.11 | 11.44 | 0.42 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 7.64 | 7.42 | 7.42 | 6.89 | 10.17 | 9.32 | 11.02 | 1.69 | 2.12 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 7.04 | 6.60 | 6.81 | 6.25 | 9.57 | 8.72 | 11.28 | 1.06 | 1.49 | 0.43 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 7.22 | 6.99 | 6.99 | 6.44 | 9.75 | 8.90 | 11.44 | 1.27 | 1.69 | 0.85 | 0.21 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 7.92 | 7.69 | 7.05 | 6.50 | 9.62 | 8.76 | 12.18 | 2.56 | 2.99 | 3.21 | 2.58 | 2.78 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 8.49 | 8.26 | 8.26 | 7.78 | 11.02 | 10.17 | 12.71 | 3.60 | 4.03 | 4.24 | 3.62 | 3.81 | 2.14 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 7.64 | 6.99 | 6.99 | 6.67 | 10.17 | 9.32 | 12.50 | 4.24 | 4.66 | 4.87 | 4.04 | 4.45 | 4.70 | 5.51 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 9.40 | 9.38 | 8.93 | 8.45 | 11.61 | 11.38 | 12.28 | 6.25 | 6.70 | 6.92 | 6.50 | 6.70 | 6.31 | 7.37 | 8.26 | | | | | | | | |
| (17) *P. undata* ABE-423 | 10.40 | 10.17 | 9.32 | 8.44 | 12.29 | 11.44 | 13.56 | 7.84 | 8.26 | 8.47 | 7.87 | 8.05 | 7.69 | 8.47 | 7.63 | 10.94 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 9.87 | 9.85 | 8.99 | 8.31 | 11.56 | 10.71 | 13.92 | 7.49 | 7.92 | 8.14 | 7.31 | 7.71 | 7.34 | 8.57 | 6.85 | 10.38 | 4.28 | | | | | | |
| (19) *N. lalandii* NUL-1 | 10.62 | 10.17 | 10.59 | 9.78 | 13.56 | 12.71 | 15.89 | 8.90 | 9.32 | 9.11 | 8.30 | 8.69 | 8.55 | 9.32 | 8.90 | 10.71 | 11.02 | 10.28 | | | | | |
| (20) *H. lugubris* ABB-20 | 13.49 | 13.25 | 13.03 | 12.56 | 15.81 | 15.60 | 16.88 | 12.39 | 12.82 | 13.03 | 12.45 | 12.61 | 12.28 | 12.61 | 12.82 | 13.29 | 12.82 | 14.04 | 11.11 | | | | |
| (21) *L. longicaudata* ATA-13 | 12.53 | 11.44 | 12.08 | 11.33 | 14.41 | 13.56 | 15.04 | 10.38 | 10.81 | 10.81 | 10.00 | 10.38 | 10.04 | 10.81 | 10.17 | 11.61 | 11.44 | 11.35 | 8.26 | 11.32 | | | |
| (22) *O. elegans* OJ-1 | 10.83 | 11.02 | 9.75 | 9.33 | 12.71 | 11.86 | 14.62 | 8.90 | 9.32 | 9.53 | 8.94 | 8.69 | 8.33 | 9.53 | 8.90 | 10.94 | 9.96 | 9.85 | 9.53 | 14.32 | 12.08 | | |
| (23) *A. andreanskyi* LN-4 | 9.13 | 8.47 | 8.47 | 8.22 | 11.65 | 10.81 | 12.92 | 5.93 | 6.36 | 6.57 | 5.74 | 5.72 | 5.77 | 6.57 | 5.51 | 7.81 | 8.05 | 8.35 | 7.20 | 10.68 | 9.32 | 6.78 | |
| (24) *L. agilis* WT-1 | 8.94 | 8.28 | 8.70 | 8.24 | 12.10 | 11.25 | 13.16 | 6.37 | 6.79 | 6.58 | 5.76 | 6.16 | 6.21 | 7.01 | 5.94 | 9.17 | 9.55 | 9.44 | 7.22 | 11.56 | 8.92 | 7.86 | 4.46 |

## ***PRLR*** **p-distance**

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 1.83 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 3.67 | 2.98 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 5.74 | 5.19 | 4.39 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 6.31 | 6.55 | 5.36 | 6.79 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 6.53 | 7.16 | 5.96 | 7.20 | 1.39 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 12.00 | 11.62 | 10.41 | 12.68 | 10.41 | 11.17 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 7.94 | 7.14 | 6.35 | 7.58 | 7.94 | 8.55 | 9.20 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 7.94 | 7.14 | 6.35 | 7.58 | 7.94 | 8.15 | 9.44 | 1.59 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 8.02 | 7.21 | 6.61 | 6.65 | 8.22 | 8.84 | 9.56 | 2.40 | 2.61 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 8.96 | 8.13 | 7.54 | 7.39 | 9.13 | 9.74 | 10.41 | 3.37 | 3.57 | 0.60 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 9.16 | 7.94 | 7.54 | 8.18 | 9.33 | 9.94 | 10.65 | 3.97 | 4.17 | 1.80 | 2.58 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 9.00 | 8.17 | 7.37 | 8.62 | 8.57 | 8.98 | 10.71 | 3.39 | 3.59 | 3.82 | 4.78 | 4.98 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 8.20 | 7.39 | 6.59 | 7.63 | 7.78 | 8.40 | 10.24 | 2.59 | 2.79 | 3.02 | 3.99 | 4.19 | 2.00 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 7.13 | 6.35 | 5.56 | 6.79 | 7.14 | 7.75 | 9.20 | 2.18 | 2.18 | 2.61 | 3.57 | 3.77 | 2.99 | 2.20 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 9.82 | 8.76 | 8.37 | 9.02 | 9.96 | 10.58 | 10.71 | 4.18 | 4.58 | 5.03 | 5.98 | 6.57 | 5.80 | 5.01 | 4.38 | | | | | | | | |
| (17) *P. undata* ABE-423 | 11.81 | 11.71 | 10.91 | 11.38 | 11.11 | 11.93 | 14.29 | 8.33 | 8.33 | 8.82 | 9.52 | 9.92 | 9.36 | 8.18 | 7.74 | 10.76 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 9.67 | 9.82 | 8.42 | 9.88 | 9.62 | 10.44 | 12.75 | 7.21 | 7.21 | 7.89 | 8.82 | 9.02 | 7.65 | 6.45 | 6.21 | 9.05 | 5.41 | | | | | | |
| (19) *N. lalandii* NUL-1 | 11.00 | 10.32 | 9.52 | 9.98 | 10.52 | 11.13 | 12.11 | 6.55 | 6.55 | 6.81 | 7.74 | 8.33 | 7.17 | 6.59 | 5.56 | 8.37 | 11.11 | 10.02 | | | | | |
| (20) *H. lugubris* ABB-20 | 12.07 | 12.15 | 10.96 | 12.22 | 11.95 | 12.57 | 13.14 | 8.57 | 8.96 | 9.05 | 9.76 | 10.76 | 9.60 | 9.02 | 8.17 | 10.40 | 11.35 | 11.47 | 8.17 | | | | |
| (21) *L. longicaudata* ATA-13 | 10.79 | 10.12 | 9.33 | 10.18 | 9.92 | 10.54 | 11.62 | 6.55 | 6.55 | 6.61 | 7.54 | 8.13 | 7.37 | 6.99 | 5.56 | 9.16 | 10.52 | 10.02 | 5.95 | 6.77 | | | |
| (22) *O. elegans* OJ-1 | 13.90 | 13.33 | 12.32 | 13.21 | 13.13 | 13.77 | 14.85 | 9.90 | 9.90 | 9.80 | 10.10 | 11.11 | 10.55 | 9.35 | 9.09 | 11.76 | 13.94 | 13.06 | 10.71 | 11.56 | 11.52 | | |
| (23) *A. andreanskyi* LN-4 | 11.68 | 11.58 | 10.78 | 11.85 | 11.38 | 11.80 | 12.20 | 7.78 | 7.98 | 8.47 | 9.38 | 9.38 | 8.02 | 7.43 | 6.99 | 9.62 | 12.57 | 11.49 | 8.98 | 12.42 | 9.78 | 12.40 | |
| (24) *L. agilis* WT-1 | 12.42 | 12.10 | 10.91 | 12.77 | 11.11 | 11.73 | 13.80 | 8.53 | 8.53 | 9.22 | 10.12 | 10.32 | 9.16 | 8.18 | 7.54 | 10.16 | 13.29 | 11.62 | 9.92 | 11.35 | 10.12 | 12.53 | 8.58 |

## *12S* p-distance

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 4.07 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 6.46 | 7.18 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 9.57 | 8.85 | 7.42 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 5.76 | 5.52 | 6.95 | 9.59 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 6.47 | 6.71 | 8.39 | 10.55 | 2.64 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 12.50 | 10.58 | 11.06 | 11.78 | 11.08 | 11.57 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 8.85 | 10.05 | 10.29 | 11.00 | 9.59 | 10.55 | 13.70 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 9.81 | 11.00 | 11.00 | 11.72 | 10.55 | 11.03 | 13.22 | 2.87 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 10.05 | 10.53 | 11.00 | 11.48 | 9.83 | 9.83 | 13.22 | 6.70 | 6.94 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 11.24 | 10.53 | 12.20 | 11.96 | 10.55 | 11.75 | 14.66 | 6.94 | 6.94 | 4.55 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 8.85 | 10.29 | 10.29 | 10.29 | 10.07 | 11.03 | 14.66 | 7.42 | 7.66 | 4.78 | 4.07 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 11.96 | 10.77 | 13.16 | 12.92 | 12.47 | 12.95 | 13.22 | 9.33 | 9.33 | 9.57 | 9.81 | 10.05 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 11.48 | 10.29 | 11.72 | 11.72 | 11.27 | 11.75 | 13.70 | 9.09 | 9.09 | 10.53 | 9.57 | 9.81 | 6.94 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 11.24 | 9.81 | 10.53 | 12.44 | 10.55 | 11.75 | 14.18 | 12.44 | 12.92 | 11.00 | 10.53 | 10.05 | 11.24 | 11.72 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 13.64 | 11.96 | 12.92 | 12.92 | 11.75 | 13.19 | 15.38 | 12.20 | 12.20 | 12.92 | 12.68 | 12.68 | 12.20 | 12.20 | 12.68 | | | | | | | | |
| (17) *P. undata* ABE-423 | 11.96 | 11.96 | 11.96 | 13.16 | 10.79 | 12.47 | 13.22 | 13.16 | 12.68 | 14.11 | 13.16 | 13.16 | 13.64 | 11.96 | 12.44 | 14.11 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 11.96 | 10.53 | 12.20 | 13.88 | 11.03 | 12.23 | 13.70 | 11.72 | 11.96 | 12.44 | 11.24 | 12.68 | 11.48 | 12.44 | 11.24 | 13.64 | 10.05 | | | | | | |
| (19) *N. lalandii* NUL-1 | 13.64 | 13.40 | 12.92 | 13.16 | 13.43 | 13.43 | 14.66 | 15.79 | 15.55 | 15.07 | 15.07 | 13.88 | 15.55 | 15.31 | 15.55 | 14.35 | 13.16 | 14.35 | | | | | |
| (20) *H. lugubris* ABB-20 | 13.16 | 12.68 | 14.83 | 14.83 | 13.43 | 13.91 | 14.18 | 14.11 | 14.35 | 14.35 | 13.64 | 14.59 | 14.59 | 14.35 | 13.64 | 16.27 | 14.59 | 12.68 | 15.31 | | | | |
| (21) *L. longicaudata* ATA-13 | 12.68 | 12.92 | 14.35 | 15.07 | 13.43 | 13.67 | 14.18 | 14.35 | 15.07 | 15.55 | 15.79 | 16.27 | 16.75 | 14.35 | 15.31 | 15.55 | 14.11 | 15.31 | 15.55 | 10.53 | | | |
| (22) *O. elegans* OJ-1 | 14.63 | 13.67 | 15.59 | 18.71 | 15.38 | 15.87 | 17.59 | 15.35 | 15.11 | 16.79 | 15.59 | 16.31 | 17.75 | 17.27 | 15.35 | 17.03 | 17.27 | 13.91 | 17.51 | 16.55 | 17.99 | | |
| (23) *A. andreanskyi* LN-4 | 11.96 | 9.81 | 11.00 | 12.68 | 10.79 | 11.99 | 11.06 | 12.20 | 12.20 | 13.16 | 13.16 | 12.92 | 12.20 | 12.68 | 10.29 | 14.35 | 12.20 | 9.57 | 14.11 | 13.16 | 13.64 | 13.43 | |
| (24) *L. agilis* WT-1 | 12.74 | 11.78 | 12.50 | 12.98 | 12.77 | 12.77 | 13.77 | 12.26 | 12.02 | 10.58 | 10.82 | 11.30 | 11.30 | 11.78 | 12.50 | 15.14 | 12.02 | 12.02 | 12.98 | 13.70 | 15.38 | 15.18 | 9.62 |

## *16S* p-distance

| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 3.33 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 5.32 | 5.99 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 5.76 | 6.65 | 5.76 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 8.89 | 9.56 | 9.56 | 9.33 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 9.53 | 10.86 | 9.76 | 9.76 | 2.67 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 13.56 | 13.56 | 13.33 | 12.22 | 16.48 | 16.22 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 12.42 | 11.97 | 12.42 | 11.75 | 13.11 | 14.19 | 12.67 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 11.75 | 11.97 | 11.97 | 11.09 | 12.67 | 13.75 | 12.00 | 3.55 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 11.31 | 11.31 | 10.20 | 9.98 | 11.78 | 12.42 | 12.22 | 5.54 | 4.21 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 11.53 | 11.31 | 10.64 | 10.20 | 12.00 | 12.64 | 12.00 | 4.66 | 3.55 | 0.89 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 12.20 | 11.75 | 11.53 | 11.31 | 12.89 | 13.53 | 13.33 | 5.99 | 4.88 | 2.88 | 2.00 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 11.53 | 12.20 | 12.20 | 11.31 | 13.78 | 13.30 | 11.11 | 7.32 | 7.32 | 6.87 | 6.43 | 8.43 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 11.97 | 12.20 | 10.86 | 11.31 | 12.89 | 13.75 | 11.78 | 6.21 | 5.76 | 4.88 | 4.66 | 6.21 | 4.88 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 11.75 | 11.31 | 10.86 | 10.42 | 12.67 | 13.08 | 12.89 | 9.76 | 9.31 | 8.43 | 7.98 | 9.09 | 10.86 | 8.65 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 11.75 | 11.97 | 12.64 | 11.53 | 13.33 | 13.97 | 14.67 | 12.20 | 11.09 | 9.31 | 9.76 | 10.42 | 12.42 | 11.09 | 11.97 | | | | | | | | |
| (17) *P. undata* ABE-423 | 12.72 | 12.95 | 11.38 | 11.61 | 12.75 | 13.84 | 14.09 | 10.49 | 9.82 | 8.26 | 8.71 | 10.49 | 11.61 | 9.15 | 10.04 | 12.50 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 11.09 | 11.09 | 10.86 | 10.64 | 13.11 | 13.08 | 12.67 | 10.20 | 9.76 | 8.87 | 8.65 | 9.53 | 10.20 | 8.87 | 9.76 | 11.75 | 6.47 | | | | | | |
| (19) *N. lalandii* NUL-1 | 11.41 | 11.41 | 10.74 | 10.07 | 13.23 | 13.42 | 13.90 | 12.53 | 11.86 | 11.63 | 11.41 | 12.30 | 12.98 | 12.08 | 11.86 | 12.98 | 13.29 | 12.75 | | | | | |
| (20) *H. lugubris* ABB-20 | 14.48 | 15.14 | 14.25 | 14.70 | 15.40 | 16.70 | 16.74 | 14.92 | 15.37 | 14.03 | 14.48 | 15.59 | 15.37 | 14.03 | 14.70 | 15.37 | 13.45 | 14.03 | 14.83 | | | | |
| (21) *L. longicaudata* ATA-13 | 11.97 | 12.64 | 12.20 | 12.20 | 13.11 | 13.75 | 14.00 | 12.20 | 10.86 | 11.09 | 11.09 | 12.42 | 12.86 | 10.64 | 9.53 | 12.42 | 12.28 | 9.98 | 10.51 | 12.47 | | | |
| (22) *O. elegans* OJ-1 | 12.64 | 12.64 | 12.86 | 13.30 | 14.44 | 14.41 | 16.89 | 13.97 | 13.53 | 13.30 | 13.53 | 13.75 | 15.08 | 14.19 | 12.42 | 14.41 | 12.95 | 13.08 | 10.51 | 16.04 | 12.64 | | |
| (23) *A. andreanskyi* LN-4 | 11.75 | 12.42 | 11.53 | 11.75 | 13.33 | 13.53 | 12.44 | 12.64 | 11.75 | 10.86 | 11.09 | 12.86 | 11.75 | 11.53 | 10.64 | 12.64 | 11.61 | 10.86 | 10.74 | 14.25 | 9.98 | 11.97 | |
| (24) *L. agilis* WT-1 | 14.06 | 14.29 | 13.39 | 14.29 | 14.99 | 16.29 | 15.44 | 13.17 | 12.28 | 11.16 | 11.16 | 11.61 | 12.28 | 11.16 | 12.28 | 13.62 | 11.69 | 12.05 | 14.64 | 13.90 | 14.06 | 13.17 | 10.49 |

| ***cyt b* p-distance** | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | (22) | (23) |
| (1) *M. suborbitalis* 1 AB-J25 | | | | | | | | | | | | | | | | | | | | | | | |
| (2) *M. suborbitalis* 2 AB-J39 | 14.17 | | | | | | | | | | | | | | | | | | | | | | |
| (3) *M. knoxii* ABM-15 | 17.76 | 18.72 | | | | | | | | | | | | | | | | | | | | | |
| (4) *M. cuneirostris* ABL-18 | 19.25 | 19.86 | 20.73 | | | | | | | | | | | | | | | | | | | | |
| (5) *I. squamulosa* 1 ABH-3 | 19.86 | 19.34 | 17.41 | 19.69 | | | | | | | | | | | | | | | | | | | |
| (6) *I. squamulosa* 2 ABH-9 | 19.69 | 20.12 | 18.20 | 20.47 | 8.66 | | | | | | | | | | | | | | | | | | |
| (7) *I. capensis* ABC-2 | 21.78 | 22.05 | 21.78 | 19.95 | 21.43 | 21.96 | | | | | | | | | | | | | | | | | |
| (8) *T. gularis* 1 ABT-1 | 22.31 | 21.78 | 22.13 | 20.03 | 20.65 | 21.61 | 21.70 | | | | | | | | | | | | | | | | |
| (9) *T. gularis* 2 ABT-3 | 23.01 | 22.31 | 22.40 | 22.05 | 21.43 | 22.57 | 21.52 | 9.01 | | | | | | | | | | | | | | | |
| (10) *T. montana* 1 ABY-2 | 22.48 | 21.17 | 21.78 | 21.43 | 20.65 | 21.52 | 20.82 | 15.05 | 15.92 | | | | | | | | | | | | | | |
| (11) *T. montana* 2 ABY-3 | 22.75 | 20.73 | 21.70 | 20.91 | 21.43 | 22.75 | 21.26 | 15.57 | 16.62 | 10.41 | | | | | | | | | | | | | |
| (12) *T. montana* 3 ABY-4 | 22.48 | 22.66 | 21.52 | 21.35 | 21.61 | 22.13 | 22.31 | 16.71 | 17.76 | 11.37 | 10.41 | | | | | | | | | | | | |
| (13) *T. essexii* ACK-1 | 23.10 | 22.31 | 23.18 | 22.48 | 24.15 | 24.06 | 20.21 | 17.67 | 17.32 | 18.37 | 18.55 | 19.95 | | | | | | | | | | | |
| (14) *T. cottrelli* ACJ-1 | 21.08 | 21.52 | 22.92 | 22.57 | 22.66 | 23.18 | 21.17 | 17.15 | 17.50 | 18.64 | 17.41 | 18.81 | 13.21 | | | | | | | | | | |
| (15) *A. australis* ABU-5 | 21.78 | 21.26 | 21.35 | 21.70 | 21.70 | 22.66 | 22.22 | 20.12 | 20.38 | 19.34 | 20.03 | 20.65 | 20.38 | 21.08 | | | | | | | | | |
| (16) *A. rupícola* ADW-5 | 23.18 | 23.45 | 23.97 | 24.93 | 25.28 | 25.46 | 23.27 | 24.06 | 24.15 | 22.57 | 22.05 | 22.83 | 24.67 | 23.97 | 21.52 | | | | | | | | |
| (17) *P. undata* ABE-423 | 22.48 | 23.01 | 22.40 | 21.78 | 21.43 | 21.96 | 20.38 | 19.69 | 19.86 | 19.42 | 18.64 | 19.86 | 19.77 | 18.81 | 21.61 | 23.97 | | | | | | | |
| (18) *P. lineoocellata* ABA-18 | 21.70 | 22.31 | 21.70 | 23.45 | 22.75 | 23.53 | 22.83 | 19.42 | 20.65 | 20.12 | 20.73 | 21.43 | 20.82 | 20.91 | 22.22 | 23.27 | 16.54 | | | | | | |
| (19) *N. lalandii* NUL-1 | 25.02 | 24.76 | 25.11 | 23.71 | 24.06 | 24.85 | 23.71 | 22.48 | 23.71 | 23.10 | 23.53 | 23.18 | 23.27 | 23.62 | 23.71 | 24.76 | 21.26 | 22.83 | | | | | |
| (20) *H. lugubris* ABB-20 | 23.88 | 23.97 | 22.92 | 24.50 | 24.67 | 24.85 | 22.75 | 23.62 | 24.41 | 23.01 | 22.92 | 23.10 | 24.58 | 25.46 | 23.45 | 24.50 | 22.13 | 23.45 | 23.45 | | | | |
| (21) *L. longicaudata* ATA-13 | 23.80 | 23.36 | 23.88 | 23.18 | 24.50 | 25.20 | 23.10 | 22.05 | 23.27 | 22.31 | 23.36 | 23.01 | 23.36 | 23.71 | 22.31 | 24.76 | 21.08 | 22.22 | 23.01 | 21.43 | | | |
| (22) *O. elegans* OJ-1 | 23.88 | 22.92 | 21.43 | 22.48 | 23.10 | 23.18 | 22.83 | 21.52 | 22.57 | 22.57 | 22.66 | 23.36 | 22.92 | 23.10 | 22.31 | 23.80 | 21.35 | 22.83 | 23.97 | 25.81 | 22.40 | | |
| (23) *A. andreanskyi* LN-4 | 21.43 | 21.78 | 22.66 | 23.01 | 23.62 | 23.18 | 23.27 | 20.82 | 21.43 | 22.31 | 22.48 | 22.75 | 22.75 | 22.13 | 21.61 | 23.71 | 22.13 | 20.47 | 23.01 | 23.62 | 23.53 | 21.61 | |
| (24) *L. agilis* WT-1 | 22.92 | 23.36 | 23.18 | 23.62 | 25.37 | 25.11 | 23.10 | 23.53 | 23.97 | 22.92 | 21.87 | 24.32 | 23.53 | 23.97 | 21.00 | 24.32 | 22.48 | 23.71 | 23.97 | 25.02 | 24.06 | 22.22 | 22.31 |

**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*. Data collected from Boulenger (1917, 1921), Schmidt (1919), Marx (1956), Broadley (1967) and Branch (1998).

| **Character** | ***Ichnotropis s.str.*** | ***Ichnotropis squamulosa*** | ***Meroles*** |
|---|---|---|---|
| chin shield | 5 pairs | 5 pairs | 4-6 pairs |
| collar | absent | absent | present or absent |
| pterygoid teeth | present | present | present or absent |
| dorsal scale rows around midbody | 28-50 rows | 42-58 rows | 42-138 rows |
| rows of ventral plates | 8-10 rows | 10-12 rows | 10-30 rows |
| occipital scale | present | usually absent | usually absent, if present then very small |
| frontonasal | single | bisected longitudinally | sometimes bisected, longitudinally in *M. knoxii* |
| subocular scale | bordering the lip | separated from lip by a labial shield | separated from lip by a labial shield |

## FIGURES

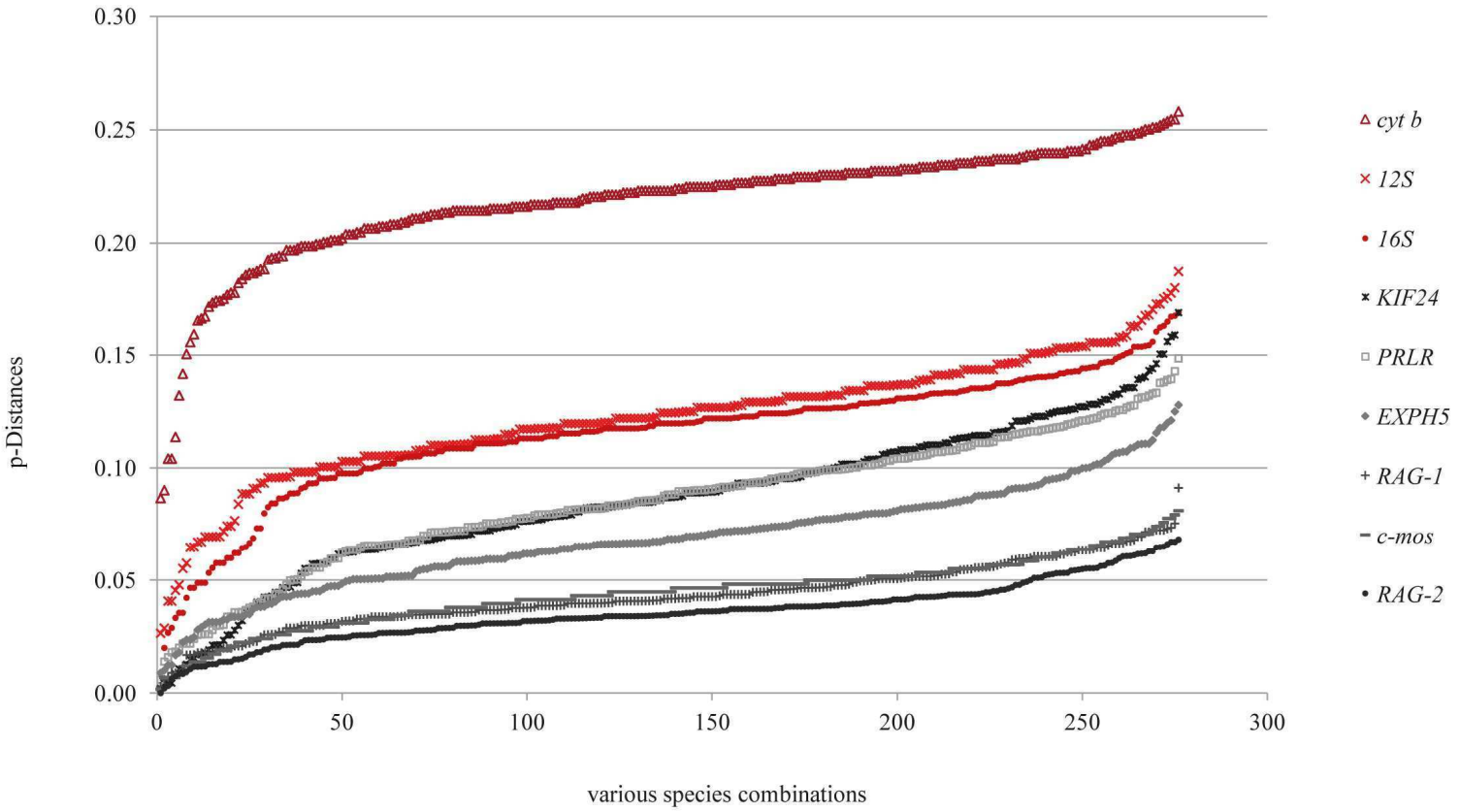


**Figure S1**. Summarizing plot (all nine resulting curves) of pairwise distances of each gene plotted in an ascending order.

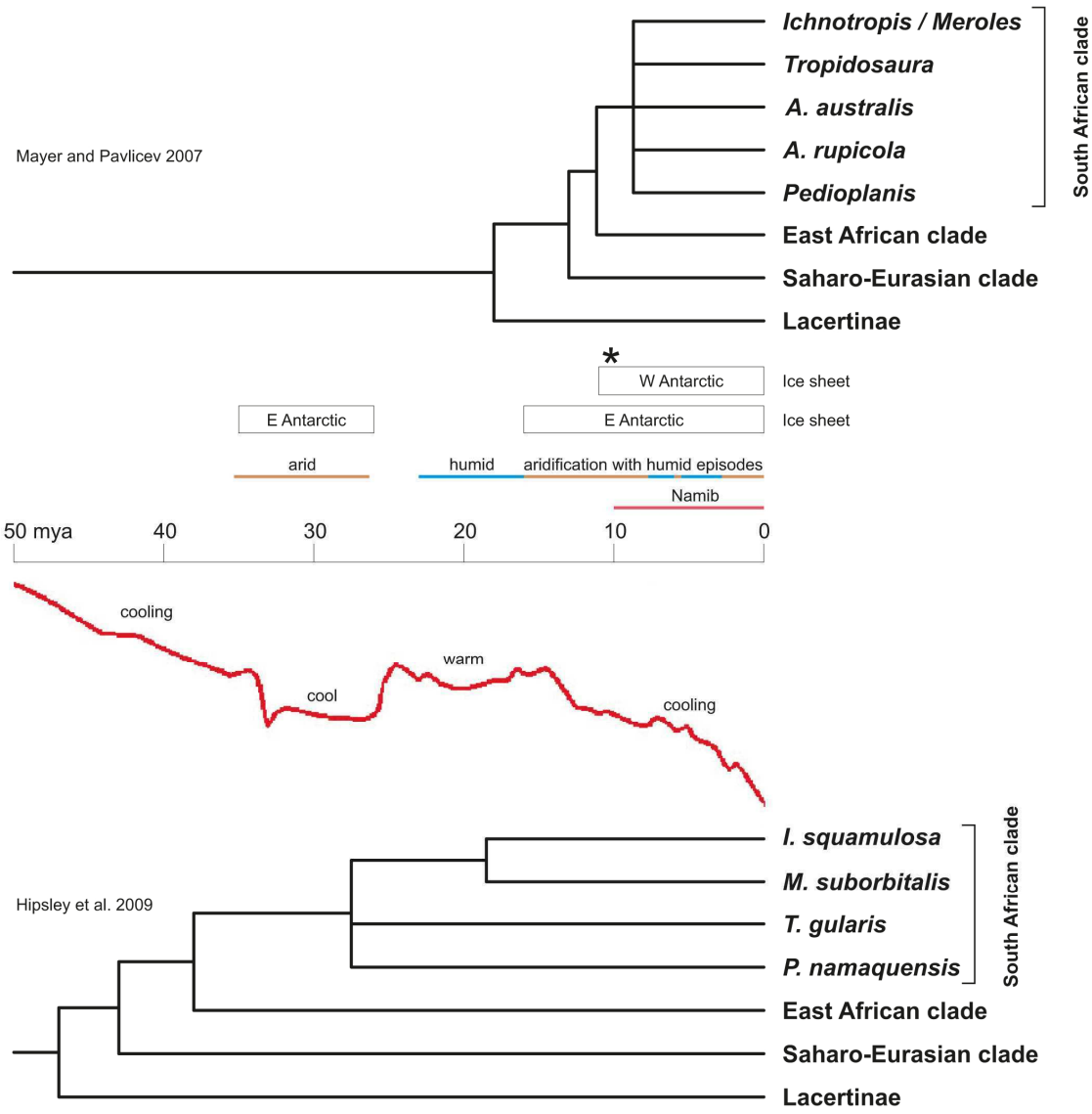


**Figure S2.** Two hypotheses of the radiation of the "South African clade". Above: hypothesis of Mayer and Pavlicev (2007) modified after the present study; below: hypothesis of Hipsley et al. (2007). The red line represents the $\delta^{18}O$ curve (Zachos et al. 2010) which reflects the general temperature trend. The asterisk indicates the starting of the Benguela current. References: Zachos et al. (2010): Cooling trend (50-33 mya); Feakins and deMenocal (2010): development of permanent Antarctic glaciations (35-26 mya); Miller et al. (1987): period of wettest and warmest conditions (23-16 mya); Lockwood (1979), Deacon (1983): reestablishment of permanent ice-caps in Eastern Antarctica (16-11 mya); Diekmann et al. (2003): formation of the West Antarctic ice sheet (11 mya); Diester-Haass et al. (2002): Benguela current (10 mya); Partridge (1993): Hyperarid Namib Desert (10 mya).

# APPENDIX II

Appendix II includes some more figures based on analyses which were performed in the course of the investigation, but not presented in the manuscript submitted. In addition, I present photos of selected taxa of Lacertidae, which were kindly provided by colleagues.

## FIGURES

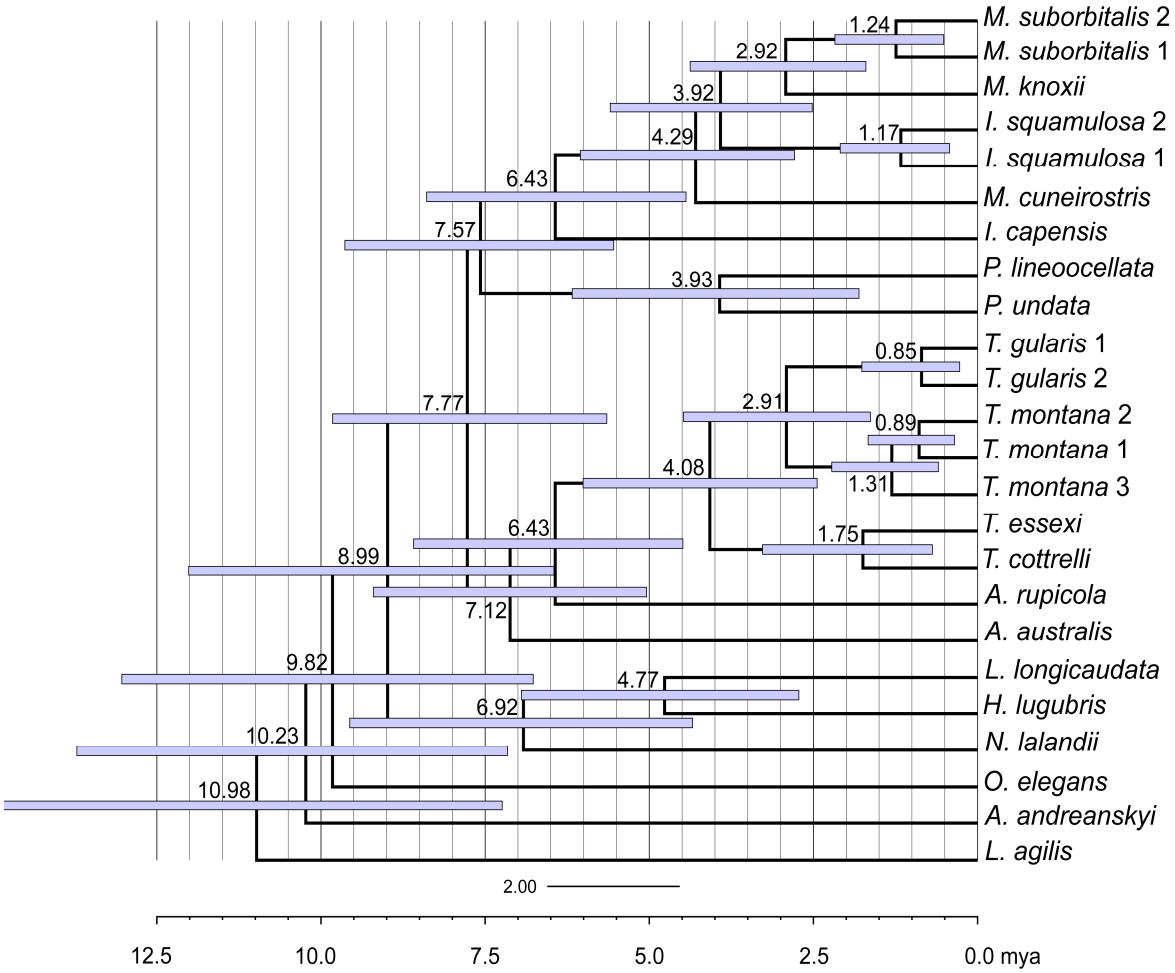


**Figure S3.** The relaxed uncorrelated lognormal clock analysis performed with BEAST. The blue bars show the widely overlapping confidence intervals.

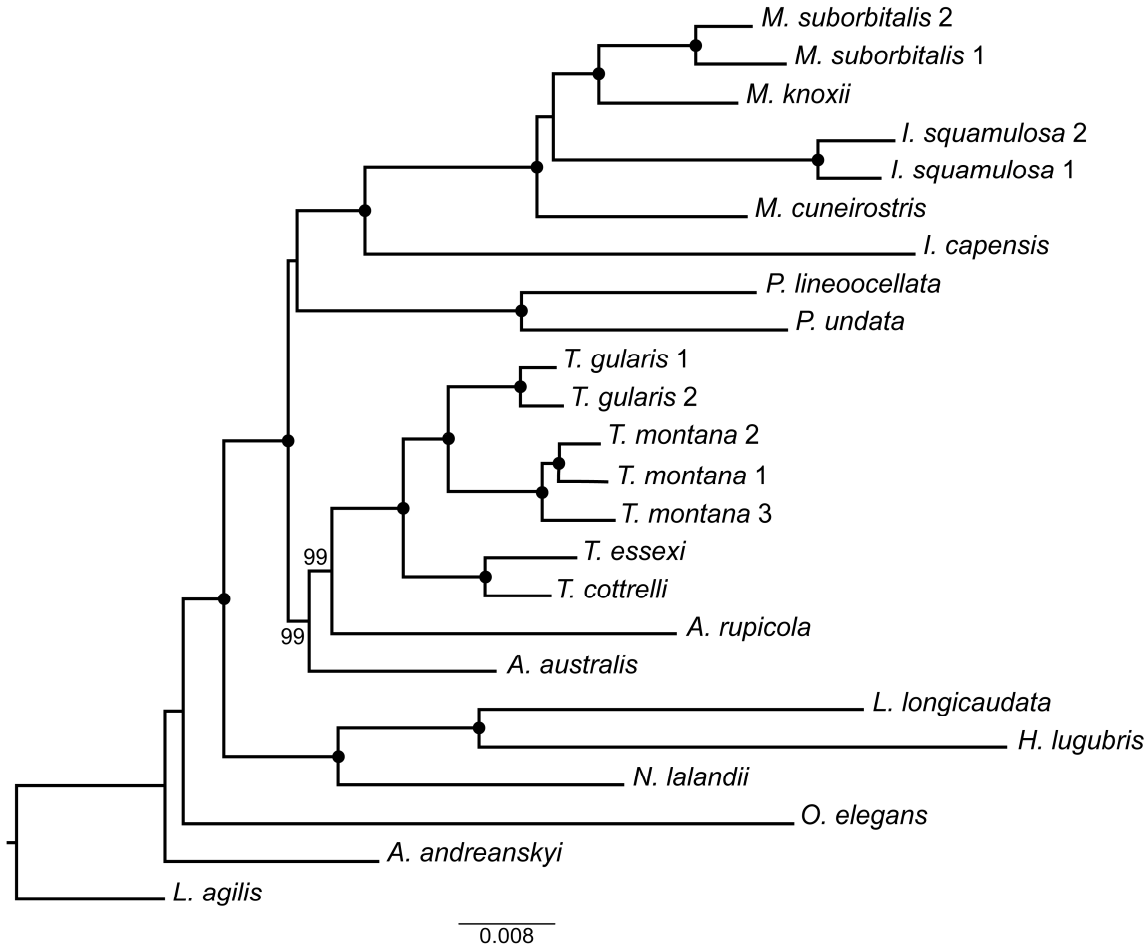


**Figure S4.** Phylogenetic tree based on the BEAST analysis showing the support values. Nodes with maximal support are marked with a black spot. Support values under 0.95 are not shown.

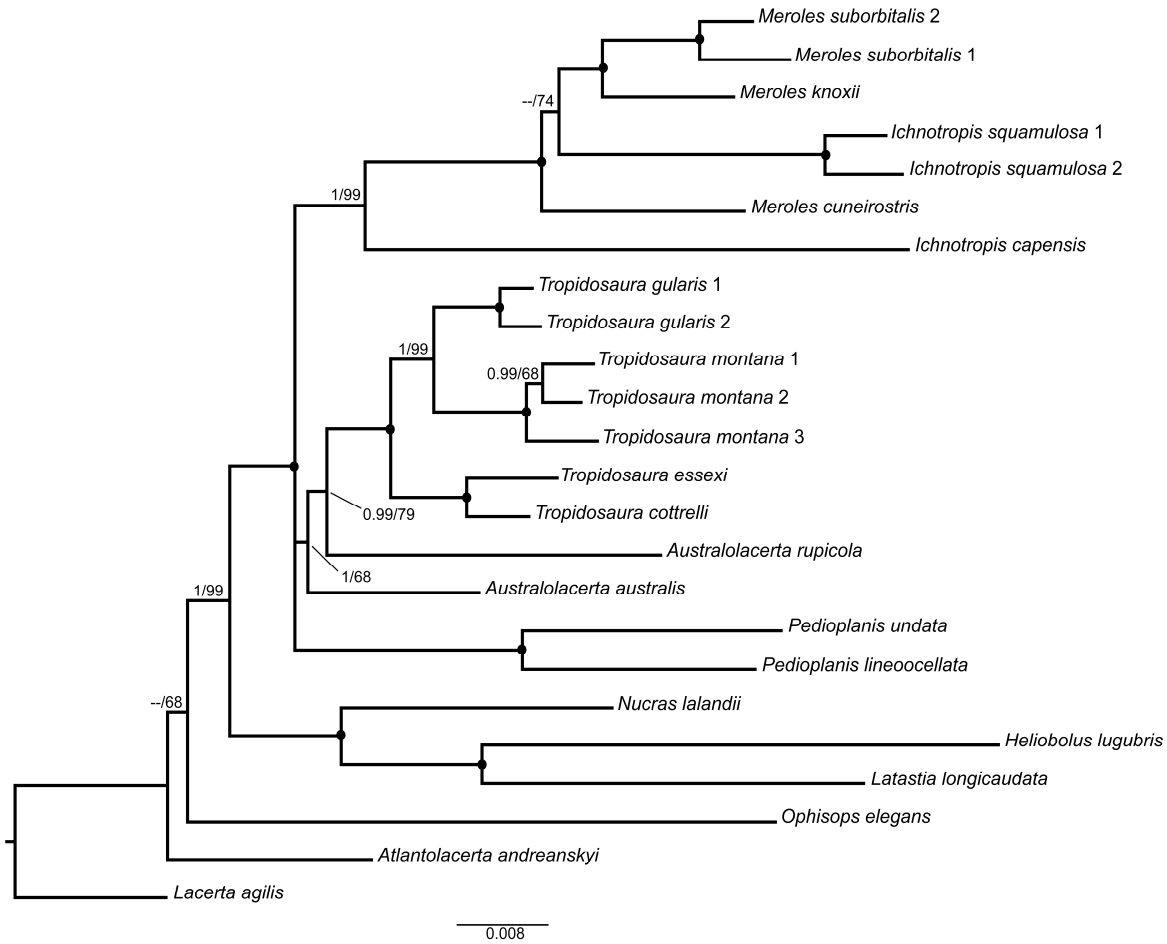


**Figure S5.** Phylogenetic BI tree based on six nuclear gene sequences (*c-mos, RAG-1, RAG-2, EXPH5, KIF24* and *PRLR*). Nodes with maximal support values from BI/ML are marked with a black spot. Support values under 0.95 (BI) and 50 % (ML) are not shown.

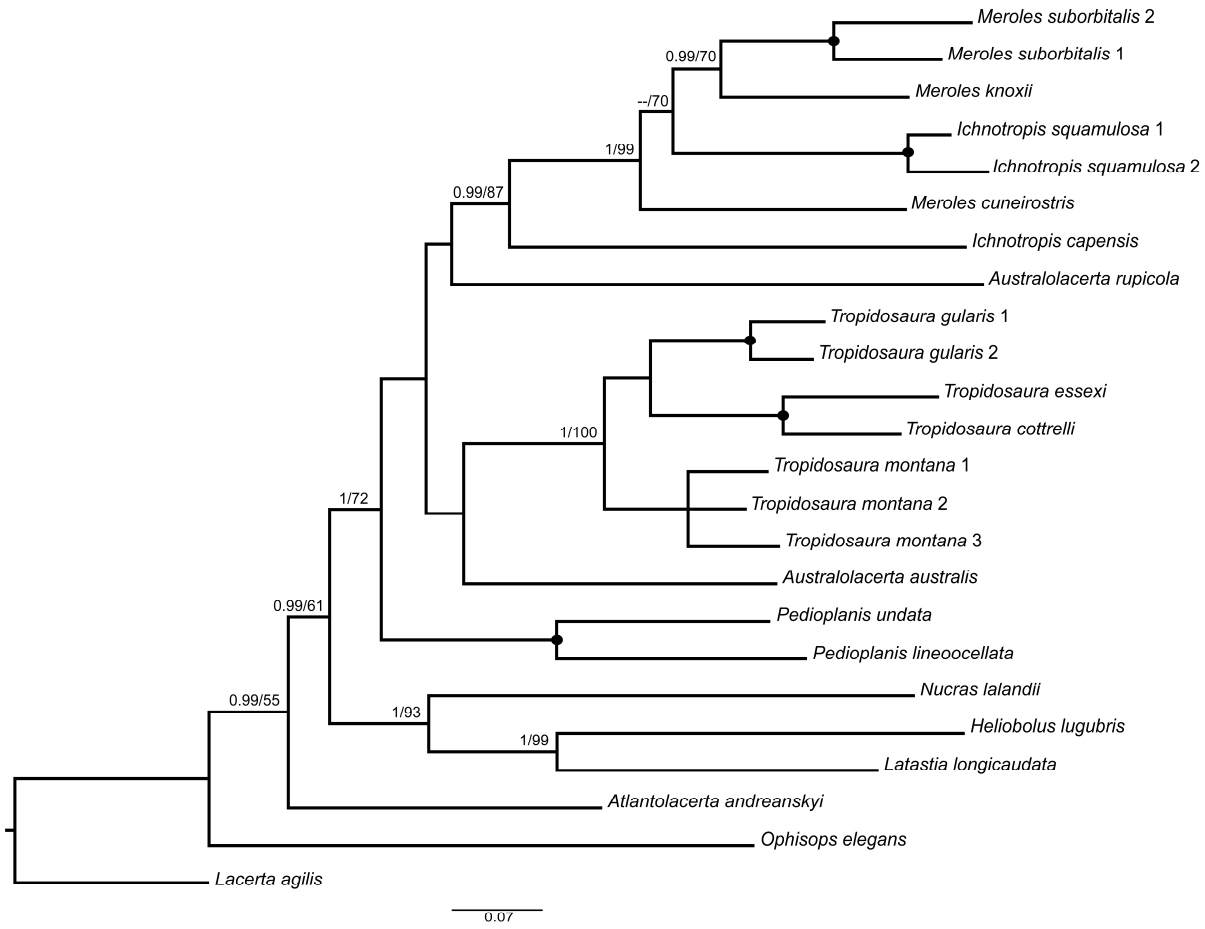


**Figure S6.** Phylogenetic BI tree based on three mitochondrial gene sequences (*12S, 16S* and *cyt b*). Nodes with maximal support values from BI/ML are marked with a black spot. Support values under 0.95 (BI) and 50 % (ML) are not shown.

## PICTURES OF REPRESENTATIVE TAXA

*Meroles knoxii*
©Sebastian Kirchhof

Habitat von *Meroles knoxii*
©Sebastian Kirchhof

*Meroles cuneirostris*
©Mirko Barts

*Ichnotropis squamulosa*
©Sebastian Kirchhof

*Australis rupicola*
©Sebastian Kirchhof

Habitat von *Australis rupicola*
©Sebastian Kirchhof

*Australolacerta australis*
©Sebastian Kirchhof

Habitat von *Australolacerta australis*
©Sebastian Kirchhof

*Pedioplanis lineoocellata*
©Sebastian Kirchhof

*Latastia longicaudata*
©Sebastian Kirchhof

*Heliobolus lugubris*
©Sebastian Kirchhof

Habitat von *Heliobolus lugubris*
©Sebastian Kirchhof

*Ophisops elegans*
©Torsten Panner

*Atlantolacerta andreanskyi*
©Pavel Smek

*Lacerta agilis*
©Johannes Hill

## DEUTSCHE ZUSAMMENFASSUNG

Eremiadinae, eine der drei Subfamilien der Lacertidae, sind in Asien und Afrika verbreitet. Frühere phylogenetische Studien deuteten darauf hin, dass eine der Hauptgruppen der Eremiadinae (der Äthiopische Clade) aus zwei Clades besteht, welche ihr jeweiliges Hauptvorkommen in Ostafrika beziehungsweise in Südafrika haben. Speziell die letztere Gruppe, welche die Gattungen *Pedioplanis, Meroles, Ichnotropis, Tropidosaura* und *Australolacerta* beinhaltet, war in der vorangegangenen molekular phylogenetischen Analyse nicht gut unterstützt.

In der aktuellen Studie wurden die phylogenetischen Beziehungen der Gattungen dieses „südafrikanischen Clades" untersucht, um zu klären, ob diese Gruppe eine gut unterstützte Gruppe ist und ob die einzelnen Gattungen monophyletisch sind. Dazu wurden Abschnitte der häufig verwendeten mitochondrialen Gene *12S rRNA, 16S rRNA* und c*ytochrome b* (zusammen 2045 bp) sowie die nukläeren Gene *c-mos* und *RAG-1, PRLR, KIF24, EXPH5* und *RAG-2* (zusammen 4473 bp) sequenziert.

Aus dem kombinierten Datensatz ergaben sich an einigen Knotenpunkten erheblich höhere Unterstützungswerte. Die Verwandtschaftsbeziehungen zwischen den fünf Hauptlinien des „südafrikanischen Clades" konnten jedoch auch mit diesem großen Datenmaterial nicht vollständig aufgelöst werden. Wir interpretieren dies als „Harte Polytomie", die auf eine schnelle Aufspaltungsfolge innerhalb des südafrikanischen Clades zurückzuführen ist. Der zusammenfassende Baum, der auf neun Genen basiert, zeigt gute Unterstützung für den „südafrikanischen Clade" sowie das Schwestergruppenverhältnis zum „ostafrikanischen Clade". Unsere Ergebnisse bestätigen die Gattung *Tropidosaura* als ein Monophylum, während die Gattung *Ichnotropis* in unseren Stammbäumen als paraphyletisch erscheint: *Ichnotropis squamulosa* scheint näher verwandt mit *Meroles* als mit *Ichnotropis capensis*. Weiters scheint auch die Monophylie der Gattung *Meroles* fragwürdig. Basierend auf unseren Studien scheint es ratsam, *Ichnotropis squamulosa* von der Gattung *Ichnotropis* in die Gattung *Meroles* zu transferieren. Aber auch die zwei Arten der Gattung *Australolacerta* (*A. australis* und *A. rupicola*) sind nur sehr entfernt verwandt, und die Gattung erscheint ebenfalls paraphyletisch zu sein.

Schließlich wurde der Versuch unternommen, die phylogenetischen Verwandtschaftsbeziehungen mit paläoklimatischen Daten zu verknüpfen und ein phylogeographisches Szenario zu entwerfen und dieses mit bereits früher postulierten Hypothesen zu vergleichen.

## ENGLISCHE ZUSAMMENFASSUNG / 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) consists 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 the present 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* and *RAG-1*, *PRLR*, *KIF24*, *EXPH* and *RAG-2* (altogether 4473 bp).

The combined data set increased the support values for several nodes considerably. However, 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 comprehensive 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 possibly paraphyletic, too.

Finally we propose a phylogeographic scenario in the context of paleoclimatic data and compare it with a previously postulated hypothesis.

# CURRICULUM VITAE

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## AUSBILDUNG

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Diplomstudium Chemie 2005 - 2006

Universität Wien

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Diplomarbeit im Bereich Evolutionsbiologie 2006 - 2012

## BERUFSERFAHRUNG

Biologie Zentrum Linz

Ferialpraktikum 2008

Naturhistorisches Museum Wien

Projektmitarbeiterin in diversen Projekten 2011 - 2012

Femtech Praktikum (1 Monat) gefördert vom

Bundesministerium für Verkehr, Innovation und Technologie 2012

## PUBLIKATIONEN

### ZEITSCHRIFTENARTIKEL

Kirchhof, S., Engleder, A., Mayer, W., Richter, K. (2011) - **Die Radiation der Lacertiden des südlichen Afrikas**. - elaphe, Rheinbach, 19 (4): 6-11.

### ABSTRACTS

Engleder Anja (2011) **Phylogenie südafrikanischer Lacertidae. -** Vortragsreihe „Molekulare Systematik“, Naturhistorisches Museum, Wien (A). - Vortrag

Engleder A., Haring E., Kirchhof S., Mayer W. (2011) **Phylogeny of South-African Lacertidae**. - NOBIS 5, Salzburg (A). - Poster

Engleder Anja (2012): **Die Lacertiden des südlichen Afrika - eine phylogenetische Studie anhand von DNA-Sequenzen**.- Jahrestagung der DGHT-AG, Gersfeld (D). - Vortrag

Engleder A., Haring E., Kirchhof S., Mayer W. (2012) **Phylogenetic relationships within the "South African Clade" of Lacertidae (Squamata). -** 7. Symposium des Departments für Evolutionsbiologie, Universität Wien (A). - Poster

## ZUSÄTZLICHE KENNTNISSE UND INTERESSEN

### SPRACHEN

Deutsch (Muttersprache)

Englisch

### EDV-KENNTNISSE

MS-Office: Word, Excel, Power Point

Adobe: Photoshop, Illustrator

sonstige
Programme: MEGA, PAUP, RAxML, BEAUTI, BEAST, Bioedit, ClustalX, jModeltest, MrBayes

### INTERESSEN

Herpetologie

Evolutionsbiologie

Phylogenetische Systematik

### MITGLIEDSCHAFTEN

Österreichische Gesellschaft für Herpetologie (ÖGH)

Network Of Biological Systematics Austria (NOBIS)

Wien, Oktober 2012